ISSN 0513-5222

Y E A S T

A Newsletter for Persons Interested in Yeast

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

JUNE 1988 Volume XXXVII, Number I

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EDITORIAL

The first issue of "YEASTS, a news letter for persons interested in yeasts" was prepared in December 1950 by Leslie R. Hedrick of the Illinois Institute of Technology (Chicago), and was mailed to 46 readers in the United States. Editorship was passed on to Emil Mrak (U.C. Berkeley) in 1952, and to Herman J. Phaff in 1954. Dr. Phaff served as Editor for the following 34 years, during which the Yeast Newsletter grew to be distributed to hundreds of subscribers in 47 countries. At first, the date of publication varied from year to year, and a voluntary subscription of $0.25 was suggested. By 1957, the regular publication of spring and fall issues was adopted, and in 1969, the Yeast Newsletter became the "Official Publication of the International Council of Yeasts and Yeast-like microorganisms." The subscription has risen slowly to its current level of $4.00 ($8.00 airmail) largely as a function of the rise in mailing costs.

I am sure that the Associate Editors and all readers of the Yeast Newsletter will join me in thanking Dr. Phaff for the kind dedication that has characterized his editorship. I hope to continue in the same tradition of promoting the informal exchange of information among researchers interested in the various facets of yeasts and yeast-like microorganisms. Several readers have sent very kind words of encouragement for which I am most appreciative. I thank one and all in anticipation of your support and cooperation.

M.A. Lachance
Editor

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

The strains listed have been added to the ATCC since October 30, 1987. Complete information on these strains may be obtained upon request from the Mycology and Botany Department of ATCC.

Name	ATCC No.	Depositor & Strain	Significance & Reference
Candida albicans	64544-64553	J.N. Galgiani, C-59, C-289, B-113, D-1079, D-1, B288, AD, B-372, D-3, B-452	Used for antifungal drug susceptibility testing (Antimicrobial Agents & Chem. 31:1343-1347, 1987)
Cryptococcus elinovii	64554	W.I. Golubev, BKM Y-2231	Type strain (J. Gen. Appl. Microbiol. 30:427-433, 1984)
Cryptococcus himalayensis	64555	W.I. Golubev, BKM Y-1645; H-3-116	Type strain (Mycologiya i Phytopathologiya 20:215, 1986)
Cryptococcus terreus	64556	W.I. Golubev, 5382 c; BKM Y2253	Type strain (Mycologiya i Phytopathologiya 20:215, 1986)
Kluyveromyces bulgaricus	64468	N. Van Huynh, IRC 101	Metabolizes lactose in whey (Process Biochem. 21:30, 1986)
Rhodotorula mucilaginosa	64684	R.K. Latta, NRC 21103	Production of acetylxylan esterase (Appl. & Environ. Microbiol. 53:2831-2834, 1987)
Rhodotorula rubra	64475	K. Kwasniewska, 2034	Biodegradation of Crystal blue (Bull. Environ. Contam. Toxicol. 34:323-330, 1985)
Saccharomyces cerevisiae	64448-64450	M. Kielland-Brandt, IVPX5-28, IVPX2-1C, IVPX1-4B	Genotypes (Carlsberg Res. Commun. 49:577-584, 1984)
Saccharomyces cerevisiae	64453	E.T. Young, 301.10a	Expresses the adh3 gene (Mol. & Cell. Biol. 5:3024-3034, 1985)
Saccharomyces cerevisiae	64454-64456	E.T. Young, 302.21, 900-17-1a, 900-17-101a	Alcohol dehydrogenase null strains (Mol. & Cell. Biol. 5:3024-3034, 1985)
Saccharomyces cerevisiae	64539-64540	P. Nagley, Mb12, J69-1B	Respiratory mutants (Biochim. Biophys. 740:88-89, 1983)
Saccharomyces cerevisiae	64665-64667	V.L. Larionov, DC 5, r2 GRF 18, GRF 18	Used for genetic transformation (Current Genetics 10:15-20, 1985)
Saccharomyces diastaticus	64511	L.I.C. de Figueroa, 1354	Can utilize dextrin as a carbon source (Appl. Microbiol. Biotechnol. 21:206-209, 1985)
Saccharomyces diastaticus	64512	L.I.C. de Figueroa, 1376	Can ferment dextrin or starch (Appl. Microbiol. Biotechnol. 21:206-209, 1985)
Schizosaccharomyces pombe	64404-64408	M. Sipiczki, 9-42, 9-46, 9-40, 9-39, 9-31	Mating type studies (Current Genetics 9:263-272, 1985; J. Gen. Microbiol. 138:1989-2000, 1982)
Sporobolomyces falcatus	64693	T. Nakase, JCM 6838; NB-264	Type isolate from dead leaf of Miscanthus sinensis (Trans. mycol. Soc. Japan 28:295-301, 1987)

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II. National Collection of Yeast Cultures, AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA. England. Communicated by P.J.H. Jackman.

