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Y E A S T

A Newsletter for Persons Interested in Yeast

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

Editorials

Anna Kocková-Kratochvílová - 1915-1992

Recently, we announced the retirement of Dr. Anna Kocková-Kratochvílová, who served for many years as Associate Editor of the Yeast Newsletter. We now announce with sadness her recent passing, on July 22 1992. An obituary has been prepared by Dr. Peter Biely.

Eighth ISY, Atlanta, Georgia

The 8th International Symposium on Yeast, held in Atlanta, Georgia, was an unqualified success. In addition to a choice selection of plenary speakers, concurrent sessions, and poster presentations, participants were treated to the warm hospitality, traditions and folklore of the American South. Congratulations to Sally Meyer for such a marvellous achievement.

Former students of Leslie Hedrick

We would be most appreciative if readers familiar with the whereabouts of John J. Bona, Kathleen Ann Killick, Rosemarie Meyer, or Elda Tsilenis could inform us of their current address. This would enable the family of the late Dr. Hedrick to return to them a second copy of their thesis.

Subscription

In order to catch up with ever increasing costs of postage, we are compelled to introduce a modest increase to the basic subscription cost. The 1993 rate will be USD$8.00 with an optional supplement of $4.00 for airmail (outside Canada or the United States). Readers are encouraged to take advantage of the savings incurred by subscribing for up to 5 years in advance.

Mailing lists

At the General Meeting of the International Commission on Yeasts, it was agreed that the Yeast Newsletter mailing list would be appended to a forthcoming issue to facilitate communication among readers. The current mailing list can be found at at the end of this issue. We have also included a list of electronic addresses of researchers interested or directly involved in the determination of the sequence of chromosome I in *Saccharomyces*. Anyone interested in this project should contact Francis Ouellette at his electronic address. YNL readers who have not yet connected their computer to an e-mail network are encouraged to do so whenever possible. The lost cost, reliability, and high speed of communication afforded by e-mail make it the unrivalled communication vehicle of the future.

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 of the ATCC.

Name	ATCC#	Depositor & Strain		Significance & Reference
Candida guilliermondii	76759	R. Indrati, Y4		Production alcohol dehydrogenase (1992. Can. J. Microbiol. **37**)
Candida parapsilosis	90018	A. Espinel-Ingroff, 7312/52.493		Taxonomy (1992. Espinel-Ingroff, A., et al.,)
Cryptococcus albidosimilis	76863	C. Kurtzman,	NRRL Y-17463	Taxonomy
Hansenula polymorpha	76760	M. Veenhuis,	mutant 125-2E	Peroxisome deficient mutant (1991. Arch. Microbiol., **156**:15-23)
Kluyveromyces bacillisporus	90019	A. Lachance,	UWO(PS)85-349.2	Taxonomy (1993. Int. J. Syst. Bacteriol. **43**: in press)
Saccharomyces castellii	76901	D. Yarrow,	CBS 4309	Taxonomy
Saccharomyces paradoxus	76856	D. Yarrow,	CBS 432	Taxonomy
	76857		CBS 2980	
	76858		CBS 5829	
	76859		CBS 7400	
Trichosporon asahii	90039	M. Smith,	CBS 2479	Taxonomy (1992. Antonie van Leeuwenhoek **61**:289-316)
Trichosporon aquatile	90044	M. Smith,	CBS 5973	Taxonomy (ibid.)
Trichosporon asteroides	90043	M. Smith,	CBS 2481	Taxonomy (ibid.)
Trichosporon coremiiforme	90042	M. Smith,	CBS 2482	Taxonomy (ibid.)
Trichosporon gracile	90038	M. Smith,	CBS 8189	Taxonomy (ibid.)
Trichosporon faecale	90041	M. Smith,	CBS 4828	Taxonomy (ibid.)
Trichosporon laibachii	90037	M. Smith,	CBS 5790	Taxonomy (ibid.)
Trichosporon loubieri	90036	M. Smith,	CBS 7065	Taxonomy (ibid.)
Trichosporon jirorecii	90047	M. Smith,	CBS 6864	Taxonomy (ibid.)
Trichosporon moniliiforme	90045	M. Smith,	CBS 2467	Taxonomy (ibid.)
Trichosporon montevideense	90035	M. Smith,	CBS 6721	Taxonomy (ibid.)
Trichosporon mucoides	90046	M. Smith,	CBS 7625	Taxonomy (ibid.)
Trichosporon ovoides	90040	M. Smith,	CBS 7556	Taxonomy (ibid.)
Yamadazyma farinosa	90009	H. Ohta,	IAM 4682	Production of (s)-(1)-frontalin (1989. Tetrahedron **45**(17):5469-5476)
Yarrowia lipolytica		C. Gaillardin,		Genetic studies
	76861	INAG 34168; 15901-4		(1991. Yeast **7**: 25-36)
	76862	INAG 33122; 21101-9		
	76982	INAG 33483		

Publications:

1. Molina, F.I., T. Inoue & S.C. Jong. 1992. Ribosomal DNA restriction analysis reveals genetic heterogeneity in *Saccharomyces cerevisiae* Meyen ex Hansen. Int. J. Syst. Bacteriol. **42**: 499-502.

2. Molina, F.I., T. Inoue & S.C. Jong. 1992. Restriction polymorphisms in the internal transcribed spacers and 5.8S rDNA of *Saccharomyces*. Curr. Microbiol. **25**: 251-255.

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

Recent acquisitions:

Cryptococcus podzolicus (Bab'eva & Reshetova) Golubev
7717 (VKM Y-2249) peat, USSR (killer strain); V.I. Golubev

Fellomyces horovitziae Spaaij et al.
7727 rotting wood, Sweden; O. Constantinescu

Filobasidiella neoformans Kwon-Chung var. *bacillispora* Kwon-Chung
7740 (CDC Y-579-92) cerebrospinal fluid, India; A.A. Padhye
7741 (CDC Y-586-92) cerebrospinal fluid, India; A.A. Padhye
7742 (CDC Y-587-92) cerebrospinal fluid, India; A.A. Padhye

Kluyveromyces piceae Weber & Spaaij
7738 rhizosphere of *Picea abies*, Germany; G. Weber
7739 rhizosphere of *Picea abies*, Germany; G. Weber

Kluyveromyces waltii Kodama
7703 either fruit or leaf of fruit tree; H.M.C. Put

Malassezia furfur (Robin) Baillon
7705 skin of Man, Netherlands; E. Guley
7706 skin of Man, Netherlands; E. Guley
7707 skin of Man, Netherlands; E. Guley
7708 skin of Man, Netherlands; E. Guley
7709 skin of Man, Netherlands; E. Guley
7710 skin of Man, Netherlands; E. Guley

Metschnikowia zobellii (van Uden & Castelo-Branco) van Uden
7704 seaweed (*Ascophyllum nodosum*), Norway; J.T. Staveland

Rhodotorula glutinis (Fres.) Harrison
7715 (VKY Y-1133) water of river, Kazachstan (killer strain);
V.I. Golubev

Saccharomyces servazzii Capriotti
7721 faeces, Germany; R. Kappe

Sporidiobolus johnsonii Nyland
7718 (VKM Y-2291) soil, USSR (killer strain); V.I. Golubev

Sporidiobolus pararoseus Fell & STatzell-Tallman
7716 (VKM Y-1632) soil, USSR (killer strain); V.I. Golubev

Sporobolomyces lactophilus Nakase et al.
7723 (JCM 7594) branch of *Abies firma*, Japan; T. Boekhout
7724 (JCM 7597) branch of *Abies firma*, Japan; T. Boekhout
7725 (JCM 7596) branch of *Abies firma*, Japan; T. Boekhout

Trichosporon mucoides Guého & M.T. Smith
7722 nail, Netherlands; S. Tan

The following paper has been published recently.

1. T. Boekhout, J. van Gool, H. van den Boogert & T. Jille. 1992. Karyotyping and G-C composition as taxonomic criteria applied to the systematics of *Tilletiopsis* and related taxa. Mycol. Res. **95**:331-342

Electrophoretic karyotypes and nucleic acid base composition were studied in strains belonging to the heterobasidiomycetous genera *Tilletiopsis*, *Tilletiaria anomala*, *Entyloma* and *Tilletia*. Chrosomosomal banding patterns and mol% G+C provided additional criteria to distinguish between species. The taxonomic position of all described taxa of *Tilletiopsis* is discussed. Further evidence is provided of an ontogenetic relationship between *Tilletiopsis*-like anamorphs and members of the *Tilletiales*.

III. Chaire de Génétique moléculaire et cellulaire, Institut National Agronomique Paris-Grignon, F-78850 Thiverval-Grignon, France. Communicated by H. Heslot.

The following book has been published recently.

1. H. Heslot & C. Gaillardin. 1992. Molecular Biology and Genetic Engineering of Yeasts. CRC Press, 393 pp.

Yeast transformation
Yeast plasmids: the 2µm circle plasmid of *S. cerevisiae*
Yeast plasmids: linear and circular plasmids of *Kluyveromyces*
Transposable elements in yeasts
Transcriptional control of gene expression
Regulation of galactose genes of *S. cerevisiae*
Protein localization and processing in yeast
- Nuclear targeting
- Vacuolar targeting
- Mitochondrial targeting
- Peroxysomal targeting
- Integral membrane proteins

Protein secretion
- Main processes
- Mating pheromones
- Killer toxins
- *S. cerevisiae* invertase
- *S. cerevisiae* acid phosphatase

The ubiquitin system and the genetic control of protein half life
Heterologous expression and secretion

IV. BKM Institute for Biochemistry and Physiology of Microorganisms, Pushchino, Moscow Region 142292, Russia. Communicated by W.I. Golubev.

The following papers have been published recently.

1. Golubev, W.I. 1991. Taxonomic evaluation of mycocins produced by the basidiomycetous yeast *Cryptococcus podzolicus*. Mikrobiologiya **60**:115-121.

Two strains of *Cr. podzolicus* have been found to produce mycocins (killer toxins) the genetic determinants of which are evidently localized in chromosomes. The mycocins have fungicidal activity at acidic pH values and act against *Cryptococcus* spp. (including *Cr. neoformans*) and few basidiomycetous fungi of tremellaceous affinity. The killing patterns of *Cr. podzolicus* mycocins and the mycocins produced by *Cr. laurentii* and *Filobasidium capsuligenum* are considered in relation to the taxonomic problems in the genera *Cryptococcus* and *Candida*.

2. Golubev, W.I. 1992. Yeasts from phyllosphere of the Far-Eastern wildlife reserve "Kedrovaya pad". Siberian Biological Journal **2**:37-42.

Numbers of propagules of the micromycetes recovered from leaves of 24 plant species in September varied between 55,000 and 550,000 per g and the proportion of yeasts varied between 1% and 70%. Among 33 yeast species isolated *Cryptococcus laurentii* and *Rhodotorula fujisanensis* were dominant in the phyllospheric populations of 18 plant species. *Candida butyri*, *C. famata*, *C. krissii*, *Kluyveromyces marxianus* var. *dobzhanskii* or *Metschnikowia pulcherrima* prevailed on the phylloplane of the other 6 plant species. Such yeasts as *Sporobolomyces roseus*, *Cr. aerius* and the yeast phase of *Tremella fuciformis* occurred in the phyllosphere of many plants whereas *C. boleticola*, *C. fennica*, *C. oleophila*, *C. scottii*, *Candida* sp., *Cr. macerans*, *Cr. uniguttulatus*, *Hanseniaspora uvarum*, *Lodderomyces elongisporus*, *Mastigomyces quercuum* comb. nov., *Met. reukaufii*, *Pichia dispora*, *P. strasburgensis*, *Rh. glutinis*, *Rh. mucilaginosa*, *Sp. shibatanus*, *T. encephala* and *Trichosporon pullulans* were recovered from one or several plant species. Among *Cr. humicolus* and *Rh. glutinis* isolates, killer strains were found. *Cr. laurentii* isolates are heterogeneous in their sensitivity to killer toxins produced by the type strains of this species and of *Cystofilobasidium bisporidii*.

3. Golubev, W. I. 1991. Taxonomic evaluation of the fungistatic mycocins produced by the yeasts of *Rhodotorula minuta* complex. Mycologiya i Phytopathologiya **25**:481-486.

Two strains of the yeast *Rhodotorula pallida* which produce mycocins active against strains of this species and of *Rh. minuta* have been identified. These mycocins have almost identical killing patterns. They are thermolabile, sensitive to proteolysis and have activity at pH 3.5-5.0. The killer phenotype is cureless, and RNA or DNA plasmids were not detected. Sensitive cells treated with the mycocin stopped budding. The *Rh. zsoltii* type strain is resistant to *Rh. pallida* mycocin, but sensitive to the mycocin produced by *Filobasidium capsuligenum*, *Rh. pallida* and *Rh. minuta* differ by lactose assimilation and maximum temperatures of growth.

4. Golubev, W. I., 1992. Antibiotic activity and taxonomic position of *Rhodotorula fujisanensis* (Soneda) Johnson et Phaff. Microbiologicheski jurnal **54**:21-26.

A killer strain of *Rhodotorula fujisanensis* produces a mycocin which is active against both ustilaginaceous yeasts and some tremellaceous ones. The mycocin has fungicidal action at pH 4.0-8.0 at temperatures below 25°C. The attempts to cure killer phenotype were unsuccessful, and RNA or DNA plasmids were not detected. The killing pattern, the composition of carotenoids and extracellular polysaccharides, and the type of coenzyme Q do not allow to assign the species studied to the genus *Rhodotorula*.

V. Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms, 1 Doroznyi 1, Moscow 113545, Russia. Communicated by G. I. Naumov.

The following papers have been accepted recently.

1. G.I. Naumov, E.S. Naumova, R.A. Lantto,[1] E.J. Louis[2] & M. Korhola[1]. 1992. Genetic homology between *Saccharomyces cerevisiae* and its sibling species *S. paradoxus* and *S. bayanus*: electrophoretic karyotypes. Yeast **8**:599-612.

[1]Research Laboratories of the Finnish State Alcohol Company (Alko Ltd), POB 350, SF-00101 Helsinki, Finland.
[2]Rosenstiel Basic Medical Sciences Research Center and Department of Biology, Brandeis University, Waltham, Massachusetts 02254-9110, U.S.A.

Chromosomal DNAs of many monosporic strains of the biological species *Saccharomyces cerevisiae*, *S. paradoxus* and *S. bayanus* were analyzed using contour-clamped homogeneous electric field electrophoresis. Southern blot hybridization with eight cloned *S. cerevisiae* genes (*ADC1*, *CUP1*, *GAL4*, *LEU2*, *rDNA*, *SUC2*, *TRP1* and *URA3*) assigned to different chromosomes was used to study homology and chromosomal location of the genes in the three sibling species. A comparative study of *Ty1*, *Ty2* and telomere-associated Y'sequences having multiple chromosomal locations was also

done. Chromosome length polymorphism was found in cultured strains of *S. cerevisiae*. Wild *S. cerevisiae* and *S. paradoxus* strains yielded chromosome banding patterns very similar to each other. The karyotype pattern of *S. bayanus* was readily distinguishable from that of *S. cerevisiae* and *S. paradoxus*. Southern blot analysis revealed a low degree of homology between the *S. cerevisiae* genes studied and the corresponding *S. paradoxus* and *S. bayanus* genes. The number of chromosomes appears to be 16 in all three species.

2. G.I. Naumov, E.S. Naumova, H. Turakainen[1] & M. Korhola.[2] 1992. A new family of polymorphic metallothionein-encoding genes *MTH1* (*CUP1*) and *MTH2* in *Saccharomyces cerevisiae*. Gene **119**:65-74.

[1]Department of Genetics, University of Helsinki, Helsinki, Finland.
[2]Research Laboratories of the Finnish State Alcohol Company (Alko Ltd), POB 350, SF-00101 Helsinki, Finland.

By pulsed-field gel electrophoresis of chromosomal DNA and hybridization with a cloned *MTH1* (*CUP1*) gene, we determined the locations of metallothionein-encoding gene sequences on chromosomes in monosporic cultures of 76 natural strains of *Saccharomyces cerevisiae*. Most of the strains (68) exhibited a previously known location for the *MTH* sequence on chromosome (chr.) VIII. Seven strains (resistant or sensitive to Cu^{2+}) showed a *MTH* sequence in a new locus, *MTH2*, on chr. XVI. One strain carried an *MTH* locus on both chromosomes VIII and XVI. Restriction fragment and Southern blot analyses showed that the two *MTH* loci were very closely related. The strains displayed heterogeneity in the size and structure of their MTH2 locus. The length of the repeat unit of MTH2 varied: a 1.9-kb or 1.7-kb unit was found, instead of the 2.0-kb unit of the MTH1 locus. The most resistant strain (resistant to 1.2 mM CuS04) contained a 0.9-kb repeat unit in addition to those of 1.9 kb and 1.7 kb. All three sensitive (to over 0.3 mM $CuS0_4$) strains with an *mth2* locus had a repeat unit of 1.9 kb or 1.7 kb, suggesting the presence of at least two copies of the MTH2 gene, with one always being in the junction area outside of the repeat unit. A monogenic tetrad segregation of 2:2 was usually found in crosses of resistant *MTH2* and sensitive *mth2* strains. Hybrids between strains with different *MTH* loci in all combinations showed low ascospore viability, suggesting that the complete lack of an *MTH* locus may lead to the death of segregants on YPD medium. The *MTH1* and *MTH2* loci were exchangeable. Strains with a high level of Cu^{2+} resistance were also resistant to Cd^{2+}. However, these two properties did not cosegregate in heterozygotic hybrids.

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

The following papers have been published recently.

1. W.T. Starmer & V. Aberdeen. 1990. The nutritional importance of pure and mixed cultures of yeasts in the development of *Drosophila mulleri* larvae in *Opuntia* tissues and its relationship to host plant shifts. In: J.S.F. Barker et al., eds., Ecological and Evolutionary Genetics of *Drosophila*, Plenum Press, New York, pp. 145-160.

The mutualistic relationship between *Drosophila* and yeasts involves a number of interesting interactions. The primary benefit received by the yeast is transportation to new habitats by the adult drosophilids and transport within the habitat by foraging larvae. The yeasts in turn provide nutritional benefits to the larvae and adult drosophilids by providing essential nutrients. In some cases the yeast can detoxify an otherwise toxic environment for the larval or adult stages. Yeasts also appear to be involved in differential habitat use (i.e., larval foraging behavior, adult feeding and oviposition behavior) and mating behavior of adults. Results presented in this paper show that mixed cultures of yeasts in the larval environment provide greater benefits in terms of developmental speed or survival as compared to single cultures of yeasts. This biculture effect was shown to be dependent on the type of host plant tissue and the yeast community specific for that tissue. Observations on the strength of the biculture effect suggest that tissues with greater chemical complexity and deficiencies result in stronger biculture effects.

2. P.F. Ganter & W.T. Starmer. 1992. Killer factor as a mechanism of interference competition in yeasts associated with cacti.[1] Ecology **73**:54-67.

[1]School of Natural and Health Sciences, Barry University, 11300 Northeast Second Avenue, Miami Shores, Florida 33161, USA.

Many yeasts in the genus *Pichia* are killers (yeasts that secrete proteins toxic to other yeasts). The genus is subdivided into complexes comprised of species with similar morphologies, physiological abilities, and percentage of (guanine + cytosine) comprising their nuclear DNA. Here, we test the hypothesis that ecological factors influence the types of toxins produced by members of two *Pichia* complexes (the *Pichia kluyveri* and *Pichia opuntiae* complexes). Members of each complex are separated by geography or host range, but all live in decaying cactus tissue. Similarities among killer toxins were investigated by testing strains from each complex in the laboratory for their ability to kill a standard set of 70 yeasts. Principal components analysis demonstrated that the killer phenotype was constant within a species, but not within the complexes. The probability that a toxin would kill a particular yeast depended on some ecological characteristic of the yeasts tested as sensitives (*i.e.* the region, host plant, or habitat from which the yeasts were collected). We further tested the hypothesis that ecological factors influence killer phenotype by analyzing within-community patterns of killing. Killer strains from two different cacti were tested for their ability to kill a subset of the yeasts actually collected from the same pockets of rotting cactus tissue from which the killer strains were collected or from rot pockets in neighboring cacti. When the yeasts tested for sensitivity were classified a priori by their neighboring cacti. When the yeasts tested for sensitivity were classified a priori by their probability of interacting with each killer strain, it was shown that the sensitivity to killer toxin was correlated with the probability of interaction. Further, the yeast community composition of a rot pocket depended on the presence or absence of a killer strain in that pocket. We demonstrated that killer toxins could change community composition by growing strains of two sensitive yeasts in the presence of a killer or a non-killer strain of *P. kluyveri*. The killer strain reached a higher cell density than the non-killer strain in the presence of sensitive yeasts. The density of sensitive yeasts grown with a killer strain was lower than that of a strain grown with a non-killer strain of *P. kluyveri*.

3. W.T. Starmer, P.F. Ganter & V. Aberdeen. 1992. Geographic distribution and genetics of killer phenotypes for the yeast *Pichia kluyveri* across the United States. Appl. Environ. Microbiol. **58**:990-997.

Representative strains (n = 61) of the yeast *Pichia kluyveri* from across the United States were studied for their ability to kill 71 other strains (representing 25 species) of yeast. This survey showed killing activity in 69% of the *P.kluyveri* strains tested. More extensive analysis of killer activity of 197 *P. kluyveri* strains against strains of five tester species showed comparable activity (67% of strains tested). This activity was shown to be equally variable within localities, within regions, and across the continent. The genetic basis of the variability was ascertained by tetrad analysis and is most likely due to alleles segregating at three epistatic loci. Evidence or the idea that killer toxins have a role in excluding other yeasts from particular habitats is discussed.

VII. Department of Applied Microbiology, Chemical Center, University of Lund, P.O. Box 124, S-22100 Lund, Sweden. Communicated by B. Hahn-Hägerdal.

The following publications have appeared recently.

1. K. Skoog & B. Hahn-Hägerdal. 1990. Effect of oxygenation on xylose fermentation by *Pichia stipitis*. Appl. Environ. Microbiol. **56**:3389-3394.