1. New NCYC Curator

Peter J.H. Jackman has been appointed Curator of NCYC in succession to Barbara Kirsop who has become Executive Director of the Microbial Strain Data Network (MSDN).

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2. Schizosaccharomyces pombe

A collection of over 300 genetically marked strains of the fission yeast Schizosaccharomyces pombe has recently been established as a collaborative project between the University of East Anglia and the NCYC with funding from the SERC. S. pombe has been used extensively been used by molecular biologists for the study of cell cycle control mechanisms, mating type switching and recombination. In addition, ion and metabolite transport coupled to plasma membrane ATPase and mitochondrial function has also been studied. Vectors have been constructed for the transformation of S. pombe and it has potential as a host for expression of heterologous eukayotic DNA. The genome of S. pombe has three linkage groups to which about 160 markers have been mapped. Because of the small number of these groups it is relatively easy to map a new mutation using mitotic haploidization and meiotic recombination techniques.

At present the collection comprises the following:

Auxotrophs: adenine, arginine, glutamate, histidine, leucine, lysine, methionine, proline, tryptophane, uracil, aromatic amino acids, phenylalanine and tyrosine mutants.
Drug resistance/sensitivity: canavanine, benomyl, cycloheximide, azaguanine and fluorouracil resistant mutants.
Mutations affecting conjugation, meiosis and sporulation: sporulation, sterility, mating type switching and vegetative iodine reaction mutants.
Cell cycle mutations: cell division cycle and nuclear division arrest mutants.
Temperature sensitive lethals and others: temperature sensitive and phosphatase mutants.

The following scientists are warmly thanked for their personal contributions to the collection:

Dr. A. Coddington, University of East Anglia, Norwich, UK.
Dr. P. Fantes, University of Edinburgh, Edinburgh, UK.
Dr. A. Goffeau, Université de Louvain, Belgium.
Dr. H. Gutz, Institut für Genetik, Braunschweig, W.Germany.
Prof. J. Kohli, University of Bern, Switzerland.
Dr. A. Nasim, National Research Council, Ottawa, Canada.
Dr. P. Nurse, Imperial Cancer Research Fund, London, UK.
Prof. Yanagida, Kyoto University , Japan.

It is anticipated that a catalogue of the collection will be available from August 1988.

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3. Contacting NCYC & NCFB by Electronic Mail

Telecom Gold/Dialcom: Telecom Gold is the electronic mail and information service provided by British Telecom. Telecom Gold is also part of the Dialcom system which provides similar services worldwide. Many companies already have mailboxes on these services. NCYC & NCFB's mailbox is on the recently established Telecom Gold system set up by the Microbial Strain Data Network which also has strain information databases of interest to microbiologists.

JANET (Joint Academic Network): JANET is the electronic network between UK Universities, Polytechnics and research institutes, and is also linked to similar academic networks worldwide such as EARN and BITNET.

NCYC & NCFB Online: NCYC & NCFB's own computer services, which provide an online identification system, NCYC catalogue and electronic mail. It is accessible from Janet or via the packet switching system (PSS) system worldwide.

Addresses:

	TELEPHONE	TELEX	FAX	JANET	TELECOM GOLD / DIALCOM
NCYC	0603 56122	975453	0603 58939	JACKMAN@UK.AC.AFRC.FRIN	10075:DBI0013
NCFB	0734 883103	265871	0734 884763	JACKMAN@UK.AC.AFRC.FRIN	10075:DBI0013

(Editor's note: From BITNET, the order of node elements must be reversed, i.e. JACKMAN@FRIN.AFRC.AC.UK).

All electronic services operate 24 hrs/day. All orders should include an order number and telephone orders should be followed by written confirmation. Please note that NCFB remains based at the AFRC Institute of Food Research, Reading Laboratory, Shinfield, Reading RG2 9AT, and orders by post should continue to be sent to that address. For further information or advice contact Dr. Jackman.

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4. Confidential Safe Deposit Service

Companies who have yeast or bacterial strains of high commercial or other importance, and who wish to have them preserved under optimal conditions for the maintenance of their properties, under secure and confidential safeguard, and yet be available on demand should note this service.

Preservation: The customer sends the strains to the NCYC/NCFB in any convenient form. No further information other than the medium requirements for growth and the pathogenicity or otherwise of the strain need be revealed to the NCFB/NCYC. The strain is then checked for viability. Bacteria are kept freeze-dried, and yeasts both freeze-dried and in liquid nitrogen in the same way as the main holdings of the collections. Viability is checked periodically in the same way as the main collections.

Security: Strains are held at the AFRC Food Research Institute sites at Norwich and Reading under secure conditions with on-site security 24 hrs/day. A duplicate collection is also maintained on a geographically separate site as a further safeguard. All information concerning the cultures is kept securely and is entirely confidential between NCYC/NCFB staff and the customer.

Access to cultures: Access is of course entirely exclusive to the customer. Inclusive in the yearly charge for this service, the customer may have up to two ampoules per strain on demand. Further ampoules can be supplied at the prevailing standard rate. Ampoules can be made available by the fastest available delivery service as required. Telephone requests from the customer are acceptable but for security purposes ampoules will only be sent to the registered customer address. Changes to this address or in the status of the company must be notified in writing. The service may be cancelled by the customer at any time and two ampoules will be returned to the depositor and all other outstanding materials and information destroyed.

Other services: Customers should note that services for the identification and typing of strains held in safe deposit are available. In particular, we are developing methods for the typing of Saccharomyces cerevisiae using 2-D electrophoretic maps of whole cell proteins and genetic fingerprinting which will be of particular interest to those wishing to know if their current working strain is the same as their safe deposit strain. It is now also possible for NCYC/NCFB to provide strains in bulk quantities of 1 kg or more in dried form. Safe deposited strains may also be conveniently converted into patent deposits when required.

5. Prices as of 1st April 1988

CULTURES (per freeze dried ampoule)	£24.00
UK non-profit institute	£12.00
BULK SUPPLY (active dried yeast 1-100 kg)	by negotiation
IDENTIFICATION (per strain)	£95.00
UK non-profit institutions	£50.00
CATALOGUE	
NCYC 2nd edition	£5.75
NCYC 3rd edition (when available)	£10.00
NCFB 3rd edition	£10.00
PATENT DEPOSIT (Budapest Treaty)	£795.00
Supply of Patent culture	£45.00
CONFIDENTIAL SAFE DEPOSIT (per culture per year)	£95.00
NCYC ONLINE (online catalogue & probabilistic identification (COMPASS), electronic mail, including handbook & unlimited use per year)	£95.00
(online catalogue only per year)	£25.00
STRAIN DATA (printout per strain)	£4.00
CONTRACT RESEARCH	by negotiation

UK customers add 15% VAT except catalogues. Postage and packing is included for UK, extra for overseas. NCFB orders should be addressed to the Reading Laboratory. For further information contact:

Dr. Peter Jackman, Curator NCYC & NCFB
AFRC Institute of Food Research,
Norwich Laboratory, Norwich NR4 7UA.
England

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

1. Recent acquisitions

Candida hinoensis, Iwasaki & Goto: CBS 7323 = WY-2 = IAM 13497, T, from soil, Tokyo, S. Goto

Candida soli, Okuma & Goto: CBS 7321 = NK-4 = IAM 13496, T, from soil, Fuchu, Tokyo, S. Goto

Candida solicola Endo et al.: CBS 7322 = WY-1 = IAM 13495, T, from soil, Hino, Tokyo, S. Goto

Candida vaccinii, Tokuoka et al.: CBS 7318 = Tokuoka 10-50A = IAM 13117, T, from flower of bilberry Vaccinium sp., Tokyo, K. Yamasato

Myxozyma geophila, van der Walt et al.: CBS 7320 = J6T, from garden soil, Chigasaki, Japan, J.P. van der Walt

Pichia galeiformis, Endo & Goto: CBS 7324 = IAM 13498 = IM-10, T, from faeces, Tokyo, S. Goto