The effect of oxygen limitation on xylose fermentation by *Pichia stipitis* (CBS 6054) was investigated in continuous culture. The maximum specific ethanol productivity (0.20 g of ethanol g dry weight^{-1} h^{-1}) and ethanol yield (0.48 g/g) was reached at an oxygen transfer rate below 1 mmol/liter per h. In the studied range of oxygenation, the xylose reductase (EC 1.1.1.21) and xylitol dehydrogenase (EC 1.1.1.9) activities were constant as well as the ratio between the NADPH and NADH activities of xylose reductase. No xylitol production was found. The pyruvate decarboxylase (EC 4.1.1.1) activity increased and the malate dehydrogenase (EC 1.1.1.37) activity decreased with decreasing oxygenation. With decreasing oxygenation, the intracellular intermediary metabolites sedoheptulose 7-phosphate, glucose 6-phosphate, fructose 1,6-diphosphate, and malate accumulated slightly while pyruvate decreased. The ratio of the xylose uptake rate under aerobic conditions, in contrast to that under anaerobic assay conditions, increased with increasing oxygenation in the culture. The results are discussed in relation to the energy level in the cell, the redox balance, and the mitochondrial function.

2. T. Lindén, J. Peetre & B. Hahn-Hägerdal. 1992. Isolation and characterization of acetic acid-tolerant galactose-fermenting strains of *Saccharomyces cerevisiae* from a spent sulfite liquor fermentation plant. Appl. Environ. Microbiol. **58**:1661-1669.

From a continuous spent sulfite liquor fermentation plant, two species of yeast were isolated, *Saccharomyces cerevisiae* and *Pichia membranaefaciens*. One of the isolates of *S. cerevisiae*, no. 3, was heavily flocculating and produced a higher ethanol yield from spent sulfite liquor than did commercial baker's yeast. The greatest difference between isolate 3 and baker's yeast was that of galactose fermentation, even when galactose utilization was induced, i.e., when they were grown in the presence of galactose, prior to fermentation. Without acetic acid present, both baker's yeast and isolate 3 fermented glucose and galactose sequentially. Galactose fermentation with baker's yeast was strongly inhibited by acetic acid at pH values below 6. Isolate 3 fermented galactose, glucose, and mannose without catabolite repression in the presence of acetic acid, even at pH 4.5. The xylose reductase (EC 1.1.1.21) and xylitol dehydrogenase (EC 1.1.1.9) activities were determined in some of the isolates as well as in two strains of *S. cerevisiae* (ATCC 24860 and baker's yeast) and *Pichia stipitis* CBS 6054. The *S. cerevisiae* strains manifested xylose reductase activity that was 2 orders of magnitude less than the corresponding *P. stipitis* value of 890 nmol/min/mg of protein. The xylose dehydrogenase activity was 1 order of magnitude less than the corresponding activity of *P. stipitis* (330 nmol/min/mg of protein).

3. J. Hallborn, M. Walfridsson, U. Airaksinen[1], H. Ojamo[1], B. Hahn-Hägerdal, M. Penttilä[1] & S. Keränen.[1] 1991. Xylitol production by recombinant *Saccharomyces cerevisiae*. Biotechnology 9:1090-1095.
[1]VTT Biotechnical Laboratory, P.0. Box 202, SF-02151 Espoo, Finland.

We obtained efficient conversion of xylose to xylitol by transforming *Saccharomyces cerevisiae* with the gene encoding the xylose reductase (XR) of *Pichia stipitis* CBS 6054. Comparison of the chromosomal and cDNA copies of the *XYL1* gene showed that the genomic *XYL1* contains no introns, and an XR monomer of 318 amino acids (35,985 D) is encoded by an open reading frame of 954 bp. The amino acid sequence of the *P. stipitis* XR is similar to several aldose reductases, suggesting that *P. stipitis* XR is part of the aldoketo reductase superfamily. *S. cerevisiae* transformed with the *XYL1* gene gave over 95% conversion of xylose into xylitol, a yield not obtainable with natural xylose utilizing yeasts.

VIII. Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, 842 38 Bratislava, Czechoslovakia. Communicated by P. Biely.

The following papers have been published by researchers of the Institute of Chemistry in the last 3 years.

1. A. Grabinska-Loniewska & E. Slaviková. 1990. Fungi in denitrification unit biocenosis. Wat. Res. **24**:565-572.

It has been found that yeasts and yeast-like microorganisms were the stable constituents in denitrification unit biocenoses fed with mineral medium with methanol, glycerol, acetic or lactic acids. Typical species for biocenosis fed with methanol, acetic acid and lactic acid were *C. boidinii*, *C. famata*, and *C. famata* and *Hansenula californica*, respectively. It is concluded that the unit mycoflora utilize C-sources for denitrification, can assimilate nitrates and fix N_2 produced during nitrate dissimilation and stimulate the growth of denitrifying bacteria.

2. B. Proksa & E. Slaviková. 1990. Chromatographic identification of yeast ubiquinones. Pharmazie **45**:936-937.

3. M. Vrsanska, J. Hirsch, P. Kovac & P. Biely. 1990. Hydrolysis of (1-3)- and (1-2)-ß-D-xylosidic linkages by an *endo*-(1-4)-ß-D-xylanase of *Cryptococcus albidus*. Carbohydrate Res. *206*:251-256.

The substrate specificity of an *endo*-(1-4)-ß-D-xylanase of the yeast *Cryptococcus albidus* was investigated using a series of methyl ß-D-xylotriosides. In addition to (1-4) linkages, the enzyme could cleave (1-3) and (1-2) linkages adjacent to a (1-4) linkage and further from the nonreducing end of the substrate. The enzyme could hydrolyse a (1-3) linkage that attached a terminal xylopyranosyl group to a (1-4)-linked xylobiosyl moiety.

4. E. Breierová, A. Kocková-Kratochvílová, J. Šajbidor & K. Ladzianska. 1991. *Malassezia pachydermatis*: properties and storage. Mycoses **34**:349-352.

Five strains of *M. pachydermatis* isolated from small animals in Bratislava were subcultured, maintained under sterile paraffin oil and preserved in liquid nitrogen. Morphological and physiological characteristics as well as the analysis of long chain fatty acids of the cell mass were determined. The results showed that the maintenance in liquid nitrogen is the most satisfactory method of preservation of pure cultures of *Malassezia pachydermatis*.

5. V. Kery, G. Kogan, K. Zajacová, K. Slamová, L. Masler & J. Alfldi. 1991. Hydrolysis of yeast cell-wall glucan by extracellular (1-3)-ß-glucanases from *Aspergillus niger*. Enzyme Microbiol. Technol. **13**:87-90.

An enzyme preparation containing (1-3)-ß-glucanase activity was prepared from a cultivation medium of industrial production of citric acid by *Aspergillus niger*. The preparation was used for enzymic hydrolysis of insoluble yeast cell-wall glucans into water-soluble fragments.

6. G. Kogan, V. Pavliak, J. Šandula & L. Masler. 1991. Structure of the pathogenic yeasts of *Candida* species - a complex insight. Carbohydrate Polymers *14*:65-76.

Cell wall D-mannans were isolated from all eight known pathogenic strains of genus *Candida*. Their structural characterization has been carried out by means of acetolysis and methylation analysis, as well as by ^{1}H- and ^{13}C-NMR spectroscopy. The results obtained point out some common features and peculiarities of the structures of the individual D-mannans. Differences observed in the structures of investigated mannans may be responsible for the differences in immunological properties of the corresponding yeast strains.

7. Z. Kossaczka, E. Machová & A. Vojtková-Lepsiková. 1991. D-xylose metabolism in *Aureobasidium pullulans*. Effects of aeration and vitamins. Appl. Microbiol. Biotechnol. **36**:375-378.

The yeast-like organisms *A. pullulans* converted efficiently D-xylose to a cell mass ($Y_{X/S}$ = 0.45 $g.g^{-1}$) with a negligible production of polyols ($Y_{P/S}$ = 0.003 $g.g^{-1}$) under the aerobic conditions. *A. pullulans* grown semiaerobically exhibited different fermentation capacity in a basal (vitaminless) medium and medium containing a mixture of 7 vitamins. It was found that under semiaerobic conditions a mixture of vitamins significantly enhanced the production of ethanol from D-xylose resulting in the 15-fold higher yield coefficient of ethanol ($Y_{E/S}$ = 0.22 $g.g^{-1}$) as compared to that achieved in the vitaminless medium. This increase of the ethanol production was accomplished at the expense of the cell mass.

8. E. Slaviková, R. Kovacovska & A. Kocková-Kratochvilová. 1991. The incidence of yeast organisms in the water of the artificial lake in Jakubov (Slovakia). Res. Mykol. **45**:103-111.

The present paper reports the results of quantitative and qualitative investigation of yeast population in fresh-water of the lake Jakubov, located in the middle of the Lowland of Zahorie. The lake is used mostly for recreational purposes during summer months. Representatives of the genera *Candida*, *Hansenula*, *Aureobasidium*, and *Rhodotorula* occurred the most frequently. The occurrence of yeast species at the beginning of the tourist season was 2 times heterogenous than after one. Isolated strains are characterized by some physiological features.

9. L. Stankovic & R. Kovacovska. 1991. Production of alditols from D-xylose by yeasts. Folia Microbiol. **36**:542-548.

Production of glycerol, tetritols, pentitols, hexitols and heptitols was tested with 193 strains of yeasts and yeast-like organisms belonging to 13 genera. According to the production of alditols, the yeast species were divided into four groups. The largest group consisted of pentitolproducing yeasts. Only few species produced glycerol, tetritol and hexitol. Production of heptitols was found mainly in sporulating yeasts.

10. J. Defaye, J.-M. Guillot, P. Biely & M. Vrsanska. 1992. Positional isomers of thioxylobiose, their synthesis and inducing ability for D-xylan-degrading enzymes in the yeast *Cryptococcus albidus*. Carbohydrate Res. **228**:47-64.

2-Thioxylobiose, 3-thioxylobiose, and 4-thioxylobiose were prepared and tested as inducers of the xylan-degrading enzymes in the yeast *Cr. albidus*. Of the three analogues only 4-thioxylobiose was found to be biologically active. 4-Thioxylobiose has a highly stimulatory effect on the synthesis of enzymes of the xylanolytic system when applied to the cells in the presence of the natural disaccharide inducer (1-4)-ß-D-xylobiose.

11. E. Machová. 1992. Induction of aldose reductase and polyol dehydrogenase activities in *Aureobasidium pullulans* by D-xylose, L-arabinose, and D-galactose. Appl. Microbiol. Biotechnol. **37**:374-377.

The induction of aldose reductase and polyol dehydrogenase activities by D-xylose, L-arabinose, D-galactose and D-glucose was studied in the yeast-like organism *Aureobasidium pullulans* CCY27-1-26. D-xylose and L-arabinose induced two distinct NADPH-dependent aldose reductases and the inducing saccharide was simultaneously the most efficient substrate for corresponding enzymatic reaction. Polyol dehydrogenase induced by D-xylose, L-arabinose and D-galactose was strictly NAD^+dependent and required only xylitol as substrate of enzymatic reaction. L-arabitol did not act as substrate for L-arabinose induced polyol dehydrogenase neither in the presence of NAD^+ and $NADP^+$.

12. E. Breierová & A. Kocková-Kratochvilová. 1992. Cryoprotective effects of yeast extracellular polysaccharides and glycoproteins. Cryobiology **29**:385-390.

Eighteen yeast strains were tested for their capacity to survive the freeze-thaw process while being cryoprotected. Cryoprotection was accomplished by combining penetrating and nonpenetrating cryoagents. Four nonpenetrating (two extracellular polysaccharides of yeast and two extracellularglycoproteins of yeast) and two penetrating agents were used together with the nutritive-rich medium. Eight different mixtures were tested. The highest survival rate was obtained with glycoproteins of *Rhodosporidium toruloides* together with DMSO and nutritive-rich medium.

13. B. Proksa, E. Slaviková & S. Uhrinová. 1992. Transformation of D-xylose by *Sporobolomyces lactosus*. Acta Biotechnol. **12**:349-350.

The new yeast species, *Sporobolomyces lactosus*, transformed D-xylose into a mixture of disaccharides. Hydrolysis of this mixture yielded D-glucose only. The main component of disaccharide mixture was α,α,-trehalose.

14. E. Slaviková, A. Grabinska-Loniewska. 1992. *Sporobolomyces lactosus*, a new species of ballistosporous yeast equipped with ubiquinone-10. Antonie van Leeuwenhoek **61**:245-248.

A new species of ballistospore-forming yeasts was recovered and its description given. *Sporobolomyces lactosus*, isolated from activated sludge treating petrochemical wastes, produces pinkish-coral to pink colored colonies, assimilates lactose and has Q-10 as the major ubiquinone.

15. E. Slaviková, R. Vadkertiová & A. Kocková-Kratochvilová. 1992. Yeasts isolated from artificial lake waters. Can. J. Microbiol. (in press).

IX. 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 published recently.

1. Webb, S.R. & H. Lee. 1992. Characterization of xylose reductase from the yeast *Pichia stipitis*: evidence for functional thiol and histidyl groups. J. Gen. Microbiol. **138**:1857-1863.

Xylose reductase (E.C.1.1.1.21) from the yeast *Pichia stipitis* NRC 2548 was purified to homogeneity via a two-step protocol employing anion-exchange and gel-filtration chromatography. The pH-activity profile revealed the presence of two ionizable groups with pK_{app} values of 5.8 and 8.1, suggesting the involvement of histidyl and thiol groups, respectively. Additional evidence supporting the involvement of these residues was provided by the use of group-specific inhibitors. The enzyme was rapidly inactivated in a pseudo-first order manner by the sulphhydryl-specific modifier p-chloromercuriphenylsulphonate (PMBS) and analysis of the order-of-reaction suggested that one essential cysteine residue was modified to effect inactivation. Treatment of the enzyme with another thiolspecific modifier, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), showed that modification of one cysteine per monomer led to 90% loss of activity, further supporting the existence of one essential cysteine residue. Inactivation by PMBS was reversed by adding 1 mM ß-mercaptoethanol. Inactivation of xylose reductase by the histidine-specific modifier diethylpyrocarbonate (DEP) followed a pseudo-first order process, and analysis of the order-of-reaction suggested that one essential histidine residue was modified to effect inactivation. Treatment of DEP-inactivated enzyme with 0.2 M neutral hydroxylamine resulted in the recovery of 45% of enzyme activity. Protection of xylose reductase from PMBS- and DEP- inactivation was provided by NADPH and NADH but not by NADP, D-xylose or DL-glyceraldehyde. This suggests that the essential histidine and cysteine residues may be involved with binding of cofactor by the *P. stipitis* xylose reductase.

X. 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. Kronlöf, J. & Haikara, A. 1992. Contamination of immobilized yeast bioreactors. J. Inst. Brew. **97**:375-380.

2. Enari, T.-M., Nikkola, M.J., Suihko, M.-L., Penttilä, M.E., Knowles, J.K.C. & LehtovaaraHelenius, P.M. 1992. Process for accelerated beer production by integrative expression in the PGK1 or ADC1 genes. Oy Panimolaboratorio-Bryggerilaboratorium Ab. U.S. Patent US 5,108,925. 28. Apr. 1992. 46 p.

3. Enari, T.-M. 1992. Genetic modification of food and beverage yeast. In: Recombinant DNA Technology I. Prokop, A. & Bajpai, R.K., eds., Annals of the New York Academy of Sciences (New York) **646**:181-192.

4. Kronlöf, J. & Linko, M. 1992. Production of beer using immobilized yeast encoding α-acetolactate decarboxylase. J. Inst. Brew. **98**:479-491.

XI. Department of Molecular Genetics and Microbiology, UMDNJ-Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Room 705, Piscataway, New Jersey 08854-5635, U.S.A. Communicated by M.J. Leibowitz.

The following two papers have been published recently.

1. Y. Liu, M. Rocourt, S. Pan, C. Liu & M. J. Leibowitz. 1992. Sequence and variability of the 5.8S and 26S rRNA genes of *Pneumocystis carinii*. Nucleic Acids Res. **20**:3763-3772.

The sequence of the coding region of the rRNA operon of rat-derived *Pneumocystis carinii* has been completed, including the genes for 5.8S and 26S rRNA. These genes show homology to the rRNA genes of yeast, and an apparent group I self-splicing intron is present in a phylogenetically conserved region. Variation in the 26S rRNA sequence was noted between *P. carinii* organisms isolated from two different sources.

2. C. Tung, Z. Wei, M.J. Leibowitz & S. Stein. 1992. Design of peptide-acridine mimics of ribonuclease activity. Proc. Natl. Acad. Sci. U.S.A. **89**:7114-7118.

A series of peptide-acridine conjugates was designed and synthesized, based on three features of the proposed catalytic mechanism of RNase A: 2'-proton abstraction by His-12, proton donation to the leaving 5'-oxygen by His-119, and stabilization of the pentacoordinated phosphorous transition state by Lys-41. The substrate binding capability of RNase A was mimicked by the intercalator, acridine. Lysine served as a linker between acridine and the catalytic tripeptide. Cleavage of target RNA was monitored by agarose gel electrophoresis and by gel-permeation chromatography. The carboxyl-amidated conjugates HGHK(Acr)-NH2, HPHK(Acr)-NH2, and GGHK(Acr)-NH2 (where Acr indicates 2-methyl-9-acridinemethylene) all had similar hydrolytic activity. The catalytic mechanism most likely involved only the abstraction of the 2'-proton and stabilization of the transition state. These RNase mimics utilized rRNA and single-stranded RNA but not doublestranded RNA and tRNA as substrates.The following paper was submitted to Yeast recently.

3. L. A. Weinstein, F. Capaldo-Kimball & M. J. Leibowitz. Genetics of heat-curability of killer virus of yeast.

The cytoplasmically-inherited M dsRNA genome segment of killer virus of *Saccharomyces cerevisiae* is heat-curable in some yeast strains but not in others. Temperature sensitivity is conferred on M1 and M2 dsRNA satellite virus segments by the L-A-HN allele of the killer helper virus genome, but not by the L-A-H allele. Both diploidy and mating type heterozygosity of the host cell are also correlated with increased virus curability.

XII. University of Agriculture, Institute of Applied Microbiology, School of Food & Biotechnology, Nussdorfer Laende 11, A-1190 Vienna, Austria. Communicated by H. Prillinger.

The following papers have been accepted recently.

1. H. Prillinger, F. Oberwinkler, C. Umile, K. Tlachac, R. Bauer, C. Doerfler & E. Taufratzhofer. In press. Analysis of cell wall carbohydrates (neutral sugars) from ascomycetous and basidiomycetous yeasts with and without derivatization. J. Gen. Appl. Microbiol.

The presence or absence of fucose, galactose, rhamnose, and xylose as well as the ratio of glucose to mannose after hydrolysis of purified yeast cell walls are valuable characters to assign yeasts or yeast states of Ascomycetes and Basidiomycetes phylogenetically. The coupling of pellicular anion exchange resins (Dionex CarboPac Pa-1) with pulsed

amperometric detection provides a simple, quick, selective, and sensitive method for the analysis of yeast cell wall carbohydrates. Phragmobasidial smut fungi of monocotyledonous (*Ustilago* s. str., *Sporisorium*) and dicotyledonous *Microbotryum*, *Sphacelotheca*) host plants cluster in two different, phylogenetically distinct yeast types, the *Microbotryum* type and the *Ustilago* type. In contrast, all smut fungi with simple holobasidia (*Entyloma*, *Melanotaenium*) from monocots and dicots investigated so far, exhibit a cell wall carbohydrate spectrum characteristic for the *Ustilago* type. *Ustilentyloma fluitans*, although a phragmobasidial smut fungus on grasses, whose smut spores and parasitic symptoms resemble *Entyloma* species, display the neutral sugar pattern of the *Microbotryum* type. The close phylogenetic relationship between the Graphiolales, Ustilaginales s. str. (phragmobasidial smuts of monocots), and Exobasidiales was substantiated further by additional strains. The presence of xylose and balanced amounts of glucose and mannose is characteristic for yeast states of the Dacrymycetaceae. The production of extracellular amyloid compound (EAC) as well as the cell wall carbohydrate pattern point to a *Tremella* type affinity of *Atractogloea stillata*, *Itersonilia perplexans*, and *Sterigmatosporidium polymorphum*. A meiosporangial evolution starting from coccal yeast basidia (*Sterigmatosporidium*) via transversely (auricularioid) septate (*Atractogloea*) to longitudinally divided phragmobasidia (*Tremella*) and simple holobasidia (*Cystofilobasidium*) was substantiated further within the *Tremella* type.

2. H. Prillinger, J. Altenbuchner, G. Laaser & C. Doerfler. In press. Yeasts isolated from Homobasidiomycetes (*Asterophora*, *Collybia*): New aspects for sexuality, taxonomy, and speciation. Exptl. Mycol.

A positive selection vector to clone fungal n-DNA in *E. coli* together with random-fragment hybridization analysis turned out to be useful tools to clarify the taxonomic relationship between the yeast and hyphal strains from Asterophora lycoperdoides and *A. parasitica* on the one hand, and to confirm conspecificity of the *Collybia* yeasts (*C. cirrata*, *C.* cf. *cookei*, and *C. tuberosa*) and to detect processes of speciation on the other. The rapid loss of sexual compatibility between the yeast and hyphal strains from *Collybia* accompanied by a distinct RFLP with a few n-DNA probes offers the possibility to investigate early stages of speciation in the dimorphic *Collybia* species. Although the species-specific intracellular yeasts isolated from two *Asterophora* species exhibited a remarkable degree of nDNA/nDNA reassociation and an identical ubiquinone (Q-9) system, conspecificity as well as a suspected partial identity of the nDNA from *Asterophora* yeasts and hyphae could be excluded. The conspecificity of the *Collybia* yeasts and hyphae, already established by morphological data and rDNA analysis could be confirmed using homologous and heterologous hybridization experiments performed with Southern blotted nDNA digested with different restriction endonucleases and hybridized with cloned 3-10 kb *Bam*H1 nDNA fragments from yeasts and hyphae as a probe. The physiological characterization of the *Collybia* yeasts displayed a remarkable variability. The qualitative and quantitative monosaccharide pattern of purified yeast cell walls exhibited some similarities to tremelloid fungi. Data are discussed in the light of taxonomy, mating-type evolution, and speciation.