Pichia hampshirensis, Kurtzman: CBS 7208 = NRRL YB-4128, T, frass of dead, cut oak (Quercus sp.), Camp Sargent, New Hampshire, USA, C.P. Kurtzman

Pichia japonica, Kurtzman: CBS 7209 = NRRL YB-2750, T, frass of Abies firma, Japan, C.P. Kurtzman

Pichia membranaefaciens, Hansen: CBS 7313 = VYAPi 01-02, CBS 7314 = VYAPi 01-04, test strains for microbial stability of sauces and dressings, resistant to 2.5% acetic acid, A. Jansen

Rhodotorula foliorum (Ruinen), Weijman et al. CBS 7306 = CSIR-Y1065, from soil, South Africa, J.P. van der Walt

Candida glabrata (Anderson), Meyer & Yarrow: CBS 7307, cycloheximide resistant, A.J. Woodgyer

Zygosaccharomyces bailii (Lindner), Guilliermond: CBS 7315 = VYASa 07-01, CBS 7316 = VYASa 07-05, test strains for microbial stability of sauces and dressings, resistant to 3% acetic acid, A. Jansen

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2. Renamed strains (in List of Cultures 1987 as S. cerevisiae) (Vaughn-Martini & Martini 1987, Antonie van Leeuwenhoek 53:77-84):

Saccharomyces bayanus Saccardo: CBS 380, T, CBS 381, CBS 431, CBS 1505, CBS 378, CBS 1604

Saccharomyces pastorianus Hansen: CBS 1538, T, CBS 1260, CBS 1486, CBS 1503, CBS 1513

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3. Publications

a. The Expanding Realm of Yeast and Yeast-like Fungi. Proceedings of an International Symposium on the Perspectives of Taxonomy, Ecology and Phylogeny of Yeasts and Yeast-like fungi. Amersfoort, The Netherlands, 3-7 August 1987. Editors: G.S. de Hoog, M.Th. Smith, A.C.M. Weijman. Studies in Mycology No. 30, Centraalbureau voor Schimmelcultures, Baarn and Delft. Amsterdam: Elseviers Science Publishers.

Contents:

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b. Guého, É., de Hoog, G.S., Smith, M.Th., Meyer, S.A. 1987. DNA relatedness, taxonomy and medical significance of Geotrichum capitatum. J. Clin. Microbiol. 25: 1191-1194.

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4. Recently completed research projects and publications in preparation:

a. The genus Nadsonia. The genus Nadsonia was revised by W.I. Golubev, M.Th. Smith, G.A. Poot, and J.L. Kock on basis of morphology, physiology, amino acid and fatty acid composition, electrophoretic patterns of some enzymes and DNA relatedness. Two species N. commutata (type CBS 6640) and N. fulvescens with two varieties, N. fulvescens var. fulvescens (type CBS 2596) and N. fulvescens var. elongata (type CBS 2594) comb. nov. are recognized.

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b. Re-examination of Candida lambica strains. From the CBS culture collection 11 strains labelled C. lambica were re-examined for their physiological properties and their G + C content. Two groups could be recognized differing in G + C content by 10%. By DNA-DNA reassociation studies, the identity of the following 6 strains with the type of C. lambica was established: CBS 603, CBS 2059, CBS 2066, CBS 2068, CBS 4550 and CBS 6662. The remaining strains deem to resemble C. ethanolica and C. rugopelliculosa. From DNA-DNA homology results the separate status of these 5 strains was shown. These 5 strains (CBS 2058, CBS 2063, CBS 2067, CBS 4325 and CBS 4326) will be described in a new taxon as C. pseudolambica sp. nov.

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c. Revision of Brettanomyces and Dekkera. The revision of Brettanomyces and Dekkera by nuclear deoxyribonucleic acid reassociation was recently finished and their imperfect-perfect relatedness was established. The result obtained suggest the following classification:

- Dekkera comprises 2 species:
  - D. anomala teleomorph of B. anomalus
  - D. bruxellensis (syn. D. intermedia) teleomorph of B. bruxellensis.
- Brettanomyces comprises 4 species:
  - B. anomalus (syn. B. claussenii; C. beyingensis)
  - B. bruxellensis (syn. B. abstinens; B. custersii; B. intermedius; B. lambicus)
  - B. custersianus
  - B. naardenensis

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IV. Department of Microbiology, CBSH, G.B. Pant University of Agriculture and Technology, Pantnagar 263145, Distt. Nainital, India. Communicated by R.S. Rana.

The following are abstracts of two Master's theses from this institution:

1. Saxena, A., Rana, R.S. 1987. Solid state alcoholic fermentation of Guava. 116 pp.

Considerable fruit is being lost every year. The disposal of such damaged fruits creates problems and causes pollution. In order to save economic losses, the solid state Alcoholic fermentation of Guava was investigated. Various parameters were optimised such as pH, water level, substrate quantity, supplementation with N, P, urea and sugar (molasses was found best), and inoculum size. This solid state alcoholic fermentation was carried out by using three standard strains and one isolated strain which are as Saccharomyces cerevisiae CDRI NTG, S. cerevisiae NCIM 3095, Candida kouse, and Pichia membranaefaciens Hansen. The pH for ethanol production was observed to be 4.5 for C. krusei and 5 for the other strains. A moisture level of 1.1.5 (w/v) was found to be appropriate for all strains. Supplementations of salts did not increase the growth efficiency and yield of ethanol. Supplementation of sugar also increased the ethanol production: 2% of sugar supplementation increased the yield up to 5.1 and 4.8% w/w for S. cerevisiae CDRI NTG and NCIM 3095. Inoculum sizes from 1.2 to 2 g dry weight of yeast reduced the fermentation time by about 5 to 10 hr with the increase of ethanol production 5.5 and 5% (w/w) for S. cerevisiae CDRI NTG and NCIM 3095 with corresponding efficiencies of 85.76 and 82.37 respectively. The results suggests that S. cerevisiae CDRI NTG is an efficient strain in comparison to other strains used during this investigation and also that solid state alcoholic fermentation is better than submerged fermentation.

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2. Dikshit, K., Rana, R.S. 1987. Solid state fermentation of banana (Musa sapientum). 96 pp.

Ethanol production from banana (Musa sapientum) through solid state fermentation by two strains of Saccharomyces was studied. Saccharomyces cerevisiae NCIM 3187 and CDRI NTG were screened. Saccharomyces cerevisiae NCIM 3187 was selected after preliminary screening for optimizing conditions for ethanol production. Total extractable sugar in the bananas was 18.7% on a wet weight basis. The ash and nitrogen contents were 0.85% and 0.165% respectively on a fresh weight basis. The ethanol yield in the case of S. cerevisiae NCIM 3187 was 7.6% (w/w) with a fermentation efficiency of 75.5% Maximum ethanol yield was obtained from 25 gm of banana impregnated with 75 mL water at pH 4.5. Nitrogen and phosphorus supplementation did not improve ethanol yields in solid state fermentation of banana cossettes.

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V. The University of Texas Health Science Center at San Antonio, Department of Pathology, and the Audie L. Murphy Memorial Veteran's Hospital, San Antonio, Texas. Communicated by M.G. Rinaldi.

The following papers have been published or accepted recently.

1. Engleberg, N.C., J.J. Johnson IV, J. Bluestein, K. Madden, and M.G. Rinaldi. 1987. Phaeohyphomycotic cyst due to the recently-described species, Phaeoanellomyces elegans. J. Clin. Microbiol. 25:605-608. (Human mycosis caused by a dematiaceous fungus possessing a prominent "black yeast" anamorph).

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2. Hadfield, T.L., M.B. Smith, R.E. Winn, M.G. Rinaldi, and C. Guerra. 1987. Mycoses caused by Candida lusitaniae. Rev. Infect. Dis. 9:1006-1012.

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3. Redding, S.W., M.G. Rinaldi, and J.L. Hicks. 1988. The relationship of oral Candida tropicalis infection to systemic candidiasis in a leukemic patient. Special Care in Dentistry (in press).

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4. Hoeprich, P.D. and M.G. Rinaldi. 1988. Candidiasis. In: Hoeprich, P.D. (Ed.). Infectious diseases. A Treatise of Infectious Processes, 4th ed., Lippincott/Harper and Row, Philadelphia, PA. (in press).

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VI. Research Institute for Viticulture and Enology, 833 11 Bratislava Matúskova 25, Czechoslovakia. Communicated by E. Minárik.

The following are summaries of papers recently accepted for publication.

1. Minárik, E., Jungová, O. 1988. Elimination of inhibiting substances by yeast ghosts and cellulose preparation in grape must fermentation (in German). Die Wein-Wissenschaft.