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

The following papers have been published or accepted for publication recently.

1. P. Holloway,[1] R.A. van Twest,[1] R.E. Subden[1] & M.A. Lachance. 1992. A strain of *Candida stellata* of special interest to oenologists. Food Res. Internat. **25**:147-146.
[1]Department of Microbiology, Guelph University, Guelph, Ontario, Canada N1G 2W1.

One of the most commonly found wild yeasts in musts of the Niagara region of Ontario, Canada, is highly fermentative and is resistant to control with SO_2. Previous attempts to classify the organism have been inconclusive. An extensive re-examination of the assimilation data and the use of clamped hexagonal electric field electrophoresis to derive a karyotype indicated that the strains are metabolic and morphological variants of *Candida stellata*.

2. H.J. Phaff, W.T. Starmer, M.A. Lachance, V. Aberdeen & J. Tredick-Kline. 1992. *Pichia caribaea*, a new species of yeast occurring in necrotic tissue of cacti in the Caribbean area. Int. J. Syst. Bacteriol. **42**:459-462 (See abstract in H.J. Phaff's communication).

3. M.A. Lachance, H.J. Phaff,[1] & W.T. Starmer.[2] In press. *Kluyveromyces bacillisporus* sp. nov., a Yeast from Emory Oak Exudate. Int. J. Syst. Bacteriol. **43**.

[1]Department of Food Science and Technology, University of California, Davis Ca 95616, USA;
[2]Department of Biology, Syracuse University, Syracuse NY 13244, USA.

Three strains of a new diploid species of *Kluyveromyces* van der Walt emend. van der Walt were isolated from exudates of Emory oak (*Quercus emoryi*) trees in Arizona. The physiological characteristics and nuclear DNA base composition (38 mol% G+C) of the isolates resemble most those of *K. africanus* and *K. delphensis*, but the three taxa are genetically unrelated as shown by DNA reassociation and rDNA restriction mapping. Morphology is typical of *Kluyveromyces*, with the difference that the mature ascospores (4 and occasionally more) are characteristically bacilliform rather than spheroidal, ellipsoidal or reniform. The mitochondrial DNA (21 mol% G+C) is present in unusually large proportions (*ca*. one half of total DNA). The type strain of *K. bacillisporus* is strain UWO(PS)85-349.2 (ATCC 90019, CBS 7720).

4. R. Shen & M.A. Lachance. In press. Phylogenetic study of ribosomal DNA of cactophilic *Pichia* species by restriction mapping. Yeast **9**.

The rDNAs of strains of the cactophilic *Pichia* species *P. amethionina*, *P. antillensis*, *P. barkeri*, *P. cactophila*, *P. caribaea*, *P. deserticola*, *P. heedii*, *P. kluyveri*, *P. norvegensis*, *P. opuntiae*, *P. pseudocactophila*, *P. thermotolerans*, and their varieties and anamorphs were mapped with 15 restriction endonucleases, and compared to *P. membranaefaciens* and *P. salictaria* as possible non-cactophilic relatives. The existence of species complexes among those taxa was confirmed. *P. membranaefaciens* was a plausible ancestral species, and its closest relative in the cactophilic group was *P. deserticola*. These two species appeared to be moderately related to *P. heedii* and to *P. barkeri*, but the latter was shown clearly to belong to the *P. kluyveri* complex, in spite of a 6 mol% G+C difference in their nuclear DNAs. *P. cactophila* and *P. pseudocactophila* ostensibly emerged from *P. norvegensis*, a facultatively cactophilic yeast. The *P. amethionina*, *P. cactophila*, and *P. opuntiae* species complexes appeared independent from one another and from all other species studied. *P. salictaria* did not appear to be related to *P. amethionina*.The following communications were presented at the 8th ISY, Atlanta, Ga, in August 1992.

5. J.E. Kaden & M.A. Lachance. 1992. Taxonomy of the *Sporopachydermia cereana* complex.

Sporopachydermia Rodrigues de Miranda 1978 is an ascomycetous yeast genus currently including *S. lactativora*, *S. cereana*, and *S. quercuum*. These three species are clearly delimited on the basis of morphological, physiological, and ecological traits. However, strains which have been identified as *S. cereana* appear to represent a complex of several species as evidenced by the significant variation observed in their base composition (Phaff, personal communication). Among 19 strains identified as *S. cereana*, 8 strains (including the type) had GC values approximating 49%, 9 strains had GC values of approximately 42%, and 2 strains had distinct values of 43.5 and 45% GC. By DNA reassociation studies, Phaff identified as many as five genetically distinct subsets among those 19 strains. These data reveal that many strains included in the taxon *S. cereana* may in fact be members of a distinct species. In an effort to elucidate the species boundaries within the *S. cereana* complex, electrokaryotyping using pulsed-field gel electrophoresis (CHEF) and novel physiological tests were performed. In addition we have identified a killer system that operates within the *S. cereana* complex, and which correlates well with mating patterns observed when strains are mixed in pairs on agar media. Chromosome numbers or patterns of chromosomal bands, growth test responses, killer susceptibility patterns, and mating reactions all failed individually to distinguish between the genetically distinct groups within *S. cereana*. However, taken together, electrokaryotypes, ß-glucoside assimilation, thallism, and maximum temperatures of growth followed a trend that opposes the 42% GC strains and the 49% GC strains. The formal description of a new species encompassing the 42% GC strains is forthcoming.

6. M.A. Lachance. 1992. *Kluyveromyces:* Systematics Since 1970.

The taxonomy of *Kluyveromyces* has been the object of intense study since van der Walt's (1970) monograph. Highlights of major developments, and the classification to be adopted in the 5th edition of THE YEASTS are outlined.

PRE-ZYGOTIC ISOLATION AND THE SPECIES CONCEPT. Reproductive isolation is easily recognized in heterothallic, haplontic yeasts. *Kluyveromyces* consists mostly of homothallic species and has served as testing ground for competing views on what constitutes a biological species in self-fertile yeasts. The focus of controversy was a group of species among which some genetic interaction was demonstrated by prototrophic selection (Johannsen 1980). Based on this criterion, van der Walt & Johannsen (1984) extended the reach of *K. marxianus* to include many formerly recognized species as either synonyms or varieties. The fusion did not take into account an important DNA reassociation study (Bicknell & Douglas 1970) which showed that hybrids between *K. fragilis* (syn. *K. marxianus*) and *K. lactis* or *K. dobzhanskii* were in fact allopolyploids, and that these 3 species were genetically distinct. van der Walt and Johannsen (1984) yet held as a separate species *K. wickerhamii*, in spite of its ability to hybridize with putative varieties of *K. marxianus*, and disregarded the weak hybridization between *K. marxianus* and *K. waltii* or *K. thermotolerans*. *K. aestuarii*, in spite of its great similarity to *K. lactis*, did not hybridize with the latter. This is surprising as *K. aestuarii* reacts to the α mating factor of *K. lactis* and forms zygotes with haploid cells of that mating type (Herman, 1970). The studies summarized above thus reaffirm the complexity of the speciation process and its consequences.

SPECIES AS GENETICALLY DISTINCT POPULATIONS. The ability to form zygotes or even to produce ascospores may not always preclude genetic isolation. In homothallic yeasts the identification of species boundaries consequently requires an appraisal of post-zygotic isolation. Sidenberg & Lachance (1986), having reviewed earlier studies in the light of their isoenzyme analyses, favoured the fusion of some taxa with *K. marxianus* and others with *K. lactis*, and the maintenance of *K. dobzhanskii* and *K. wickerhamii* as separate species. They further proposed the retention of the varietal epithet *drosophilarum* for lactose-negative members of *K. lactis*. This was met with strong criticism by Naumov (1988), who preferred the preservation of *K. drosophilarum* and *K. phaseolosporus* (another synonym of *K. lactis*) as separate species, and deplored the integration of *K. vanudenii* into the variety *drosophilarum*, and not *lactis*. The more recent DNA reassociation studies of Fuson et al. (1987) and Vaughan Martini & Martini (1987) not only confirmed the species delineation suggested by Sidenberg & Lachance (1986), but lent further assurance that *K. lactis* is genetically complex, with no obvious boundary between its possible varieties. I shall therefore retain *K. lactis* as a species with two varieties pending clarification of their inter-relationships.

IS *KLUYVEROMYCES* A "GOOD" GENUS? Were it to be demonstrated that the species currently assigned to *Kluyveromyces* in fact constitute a polyphyletic assemblage, serious consideration for taxonomic realignments might be fitting. Common sense and the need for nomenclatural stability both dictate that phenetically homogeneous, paraphyletic genera should be considered acceptable, and monophyletic genera, indissoluble. *Kluyveromyces* contains 3 phenetic groups, originally identified by Poncet (1973) by factor analysis. Group A comprises nutritionally restricted species, and group B holds species that resemble *K. lactis* and *K. marxianus*. Group C is limited to *K. thermotolerans* and *K. waltii*, both of which share many features with *Zygosaccharomyces*, and might be advantageously transferred to that genus when sufficient evidence is at hand. At present, no evidence exists for polyphyly (or paraphyly) in groups A and B considered as a whole, and even less so in group A alone. A proposal by Naumov (1987) would retain some group A species in *Kluyveromyces*, transfer other group A species with group B and C species to *Zygofabospora*, and erect a third genus to accommodate *K. phaffii* (a group A species). Clearly, I cannot concur with his proposal.

XIV. Department of Food Science and Technology, University of California, Davis, CA 95616, U.S.A. Communicated by H.J. Phaff.

The following paper has appeared recently.

1. H.J. Phaff, W.T. Starmer,[1] M.A. Lachance, V. Aberdeen[1] & J. Tredick-Kline. 1992. *Pichia caribaea*, a new species of yeast occurring in necrotic tissue of cacti in the Caribbean area. Int. J. Syst. Bacteriol. **42**:459-462. [1]Department of Biology, Syracuse University, Syracuse, New York 13212.

We describe *Pichia caribaea*, a new species of yeast which is closely related to *P. amethionina*. *P. caribaea*, of which 92 strains were isolated, is heterothallic and occurs in nature in both the haploid state and the diploid state. It

produces asci with four hat-shaped spores, which are gradually released upon maturity. *P. caribaea* occurs in rotting tissue of cereoid and opuntia cacti on various islands in the greater Caribbean area and on coastal land masses surrounding it. It resembles *P. amethionina* var.*pachycereana* in its assimilation pattern of carbon compounds but differs in its ability to ferment glucose strongly. The DNAs of *P. caribaea* and the two described varieties of *P. amethionina* slow about 40% complementarity. The type strain of *P. caribaea* is strain UCD-FST 81-52 (= ATTC 75713 = CBS 7592).

XV. Laboratoire de Microbiologie et de Technologie des Fermentations, Institut des Produits de la Vigne, INRA - 2 Place Viala, 34060 Montpellier Cedex 01, France. Communicated by P. Barre.

The aims of our laboratory (Director : P. Barre) are to cover all the aspects of alcoholic fermentation by *Saccharomyces cerevisiae* in oenological conditions. Research activities will be focused on four topics: (1) genome of wine industrial yeasts (F. Vézinhet); (2) Metabolic physiology of *Saccharomyces cerevisiae* in oenological conditions (J.M. Salmon); (3) Improvement of industrial *Saccharomyces cerevisiae* strains by classical genetics and molecular biology (S. Dequin, B. Blondin, C. Riou, V. Galéote, F. Vézinhet); (4) Microbial engineering (J.M. Sablayrolles).The following papers have been published since 1988:

Topic (1)

1. Blondin B. & Vézinhet F. 1988. Identification de souches de levures oenologiques par leur caryotypes obtenus en électrophorèse en champ pulsé. Revue Française d'Oenologie **28**:7-11.

2. Vézinhet F., Blondin B. & Hallet J.N. 1990. Chromosomal DNA patterns and mitochondrial DNA polymorphism as tools for identification of oenological strains of *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. **32**:568-571.

3. Aizac T., Delteil D. & Vézinhet F. 1991. Etude écologique de souches de levures contaminantes. Application de la technique d'identification par caryotypes. Revue Française d'Oenologie, **31**:16-20.

4. Vézinhet F., Hallet J.N., Valade M. & Poulard A. 1992. Ecological survey of wine yeast strains by molecular methods of identification. Amer. J. Enol. Vit. **43**:83-86.

5. Bidenne C., Blondin B., Dequin S. & Vézinhet F. 1992. Analysis of the chromosomal DNA polymorphism of wine strains of *Saccharomyces cerevisiae*. Curr. Genet. **22**:1-7.

Topic (2)

6. Salmon J.M., Gancedo C. & Pinon R. 1989. Isolation and characterization of mutants of *Saccharomyces cerevisiae* able to sporulate in the presence of glucose. J. Gen. Microbiol. *135*:203-209.

7. Salmon J.M. 1989. Effect of sugar transport systems inactivation in *Saccharomyces cerevisiae* on sluggish and stuck oenological fermentations. Appl. Environ. Microbiol. **55**:593-598.

8. Salmon J.M. 1991. Determination of malolactic enzyme activity using an immobilized L-lactate oxidase probe. Biotechnol. Techn. **5**:383-388.

9. Mauricio J.C. & Salmon J.M. 1992. Rapid spectrophotometric determination of the exponential constant of ethanol-enhanced proton diffusion in yeasts. Biotechnol. Techn. **6**:27-32.

10. Sentenac H., Bonneaud N., Minet M., Lacroute F., Salmon J.M., Gaymard F. & Grignon C. 1992. Cloning and expression in yeast of a plant potassium ion transport system. Science **256**:663-665.

11. Mauricio J.C. & Salmon J.M. 1992. Apparent loss of sugar transport activity in *Saccharomyces cerevisiae* may mainly account for maximum ethanol production during alcoholic fermentation. Biotechnol. Lett. **14**:577-582.

12. Salmon J.M., Vincent O., Mauricio J.C., Bely M. & Barre P. 1993. Sugar transport inhibition and apparent loss of activity in *Saccharomyces cerevisiae* as a major limiting factor of oenological fermentations. Amer. J. Enol. Vit. **44**: (in press).

Topic (3)

13. Vézinhet F. 1989. Obtention par recombinaison intra-génomique de clones non moussants à partir d'une souche de levure d'intérêt oenologique *Saccharomyces cerevisiae*. Sciences des aliments **9**:253-265.

14. Saulnier L., Mercereau T. & Vézinhet F. 1991. Mannoproteins from flocculating and non flocculating *Saccharomyces cerevisiae* yeasts. J. Sci. Food Agric. **54**:275-286.

15. Vézinhet F., Blondin B. & Barre P. 1991. Mapping of the *FLO5* gene of *Saccharomyces cerevisiae* by transfer of a chromosome during cytoduction. Biotechnol. Lett. **13**:47-52.

16. Stucka R., Dequin S., Salmon J.M. & Gancedo C. 1991. DNA sequences in chromosome II and VII code for pyruvate carboxylase isoenzymes in *Saccharomyces cerevisiae*: analysis of pyruvate carboxylase deficient strains. Mol. Gen. Genet. **229**:307-315.

Topic (4)

17. Sablayrolles J.M. & Barre P. 1989. Effect of anisothermal conditions on the kinetics of alcoholic fermentations by *Saccharomyces cerevisiae* in oenological conditions. Bioprocess Engin. **4**:139-143.

18. Sablayrolles J.M. & Barre P. 1989. Pilotage automatique de la température de fermentation en conditions oenologiques. Sciences des Aliments **9**:239-251.

19. Bely M., Sablayrolles J.M. & Barre P. 1990. Automatic control of assimilable nitrogen addition during alcoholic fermentation in oenological conditions. J. Ferment. Bioengin. **70**:1-6.

20. Bely M., Sablayrolles J.M. & Barre P. 1990. Description of alcoholic fermentation kinetics: its variability and interest. Amer. J. Enol. Vit. **41**:319-324.

21. Sablayrolles J.M. & Barre P. 1992. Use of a coulter-counter to study the alcoholic fermentation in oenological conditions. Biotechnol. Techn. **6**:15-18.

22. Dubois C., Sablayrolles J.M., Salmon J.M. & Barre P. 1992. Étude de fermenteurs à cellules floculantes pour la reprise de vins en arrêt de fermentation. Science des aliments **3**: (in press).

23. Sablayrolles J.M. & Barre P. 1993. Kinetics of alcoholic fermentation in anisothermal oenological conditions. I. Influence of temperature evolution on the instantaneous rate of fermentation. Amer. J. Enol. Vit. **44**: (in press).

24. Sablayrolles J.M. & Barre P. 1993. Kinetics of alcoholic fermentation in anisothermal oenological conditions. II. Predictions from the kinetics in isothermal conditions. Amer. J. Enol. Vit. **44**: (in press).

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

The following papers have recently appeared or are in press:

1. Pretorius, G.H.J. & Muller H.E. 1992. Conservation of binding site specificity of three yeast DNA binding proteins. FEBS Lett. **298**:203-205.

2. Meyer, P.S., Du Preez, J.C. & Kilian, S.G. 1992. Isolation and evaluation of yeasts for biomass production from bagasse hemicellulose hydrolysate. System. Appl.Microbiol. **15**:161-165.

3. Meyer, P.S., Du Preez, J.C. & Kilian, S.G. 1992. Cultivation of *Candida blankii* in simulated bagasse hemicellulose hydrolysate. J. Ind. Microbiol. **9**:109-113.

4. Meyer, P.S., Du Preez J.C. & Kilian, S.G. 1992. Effect of temperature and pH on *Candida blankii* in chemostat culture. World J. Microbiol. Biotechnol. **8**:434-438.

5. Meyer, P.S., Du Preez, J.C. & Kilian S.G. 1992. Chemostat Cultivation of *Candida blankii* on sugar cane bagasse hemicellulose hydrolysate. Biotechnol. Bioeng. **40**:353-358.

6. Horn, C.H., Du Preez, J.C. & Kilian, S.G. 1992. Amylase production by a *Schwanniomyces occidentalis* mutant in chemostat culture. Appl. Microbiol. Biotechnol. **37**:147-151.

7. Horn, C.H., Du Preez, J.C. & Kilian, S.G. 1992. Fermentation of grain sorghum starch by co-cultivation of *Schwanniomyces occidentalis* and *Saccharomyces cerevisiae*. Bioresource Technology **42**:27-31.

8. Horn, C.H., Du Preez, J.C. & Kilian, S.G. 1992. Protein enrichment of grain sorghum by submerged culture of the amylolytic yeasts *Schwanniomyces occidentalis* and *Lipomyces kononenkoae*. World J. Microbiol. Biotechnol. **8**:416-422.

9. Albertyn, J., Van Tonder, A. & Prior, B.A. 1992. Purification and characterization of glycerol-3-phosphate dehydrogenase of *Saccharomyces cerevisiae*. FEBS Lett. **308**:130-132.

10. Augustyn. O.P.H., Kock, J.L.F. & Ferreira, D. 1992. Differentiation between yeast species, and strains within a species, by cellular fatty acid analysis. 5. A feasible technique? System. Appl. Microbiol. **15**:105-115.

11. Botha, A, Kock, J.L.F., Coetzee, D.J., Van Dyk, M.S., Van der Berg, L. & Botes, P. 1992. Yeast eicosanoids. I. The distribution and taxonomic value of cellular fatty acids and arachidonic acid metabolites in the dipodascaceae and related taxa. System. Appl. Microbiol. **15**:148-154.

12. Botha, A., Kock, J.L.F., Coetzee, D.J., Linde, N.A. & Van Dyk, M.S. 1992. Yeast eicosanoids. II. The influence of non-steroidal anti-inflammatory drugs on the life cycle of *Dipodascopsis*. System. Appl. Microbiol. **15**:155-160.

13. Coetzee, D.J., Kock, J.L.F., Botha, A., Van Dyk, M.S., Smit, E.J. & Botes, P.J. 1992. Yeast eicosanoids. III. The distribution of arachidonic acid metabolites in the life-cycle of *Dipodascopsis uninucleata*. System. Appl. Microbiol. **15**:311-318.

14. Jansen van Rensburg, E.L., Kock, J.L.F., Botha, A., Coetzee, D.J. & Botes, P.J. 1992. The characterization of lipomycetaceous yeasts from South African soils. S. Afr. J. Sci. **88**:390-390.

15. Meyer, P.S., Du Preez, J.C., B.D. Wingfield & Kilian, S.G. In press. Evaluation of *Candida blankii* hybrids for biomass production. J. Biotechnol.

The cell size of a diploid *Candida blankii* isolate (UOVS-64.2), a yeast with potential for use in single cell protein production from hemicellulose hydrolysates, was increased by intraspecific protoplast fusion of auxotrophic mutants produced by UV irradiation followed by nystatin enrichment. Protoplast fusion yielded an isolate (UOVS-P2) which had a mean cell volume of approximately three times that of the parental strain. This isolate was not a true tetraploid but rather an aneuploid strain. Its maximum specific growth rate was only 40% that of the parental strain and the cell and protein yields were also appreciably lower. During continuous cultivation in simulated bagasse hemicellulose hydrolysate, isolate UOVS-P2 proved unstable and a spontaneous reduction in ploidy occurred. A new isolate (UOVS-PB2) originating from this culture had a 43% higher mean cell volume and contained approximately 13% more DNA than the parental strain (UOVS-64.2). In simulated hemicellulose hydrolysate the cell and protein yields of isolate UOVS-PB2 were slightly lower than those of isolate UOVS-64.2, although these yields were similar in shake flask cultures.

16. Van Zyl, P.J., Kilian, S.G. & Prior, B.A. In press. Physiological responses of *Zygosaccharomyces rouxii* to osmotic stress. Appl. Microbiol. Biotechnol.