Yeast ghosts and microcrystalline cellulose are able in laboratory conditions to decrease considerably the inhibiting action of fungicide residues on the fermentation of grape must. Higher alcohol levels and lower rest sugar concentration in the wine may be achieved. The volatile acid content is usually decreased. The consequence of the adsorption ability of these substances may be also used in the fermentation of grape must with high sugar content.

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2. Minárik, E. Jungová, O. 1988. Further experience with the application of biosorbents in grape must fermentation under unfavorable conditions (in Slovak). Kvasny prumysl, Prague 34.

Yeast ghosts and microcrystalline cellulose guarantee an essential decrease or full elimination of the inhibiting effect of fungicide residues on yeasts in the course of grape must fermentation. The fermentation start is speeded up, the course of fermentation is proceeding smoothly without delay, and more completely compared with musts containing fungicide residues but no biosorbents. Higher alcohol content, lower residual sugar and volatile acid concentrations in the wine are the result of the application of biosorbents. The importance of biosorbents in the fermentation of grape must under unfavourable fermentation conditions is underlined.

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VII. Department of Applied Microbiology and Food Science, University of Saskatchewan, and Plant Biotechnology Institute, National Research Council, Saskatoon, Saskatchewan, Canada S7N 0W9. Communicated by J.W.D. GrootWassink.

The following are abstracts of recently published or accepted papers.

1. Tsang, E.W.T., GrootWassink, J.W.D. 1988. Extraordinarily rapid appearance of a β-fructofuranosidase (Exo-inulase)-hyperproduction mutant in continuous culture of Kluyveromyces fragilis. J. Gen. Microbiol. 134:679-688.

A wild-type strain of the yeast Kluyveromyces fragilis was grown in continuous culture on inulin-, fructose- and glucose-limited media. In the presence of organic nitrogen, all three carbon sources supported an extremely rapid changeover to a homogeneous mutant cell population that exhibited hyperproduction of β-fructofuranosidase (exo-inulase). The non-specific role of the carbon substrate in the takeover suggested co-regulation of inulase synthesis and early glycolytic pathway enzymes such as hexokinase, resulting in gratuitous hyperproduction of inulase. The rate of mutant appearance increased linearly with a decrease in dilution rate as well as carbon/nitrogen ratio, implying that the responsible forces were related to the residual concentration of the limiting carbon substrate. Under optimum conditions, mutant cells were detected within only 40 h (2.9 generations) from the start of continuous culture, at least 6.5 times faster than predicted. The maximum rate of displacement was as high as 54% per generation, or more than 12 times greater than predicted from the differential growth rate between the wild-type and mutant strain. These extraordinarily rapid takeovers point to a very fast and ongoing genetic change triggered by growth under severe stress.

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2. Tsang, E.W.T., GrootWassink, J.W.D. Stability of exo-inulase production on lactose in batch and continuous culture of a Kluyveromyces fragilis hyperproducing mutant. (In print: Enzyme and Microbial Technology)

The maintenance of hyperproduction of an exo-inulase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26 by a mutant of the yeast Kluyveromyces fragilis was examined in continuous culture on lactose. After the usual initial phenotypic adaptation with activities reaching up to ~35 U/mg dry cell weight (i.e. ~4%, w/w), cultures lacking detectable residual lactose exhibited a rapid loss of enzyme yield. The original hyperproducing strain was displaced by a wild-type cell population with twice the affinity for lactose, resulting from increased lactase (β-D-galactoside galactohydrolase, EC 3.2.1.23) activity. The displacement could be prevented by shifting from carbon to nitrogen limitation, using a large excess of lactose and a dilution rate high enough to leave residual lactose in the culture. These conditions were consistent with those prevailing in lactose-limited batch culture which also showed complete genetic stability. Although the exo-inulase level in batch culture (19 U/mg) was higher than in continuous culture (12 U/mg), the volumetric productivity of the latter was several fold greater.

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VIII. PROIMI, Avenida Belgrano y Pasaje Caseros, 4000, S.M. de Tucuman, Argentina. Communicated by J.F.T. Spencer.

The following paper has been submitted recently for publication.