When cell suspensions of *Zygosaccharomyces rouxii* were subjected to osmotic shock with NaCl, the cell volume decreased sharply and plasmolysis was observed. The cell subsequently recovered and volumes similar to those of cells growing at the respective water activity (a_w) values were found. Cycloheximide prevented cell recovery, indicating the involvement of protein synthesis in the recovery process. The intracellular glycerol concentration of *Z. rouxii* incubated in the presence of [^{14}C]glycerol increased from 13 to 96 mmol/l during the initial 20 min after an upshock from 0.998 a_w to 0.96 a_w. All the intracellular glycerol was labelled and therefore derived from the medium. Labelled glycerol was subsequently utilized and replaced by unlabelled glycerol produced by the cell within 90 min. The initial increase in glycerol concentration following the upshock was confirmed by ^{13}C nuclear magnetic resonance (NMR) spectroscopic studies of cell extracts. The combined dihydroxyacetone and dihydroxyacetone phosphate concentrations fluctuated during this period, whereas glycerol3-phosphate initially increased and then remained constant. This indicates that the production of glycerol is regulated. Decreases in ATP and polyphosphate levels were observed following osmotic upshock and may reflect a greater demand for ATP during the period of adjustment to decreased aw. The changes in cell volume and in ATP concentration following osmotic upshock may serve as osmoregulatory signals in *Z. rouxii* as was suggested previously for other microorganisms.

17. van der Walt J.P. In press. The Lipomycetaceae, a model family for phylogenetic studies. Antonie van Leeuwenhoek.

The Lipomycetaceae (Endomycetales) are known from the genera *Dipodascopsis*, *Lipomyces* and *Zygozyma* with budding anamorphic states in *Myxozyma*. The family is easily recognized culturally and physiologically but is phenotypically and ecologically extremely diverse. This natural taxon is phylogenetically distinct from the Saccharomycetaceae, but probably related to the Dipodascaceae. The possible evolution of the lipomycetaceous anamorphs is discussed.

18. van der Walt, J.P., Brewis, E.A. & Prior B.A. In press. A note on the utilization of aliphatic nitriles by yeasts. System. Appl. Microbiol.

Nine yeast strains of ascomycetous affinity were examined for their ability to utilize a series of aliphatic mono- and dinitriles as well as their corresponding amides as sole source of nitrogen. The results indicate that while some species failed to utilize these nitriles, this property nonetheless, does not appear to be uncommon among these microfungi. The consistently coincidental utilization of nitriles and their corresponding amides indicates that the hydrolysis involves a two-step reaction mediated by nitrilehydratase and amidase. The data suggest that some of the nitrile hydratases might be substrate and strain specific.

20. Botha A., Kock, J.L.F., Van Dyk, M.S., Coetzee, D.J., Augustyn, O.P.H. & Botes, P.J. In press. Yeast eicosanoids. IV: Evidence for prostaglandin production during ascosporogenesis by *Dipodascopsis tóthii*. System. Appl. Microbiol.

Using labelled arachidonic acid as prostaglandin precursor, thin layer chromatography, and scintillation counting, we have found evidence that two prostaglandins, PGE_2 and PGF_{2a}, are produced during ascosporogenesis in *Dipodascopsis tóthii*. We have also demonstrated that a small yeast population density in liquid medium is essential for ascosporogenesis and prostaglandin production in this yeast.

21. Kock, J.L.F. & Ratledge, C. In press. Changes in lipid composition and arachidonic acid turnover during the life cycle of the yeast *Dipodascopsis uninucleata*. J. Gen. Microbiol.

Ungerminated ascospores of *Dipodascopsis uninucleata* contained 18 times more lipid (5.5% dry wt) than germinated cells and comprised 58% (w/w) glycolipids, 28% (w/w) neutral lipids (mainly triacylglycerols) and 14% (w/w) phospholipids (mainly phosphatidylcholine and phosphatidylethanolamine). During germination the absolute amounts of all three lipid fractions fell sharply but, during the subsequent initiation of hyphal growth, the amount of phospholipids increased. As these hyphae began to differentiate for the sexual stage of the life cycle, the amount of neutral lipid then increased. The fatty acyl groups of the glyco-, neutral- and phospho-lipid fractions throughout the life cycle were mainly palmitate (16:0), oleate (18:1) and linoleate (18:2). The percentage of 16:0 remained constant during the life cycle while the relative amounts of 18:2 plus α-linolenate (18:3) in the glyco-, neutral- and phospho- lipid fractions first increased during initiation of growth and then decreased during the onset of differentiation. The opposite trend occurred with 18:1. When [^{3}H]arachidonic acid (ARA) and [1-^{14}C]18:1 were fed separately to *D. uninucleata*, both were rapidly incorporated into phospholipids. Highest incorporation of ARA was in the growth phase and, during the onset and remainder of the differentiation phase, the amount of ARA decreased in this fraction. Incorporation of 18:1 increased during growth and differentiation with a significant proportion (49% to 57%) being incorporated into triacylglycerols compared to a much a smaller proportion (12% to 17%) of ARA. During the differentiation phase, the majority (55%) of [^{3}H] ARA was incorporated into a complex of metabolites, including possible eicosanoids and prostaglandin-like compounds. Only 11% of [1-^{14}C]18:1 was incorporated into this fraction. The role of this fraction is discussed in relation to the possible conversion of ARA into prostaglandin materials both of structural/functional significance to the yeast and also of some economic interest.

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

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

Neutral trehalase (EC 3.2.1.28) is a trehalose hydrolyzing enzyme of the yeast *Saccharomyces cerevisiae*, (App, H. and Holzer, H. (1989) *J.Biol.Chem*., **264**, 17583-17588). The gene of neutral trehalase was cloned by complementation of a neutral trehalase deficient yeast mutant which was obtained by ethyl-methanesulfonate mutagenesis. Three mutants without detectable neutral trehalase activity were obtained and characterized by tetrad analysis and found to belong to the same complementation group. The mutants were transformed with a *S. cerevisiae* genomic library in YEp24. Two overlapping plasmids were isolated, containing the neutral trehalase gene *NTH1* with an open reading frame of 2079 bp, encoding a protein of 693 amino acids, corresponding to a molecular weight of 79,569 Da. Several putative TATA boxes were found in the 5' nontranslated region of the *NTH1* gene. In positions -652 to -641 a possible binding sequence for the MIG1 protein, a multicopy inhibitor of the GAL1 promotor, which also binds to the promotor sequences of the *SUC2* and the *FBP1* gene, was found. The start codon of the neutral trehalase is located about 2500 bp upstream of the centromere 4 consensus sequence elements I, II, and III (Mann, C., and Davis, R.W. (1986) *Mol. Cell.Biol*. **6**, 241-245). Vicinity to a centromere is known to have a depressing influence on the number of plasmid copies per cell. This probably explains why transformation with pNTH does not lead to overexpression of neutral trehalase. The four consensus sequences AATAAA contained in the centromeric elements and reconfirmed by our sequencing data might be polyadenylation signals for *NTH1*-mRNA transcription termination. Northern blot analysis yielded a single mRNA species of approximately 2.3 kb. The neutral trehalase protein has a putative cAMP-dependent phosphorylation consensus sequence *RRGS* from amino acid positions 22-25. Therefore the previously described activation of neutral trehalase by cAMP dependent phosphorylation is probably due to phosphorylation of serine 25. Four potential N-glycosylation sites (*Asn-X-Ser/Thr*) occur in the open reading frame of the neutral trehalase gene. However, no evidence for glycosylation could be detected in western blots of the extracted protein. The amino acid sequence of neutral trehalase from *S.cerevisiae* pointed to significant similarity in three domains with the osmoregulated *treA* gene encoding the periplasmic trehalase from *E. coli* K12 (Gutierrez, C., Ardourel, M., Bremer, E., Middendorf, A., Boos, W., and Ehmann, U., (1989). *Mol. Gen. Genet*. **217**, 347-354) and in four domains to the trehalase gene of rabbit small intestine (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). These domains might be part of the catalytic center of these trehalases. In order to study the biological function of the neutral trehalase, an isogenic disruption mutant, with the *URA3* gene inserted, was constructed. It could be shown, that trehalose, accumulated after heat stress, is degraded by neutral trehalase, not acid trehalase.

XVIII. Department of Molecular Cell Biology, Biotechnology Centre, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands. Communicated by F.M. Klis.

The following paper was accepted for publication recently.

1. M.P. Schreuder, S. Brekelmans, H. van den Ende & F.M. Klis. In press. Targeting of a heterologous protein to the cell wall of *Saccharomyces cerevisiae*. Yeast **9**.

The sexual adhesion protein of *Saccharomyces cerevisiae* MATα cells, α-agglutinin, could not be extracted from the cell wall with hot SDS, but became soluble after digestion of the cell wall with laminarinase. This indicates that it is intimately associated with cell wall glucan. A fusion protein was constructed consisting of the signal sequence of yeast invertase, guar α-galactosidase, and theC-terminal half of the α-agglutinin. Most of the fusion protein was incorporated in the cell wall. A small amount could be extracted with SDS, but most of it could only be extracted with laminarinase. On the other hand, cells containing a construct consisting of the signal sequence of invertase and α-galactosidase released most of the α-galactosidase into the medium and all cell wall associated α-galactosidase was released by SDS. Labelling with antibodies showed that the α-galactosidase part of the fusion protein was exposed on the surface of the cell wall. The results demonstrate that the C-terminal half of the α-agglutinin contains the information needed to incorporate a protein into the cell wall.

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

The following paper has been accepted recently.

1. F. Yanagida, F. Ichinose, T. Shinohara & S. Goto. 1992. Distribution of wild yeasts in the white grape varieties at Central Japan. J. Gen. Appl. Microbiol. (in press).

The distribution of wild yeasts was studied in 11 samples of grape varieties at 5 localities of different climatic conditions of Nagano and Yamanashi Prefectures in Central Japan. The yeast populations in 6 healthy samples ranged from 0.007 to 6.0 x 10 CFU/ml. In five samples of slightly injured grapes, populations were 110 to 1100 x 10 CFU/ml. Two hundred and sixty eight isolates were assigned to 12 species belonging to 4 genera. In 6 samples of grape varieties, *Kloeckera apiculata* predominated. In contrast, in the other 5 samples of 3 grape varieties, basidiomycetous yeasts (*Cryptococcus*, *Rhodotorula*, and other genera) predominated. Generally, basidiomycetous yeasts predominated in a late crop varieties from climatic regions, and ascomycetous yeasts (*K. apiculata*) predominated in grape varieties from mild climate regions.

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

The following paper was recently accepted for publication.

1. Koshinsky, H.A., R.H. Cosby & G.G. Khachatourians. In press. Effects of T-2 toxin on ethanol production by *Saccharomyces cerevisiae*. Biotechnol. Appl. Biochem.

A trichothecene mycotoxin, T-2 toxin, inhibits several aspects of cellular physiology in *Saccharomyces cerevisiae*, including protein synthesis and mitochondrial functions. We have studied growth of, glucose utilization by, and ethanol production by *S. cerevisiae* and show that they are inhibited by T-2 toxin between 20 to 200 µg/mL in a dose-dependent manner. At 200 µg/mL, T-2 toxin causes cell death. The apparent inhibition of ethanol production was found to be the result of growth inhibition. On the basis of biomass or glucose consumption, T-2 toxin increased the amount of ethanol present in the culture. This suggests that T-2 inhibits oxidative but not fermentative energy metabolism by inhibiting mitochondrial function and shifting glucose catabolism towards ethanol formation. As T-2 toxin does not directly inhibit ethanol production by *S. cerevisiae*, this system could be used for ethanol production from trichothecene-contaminated grain products.

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

The following papers have been published recently.

1. G. Weber, F. Spaaij & J.P. van der Walt[1]. 1992. *Kluyveromyces piceae* sp. nov., a new yeast species isolated from the rhizosphere of *Picea abies* (L.) Karst. Antonie van Leeuwenhoek **62**:239-244.
[1]Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein 9300, South Africa.

Two strains of an undescribed species of the genus Kluyveromyces were recovered from the rhizosphere of spruce. A description of the new species, *Kluyveromyces piceae*, is given and itsclassification on basis of ascospore shape, substrate utilization, G+C-content, DNA-DNA-reassociation data and habitat specificity is discussed.

2. F. Spaaij, G. Weber, and J.P. van der walt.[1] 1992. *Myxozyma sirexii* sp. nov. (Candidaceae), a new yeast isolated from frass of the woodwasp *Sirex juvencus* L. System. Appl. Microbiol. **15**:427-431.
[1]Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein 9300, South Africa.

Three strains of an undescribed species belonging to the genus *Myxozyma* were recovered from an insect habitat. The new species differs from the other accepted species of the genus in its carbon assimilation pattern, mol% G+C and low DNA-DNA homology. A description of the new species, *Myxozyma sirexii*, is given, and a key to the genus *Myxozyma* is provided.

XXII. Research Institute for Viticulture and Enology, Bratislava, Matúškova 25, Czecho-Slovakia 833 11. Communicated by E. Minárik.

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

1. Krásny, Š., Malík, F. & Minárik, E. 1992. Immobilized cells in wine Processing, Part 2: Use of immobilized cells for secondary fermentation (in German). Die Wein-Wissenschaft **47**:53-55.

The second part of a series of papers on immobilized systems in wine making deals with their utilization in secondary fermentation. Conditions required on properties of the immobilized system for its applicability in the process of secondary fermentation are characterized. The recent state of this problem is given from the point of view of classic and tank technology of sparkling wine production.

2. Krásny, Š., Malík, F. & Minárik, E. 1992. Immobilized cells in wine processing. Part 3: Immobilized cells In the process of biological acid decomposition of the wine (in German). Die Wein-Wissenschaft **47** (in press).

The paper deals with the utilization of immobilized cells in the process of deacidification of wines. Perspectives of immobilized special systems in this process with free and immobilized cells are evaluated. Systems of immobilized lactic acid bacteria and yeasts of the genus *Schizosaccharomyces* are elucidated.

3. Kyaeláková, M. 1992. The influence of fungicide residues used in wine protection on the inhibition of yeasts (in Czech). Thesis. Faculty of Horticulture, Univ. of Agriculture, Brno-Lednice, 193 pp.

Five fungicide preparations (Euparen, Curzate SM, Novozir MN, Ridomil Plus 48 WP and Rowral 50 WP had been tested on their inhibitory effect on two *Saccharomyces cerevisiae* strains (Bratislava 1, Myslenice 1) known as resistant to fungicide residues in grape must fermentations. Euparen (dichlofluanid) inhibited growth fermentation activity of both strains already at 0.1 $mg.l^{-1}$. Curzate SM (cymoxanil) caused light inhibition at 20-30 $mg.l^{-1}$. The same was observed with Novozir MN 80 (mancozeb). Ridomil Plus 48 WP (metalaxil) showed only light inhibition at concentrations of 50-100 $mg.l^{-1}$. The yeasts tolerated relatively high concentrations of Rowral 50 WP up to 250 $mg.l^{-1}$. Technological consequences connected with the reduction of fungicide residues by different active measures in wineries are dealt with in detail.

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

The following are recent or incipient publications.

1. Barnett, J.A. 1992. The taxonomy of the genus *Saccharomyces* Meyen ex Reess: a short review for non-taxonomists. Yeast **8**:1-23.

2. Barnett, J.A. 1992. Yeast exploitation and research. [Book review: *Saccharomyces*, edited by M.F. Tuite & S.G. Oliver, plenum Press 1991]. Trends Biotechnol. **10**:103-104.

3. Barnett, J.A. 1992. Some controls on oligosaccharide utilization by yeasts: the Kluyver effect. FEMS Microbiol. Lett. **100** (in the press).

4. Entian, K.-D. & Barnett, J.A. 1992. Regulation of sugar utilization by *Saccharomyces cerevisiae*. Trends Biochem. Sci. (in the press).

XXIV. Department of Biochemistry and Molecular Biology, School of Medicine, The University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, Kansas 66160-7421, USA. Communicated by W. N. Arnold.

The following book was published recently.

1. W.N. Arnold. 1992. Vincent van Gogh: chemicals, crises, and creativity. Birkhäuser, Boston, 320 pp. ISBN 0-8176-3616-1.

When van Gogh committed suicide in 1890 his art work was acknowledged by only a small cadre of friends and followers. Today, his work is universally embraced and he is on everybody's list of outstanding artists. The intervening number of years is matched by almost as many publications attempting a posthumous diagnosis of his underlying problems and trying to understand his jagged life. Medical explanations for van Gogh's often bizarre behavior have included epilepsy, schizophrenia, manic depressive psychosis, and more. Various authors have speculated on the influence of digitalis poisoning, cataracts, and glaucoma upon the artist's perception. Psychoanalysts have had a field day trying to rationalize the artist's suicide at the height of his creative power. Papers from Japan and the United States even claimed that van Gogh suffered from Ménière's disease, an inner ear disorder causing vertigo, and thus tried to explain the ear-cutting incident. However, in this scholarly, yet very readable work, Dr. Arnold identifies acute intermittent porphyria, an inherited disease arising from a partial deficiency of a liver enzyme, as the underlying problem. This unifying hypothesis accommodates the age of onset, the lucidity and productivity between medical crises, and all of the well-documented signs and symptoms revealed in Vincent van Gogh's voluminous correspondence with family and friends. The importance of the exacerbation factors of alcohol abuse in the form of absinthe and malnutrition are also developed in depth. The chemical and medical themes that run through the book are vital to the thesis, but the graded and progressive development of each topic will appeal to the broad audience interested in Vincent van Gogh. This is a thorough analysis in the light of modern chemical and biochemical findings. The result is a new understanding of the man, as well as new insights into the sources of creativity in general.

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

The following papers have been published since our last report.

1. K.C. Thomas & W.M. Ingledew. 1992. Production of 21% (v/v) ethanol by fermentation of very high gravity (VHG) wheat mashes. J. Indust. Microbiol. **10**:61 -68.

Wheat mashes containing very high concentrations of fermentable sugars were fermented with active dry yeast with and without yeast extract as nutrient supplement. At a pitching rate of 76 million cells per g of mash, an ethanol yield of 20.4% (v/v) was obtained, but to achieve this, yeast extract as nutrient supplement was to be added to the wheat mash. When the pitching rate was raised to 750 million cells per g of mash the ethanol yield increased to 21.5% (v/v) and in this case there was no need for nutrient supplementation. The efficiency of conversion of sugar to ethanol was 97.6% at the highest pitching rate; it declined slightly with decreasing pitching rate. It is suggested that the high yield of ethanol at high pitching rates in the absence of any added nutrient supplement resulted through the release of nutrients from cells which lost viability and lysed When no significant loss of viability was observed as when low pitching rates were used nutrient supplement was required to obtain maximum yield of ethanol.

2. K.C. Thomas & W.M. Ingledew. 1992. The relationship of low lysine and high arginine to efficient ethanolic fermentation of wheat mash. Can. J. Microbiol. **38**: 626-634.

Very high gravity wheat mashes containing 20 or more grams of carbohydrates per 100 mL were fermented completely by *Saccharomyces cerevisiae* even though these mashes contained low amounts of assimilable nitrogen. Supplementation of wheat mashes with various amino acids or with yeast extract, urea or ammonium sulfate reduced the fermentation time. Lysine or glycine added as single supplements, however, inhibited yeast growth and fermentation. With lysine, yeast growth was severely inhibited and a loss of cell viability as high as 80% was seen. Partial or complete reversal of lysine-induced inhibition was achieved by the addition of a number of nitrogen sources. All nitrogen sources that relieved lysine-induced inhibition of yeast growth also promoted uptake of lysine and restored cell viability to the level observed in the control. They also increased the rate of fermentation. Experiments with minimal media showed that for lysine to be inhibitory to yeast growth, assimilable nitrogen in the medium must be either absent or present in growth-limiting concentrations. In the presence of excess nitrogen, lysine stimulated yeast growth and fermentation. Results indicate that supplementing wheat mash with other nitrogen sources increases the rate of fermentation not only by providing extra nitrogen, but also by reducing or eliminating the inhibitory effect of lysine on yeast growth.

3. R. McCaig, J. McKee, E.A. Pfisterer, D.W. Hysert, E. Munoz & W.M Ingledew. 1991. Very High Gravity Brewing - Laboratory and Pilot Plant Trials. J. Amer. Soc. Brew. Chem. **50**:18-26

Transfer to the brewing industry of laboratory-scale, very high gravity (VHG) fermentation technology resulted in a series of experiments which have shown, under pilot plant conditions, that worts up to 24° Plato can be fermented within acceptable time frames to produce beers with over 11 % v/v ethanol. Freshly harvested commercial pitching yeast slurry was used in all cases. Extensive chemical analyses and sensory evaluation have indicated that the beers, after dilution to 5% ethanol, were cleaner and more physically stable than control beers. Ester levels in the VHG beers were elevated but after dilution, the concentrations were comparable to commercial products. Organoleptically, beers in the 18 to 20° Plato range were preferred by most taste panelists.

4. M. Whiting, M. Chrichlow, W.M. Ingledew & B. Ziola. 1992. Detection of *Pediococcus* spp. in Brewing Yeast by a Rapid Immunoassay. Appl. Environ. Microbiol. **58**:713-716.

A membrane immunofluorescent-antibody test was developed to detect diacetyl-producing *Pediococcus* contaminants in brewery pitching yeast (yeast [*Saccharomyces cerevisiae*] slurry collected for reinoculation). Centrifugations at 11 and 5100 x g separate yeast cells from bacteria and concentrate the bacteria, respectively. Pelleted bacteria

resuspended and trapped on a black membrane filter are reacted with monoclonal antibodies specific for cell surface antigens and then with fluorescein-conjugated indicator antibodies. Whether pitching yeast is contaminated with pediococci at 0.001% is determined in <4 hours. The sensitivity of the assay is 2 orders of magnitude below the *Pediococcus* detection limit of direct microscopy.

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

The following papers have been accepted or submitted for publication recently.

1. E.C.A. Eleutherio, P.S. de Araujo[1] & A.D. Panek. In press. Role of the trehalose carrier in dehydration resistance of *Saccharomyces cerevisiae*. Biochim. Biophys. Acta. [1]Departamento de Bioquimica, Instituto de Quimica, Universidade de São Paulo, Brasil.

Yeast cells are well known for their ability to survive complete dehydration, a phenomenon that is directly linked with the presence of the sugar trehalose in these cells. This sugar apparently endows the cells with the capacity to survive dehydration. Previous studies on in vitro models showed that trehalose must be present on both sides of the bilayer to stabilize dry membranes. The present report demonstrates that a specific trehalose carrier seems to enable the sugar to protect the yeast cell membrane by translocating trehalose from the cytosol to the extracellular environment. *Saccharomyces cerevisiae* mutant strains which lack the trehalose carrier did not survive after dehydration although they accumulated endogenous trehalose. Furthermore, when carrier mutants were dehydrated in the presence of exogenous trehalose the cells became more resistant showing increased survival.

2. M.J.S. Ribeiro, A.D. Panek & J.T. Silva[1]. Trehalose metabolism in *Saccharomyces cerevisiae* during heat shock. [1]Departamento de Biologia Celular e Molecular, Instituto de Biologia, Universidade Federal Fluminense, 24130, Niterói, RJ, Brasil.

When different strains of *Saccharomyces cerevisiae* grown at 23°C were transferred to 36°C, trehalose and glycogen were accumulated. Glycogen accumulation was less extensive and its synthesis started at least 15 minutes after initiation of trehalose synthesis. The steady-state intracellular concentration of trehalose increased together with the activities of the enzymes trehalose-6-phosphate synthase, UDPG-pyrophosphorylase, phosphogluco-mutase and trehalase. A small but significant change was observed in hexokinase activity. Our results directly implicate isoform PII of hexokinase and the minor isoform of phosphoglucomutase in the pathway of trehalose formation during heat shock. We also showed that the major isoform of phosphoglucomutase increased in activity but was not essential for trehalose accumulation. Studies with the glucose uptake system indicated that trehalose accumulation could be primarily determined by intracellular availability of substrates due to the increase in the rate of glucose uptake. The increase in uptake appears to have two components: a kinetic effect of temperature upon glucose transporters and an increase in the numbers of molecules of the transporters, probably mediated by synthesis "de novo".

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

The following papers have been published recently or are in press

1. Miklos, I. & Sipiczki, M. 1991. Breeding of a distiller's yeast by hybridization with a wine yeast. Appl. Microbiol. Biotechnol. **35**:638-642.

Hybrids were constructed between auxotrophic mutants of a heterothallic distiller's strain and a homothallic wine yeast. The hybridization resulted in a significant increase in both ethanol production and tolerance against exogenous ethanol. The hybrids were heterogenous in ploidy, probably due to segregation of aneuploids during culturing. Sporulation of the

hybrids broke down the high productivity, producing spore clones that were mostly of various intermediate levels of performance. However, a meiotic product superior to both crossing partners was also found. The results demonstrate that fermentation capacity can be improved by crossing with a low performance strain.

2. Grallert, B., Sipiczki, M. 1991. Common genes and pathways in the regulation of the mitotic and meiotic cell cycles of *Schizosaccharomyces pombe*. Curr. Genet. **20**:199-204.

Cell division cycle mutants defective in G1; DNA replication or nuclear division were tested for sporulation at semirestrictive temperatures. In *cdc1-7*, *cdc5-120*, *cdc17-L16* and *cdc18-46* no abnormalities were observed; *cdc1O-129*, *cdc20-M1O*, *cdc21-M6B*, *cdc23-M36* and *cdc24-M38* formed four-spored asci but with a low efficiency; *cdc22-M45* was completely defective in meiosis, but could conjugate and formed zygotes with a single nucleus. Mutants defective in the mitotic initiation genes *cdc2*, *cdc25* and *cdc13* were blocked in meiosis II. None of the *wee1-50*, *adh.nim1*$^+$ and *win1*$^+$ alleles had any affect on sporulation, suggesting that their interactions with *cdc25* and *cdc2* are specific to mitosis. The meiotic function of *cdc13* is TBZ-sensitive and probably exerted downstream of *cdc2*. Single mutants in *cut1* or *cut2* did not effect sporulation, whereas the double mutant *cut1 cut2* formed two-spored asci. The results demonstrate that the cell division cycle and the meiotic developmental pathway share common genes and regulatory cascades.

3. Leupold, U., Sipiczki, M. 1991. Sterile UGA nonsense mutants of fission yeast. Curr. Genet. **20**:67-73.

Eight sterile mutants, which regain their fertility upon activation of an inactivated UGA suppressor allele of the serine tRNA gene *sup3*, are shown to carry UGA nonsense alleles of two established *ste* genes, *ste1* (one mutant) and *ste6* (two mutants), and of two novel genes, *ste9* (four mutants) and *ste10* (one leaky mutant of *ras1*$^-$/*ste5*-like cell morphology). The mutant alleles of *ste1* and *ste9* lead to a defect in both conjugation and meiosis, whereas those of *ste6* and *ste10* affect mating only. Two of the four genes map to chromosome 1, *ste1* in the left arm 6 cM distal of *ura1*, and *ste9* in the right arm 3 cM distal of *ade2*. The *ste10* and *ste6* genes are located in the right arms of chromosomes II and III, respectively, the former 4 cM distal of *trp1* and the latter 1 cM proximal or distal of *trp3*.

4. Leupold, U., Sipiczki, M., Egel, R. 1991. Pheromone production and response in sterile mutants of fission yeast. Curr. Genet. **20**:79-85.

Genetically heterothallic strains of various sterile mutants were assayed for residual production of the corresponding mating pheromone as well as responsiveness towards the opposite pheromone. No sexual activities were detected in *ste11* strains (previously referred to as *aff1* or *steX*, which we show are allelic), whilst the production of M factor was unaffected by *ste1* to *ste10* mutations. P factor production was still possible in class I *ste* mutants (*ste5*, *ste6* and *ste10*), which also allow meiosis in diploid strains. With the exception of the leaky *ste10-F23* mutant, no changes in cell morphology were induced by exposure to opposite pheromone in the ste mutant strains.

5. Sipiczki, M., Grallert, B., Miklos, I. In press. Mycelial and syncytial growth in *Schizosaccharomyces pombe* induced by novel septation mutations. J. Cell. Sci. (in press).

Mutation in the gene *sep1*$^+$ of the unicellular fission yeast *S. pombe* impairs cell separation after cytokinesis and confers a branching mycelial morphology. The mutant is not defective in cell wall ß-glucanase activity but shows increased sensitivity to Ca^{2+} and Mg^{2+} and increased resistance to the microtubule inhibitor benomyl. The mycelial growth of *sep1-1* provides a convenient method for the examination of the polar growth pattern and for pedigree analysis as demonstrated by the segregation of mating types in the homothallic microhyphae. *sep1* is closely linked to *ade1* (0.94 cM) on the right arm of chromosome II. The ts mutation *spl1-1* confers bent cell shape and causes aberrant septum formation at the restrictive temperature. *sep1*$^+$ and *spl1*$^+$ perform closely related functions as their mutant alleles interact with each other and with another septation mutant *cdc4-8*. These functions may overlap with certain cytoskeletal processes and with the determination of cell polarity because the triple mutant forms huge multi-nucleate syncytia with promiscuous branching and rare septum formation.

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

The following thesis has been defended recently.

1. C. Verduyn. 1992. Energetic aspects of metabolic fluxes in yeasts. Ph.D. Thesis, Delft University of Technology. 158 pp.

This thesis deals with the bioenergetics of yeasts. Bioenergetics are most easily studied in anaerobic chemostat cultures, which allow quantification of the biomass yield on ATP via analysis of product formation. Due to a general lack of data on anaerobic chemostat culture of yeasts, it was decided to study the physiology of *S. cerevisiae* CBS 8066 in anaerobic glucose-limited chemostat cultures (Chapter 2). The biomass yield declined with increasing growth rate. The yield of strain 8066 was lower than that of strain H1022. The latter strain did not produce acetate, which correlated with a relatively high level of acetyl-CoA synthetase. The biomass yield of both strains was negatively affected by the presence of weak acids or growth at a low extracellular pH.

In Chapter 3, the basic data from Chapter 2 have been used to evaluate the energetics of *S. cerevisiae* in anaerobic glucose-limited cultures. The observed Y_{ATP} (g biomass produced per mol ATP formed) was dependent on the biomass composition, the presence of weak acids, the provision of an adequate amount of fatty acids and the extracellular pH. The maximum observed Y_{ATP} was 16 g biomass.(mol ATP formed)$^{-1}$, which is much higher than previous values obtained with batch cultures.

Chapter 4 evaluates the growth yields of yeasts in general under aerobic conditions. It is shown that the ATP requirement for biomass formation and the efficiency of energy transduction during respiration (P/O ratio) affect the biomass yield to a major extent. Various methods to calculate the P/O-ratio are also discussed in this chapter. The P/O-ratio may be calculated when the experimental Y_{ATP} is known. Unfortunately, Y_{ATP} cannot be determined directly in aerobic cultures. A theoretical Y_{ATP} can be calculated, but it is much higher than experimental Y_{ATP} as observed in anaerobic cultures. This problem is usually solved in the literature by multiplying the theoretical Y_{ATP} with a fixed factor to arrive at an experimental Y_{ATP}. However, an alternative method would be to assume a fixed difference between theoretical and experimental Y_{ATP} requirements. It can then be calculated that, assuming a fixed difference, P/O-ratios are obtained which are more or less independent of the growth substrate.

A further point of discussion in the literature is the relation between bioenergetics and the growth rate of *S. cerevisiae* in aerobic glucose-limited cultures. The physiology of *S. cerevisiae* CBS 8066 as a function of the growth rate was first studied (Chapter 5). A fully respiratory metabolism was observed at growth rates < 0.39 h^{-1}. At D > 0.39 h^{-1}, the qO_2 became more or less constant and alcoholic fermentation occurred. However, between D = 0.32 and 0.39 h^{-1}, a disproportionate increase in specific oxygen uptake rate was observed, which coincided with the appearance of acetate. An enzymic study showed that acetate accumulation could be explained mainly by glucose repression of acetyl-CoA synthetase. The latter enzyme converts acetate into acetyl-CoA, which can subsequently be fed into the TCA cycle.

In Chapter 6, data from the previous chapter are used to calculate the energetics of *S. cerevisiae* as a function of the growth rate. Two approaches were compared. When it is assumed that Y_{ATP} is constant, it can be calculated that the effective P/O-ratio decreases 2-3 fold with increasing D. Assuming a constant P/O-ratio, a significant decrease in Y_{ATP} was calculated. In recent literature the first method, i.e. constant Y_{ATP} is commonly employed. However, considering the increase in protein (an 'expensive' item in terms of ATP) and the appearance of various by-products with increasing dilution rates, it is concluded that in bioenergetic calculations a constant P/O-ratio should be considered.

As has already been mentioned several times, weak acids affect the energetics of microorganisms. Added acids act as proton conductors, resulting in an acidification of the cytosol. This has to be countered by expulsion of protons by hydrolysis of ATP via the plasma membrane ATPase. In order to provide this ATP, increased respiration is required. The effect of benzoate on metabolic fluxes in aerobic cultures was studied in detail in Chapter 7. Addition of benzoate to *S. cerevisiae* at low dilution rates resulted in an increase in qO_2 to values as high as 19-21 mmol.g^{-1}.h^{-1}. An enzymic study showed that the resulting high glycolytic and respiratory fluxes were not associated with increased amounts of some key enzymes. Hence it seems likely that fluxes are regulated to major extent by concentrations of metabolites.

Under aerobic conditions, oxygen functions as the terminal electron acceptor in the respiratory chain. However, hydrogen peroxide can also function as an electronacceptor, via the mitochondrial enzyme cytochrome c peroxidase. It

accepts electrons at the level of cytochrome c, that is before the proton translocating cytochrome oxidase. Hence it can be expected that addition of hydrogen peroxide to the medium will result in a reduced yield. This was indeed found in experiments with a catalase-negative mutant of *H. polymorpha* (Chapter 8). Addition of hydrogen peroxide resulted in elevated levels of cytochrome c peroxidase. Furthermore the functioning of hydrogen peroxide as an electron acceptor was confirmed in experiments in which ethanol was oxidized under anaerobic conditions in the presence of hydrogen peroxide by catalasenegative *H. polymorpha* (Chapter 9).

Finally, a 'short' review of factors affecting the biomass yield is presented in Chapter 10. Many of these factors have already been discussed in previous chapters. As such, this chapter thus provides a general summary of Chapters 2 to 9. Furthermore some attention is paid to the effect of salts, temperature, and partial gas pressures on the biomass yield. The last section of this chapter discusses the relation between the energy content of the carbon source and the biomass yield. For this purpose, *C. utilis* was grown on a number of carbon sources and the biomass yield, expressed as g biomass per g substrate carbon, was calculated, as well as the percentage assimilation of the carbon source. It is concluded that the biomass yield is mainly determined by the biochemical routes leading to central metabolic precursors, rather than by the energy content of the substrate.Recent publications.

2. C. Verduyn, E. Postma, W.A. Scheffers & J.P. van Dijken. 1992. Effect of benzoic acid on metabolic fluxes in yeasts: continuous-culture study on the regulation of respiration and alcoholic fermentation. Yeast **8**:501-517.

Addition of benzoate to the medium reservoir of glucose-limited chemostat cultures of *Saccharomyces cerevisiae* CBS 8066 growing at a dilution rate (D) of 0.10 h^{-1} resulted in a decrease in the biomass yield, and an increase in the specific oxygen uptake rate ($q0_2$) from 2.5 to as high as 19.5 mmol $g^{-1}h^{-1}$. Above a critical concentration, the presence of benzoate led to alcoholic fermentation and a reduction in $q0_2$ to 13 mmol $g^{-1}h^{-1}$. The stimulatory effect of benzoate on respiration was dependent on the dilution rate: at high dilution rates respiration was not enhanced by benzoate. Cells could only gradually adapt to growth in the presence of benzoate: a pulse of benzoate given directly to the culture resulted in wash-out.

As the presence of benzoate in cultures growing at low dilution rates resulted in large changes in the catabolic glucose flux, it was of interest to study the effect of benzoate on the residual glucose concentration in the fermenter as well as on the level of some selected enzymes. At D = 0.10 h^{-1}, the residual glucose concentration increased proportionally with increasing benzoate concentration. This suggests that modulation of the glucose flux mainly occurs via a change in the extracellular glucose concentration rather than by synthesis of an additional amount of carriers. Also various intra-cellular enzyme levels were not positively correlated with the rate of respiration. A notable exception was citrate synthase: itslevel increased with increasing respiration rate.

Growth of *S. cerevisiae* in ethanol-limited cultures in the presence of benzoate also led to very high qO_2 levels of 19-21 mmol $g^{-1}h^{-1}$. During growth on glucose as well as on ethanol, the presence of benzoate coincided with an increase in the mitochondrial volume up to one quarter of the total cellular volume. Also with the Crabtree-negative yeasts *Candida utilis*, *Kluyveromyces marxianus* and *Hansenula polymorpha*, growth in the presence of benzoate resulted in an increase in qO_2 and, at high concentrations of benzoate, in aerobic fermentation. In contrast to *S. cerevisiae*, the highest qO_2 of these yeasts when growing at D = 0.10 h^{-1} in the presence of benzoate was equal to, or lower than the qO_2 attainable at μ_{max} without benzoate. Enzyme activities that were repressed by glucose in *S. cerevisiae* also declined in *K. marxianus* when the glucose flux was increased by the presence of benzoate.

The maximal aerobic fermentation rate at D = 0.10 h^{-1} of the Crabtree-negative yeasts at high benzoate concentrations was considerably lower than for *S. cerevisiae*. This is probably due to the fact that under aerobic conditions these yeasts are unable to raise the low basal pyruvate decarboxylase level: cultivation without benzoate under oxygen-limited conditions resulted in rates of alcoholic fermentation and levels of pyruvate decarboxylase comparable to those of *S. cerevisiae*.

3. C.C.M. van Leeuwen[1], R.A. Weusthuis, E. Postma[2], P.J. van den Broek[1] & J.P. van Dijken. 1991. Maltose/proton co-transport in *Saccharomyces cerevisiae*. Comparative study with cells and plasma membrane vesicles. Biochem. J. **284**:441-445.

[1]Department of Medical Biochemistry, Sylvius Laboratory, P.0. Box 9503, 2300 RA Leiden. [2]Zaadunie B. V., Westeinde 62, 1600 AA Enkhuizen, The Netherlands.

Maltose/proton co-transport was studied in intact cells and in plasma membrane vesicles of the yeast *Saccharomyces*

cerevisiae. In order to determine uphill transport in vesicles, plasma membranes were fused with proteoliposomes containing cytochrome c oxidase as a protonmotive force-generating system. Maltose accumulation, dependent on the electrical and pH gradients, was observed. The initial uptake velocity and accumulation ratio in vesicles proved to be dependent on the external pH. Moreover, kinetic analysis of maltose transport showed that V_{max} values greatly decreased with increasing pH, whereas the K_m remained virtually constant. These observations were in good agreement with results obtained with intact cells, and suggest that proton binding to the carrier proceeds with an apparent pK of 5.7. The observation with intact cells that maltose is co-transported with protons in a one-to-one stoichiometry was ascertained in the vesicle system by measuring the balance between proton-motive force and the chemical maltose gradient. These results show that maltose transport in vesicles prepared by fusion of plasma membranes with cytochrome c oxidase proteoliposomes behaves in a similar way as in intact cells. It is therefore concluded that this vesicle model system offers a wide range of new possibilities for the study of maltose/proton co-transport in more detail.

4. T.J. Wenzel[1], M.A. van den Berg[1], W. Visser, J.A. van den Berg[1] & H.Y. Steensma. 1992. Characterization of *Saccharomyces cerevisiae* mutants lacking the E1α subunit of the pyruvate dehydrogenase complex. Eur. J. Biochem. **209**, 697-705. [1]Department of Cellbiology and Genetics, Leiden University, The Netherlands

Pyruvate dehydrogenase mutants of *Saccharomyces cerevisiae* were isolated by disruption of the *PDA1* gene. To this end, the *PDA1* gene encoding the E1a subunit of the pyruvate dehydrogenase complex was replaced by the dominant *Tn5ble* marker. Disruption of the *PDA1* gene abolished production of the E1a subunit and pyruvate dehydrogenase activity. Two additional phenotypes were observed in the Pdh^- mutants: (a) a reduced growth rate in glucose medium which was partially complemented by the amino acid leucine; (b) an increase in formation of petites which lack mitochondrial DNA [rho°], during growth on glucose. Both phenotypes were shown to be a result of inactivation of the *PDA1* gene. Explanations for these phenotypes are discussed.

5. T.J. Wenzel, A. Migliazza, H.Y. Steensma & J.A. van den Berg. 1992. Efficient selection of phleomycin-resistant *Saccharomyces cerevisiae* transformants. Yeast **8**:667-668. Department of Cell Biology and Genetics, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

The recently described dominant yeast marker *Tn5ble* confers phleomycin resistance on the yeast *Saccharomyces cerevisiae* (Gatignol, Baron and Tiraby, 1987. Mol. Gen. Genet. **207**, 342-348). Incubation in non-selective medium prior to selection is critical, however, for getting phleonmycin-resistant transformants. A 6-h incubation period was found to give optimal transformation frequencies, up to 103 transformants/µg plasmid, comparable to selection for uracil prototrophy (Ura^+).

6. G.P.H. van Heusden, T.J. Wenzel, E.L. Lagendijk, H.Y. Steensma & J.A. van den Berg. 1992. Characterization of the yeast *BMH1* gene encoding a putative protein homologous to mammalian protein kinase II activators and protein kinase C inhibitors. **302**:145-150. Department of Cell Biology and Genetics, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

We describe the identification and characterization of the *BMH1* gene from the yeast *Saccharomyces cerevisiae*. The gene encodes a putative protein of 292 amino acids which is more than 50% identical with the bovine brain 14-3-3 protein and proteins isolated from sheep brain which are strong inhibitors of protein kinase C. Disruption mutants and strains with the *BMH1* gene on multicopy plasmids have impaired growth on minimal medium with glucose as carbon source, i.e. a 30-50% increase in generation time. These observations suggest a regulatory function of the bmh1 protein. In contrast to strains with an intact or a disrupted *BMH1* gene, strains with the *BMH1* gene on multicopy plasmids hardly grew on media with acetate or glycerol as carbon source.

7. S.G. Oliver et al. (many authors). 1992. The complete DNA sequence of yeast chromosome III. Nature **357**:38-46.

The entire DNA sequence of chromosome III of the yeast Saccharomyces cerevisiae has been determined. This is the first complete sequence analysis of an entire chromosome from any organism. The 315-kilobase sequence reveals 182 open reading frames for proteins longer than 100 amino acids, of which 37 correspond to known genes and 29 more show some similarity to sequences in databases. Of 55 new open reading frames analyzed by gene disruption, three are essential genes; of 42 non-essential genes that were tested, 14 show some discernible effect on phenotype and the remaining 28 have no overt function.

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

The following papers have been published recently.

1. G.I. Naumov, E.S. Naumova, R.A. Lantto[1], E.J. Louis[2] and M. Korhola[1]. 1992. Genetic homology between *Saccharomyces cerevisiae* and its sibling species *S. paradoxus* and *S. bayanus*: electrophoretic karyotypes. Yeast **8**:599-612 (See abstract under G.I. Naumov's communication).

2. G.I. Naumov, E.S. Naumova, H. Turakainen,[1] and M. Korhola.[2] 1992. A new family of polymorphic metallothionein-encoding genes *MTH1* (*CUP1*) and *MTH2* in *Saccharomyces cerevisiae*. Gene **119**:65-74 (See abstract under G.I. Naumov's communication).

The following papers were presented at the 8th ISY in Atlanta, Georgia, USA, August 1992.