Spencer, J.F.T.[1], Spencer, D.M.[1], Schiappacasse, M.C.[2], Heluane, H., Reynolds, N.[3], de Figueroa, L.I. Two new methods for recovery and genetic analysis of hybrids after fusion of yeast protoplasts. [1]Visiting Scientists from Goldsmiths' College, University of London, [2]Research Fellow from Escuala de Ingenieria, Faculdad de Ingenieria, Universidad Catolica de Valparaiso, Chile, [3]Department of Biological Sciences and Environmental Health, Thames Polytechnic, Wellington Street, London SE18 6PF, England.

A method for regeneration of yeast protoplasts and fusants in a gelatin-agar mixture, followed by total recovery of the regenerated cells from the gelatin-agar mixture and isolation of the fusants is described. A one-step method for obtaining intergeneric fusants in which the greater part of the genome is derived from Saccharomyces cerevisiae, and in which the fusant can be sporulated directly and tetrad analysis carried out without construction of further hybrids, is also described.

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

We have recently achieved in our laboratory the functional relocation to the yeast nucleus of a mitochondrial gene encoding a hydrophobic membrane protein. The following are summaries of papers (1-3) that have been published concerning the expression outside mitochondria of subunits 8 and 9 of the mitochondrial ATP synthase complex of Saccharomyces cerevisiae using chemically synthesized nuclear-compatible versions of their mitochondrial genes, together with the import of these proteins into mitochondria. A review (4) of the biochemical genetics of the F_0-membrane sector of mitochondrial ATP synthase has also appeared, in which the enzyme from S. cerevisiae features prominently.

1. Gearing, D.P., Nagley, P. 1986. Yeast mitochondrial ATPase subunit 8, normally a mitochondrial gene product, expressed in vitro and imported back into the organelle. EMBO J. 5: 3651-3655.

Subunit 8 of yeast mitochondrial F_0F_1-ATPase is a proteolipid made on mitochondrial ribosomes and inserted directly into the inner membrane for assembly with the other F_0 membrane-sector components. We have investigated the possibility of expressing this extremely hydrophobic, mitochondrially encoded protein outside the organelle and directing its import back into mitochondria using a suitable N-terminal targeting presequence. This report describes the successful import in vitro of ATPase subunit 8 proteolipid into yeast mitochondria when fused to the targeting sequence derived from the precursor of Neurospora crassa ATPase subunit 9. The predicted cleavage site of matrix protease was correctly recognized in the fusion protein. A targeting sequence from the precursor of yeast cytochrome oxidase subunit VI was unable to direct the subunit 8 proteolipid into mitochondria. The proteolipid subunit 8 exhibited a strong tendency to embed itself in mitochondrial membranes, which interfered with its ability to be properly imported when part of a synthetic precursor.

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2. Nagley, P., Farrell, L.B., Gearing, D.P., Nero, D., Meltzer, S., Devenish, R.J. 1988. Assembly of functional proton-translocating ATPase complex in yeast mitochondria with cytoplasmically synthesized subunit 8, a protein normally encoded within the organelle. Proc. Natl. Acad. Sci. USA 85:2091-2095.

A mitochondrial gene from Saccharomyces cerevisiae encoding a hydrophobic membrane protein, subunit 8 or the F_0/F_1-type mitochondrial ATPase complex, has been functionally replaced by an artificial nuclear gene specifying an imported version of this protein. The experiments reported here utilized a multicopy expression vector (pLF1) that replicates in the nucleus of yeast cells and that carries an inserted DNA segment specifying a precursor protein (N9/Y8) consisting of subunit 8 fused to an N-terminal cleavable transit peptide (the leader sequence from Neurospora crassa ATPase subunit 9). The successful incorporation of the imported subunit 8 into functional ATPase complexes after transformation with pLF1 expressing N9/Y8 was indicated by the efficient genetic complementation of respiratory growth defects of aap1 mit$^-$ mutants, which lack endogenous subunit 8. The reconstitution of ATPase function was confirmed by biochemical assays of ATPase performance in mitochondria and by immunochemical analyses that demonstrated the assembly of the cytoplasmically synthesized subunit 8 into the ATPase complex. Reconstitution of ATPase function required the cytoplasmically synthesized subunit to have a transit peptide. The strategy for importation and reconstitution developed for subunit 8 leads to a systematic approach to the directed manipulation of mitochondrially encoded membrane-associated proteins that has general implications for exploring membrane biogenesis mechanistically and evolutionarily.

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3. Farrell, L.B., Gearing, D.P., Nagley, P. 1988. Reprogrammed expression of subunit 9 of the mitochondrial ATPase of Saccharomyces cerevisiae: Expression in vitro from a chemically synthesized gene and import into isolated mitochondria. Eur. J. Biochem. 173:131-137.