3. M. Korhola. 1992. Distilling Yeasts. pp. 29-30

The ALK0554 distiller's yeast strain currently used at Alko Ltd was developed by continuous culture selection over 7 months (about 300 generations) for increased ethanol tolerance and thus faster fermentation and yielded a 20% improvement in ethanol production rate and tolerance (Korhola, 1983). The basic idea underlying the selection system was to allow the yeast cells to accumulate the necessary mutations to make them more ethanol tolerant than the starting strain under the conditions used.

Now we have observed (Jalava, 1990) that ALK0554 differs from the starting strain MK270 in having an inferior ability to grow on galactose. We have tried to determine the reasons for the slow induction of galactose utilization by genetic and biochemical studies (Lantto and Korhola, 1992), but the relationship of the Gal^- phenotype and ethanol tolerance is thus far unexplained. Others (Naumov and Gudkova, 1979) have observed that certain wine yeasts had accumulated *gal2*, *gal4* and *gal7* mutations. Alcohol fermentation tanks are an open system, the raw materials are not completely sterile, and the yeast used may contain bacterial contaminants. Therefore, the fermentations always result in the production of some acetic acid and lactic acid by contaminating lactic acid bacteria. Recently we have chosen a new distiller's yeast with high acid and temperature tolerance. The strain was originally isolated from Finnish rye bread sour dough, where conditions of acidity prevail. The fermentation characteristics of the new yeast has been compared to ALK0554 and to some other distiller's yeasts. The new strain is especially aimed at use in plants with continuous grain alcohol fermentation. One of the most sought after characteristics of distiller's yeasts is having an optimal fermentation temperature that is as high as possible. We have determined the maximum temperatures of growth for about 600 *Saccharomyces cerevisiae* strains (Väisänen and Korhola, 1992). Overall there is a remarkable homogeneity among these yeasts; nevertheless, a general correlation was found between the order of the average T_{max} values for these industrial yeasts and the source of isolation, *i.e.* the process use temperature. The average T_{max} temperatures were: 40.5°C for distiller's yeasts, 39.9°C for baker's yeasts, 37.6°C for wine yeasts, and 37.2°C for brewer's yeasts. Since we have worked for a long time on the development of new melibiose-utilizing (mel^+) industrial yeasts (Liljeström et al., 1992), and since even marginal solutions to the industrial effluent problems are welcomed, we have tested some of the genetically engineered MEL-yeast constructs in beet molasses alcohol fermentations at the laboratory scale. The results showed that some of the constructs give a 1-2 percentage point yield increase in normal beet molasses fermentations by fully utilizing the raffinose sugar. Thus, beet molasses alcohol distilleries could increase their alcohol yield and decrease their effluent load by utilizing these Mel^+ yeasts instead of the conventional distiller's yeasts (Mel^-).

4. R.A. Lantto and M. Korhola. 1992. Characterization of the galactose slow growing phenotype of a distillers' yeast. p. 201.

Our distillers' yeast has been isolated from a continuous culture of a commercial bakers' yeast strain after six months incubation in a synthetic medium containing 10% glucose and increasing concentrations [up to 120 (v/v)] of ethanol (1). One of the noticeable new features of the isolate was a weakened ability to grow on galactose. The induction of growth of the isolate was very slow (66 hrs) in YNB + 2% galactose medium compared to the starting strain (10 hrs)(2). Accumulation of *gal2*-, *gal4*,- and *gal7*-mutations in wine making has been reported (3). The purpose of our study is to characterize both genetically and biochemically the mutations causing the slow growing phenotype in our distillers' yeast. Haploid random spore segregants can be grouped in four categories concerning the growth on galactose: 1) wild type, 2) no growth on galactose either in respiratory sufficient (RS) or in respiratory deficient (RD) conditions, 3) delayed induction of growth on galactose when RS and 4) very slow growth on 0.5% galactose but wild type growth on 5% galactose when RS. Mutations seem to be pleiotropic affecting the utilization of different glucose repressible sugars such as raffinose, maltose and glycerol and resembling known *imp*- and *snf*-mutations. Possible changes in the activities of some galactose pathway enzymes are also discussed.The following paper was presented at the 16th International Conference on Yeast Genetics and Molecular Biology, Vienna, Austria, August 1992.

5. H. Turakainen[1], G. Naumov[2], P. Kristo[1] and M. Korhola[1]. 1992. Mapping and sequencing of the Saccharomyces cerevisiae *mel* gene family genes *MEL1-MEL10* Yeast **8** (Spec. Iss.): S659. [1]Department of Genetics, University of Helsinki, Arkadiankatu 7, SF-00100 Helsinki, Finland. [2]All-Union Scientific Research Institute for Genetics and Selection of Industrial Microorganisms, 1 Dorozhnyi 1, Moscow 113545, Russia.

The *MEL* gene family of *Saccharomyces cerevisiae* includes at least ten structural genes for melibiose-hydrolyzing enzyme α-galactosidase: *MEL1-MEL10. MEL* genes have been mapped by electrokaryotyping on the following chromosomes: *MEL1*, II (Vollrath et al. 1988); *MEL2*, VII; *MEL3*, XVI; *MEL4*, XI; *MEL5*, IV; *MEL6*, XIII; *MEL7*, VI; *MEL8*, XV; *MEL9*, X/XIV; *MEL10*, XII (Naumov et al. 1990, 1991). In the present study we have physically and to some extent genetically mapped *MEL2-MEL10* within the chromosomes and found that also these genes, like other fermentation genes in *S.cerevisiae*, map to the ends of chromosomes. The production of small chromosome fragments from the *MEL2-MEL10* and from *MEL1* (2L) strain with same fragmentation plasmids strongly suggested that also *MEL2-MEL10*, like *MEL1*, were located in the left end of chromosomes. Genetic mapping by tetrad analysis of a gene near the telomere meets additional difficulties due to lack of marker genes in that area. We used *GAL4* locus (16R) to genetically map *MEL3*. No cosegregation was observed between these two genes which gave additional evidence for the location of *MEL3* in the left end of the chromosome. We did not try to map the other *MEL* genes. Extensive sequence homology of the *MEL1-MEL10* genes has been demonstrated by Southern blot hybridization of restriction endonuclease-digested genomic DNA (Naumov et al. 1990, 1991). The primary results from sequencing of *Eco*Rl-fragments containing *MEL2-MEL10* genes has shown that the genes are very highly homologous also in 5'- and 3'-flanking regions of the genes. The sequence of the 5'-flanking region beginning from the cloning *Eco*RI site (-512) suggested that *MEL2* was more closely related to *MEL1* than were *MEL3-MEL10*. The 3'-flanking sequences showed almost identical regions in the end of cloned EcoRl-fragments (5 kb downstream from the stop codon in *MEL4* and *MEL8* genes, and 3 kb downstream in *MEL5*, *MEL7* and *MEL9* genes).

6. O. Vuorio, J. Londesborough & N. Kaikkinen[1]. 1992. Trehalose synthase: purification of the intact enzyme and cloning of the structural genes. Yeast **8**(Spec. Iss.):S626. [1]Institute of Biotechnology, Helsinki University, Valimotie 7, SF00380, Helsinki, Finland.

Previously we have reported the purification from baker's yeast of a proteolytically modified complex of trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) that contained a short polypeptide and a truncated long polypeptide [1]. Here we report the purification of the intact complex. The TPS activity of this intact protein can be increased several-fold by the so-called TPS-activator. This activatory protein has now been identified as the glycolytic enzyme, phosphoglucoisomerase. We have also found fructose-6-phosphate to be a strong activator of TPS, especially at physiological phosphate concentrations.

We have cloned two genes which code for the assumed two subunits of trehalose synthase. One, *TSS1*, codes for the

short, 57kDa polypeptide, and the other, *TSL1*, codes for the longer polypeptide, which has an intact molecular weight of about 130kDa. The 5'-terminal sequence of *TSL1* has not yet been determined, but the known 75% shows close homology to *TSS1*. A 1.5 kilobase sequence from the middle of TSL1 is 37% identical at the amino acid level with the entire *TSS1* coding sequence. Surprisingly, *TSS1* is identical with the *CIF1* [2] and *FDP1* [3] genes, alleles which have been recently cloned and sequenced. Apparently, the small subunit of trehalose synthase may be involved in the early steps of glucose-induced cAMP signalling.

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

The following publication has appeared recently.

1. Bailis, A. M., Arthur, L. & Rothstein, R. 1992. Genome rearrangement in *top3* mutants of *Saccharomyces cerevisiae* requires a functional *RAD1* excision repair gene. Molec. Cell. Biol., **12**:4988-4993.

Saccharomyces cerevisiae cells that are mutated at *TOP3*, a gene that encodes a protein homologous to bacterial type I topoisomerases, have a variety of defects including reduced growth rate, altered gene expression, blocked sporulation, and elevated rates of mitotic recombination at several loci. The rate of ectopic recombination between two unlinked, homeologous loci, *SAM1* and *SAM2,* is six-fold higher in cells containing a *top3* null mutation than in wild-type cells. Mutations in either of the two other known topoisomerase genes in yeast, *TOP1* and *TOP2*, do not affect the rate of recombination between the *SAM* genes. The *top3* mutation also changes the distribution of recombination events between the *SAM* genes, leading to the appearance of novel deletion/insertion events in which conversion tracts extend beyond the coding sequence, replacing the DNA flanking the 3' end of one *SAM* gene with non-homologous DNA flanking the 3' end of the other. The effects of the *top3* null mutation on recombination are dependent on the presence of an intact *RAD1* excision repair gene as both the rate of *SAM* ectopic gene conversion, and conversion tract length were reduced in *rad1top3* mutant cells compared to *top3* mutants. These results suggest that a *RAD1*-dependent function is involved in the processing of damaged DNA that results from the loss of Top3 activity, targeting such DNA for repair by recombination.

XXXI. Laboratory of Microbiology, Gulbenkian Institute of Science, 2781 Oeiras Codex, Portugal. Communicated by A. Madeira-Lopes, I. Spencer-Martins & M.C. Loureiro-Dias.

The following are summaries of papers published or accepted since our last communication.

1. M. Kobatake, N.J.W. Kreger-van Rij, T. Placido & N. van Uden. 1992. Isolation of proteolytic psychrotrophic yeasts from fresh raw seafoods. Lett. Appl. Microbiol. **14**:37-42.

A total of 103 cultures of yeasts were isolated from seven kinds of fresh raw seafoods. The isolates comprised six genera, *Candida*, *Cryptococcus*, *Debaryomyces*, *Rhodotorula*, *Sterigmatomyces* and *Trichosporon*, and included 21 different species. All the isolates were psychrotrophic yeasts. Proteolytic activities of 50 psychrotrophic strains were studied by use of skim milk within the temperature range of 2°C. All the strains showed various degrees of proteolysis. In particular, *Candida lipolytica, Trichosporon pullulans* and *Candida scottii* were active species at lowtemperatures. Sensory spoilage due to the proteolytic yeasts were observed in mackerel homogenates stored at 10°C. *C. lipolytica* inoculated homogenates caused spoilage with ammoniacal odours after 1 week of storage. Values of total volatile basic nitrogen at 10°C were highest with *C. lipolytica* among 35 strains tested, followed by *Tr. pullulans*, *Trichosporon cutaneum*, *C. scottii*, *Rhodotorula glutinis* and *Cryptococcus luteolus*. Proteolytic psychrotrophic yeasts were widely distributed in raw seafoods.

2. D. Marquina, C. Peres, F,V. Caldas, J.F. Marques, J.M. Peinado & I. Spencer Martins. 1992. Characterization of the yeast population in olive brines. Lett. Appl. Microbiol. **14**:279-283.

Yeasts were isolated from spontaneous fermentations of olives in brines. Ascomycetous species dominated the yeast flora (>90%) and among them *Pichia membranaefaciens* and related species. Some components of the olives were tested as substrates for growth. Killer activity was observed in approximately half of the isolates, and the wider spectra were displayed by strains of *Pichia anomala*.

3. G. Giménez-Jurado. 1992. *Metschnikowia gruessii* sp. nov., the teleomorph of *Nectaromyces reukaufii* but not of *Candida reukaufii*. System. Appl. Microbiol. **15**:432-438.

One strain identified by conventional phenetic methods as *Metschnikowia reukaufii* did not hybridize with a species-specific probe produced in our laboratory. Molar % G+C composition and a relative heteroduplex formation of 7% with the type strain of *M. reukaufii* revealed that they did not belong to the same taxon. Comparisons with the type strain of *Candida reukaufii* (CBS 1903) and the authentic strain of *Nectaromyces reukaufii* (CBS 611) showed no similarity with the former (22% reassociation) and conspecificity with the latter (97% reassociation). Low nuclear DNA reassociation values with all the type strains of other *Metschnikowia* species provided sufficient evidence for proposing a novel species, *Metschnikowia gruessii* that represents the teleomorph of *Nectaromyces reukaufii* but not of *Candida reukaufii*. It differs from the type strain of *M. reukaufii* in its cell and ascus morphology, assimilation of trehalose and maximum temperature for growth; and from the other described *Metschnikowia* species by additional characteristics. More strains of *Metschnikowia gruesii* were repeatedly isolated from flowers in the natural park of Arrabida, Portugal.

4. A. Madeira-Lopes. 1992. Thermobiology of yeasts; a sketch, In: Profiles on Biotechnology (T.G. Villa and J. Abalde, Eds.), pp, 155-172, Univ. Santiago de Compostela, Spain.

Two distinct categories of temperature profiles (that is, the conjunct display of the Arrhenius plots of specific rates of growth and thermal death) have been found in yeasts. While the associative profiles are characterized by an interference of thermal death with growth, in a temperature range between the optimum and the maximum temperatures for growth, the dissociative profiles exhibit a no-growth, no-death temperature zone, separating the maximum temperature for growth from the minimum temperature of thermal death. The presence of metabolites, antibiotics or preservatives has been observed to produce effects on kinetic, thermodynamic and energetic parameters of growth and death of medically-important yeasts, as well as of industryprone yeasts, which are portrayed on an organisms temperature profile. Those agents may displace a profile "en bloc" to lower temperatures, shrink the temperature range of growth to intermediate temperatures, or transform an associative profile into dissociative. The hypothesis that the targets of thermal death (and, conceivably, of high enthalpy death in general) are mitochondrially determined has been supported by several observational pieces.

5. A. Fonseca. 1992. Utilization of tartaric acid and related compounds by yeasts: Taxonomic implications. Can. J. Microbiol. **38** (in press).

A survey of yeasts capable of growing on L(+)-tartaric acid as the sole source of carbon and energy showed that this organic acid is assimilated by a significant number of species of basidiomycetous affinity and is seldom utilized by ascomycetous yeasts. This conclusion was further supported by the fact that among approximately 100 isolates from various natural substrates using selective media with L(+)-tartaric acid only one strain of ascomycetous affinity was obtained. In a more comprehensive survey 442 yeast strains belonging to 138 species, mostly of basidiomycetous affinity, were also screened for the assimilation of different aldaric acids: D(-)-tartaric acid, meso-tartaric acid, L(-)-malic acid, D(+)-glucaric acid (saccharic acid) and galactaric acid (mucic acid). L(+)-Tartrate was the most frequently utilized tartaric acid isomer (55% of the total number of strains of basidiomycetous affinity belonging to either the Tremellales/Filobasidiales or the Ustilaginales) when compared with the D(-) and *meso* forms which were assimilated by 12 and 18% of the total number of strains, respectively (mainly of tremellaceous species). Saccharic acid was utilized by about 75% of the total number of species of Tremellales affinity and by less than 20% of the ustilaginaceous species. Assimilation of mucic acid occurred in more than 50% of the tremellaceous species and only in 5% of the species related to the Ustilaginales. These tests, not used in standard yeast identification sets, appear to contribute to distinguishing taxa at or above the species level.

XXXII. Instituto de Biotecnologia, Universidade de Caxias do Sul, Caixa Postal 1352, 95070 Caxias do Sul, RS, Brazil. Communicated by J. L. Carrau.

The following abstracts describe recent work from our laboratory.

1. Griguol,[1] R.I. & J.L.Carrau. Detection of killer/neutral/sensitive yeasts at the native flora of wine districts of Cordoba and La Rioja, Argentina.

[1]Cooperativa la Riojana, Chilecito, La Rioja/Inst. de Invest. "Dr. F. Oreglia", Fac. Enologia/UJAM, Mendoza, Argentina.

In the present communication we show the initial results obtained in the identification of killer, neutral and sensitive phenotypes of yeasts in vineyards and wine industries of Cordoba and La Rioja. A total of 50000 clones were evaluated, being 5000 from a wine industry of Cordoba and 45000 from three industries of La Rioja. In both regions vineyard and wine industry samples were evaluated. Killer clones were only detected in the wine industries after the beginning of fermentation. Outnumbering by native killer yeasts was observed during the fermentation process. Even when high volumes of dry yeast were inoculated, native killers outnumbered the industrial strain at the end of the fermentation.

2. S. Echeverrigaray, F.C.A. Tavares,[1] L.H. Gomes[1] & F.C. Boscariol.[1] 1991. Inhibition of yeast growth by herbicides.

[1]Dpto. Genética, ESALQ-Universidade de São Paulo, Piracicaba, SP, Brazil. Ciência e Cultura **43**:457-459.

Yeast growth inhibition was evaluated in different media in the presence of fourteen herbicides. Nine herbicides at high concentrations were effective against yeasts. Variable response to the kind and dosage of herbicides was observed among and within yeast species. The most effective herbicides were Dual (metolachlor) and Propanin (propanil). The results suggested that at the recommended agricultural dosages, the herbicides tested would have a minimal impact on natural yeast populations.

3. S. Echeverrigaray, L.H. Gomes[1] & F.C.A. Tavares.[1] Metolachlor resistance in *Saccharomyces cerevisiae*.

[1]Depto. Genética, ESUQ-Universidade de São Paulo, Piracicaba, SP, Brazil.

Metolachlor, an α-chloroacetamide herbicide, inhibits growth of yeast *Saccharomyces cerevisiae* on complete, minimal and non fermentative media. Spontaneous and induced mutants resistant to Metolachlor were isolated. All of the resistance mutations segregated 2:2 in tetrads, 70% were recessives, 16.6% were semi dominants and 13.4% were dominants. A semi dominant spontaneous resistant mutation (*MTC1*) was mapped in chromosome XV at 33.3 cM from *ade2* and 31.7 cM from *his3*, and a recessive mutation (*mtc2*) was located in chromosome IV. Other than the main effect of Metolachlor that inhibits yeast growth in complete medium, a secondary effect of the herbicide was detected on yeast respiration.The following papers were presented at the 5th Latin American Congress on Enology and Viticulture, Montevideo, Uruguay.

4. M. Soubihe,[1] L.H. Gomes,[1] S. Echeverrigaray, & F.C.A. Tavares.[1] Identification of industrial yeast strains by electrophoretic analysis of isozymes and proteins.

[1]Dept. Genética, ESALQ- Universidade de São Paulo, Piracicaba, SP, Brazil.

Several techniques as serological analysis, electrophoresis of proteins and DNA have been used with success in the identification and characterization of yeast strains applied on wine making, brewing, baking and distillation industry. In the present report we evaluated the efficiency of the electrophoretic analysis of total proteins and isozymes for the identification of the five yeast strains commonly used in Brazilian fuel alcohol industries. The α-esterase analysis showed three different patterns allowing the identification of the strains M606 and Itaiquara. Three patterns were also

obtained for lactate-dehydrogenase individualizing the strains M300A and Itaiquara. The Fleischmann strain showed a unique pattern for 6-phosphoglucoisomerase. Total protein electrophoretic analysis allowed the rapid characterization of M606 and Itaiquara strains. The quantitative evaluation of total protein electrophoretic profiles showed significant differences on all the strains.

5. S. Echeverrigaray, F.C.A. Tavares,[1] U. Lavi,[2] O. Gal[3] & J. Hillel.[3] DNA fingerprinting applied to yeast identification.

[1]Depto. Genética, ESALQ-Universidade de São Paulo, Piracicaba, SP, Brazil;
[2]Agric. Res. Org., Bet Dagan, Israel;
[3]Dept. Genetics, Fac. Agriculture, Hebrew Univ. Jerusalem, Rehovot, Israel.

The classification and identification of yeasts has traditionally relied on biochemical, morphological and physiological criteria, which often fail to identify yeast strains within a species, limiting their potential on industrial yeast strains mini satellite probes which cross-hybridize to hypervariable regions provided a highly polymorphic system of molecular genetic markers widely used in individuals identification in animals, plants and microorganisms. In the present communication we report the use of minisatellite probes (33.6; 33.15; M13 and R18.1) in the identification of *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* strains. The most useful probe for DFP analysis of yeasts was R18.1, originated from a bovine genomic library. This probe yielded 14 to 27 distinguishable bands in each profile, allowing the evaluation of bandsharing frequencies: 0.40 to 0.44 between strains of *S. cerevisiae*; 0.11 to 0.18 between *S. cerevisiae* and *K. marxianus* and 0.70 between the parental strain KM9 and the hybrid MM1 (*K. marxianus*).

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

Recent publication.

1. Y. Yamada, K. Maeda, T. Nagahama, I. Banno & M.A. Lachance. 1992. The phylogenetic relationships of the genus *Sporopachydermia* Rodrigues de Miranda (Saccharomycetaceae) based on the partial sequences of 18S and 26S ribosomal RNAs. J. Gen. Appl. Microbiol. **38**:179-183.

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

XXXIV. Department of Biology, Faculty of Medicine, Masaryk University Brno, Joštova 10, 662 44 Brno, Czechoslovakia. Communicated by M. Kopecká.

Recently published papers.

1. M. Gabriel, M. Kopecká & A.. Svoboda. 1992. Structural rearrangement of the actin cytoskeleton in regenerating protoplasts of budding yeasts. J. Gen. Microbiol. **138**:229-239.

In *Saccharomyces cerevisiae* cells the actin cytoskeleton is present as actin dots in the bud and around the septum, i.e. in areas of intensive cell wall synthesis, and as actin cables, which are loose bundles along the longitudinal cell axis. However, the apparently asymmetrical pattern of actin no longer persisted after protoplasting, when the cables disappeared and dots were evenly distributed under the whole protoplast surface. This pattern was maintained during regeneration of a new cell wall all over the protoplast surface, thus providing evidence of a relationship between the new wall formation and the presence of a regular arrangement of actin dots. The completed cell wall allowed the protoplast to bud and produce a normal daughter cell. However, before the walled protoplast began to bud, actin dots accumulated at the site of bud emergence and actin cables appeared, extending to the cytoplasm. Later, actin dots accumulated in the growing bud, forming a ring in the neck, and actin cables passed to the bud. Completion of the protoplast-to-cell reversion was preceded by restoration of the normal actin cytoskeleton.

2. M. Kopecká & M. Gabriel. 1992. The influence of Congo red on the cell wall and (1-3)-ß-D-glucan microfibril biogenesis in *Saccharomyces cerevisiae*. Archiv. Microbiol. **158**:115-126.

Congo red was applied to growing yeast cells and regenerating protoplasts in order to study its effects on wall biogenesis and cell morphogenesis. In the presence of the dye, the whole yeast cells grew and divided to form chains of connected cells showing aberrant wall structures on both sides of the septum. The wall-less protoplasts in solid medium with the dye exhibited an abnormal increase in volume, regeneration of aberrant cell walls and inability to carry out cytokinesis or protoplast reversion to cells. In liquid medium, the protoplast synthesized glucan nets composed mainly of thin, fibrils orientated at random, whereas normally, in the absence of dye, the net consist of rather thick fibrils, 10 to 20 nm in width, assembled into broad ribbons. These fibrils are known to consist of triple 6/1 helical strands of (1-3)-ß-D-glucan arranged laterally in crystalline packing. The thin fibrils (c. 9 to 4 nm wide) can contain only a few triple helical strands (c. 1.6 nm wide) and are supposed to be prevented from further aggregation and crystallization by complexing with Congo red on their surfaces. Some loose triple 6/1 helical strands (native elementary fibrils) are also discernible. They represent the first native (1-3)-ß-D-glucan elementary fibrils depicted by electron microscopy. The effects of Congo red on growth and the wall structure in normal cells and regenerating protoplasts in solid medium can be explained by the presence of a complex which the dye forms with (helical) chain parts of the glucan network and which results in a loss of rigidity by a blocked lateral interaction between the helices.

Invited lecture, 4th International Symposium on Yeasts, August 23-28, 1992, Atlanta, Georgia, USA.

3. M. Kopecká & M. Gabriel. 1992. Electron microscopy of the yeast cell wall.

Posters, 6th Cell Wall Meeting, August 25-24, 1992, Nijmegen, the Netherlands.

4. M. Gabriel & M. Kopecká. Morphogenesis of the cell wall in yeasts investigated in actin mutants and their protoplasts.

5. A. Svoboda & O. Nečas. Cell wall formation in protoplasts of yeast secretory mutants

Posters, 16th International Conference on Yeast Genetics and Molecular Biology, August 15-21, 1992, Vienna, Austria.

6. M. Gabriel & M. Kopecká. A single mutation in the actin gene can dissociate nuclear division from cytokinesis in the cell cycle of budding yeasts.

7. M. Havelková & I. Pokorná. Aberrant nuclear cycles induced by prolonged growth in yeast protoplasts without cell walls.

8. A. Svoboda & I. Pokorná. Changes in the cytoskeleton associated with conjugation and sporulation in *Schizosaccharomyces japonicus* var. *versatilis*.

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

The following article has appeared since the last issue of the Yeast Newsletter.

1. Seghueila, L., Lambrechets C., Boze H,, Moulin G., Galzy P. 1992. Purification and properties of the phytases from *Schwanniomyces castellii*. J. Ferment. Biotechnol. **74**:1-6.

XXXVI. Laboratorio de Ecologia Microbiana e Taxonomia & Laboratorio de Leveduras, Coleção de Culturas, Departameto de Microbiologia Geral, Instituto de Microbiologia, CCS, Bloco I, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, 21941, Brasil. Communicated by A.N. Hagler and L.C. Mendonça-Hagler.

We would like to thank Elena Naumova and Gennadi Naumov for their many contributions to our laboratories during their visit between March and August of 1992.

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

1. Morais, P.B., A.N. Halger, C.A. Rosa, L.C. Mendonça-Hagler, A.N. Hagler & L.B. Klaczka. 1992. Yeasts associated with *Drosophila* in tropical forests of Rio de Janeiro, Brazil. Can. J. Microbiol. **38**: (in press).

The distribution and diversity of yeast species vectored by and from the crop of eight groups of species of *Drosophila* is described for two rain forest sites and an urban wooded area in Rio de Janeiro, Brazil. The typical forest *Drosophila* groups *guarani*, *tripuctata* and *willistoni* showed a higher diversity of yeasts than the cosmopolitan *melanogaster* flies, suggesting different strategies of utilization of substrates. Apiculate yeasts, including *Kloeckera apis*, *Kloeckera javanica* and *Kloeckera japonica* were the prevalent species. *Geotrichum* spp. and *Candida citrea* were also frequent isolates in the forest sites. Our results suggested that the flies carried yeasts from their feeding substrates. Most of the yeasts were strong fermenters and assimilated few compounds, usually sucrose, cellobiose and glycerol. This indicated a preference of the flies for food sources like fruits. Some yeasts were primarily isolated from one group of *Drosophila*; for example *Kloeckera javanica* from *melanogaster*, *Debaryomyces vanriji* var. *yarrowii* from *tripunctata* and *Kluyveromyces delphensis* from *willistoni* flies. These associations and differences in the yeast communities among the fly groups suggested a differentiation of diets and specialization of the yeast-*Drosophila* association in the tropical forests.

2. Morais, P.M., A.N. Hagler, C.A. Rosa & L.C. Mendonça-Hagler. 1992. High maximum growth temperature apiculate yeasts from *Drosophila* of Rio de Janeiro. Rev. Microbiol. (São Paulo) **23**:163-166.

Some strains of *Kloeckera africana*, *K. apis*, *K. japonica* and *K. javanica* isolated from *Drosophila* in forests and an urban wooded area in Rio de Janeiro, RJ grew at temperatures that exceeded those reported for these apiculate yeasts. These isolates represent new biotypes that may have been selected by high environmental temperatures frequently encountered in the tropical climate of Rio de Janeiro, Brazil.

3. Rosa, C.A., A.N. Hagler, R. Monteiro, L.C. Mendonça-Hagler, and P.B. Morais. 1992. *Clavispora opuntiae* and other yeasts associated with the moth *Sigelgaita* sp. in the cactus *Pilosocereus arrabidae* of Rio de Janeiro, Brazil. Antonie van Leeuwenhoek. In press

Clavispora opuntiae was the prevalent yeast associated with the feeding sites of *Sigelgaita* sp. larvae in the cactus *Pilosocereus arrabidae*. Also associated with this habitat were *Candida arrabidae*, *Pichia cactophila*, *Pichia barkeri*, *Candida* sp. A, *Geotrichum* sp., *Geotrichum sericeum* and the yeastlike organisms *Prototheca zopfii* and *Acremonium* sp. Atypical yeast biotypes were isolated that may represent new species of *Pichia*, *Sporopachydermia* and *Candida*. Mating types of *Clavispora opuntiae* were at a ratio $70h^+$ to 3 h^- and reduced levels of sporulation suggested low pressure for sexual reproduction in this habitat. *Sigelgaita* sp. probably was not an important vector for *Clavispora opuntiae* because it was not isolated from an adult or eggs of this moth.

4. Naumov, G.I., E.S. Naumova, L.C. Mendonça-Hagler, and A.N. Hagler. 1992. Taxogenetics of *Pichia angusta* and similar methylotrophic yeasts. (Minireview) Ciencia e Cultura **44**: in press.

Methanolotrophic yeasts are classified in the genera *Candida* and *Pichia* and have potential applications in biotechnology because of the possibility of allowing the use of methanol to produce products. The thermotolerant methanol assimilating yeast *Pichia angusta* (Tennisson, Hall, et Wickerham) Kurtzman (syn. *Hansenula polymorpha*, *Hansenula angusta*) has considerable intraspecific genetic heterogeneity. Isolates from cacti have been shown by DNA/DNA reassociation to be genetically homogenous independent of geographical origin. However GC% and DNA/DNA reassociation data of *P. angusta* isolates from soil, water and orange juice show differentiation from cactus strains at least at the variety level. Classical genetics also shows a degree of genetic isolation between two strains that suggests the existence of sibling species. Some other methanol assimilating *Pichia* species are similar to *P. angusta* in phenotype and DNA base composition.

5. Mendonça-Hagler, L.C., A.N. Hagler, and C.P. Kurtzman. 1992. Phylogeny of *Metschnikowia* species estimated from partial rRNA sequences. Inter. J. Syst. Bacteriol. **43**: in press.

Phylogenetic relationships of species assigned to the genus *Metschnikowia* were estimated from extent of divergence among partial sequences of rRNA. The data suggest that aquatic (*M. australis*, *M. bicuspidata*, *M. krissii*, and *M. zobellii*) and terrestrial species (*M. hawaiiensis*, *M. lunata*, *M. pulcherrima*, and *M. reukaufii* form two groups within the genus. *M. lunata* and *M. hawaiiensis* are well separated from other members of the genus and *M. hawaiiensis* may be sufficiently divergent that it could be placed into a new genus. Species of *Metschnikowia* are unique compared to other ascomycetous yeasts because they have a deletion in the large subunit rRNA sequence that includes nucleotides 434-483.

6. Hagler, A.N., L.C. Mendonça-Hagler, C.A. Rosa and P.B. Morais. 1992. Yeasts as an example of microbial diversity of Brazil. Estrutura, Funcionamento e Manejo de Ecossistemas. UFRJ, Rio de Janeiro, in press.

The high degree of biodiversity among microorganisms is noted and some of the problems involved in studies of microbial communities are presented using yeasts as an example. Considerable data are available on yeasts of temperate regions and substantial collections of yeasts from these studies exist, but studies of yeasts in tropical environments have been rare and mostly focused on medical interests. The diversity and distribution of yeasts in animals, aquatic and terrestrial environments including sand dune (restinga) ecosystems, *Drosophila* of rain forests, and fruits in Brazil are reviewed. These studies have shown different habitats to have characteristic yeast communities and these include many new biotypes, some probably representing new species.

7. Costa, A.M., M.L. Sanches Nunes, M.D.M. van Weerelt, G.V. Fonseca Faria, L.C. Mendonça-Hagler, and A.N. Hagler. 1991. Microbial quality of the mussel *Perna perna* extracted from Guanabara Bay (RJ, Brasil). In Magoon et al. eds. Costal Zone 91, Vol. 4:3259-3267.

Mussels (Perna perna) concentrated microorganisms including yeasts, coliforms, filamentous fungi and heterotrophic bacteria from the water of their environment. Yeast counts were about 100 times higher in whole mussel tissue homogenates than in seawater at the site of their collection. Most of these microbial populations were in the intestine of the mussels.

8. Guimaraes, V.F., M.A. Araujo, L.C. Mendonça-Hagler and A.N. Hagler. 1992. *Pseudomonas aeruginosa* and other microbial indicators of pollution in fresh and marine waters of Rio de Janeiro, Brazil. Environmental Toxicology and Water Quality, in press.

Counts of *Pseudomonas aeruginosa* had a high positive correlation with counts of coliforms, fecal streptococci, presumptive pathogenic yeasts and heterotrophic bacteria in surface waters of Rio de Janeiro. The use of a 40°C

incubation temperature for yeast counts was highly selective for pollution associated yeasts, especially the opportunistic pathogens *Candida tropicalis*, *Candida krusei*, and *Candida parapsilosis*. Microbial indicators of fecal pollution were not effective for noting the presence of *P. aeruginosa* in freshwaters.

The following undergraduate monographs have recently been defended.

9. Georgia, Maria de Oliverira Franco, B.Sc. 1992. Leveduras e coliformes associados a Bromelia *Quesnella quesnellana* no Manquezal de Coroa Grande, Baia de Sepetiba-RJ. Inst. Biologia UFRJ. 48+, VII pp.

Water was collected from 22 plants of the bromeliad species *Quesnelia quesneliana* from the mangrove area near Coroa Grande, Sepetiba Bay, Rio de Janeiro, Brazil. Total coliform counts ranged from 1,100 to 2,400,000/100 ml and fecal coliforms from 8 to 54,000/100 ml. 37 of 90 cultures isolated from fecal coliform tests were identified as *Escherichia coli*. The most frequent yeast isolates were *Aureobasidium pullulans*, *Candida famata*, *Candida colliculosa*, *Candida quilliermondii*, *Cryptococcus laurentii*, *Cryptococcus albidus*, and *Rhodotorula rubra*. The sanitary significance of the coliform counts in these waters was questioned because the yeast community was typical of plant associated communities and mostly lacking yeasts typical of fecal contamination.

10. Simone, Reis Santos. B.Sc. 1992. Communidades de leveduras associadas a nectarios extraflorais de *Senna australis* e *Senna bicapsularis* (Leguminosae) e Insetos Vetores na Restinga de Barra de Marica (RJ).

Extra floral nectaries had yeast communities that mostly were the same as those of insect visitors. This community was mostly formed by basidiomycetous yeasts that were non-fermentative, grew on a large variety of carbon sources, assimilate nitrate, produce pigments and starch like compounds, and are osmotolerant. Of the insects sampled, 76% vectored yeasts with *Candida antarctica*, *Rhodotorula glutinis*, and *Pichia* sp. the most frequent. Nectaries from *S. australis* plants in flower were more frequently colonized by yeasts and with more diverse communities including *Aureobasidium albidus* the most frequent.

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

The following article, whose abstract was given in the last issue, is now in press.

1. Maleszka, R. & G.D. Clark-Walker. 1993. Yeasts have a four-fold variation in ribosomal DNA copy number. Yeast **9**:53-58.

Obituary

Dr. Anna Kocková-Kratochvílová

It is with great sadness that we have to announce the death of Professor Dr. A. Kocková-Kratochvílová, DrSc., a prominent scientist of the Institute of Chemistry, Slovak Academy of Sciences in Bratislava, Czechoslovakia, and also a former associate editor of the Yeast Newsletter. She passed away on July 22, 1992, at the age of 77, after a prolonged illness. Her death is a great loss to Czechoslovak science and the international yeast research community. She belonged to those Czechoslovak scientists who gained international reputation under conditions of the previous system in the country, as a result of her enormous

working activity and total devotion to scientific work. Her most important achievement is the Czechoslovak Collection of Yeast and Yeast-like Microorganisms, which she founded and directed for several decades. The Collection is one of the largest in Central Europe and represents a source of strains for research and fermentation industry. She contributed more than 300 original papers to yeast taxonomy, ecology and biochemistry. She wrote several textbooks and monographs, published several Yeast Catalogues. Her last title published in English was "Yeasts and Yeast-like Organisms" (VCH Publishers). Her last book "Taxonomy of Yeasts and Yeast-like Microorganisms", which was completed only recently and published in Slovak, awaits its translation into foreign languages.

During her whole life Dr. Kocková-Kratochvílová was extremely active in promoting international scientific contacts and cooperation. She was the founder of the tradition of general and specialized international symposia. She organized the First International Symposium on Yeasts in 1964 in Smolenice Castle near Bratislava. She was also in charge of the Second International Symposium on Yeasts held in 1966 in Bratislava. During this conference she initiated the creation of the International Commission for Yeasts which became the driving force of international activities of yeast researchers in the years to come. Dr. Kocková-Kratochvílová served as the first chairman of this international body (1966-l969). She also was an outstanding teacher. She lectured at various Universities at home and abroad. She supervised 16 PhD students and more than 20 graduate students. Her meritorious work lives in her pupils.

An unlimited devotion of Dr. Kocková-Kratochvílová to science, her unbelievable energy and enthusiasm, her love for nature, will remain an example for all those who knew her.

Peter Biely

International Commission on Yeasts

General Meeting of the International Commission on Yeasts, August 27, 1992, Atlanta, Georgia (8th ISY)

Minutes of the meeting of the International Commission on Yeasts and Yeast-like Fungi, held August 27, 1992, 12:30 p.m., at Georgia State University, Atlanta, Ga, USA. ICY Chair Alessandro Martini presiding.

COMMISSIONERS IN ATTENDANCE: D.R. Berry (U.K.), T. Deak (Hungary), J.C. du Preez (South Africa), G.H. Fleet (Australia), G.S. de Hoog (The Netherlands), P. Galzy (France), M. Korhola (Finland), C.P. Kurtzman (U.S.A.), B.F. Johnson (Canada), M.A. Lachance (Yeast Newsletter), A. Martini (Italy), S.A. Meyer (U.S.A.), H.J. Phaff (Honorary), B. Prior (South Africa), I. Russell (Canada), M.Th. Smith (The Netherlands), J.F.T. Spencer (U.K.), and G.G. Stewart (Canada).

1. MINUTES OF THE LAST MEETING. The minutes of the previous meeting were accepted as written.
2. MEMBERSHIP. Alessandro Martini introduced the topic of membership on the Commission (each country can have three members). A list of new members was proposed.[1] The list was accepted by all members in attendance. New members will be notified and given six months to reply. The resignation of Dr. A.H. Rose was accepted.
3. NOMINATIONS FROM SOCIETIES. Bernard Prior suggested that National Societies propose members for the Commission. Alessandro Martini stated that this would not work well in his country, and possibly not in others. Clete Kurtzman questioned which of several national societies would make the recommendations and Herman Phaff questioned who would make the final decision.
4. FUTURE MEETINGS - ISSY. The next Specialized Symposium on Yeasts will be held August 23-26, 1993 in Arnhem, The Netherlands. Topic: "Metabolic Compartmentation in Yeasts".
   PROPOSED ISSY
   1994 - New Delhi, India - Topic: Pathogenic Yeasts.
   1995 - St. Petersburg, Russia - Topic: Not determined; possibly be ecology.
5. NEXT GENERAL SYMPOSIUM - ISY-9 - 1996. Alessandro Martini reported that a proposal was received that recommended a joint meeting with the International Biotechnology Symposium, August 25-30, 1996 in Sydney, Australia. Graham Fleet enthusiastically gave his endorsement for the meeting to be held in Sydney. He said there was strong backing from industry and support was available. Although there are many topics overlapping with the Biotechnology group, the ISY

would remain independent. He pointed out that there is growing interest in yeasts in Asia and Southeastern Asia and we would attract people from this area.

David Berry said that John Johnston (Scotland) considered to propose that the next general symposium be held in Edinburgh, but that this could be reconsidered in terms of a Specialized Symposium, perhaps in 1995. Possible topics: Physiology, genetics, growth and differentiation.

Graham Fleet added that the International Bacteriology and Mycology meeting was going on a 3-year cycle, the next meeting to be held in Israel.

(Note: The 1992 Directory of IUMS states that the 7th International Congress of Bacteriology & Mycology will be held in Prague, Czechoslovakia, July 3-8, 1994 and that the 8th International Congress will be held in Jerusalem, Israel, August 19-26, 1996. S.A.M.)

The Yeast Genetics meetings will convene in odd numbered years: 1993 U.S.A., 1995 Portugal, and 1997 Italy.

It was agreed that the next International Symposium on Yeasts will be held in Sydney, Australia, 1996.

6. ICY ARCHIVES. Alessandro Martini stated that Archives for the International Commission on Yeasts and Yeast-like Organisms should be organized. Numerous documents, books, and letters should be preserved and maintained. Previous organizers of meetings should be contacted for information on these meetings.
7. NAME OF THE ICY. The name INTERNATIONAL COMMISSION ON YEASTS AND YEAST-LIKE ORGANISMS was discussed. Different records show several different versions, and some discrepancies exist. It was unanimously decided that the name should be shortened to INTERNATIONAL COMMISSION ON YEASTS. The secretary of the International Union of Microbiological Societies will be informed of the decision.
8. DECLINING ATTENDANCE. The question of attendance at our meetings was discussed. The need to bring in younger people, namely students, to keep our organization growing and dynamic was emphasized.

   André Lachance suggested the possibility of offering scholarships to students for attending the meetings.
9. ICY DIRECTORY. Alessandro Martini had requested names of yeast researchers to be sent, but few were received. He is still willing to collect names and disperse the information. It was agreed that the Yeast Newsletter mailing list should be appended to a forthcoming issue, and that additional names of 'yeast students and postdocs' should be sent to André Lachance for inclusion in the Yeast Newsletter.
10. ELECTION OF THE NEXT ICY CHAIR FOR 1992-96. Sally Ann Meyer, Chair of the Organizing Committee of ISY-8 was elected unanimously.
11. MISCELLANEOUS BUSINESS. Alessandro Martini reported on a problem brought to his attention regarding the pronunciation of Latin names of the yeast species. He volunteered to contact a Latin scholar to learn the correct pronunciation and to pass this information on to the rest of us in the future. Members expressed their appreciation for his willingness to do so.

Meeting adjourned at 1:35 p.m.

Sally A. Meyer

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[1]List of newly proposed ICY members (Nominating members are given in parentheses)

BEKER, Martin, Latvia (N. Elinov & R. Sentandreu, ISSY XV)
RAPOPORT, Alexander, Latvia (T. Lachowicz and R. Sentandreu, ISSY XV)
BIELY, Peter, Czechoslovakia (E. Minarik for A. Kocková-Kratochvílová, ISSY XIV)
CALLEJA, Gode B., The Philippines (B.F. Johnson, ISSY XIV)
SPENCER-MARTINS, Isabel, Portugal (S.A. Meyer and A. Martini)
LOUREIRO-DIAS, Maria C., Portugal (W.A. Scheffers, ISSY XIII)
OLSEN, Ingar, Norway (A. Stenderup, ISSY XIV)
SANDVEN, Per, Norway (A. Stenderup, ISSY XIV)
OXENBOLL, Karen M., Danmark (A. Stenderup, ISSY XIV)
RASPOR Peter, Slovenia (V. Johamides, ISSY XV)
PRILLINGER Hans-Joerg, Austria (H. Klaushofer, ISSY XIV)
SCHENBERG, Ana C. Brazil (S.A. Meyer)
MENDONÇA-HAGLER, Leda C., Brazil (A. Martini)
LI Ming-Xia, Popular Republic of China (C.P. Kurtzman, ISSY XIV)
MAGORNAYA, S.S., Ukraine (Smirnov, President, Ukrainian Acadeny of Sciences)
SIBIRNY Andrei, Ukraine (G. Shawlowsky, ISSY XV)
PRASAD R., India (A. Martini, ISSY XIV)
KULAEV, I. S., Russia (N.P. Elinov, ISSY XV)
MOORE, Roy, United Kingdom (J.F.T. Spencer & A. Martini)
SPENCER, Frank, (changing country representation from U.K. to Argentina)
DE FIGUEROA, Lucia I., Argentina (J.F.T. Spencer).

Recent meetings

21st Annual Yeast Conference of the Czechoslovak Commission for Yeasts, Bratislava, Czechoslovakia, February 18-20, 1992.

The tradition of the annual yeast meetings of the Czechoslovak Commission for Yeasts held in Smolenice Castle continues. On February 18-20, 1992, the Commission in collaboration with the Institute of Chemistry, Slovak Academy of Sciences in Bratislava, organized the 21st Annual Yeast Conference. The scientific program was concentrated on three topics: Industrial Yeasts, Yeast Genetics, and Structure and Biological Activity of Yeast Polysaccharides.

Plenary lectures on industrial yeasts:

B. Pardonová: Brewing yeasts and practice.

F. Malík, S. Michalčáková: Industrially important strains of winery yeasts.

J. Pásková, J. Langpaulová: Production strains in brewing and bakery.

B. Janderová, O. Bendová: Construction of industrial yeast strains.

R. Zeman: Biosynthesis of phenylacetylcarbinol by yeasts.

J. Vala: Trends in better utilization of yeast biomass.

Plenary lectures in Yeast Genetics:

V. Vlčková: Yeasts as models to study genotoxicity of chemicals.

J. Brozmanová: DNA repair in yeasts.

J. Šubík: Genetic and physical methods of gene mapping.

L. Šabová: Regulation of the transcription of genes coding for mitochondrial proteins.

M. Šipicky: Molecular cloning in *Schizosaccharomyces pombe*.

I. Janatová: Vector systems for non-traditional yeasts.

B. Janderová, O. Bendová: Artificial yeast chromosomes.

Z. Pálková: Expression of foreign genes in yeasts.

J. Kolarov: Isogenes of mitochondrial proteins.

S. Michalčaková: Yeast killer systems.

F. Půta: Vectors for positive selection.

T. Ruml: Cloning of the gene coding for the HIV virus envelope protein in the cells of *Saccharomyces cerevisiae*.

V. Vondrejs: Autogenomic libraries as an evolutionary abbreviation.

Plenary lectures on yeast polysaccharides:

T. Trnovec: Immunomodulating polysaccharides.

A. Tomšíková, D. Vraná, L. Kotál: Inhibitory effect of glucan-chitin on the adherence of *Candida albicans* to epithelial cells.

D. Chorvatovičová, J. Navarová: Protective effect of glucans against mutagens.

J. Navarová, D. Chorvatovičová: Biochemical aspects of the protective effect of glucans.

L. Slováková, V. Šubíková, J. Šandula: The effect of yeast cell wall components on the hypersensitive plant reaction after infection with viruses.

M. Kopecká, J. Šandula, J. Navarová: Electron microscopic study of yeast cell wall and cell wall glucans.

J. Šandula: Structure and biological properties of cell wall polysaccharides of the genera *Saccharomyces* and *Candida*.

J. Šajbidor: Lipids of *Saccharomyces cerevisiae*.

The following posters were exhibited at the conference:

V. Stollárová: Application of the knowledge of yeast ecology in high-school biology courses.

E. Breierová: Trehalose as a cryoprotective agent.

E. Breierová, J. Šajbidor: Composition of fatty acids in lipids from xylose-utilizing yeasts.

J. Šajbidor, E. Breierová: Osmotic pressure effect on fatty acid composition in a *Dipodascus* sp.

K. Dercová, S. Baláž, D. Müncnerová, L. Haluška: Biodegradation of polychlorinated phenols by yeasts.

V. Farkaš, J. Haplová: Solubilization of glucan-synthase from *Saccharomyces cerevisiae*.

E. Machová, L. Šoltés: Depolymerization of *Saccharomyces cerevisiae* glucan by ß-1,3-glucanase from *Trichoderma reesei*.

M. Vršanska, P. Biely, J. Defaye: Induction of the xylanolytic system of *Cryptococcus albidus* by thioanalogues of xylobiose and methyl-ß-D-xyloside.

E. Slaviková, R. Vadkertiová: Yeasts producing carotenoid pigments and their occurrence in calm waters.

F. Cvrčková, B. Zikánová, B. Pardonová: Chlorosulfurone resistant brewers yeasts and the diacetyl production.

M. Gabriel, M. Kopecká, A. Svoboda: Protoplasts of *Saccharomyces cerevisiae* DBY 1690 $ACT1^{+}/ACT1^{+}$ in studies of the role of the actin cytoskeleton in cell morphogenesis.

D. Filipp, G.L. Kogan: Use of artificial yeast chromosomes for cloning of long DNA fragments of *Drosophila*.

M. Grešík, M. Rose, K. Melcher, K.-D. Entian: Isolation and characterization of the *SER2* gene coding for 3-phosphoserinephosphatase in *Saccharomyces cerevisiae*.

T. Ruml, D. Bedwell, E. Hunter: Formation of the gag proteins and capsid Mason-Pfizer ape virus in *Saccharomyces cerevisiae*.

M. Janitor, J. Šubík: Construction and properties of a *Saccharomyces cerevisiae* recombinant used for the investigation of nucleus-cytoplasm relations in eukaryotes.

F. Jurský: Replication ability of the ARS sequences of *Saccharomycopsis fibuligera* in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Kluyveromyces lactis*.

J. Nosek, P. Griac, D. Filipp: Physical characterization of the genome of *Dipodascus magnusii*.

M. Obernauerová, J. Šubík: Effect of ofloxacine on mitotic stability of plasmids and chromosomes in yeasts.

G. Kogan, J. Šandula: Structural features of a glucomannan from *Candida utilis*.

At a meeting of the Czechoslovak Commission for Yeasts that took place during the conference it was decided that the 22nd Annual Yeast Conference will be organized in Smolenice, February 17-19, 1993, and that its scientific program will be devoted mainly to genetics and molecular biology of yeasts, yeast cytoskeleton and industrially important yeasts.

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

Yeast Biology Symposium, Haryana Agricultural University. Hisar 125004, India.

The two-day National Symposium on Yeast Biology, held at HAU on 20-21 Feb, 1992, was attended by about 150 delegates from India and Abroad. Besides representatives from research institutions and industries, the participants in the National Symposium included Professor David R. Berry from UK, Professor Peter Munze from Switzerland, Professor Yasuyuki Uzuka from Japan and Professor Gugnani from Nigeria, who shared their experiences in the alcohol industry, basic genetics and for production of fats and flavours with their Indian counterparts. The Symposium provided an excellent opportunity for interaction between research scientists, representatives from the yeast industry and concerned specialists.

The Symposium was opened by the Vice-Chancellor, Dr. A.L. Chaudhary. In his address, He said that yeast has been studied as an integral part of the animal and plant sciences since time immemorial. In India yeast is used by 218 distilleries, 50 breweries, 450 sugar factories together with a large number of pharmaceutical industries and baking units. compared to this large scale use of yeast, basic and applied research is carried out by only a few universities with the result that there is little interaction between researchers and technologists in industry. The Vice-Chancellor expressed happiness that the Indian Yeast Group (IGY), which as the coordinating body of yeast researchers, was initially formed through the initiative of the HAU scientists, and has played a significant role in promoting yeast research in the country.

The participants reviewed the research work done on yeast organism and had detailed discussions on many different aspects. Prof. Dalel Singh, the leader of the team which was awarded a medal by The Indian Sugar Technologists' Association for its outstanding work in fermentation technology, made a significant contribution towards the success of the National Symposium as its Organising Secretary.

Valedictory session

Dr. Har Swarup Singh, Lt. Governor of Pondicherry and the former Vice-Chancellor of HAU began his address appreciating the research work done in Yeast Biology by the Indian Yeast Group and the special contribution made by CCS HAU, Dr. Singh said that by organising four National Symposia in a short span of 7 years, the group has established itself on sound footing. Dr. Singh advised the yeast researchers to focus their attention on achieving further excellence. Prof. P. Tauro, Dean, Post-Graduate studies, while welcoming the Lt. Governor, observed that although progress has been made in improving the fermentation process through improvement in yeast strains and modification of technology, much more needs to be done to overcome the problem of high temperature on fermentation efficiency, by-product formation and on the reduction of volume of the effluent.

The meeting also reviewed the progress made in basic research using Yeast as the model system. The progress in studies on the cell cycle, the use of yeast for cloning heterologous genes and the production of new proteins required for vaccine/haemoglobin production, the possibilities of externally manipulating yeast genome etc were briefly reviewed.

The future strategies, it was pointed out, should not revolve solely around the work on *Saccharomyces cerevisiae* but should be expanded to include other yeasts such as

Candida, *Kluyveromyces*, and *Hansenula* etc and their enzyme systems for the use of alternative sugar sources for fermentation, for biomass production and for the production of new fats and flavours. Dr. R.K. Vashishat, Convener of the Symposium highlighted the activities of the Indian Yeast Group. The Dean, College of Basic Sciences and Humanities, Dr. Randhir Singh welcomed the distinguished delegates and the Chief Guest.

Prof. D. Singh selected as National Coordinator IYG.

Keeping in view the contribution made and services rendered by Prof. Dalel Singh, Organising Secretary of the National Symposium, he was selected as the National Coordinator of the Indian Yeast Group. The next meeting of IYG will be held in Bombay.

Prof. Dalel Singh
Dept. of Microbiology
Haryana Agricultural University

Eight International Symposium on Yeasts, Atlanta, Georgia, August 1992

I wish to thank all of you who attended the 8th ISY in Atlanta, GA, USA, August 23-28, 1992 for the wonderful meeting that you generated. I think it was terrific, both scientifically and socially (although Hurricane Andrew did put a 'damper' on the laser show). After 4 years of planning the Symposium and wondering how it would turn out, I was very pleased with the results, but also very sad to see the week disappear so quickly. My special thanks to Sally Siewert who did so much to make ISY-8 a wonderful experience and a successful meeting - working with her was a pleasure; also to Pat Dickinson and the staff of the Division of Continuing Education at Georgia State University for all they did to make the meeting run smoothly. I sincerely hope you all enjoyed the ISY-8 as well as the southern hospitality of Atlanta.

Sally A. Meyer, ISY-8 Organizing Committee Chair

Forthcoming meetings

Yeast Genetics and Molecular Biology Meeting, University of Wisconsin, Madison, Wisconsin, Une 8-13 1992

Organized under the patronage of the Genetics Society of America, the 1993 meeting on yeast genetics and molecular biology will be heldJune 8 to 13, 1993, at the University of Wisconsin, on the shore of Lake Mendota. The main areas of interest will be cell biology, cell growth and development, chromosome stucture and maintenance, genetic alterations, and gene expansion. The dealine for submission of abstracts is February 8 1993, and advance registration must be received before April 13 1993. **For further information, contact, as soon as possible:**

Anne Marie Langevin, Meetings Manager
GSA Administrative Office
9560 Rockville Pike
Bethesda, Maryland
U.S.A. 20814-3998

Phone: 301 571-1825

16th International Specialized Symposium on Yeasts, Metabolic Compartmentation in Yeasts. August 23-26 1993, Arnhem, The Netherlands.

The symposium programme will include lecture and poster sessions on the various metabolic compartments in yeast cells. Strong emphasis will be on trafficking and transport across membranes of metabolites and exchange of 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 programme.

Location: Contrary to an earlier announcement, the meeting will be held at the **Papendal Congress Centre, Arnhem, The Netherlands.**

This new congress accommodation is situated in a beautiful rural environment. It offers hotel accommodation and car parking facilities for approximately 200 participants.The 16th International Specialized Symposium on Yeasts 1993 will be organized in Rotterdam, The Netherlands.

Organizing Committee: W.A. Scheffers (chairman), Delft University of Technology, Delft; W.R. de Boer, Gistbrocades, Delft; J. Maat, Unilever Research Laboratory, Vlaardingen, and Free University, Amsterdam; R. van den Berg, Heineken Breweries, Zoeterwoude; J.P. van Dijken, Delft University of Technology, Delft.

Programme outline: Morning and afternoon sessions will consist of lectures by invited speakers. In the evening, poster sessions and workshops are being planned. A selection will be made from submitted abstracts for oral presentation in workshops.

Preliminary programme: Individual sessions will focus on biochemical, genetic and physiological aspects of the following metabolic compartments: cell wall and plasma membrane; mitochondrion; peroxisome; vacuole, Golgi and endoplasmic reticulum; nucleus. In each session, state-of-the-art reviews will be presented by invited speakers. Emphasis will be on biogenesis, structure and function. In the final session, the central topic is the integration of the function of the various metabolic compartments in overall cellular metabolism.

Invited speakers and provisional titles of their lecture include:

W. Harder (Delft/Groningen, The Netherlands) The yeast cell as a microcosm.

J.G.H. Wessels (Groningen, The Netherlands) Cell wall and cell membrane: biogenesis, structure and function

R. Lagunas (Madrid, Spain) Solute transport across the plasma membrane.

J.P. van Dijken (Delft, The Netherlands) The mitochondrion: structure and function.

H. Neupert (München, Germany) Biogenesis of the mitochondrion.

M. Veenhuis (Groningen, The Netherlands) The peroxisome: structure and function.

W.H. Kunau (Bochum, Germany) Biogenesis of the peroxisome.

A. Wiemken (Basel, Switzerland) The vacuole: structure and function.

S.D. Emr (La Jolla, USA) The mechanism and control of protein sorting to the vacuole.

J.A. van den Berg (Delft/Leiden, The Netherlands) The nucleus and the nucleolus: structure and function.

H.A. Raué (Amsterdam, The Netherlands) Nucleo-cytoplasmic transport

A. Goffeau (Louvain-la-Neuve, Belgium) ATPases in cellular metabolism.

J. Thevelein (Leuven, Belgium) Nutrient-induced signal transduction in connection with sugar transport and H^+-ATPase activity.

H.V. Westerhoff (Amsterdam, The Netherlands) Dynamics of the metabolic interaction between compartments.

W.N. Arnold (Kansas City, USA) The periplasmic space as a metabolic compartment.

W.N. Konings (Groningen, The Netherlands) The plasma membrane in relation to cell metabolism in yeasts.

G. Schatz (Basel, Switzerland) The mitochondrion in the overall cellular metabolism.

I.J. van der Klei (München, Germany) The peroxisome in the overall cellular metabolism.

D. Wolf (Stuttgart, Germany) The vacuole in the overall cellular metabolism.

C. Ratledge (Hull, United Kingdom) Integration of compartmentalized metabolic functions in the yeast cell.

Official language: The official language for the symposium will be English.

Registration and call for abstracts: The number of participants will be limited to 200, hence early registration is recommended. The second circular, containing the final programme and registration forms will be sent in February 1993 to those who have made the request (as soon as possible).

Registration fee: The subsidized symposium fee will be Dfl. 850. if paid before May 1 1993, and Dfl. 1050. after that date. Included in the fee are: access to all symposium activities; symposium programme and abstract book; coffee and tea during breaks; and all lunches and dinners during the symposium. Not included in the fee is hotel accommodation at Papendal. Rates for accommodation vary from Dfl. 37.50 to Dfl. 130. per night

per person, breakfast included. Additional accommodation before and/or after the symposium may be arranged upon request. Car parking at Papendal is free. It is assumed that participants will stay for the duration of the symposium and the full fee will be charged irrespective of the time of attendance. **For further information, please contact:**

W.A. Scheffers,
Kluyver Laboratory of Biotechnology,
Julianalaan 67,
NL-2628 BC Delft, The Netherlands.

Telephone: (..31) 15 782411
Fax: (..31) 15 782355
or (..31) 15 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); J.R. Graybill (USA); and M. Moroni (Italy). The conference is 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: Molecular biology and biochemistry. Taxonomy, ecology and epidemiology. Pathogenesis and immuno-response. Clinical manifestations in humans and animals. Diagnosis. Antifungals: Laboratory aspects and animal models. Therapy and management. Each session will include state of the art lectures followed by relevant oral presentation and posters. **Contact:**

Dr. M.A. Viviani,
Istituto di Igiene e Medicina Preventiva
Universita di Milano
Milano, Italy

FAX 39-2-55191561

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

The 4th European Congress of Cell Biology will be held in Prague, Czechoslovakia, June 26 to July 1, 1994.

To receive additional information, contact:

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

M. Kopecká

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

Site and date: The 7th International Symposium on the Genetics of Industrial Microorganisms will be held at the Palais des Congrès, Montreal, Québec, Canada on June 26 - July 1, 1994.
Organizing committee: Claude Vézina, Chairman, BioChem Pharma Inc., Laval; Graham G. Stewart, Co-Chairman, John Labatt Limited, London (also Co-Chairman Scientific Program Committee); Michael DuBow, Vice-Chairman, McGill University, Montreal; Julian Davies, Co-Chairman,Scientific Program Committee, University of British Columbia, Vancouver; Brigitte Lebreton, Local Arrangements, CITEC, Montréal; Jim Germida, Member, University of Saskatchewan, Saskatoon; Ted Medzon, Member, University of Western Ontario, London; David Y. Thomas, Member, National Research Council Canada, Biotechnology Research Institute, Montréal; Nicole Léger, Symposium Manager, National Research Council Canada, Ottawa; Laurier Forget, Member, National Research Council Canada, Ottawa.

Scientific program: A broad range of topics will be discussed in the six days of oral and poster presentations. The following is a preliminary list of the major topics: Recent advances in antibiotics; screening for new activities from microbes and improving production; microbial genetics of infectious agents (mycobacteria, fungi, yeasts, etc.); bioremediation; secretion systems, their roles in industry; food microbiology; biotransformations; yeast (traditional and novel applications); downstream processing systems regarding genetically manipulated organisms; global regulatory systems in industrial microorganisms.

The second circular will be published in the Fall of 1993 and will contain the call for papers, special format paper for typing the abstract, a final list of topics, the theme and Symposium programs, the social events, the tours, and registration and accommodation information. **In order receive the second circular, please contact, as soon as possible:**

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

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

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

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

M. Kopecká

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

An invitation to the 10th International Biotechnology Symposium, August 25-30, 1996, Sydney, Australia.

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

To join the mailing list for the Symposium, contact:

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

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

Brief News Items

Change of address: H. Prillinger

In March 1992 I moved to the following address:

Dr. H. Prillinger
University of Agriculture
Institute of Applied Microbiology
School of Food & Biotechnology
Nussdorfer Laende 11
A-1190 Vienna, Austria.

Change of address: P. F. Ganter

I have recently moved to Tennessee State University.

Dr. P. F. Ganter
Biology Department, Tennessee State University
3500 John Merritt Blvd.
Nashville, Tn 37209-1661

Phone 615 320-3320

Scientist Position, INRA Paris-Grignon

We are searching for a qualified scientist for which the Institut National Agronomique (INRA) is about to open a competition. The successful candidate will coordinate scientific activities developing around the yeast culture collection at the Grignon Research Institute, will be in charge of the collection personnel (1 research assistant and 2 technicians), and will be responsible for conducting research in the areas of genetic instability mechanisms for industrial strains; molecular characterization and taxonomy. **Those interested should contact:**

Dr. C. Gaillardin
Laboratoire de Génétique Moléculaire et Cellulaire
INRA-CRNS
Centre de Biotechnologie Agro-Industrielle
78850 Thiverval-Grignon
France

Telephone 33 1 30815452
Fax 33 1 30815457

Change of address: Robert H. Haynes

Robert H. Haynes, Department of Biology, York University, moved recently to Palo Alto, California, to become President and Editor-in-Chief of Annual Reviews, Inc. He will remain affiliated with York University as Distinguished Research professor Emeritus. Annual Reviews, a nonprofit scientific publisher, was founded at Stanford University by the Canadian biochemist, Professor J. Murray Luck, a man still intellectually active and physically vigorous at age 93. It is an amazing coincidence that both Dr. Luck and Dr. Haynes are graduates, 33 years apart, of the Brantford (Ontario) Collegiate Institute! 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, appeared in 1932 and has been published every year since then. 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.

Dr. Robert H. Haines
Annual Reviews Inc.
4139 El Camino Way
PO. Box 10139
Palo Alto, California 94303-0897 USA

Telephone 415-493-4400
Fax 415-855-9815

Search for *S. cerevisiae* strains that form LACY colonies

I would like to locate yeast strains that form colonies with a distinctive "lacy" colony morphology, i.e. colonies with distinct ridges. Our interest in such strains is that their colony morphology might be dependent on the functioning of some of the *BUD* genes (Chant & Herskowitz. 1991. Cell **65**:1203-1212), in which case we would have a convenient colony morphology assay for *BUD* gene function. For these strains to be of use to us, they would have to cross with our *S. cerevisiae* strains.

Anyone with information should contact me at the following address:

Dr. Ira Herskowitz
Department of Biochemistry & Biophysics
Room S-964
University of California, San Francisco
San Francisco, California 94143-0448

FAX 415 476-0943

Yeast Researcher Electronic Mail Addresses

I am in the process of getting a list of Electronic Mail addresses of people who are interested in yeast biology. This list will have several purposes, but the major objective is to get electronic communication going within the yeast researcher community. Things that I hope will be gained from this group is information about stocks and strains, and a general exchange of data.

If you want to be added to this directory, please send me an email message indicating your name as you would like to see it in the directory. Usually one given name and other initials with the the last name. I thank you for your interest in this project, and please stay tuned as there will more to come.

The document listed in the following pages can be retrieved by anonymous FTP from ftp.biol.mcgill.ca in the /pub/email_list directory.

B.F. Francis Ouellette
Manager, Yeast Chromosome I project
Department of Biology
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Montréal, Québec, Canada

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