A synthetic gene has been designed and constructed by total chemical synthesis as a first step in the functional relocation from the mitochondrion to the nucleus of a gene encoding subunit 9 of the yeast mitochondrial ATPase complex. This gene (NAP9) incorporates codons frequently used in nuclear genes of Saccharomyces cerevisiae and additionally includes a series of unique restriction enzyme cleavage sites to facilitate future systematic manipulations of the gene and its protein product. Following the expression of the NAP9 gene by transcription and translation in vitro, a radiolabelled protein was produced which displayed a gel electrophoretic mobility and solubility in chloroform/methanol characteristic of the authentic subunit 9 proteolipid encoded in vitro by the mitochondrial oli1 gene. In order to achieve import into mitochondria of yeast subunit 9, a fusion was made between NAP9 gene and DNA encoding the cleavable presequence of the nuclearly encoded precursor to subunit 9 from Neurospora crassa. Following expression in vitro, the resultant fusion protein was imported and appropriately processed by isolated yeast mitochondria. The import of yeast subunit 9 was less efficient than that observed in parallel import experiments with yeast subunit 8 attached to the same presequence or with the naturally occurring intact N. crassa subunit 9 precursor. Yeast subunit 9 lacking a leader sequence is not imported into mitochondria but, unlike subunit 8, it does not embed itself into the outer membrane, in spite of its highly hydrophobic character.

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4. Nagley, P. 1988. Eukaryote membrane genetics: The F_0 sector of mitochondrial ATP synthase. Trends Genet. 4:46-52.

The set of integral membrane proteins of the proton-translocating F_0 sector of mitochondrial ATP synthase provides a focal point for eukaryote membrane genetics from molecular, cell biological and evolutionary perspectives. Detailed analysis of yeast mutants has elucidated the assembly and functions of three mitochondrially encoded F_0 sector subunits, whereas work on filamentous fungi and mammals has revealed that one of these subunits is encoded in the nuclear genome and delivered to the mitochondrial inner membrane following import into the organelle. These two aspects of membrane biogenesis have now been integrated through the deliberate relocation of a yeast mitochondrial gene to the nucleus; a functional mitochondrial ATP synthase complex has thus been assembled using an F_0 sector subunit introduced into the membrane from the non-natural side.

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X. Institut für Mikrobiologie, Technische Hochschule Darmstadt, Schnittspahnst. 10, D-6100 Darmstatadt, Federal Republic of Germany. Communicated by F.K. Zimmermann.

The following are summaries of work recently accomplished at the institute.

1. Gozalbo, D., Hohmann, S. The naturally occurring silent invertase gene $suc2^{o}$ contains a stop codon that is occasionally read through.

There are two alternative alleles of the invertase structural gene locus SUC2 in strains of Saccharomyces cerevisiae: either the active allele SUC2 which codes for an active invertase or $suc2^{o}$ which is transcribed but does not lead to the formation of detectable amounts of invertase (R. Taussig and M. Carlson: Nucl. Acid Res. 11:1943, 1983). Allele $suc2^{o}$ was cloned and sequenced. An amber termination codon was identified at position 233 within the 532 codon coding region. Surprisingly, transformants carrying $suc2^{o}$ on plasmid YRp7 fermented sucrose and showed a residual invertase activity of 50 mU/mg protein as compared to 500-800 mU/mg protein in normal SUC2 strains. This suggested an occasional read through of the amber stop codon. Artificial hybrid alleles with a 5'-$suc2^{o}$ and a 3'-SUC2 sequence, construct 1, and vice versa, construct 2, were constructed using fragments delimited by the central BamH1 site common to both alleles. The amber codon is located in the 5'-fragment. Transformants with construct 1 showed twice the invertase activity of transformants carrying the pure $suc2^{o}$ allele. Transformants carrying construct 2 showed half the invertase activity of transformants with the complete SUC2 allele. This suggests that the 3'-half of $suc2^{o}$ codes for an invertase amino acid sequence which reduces the enzyme activity compared to the SUC2 3'-prime half. Moreover, read through of the amber codon is detectable only when $suc2^{o}$ is present in larger copy numbers. The read through product can be detected after electrophoresis of crude extracts in native acrylamide gels and staining for invertase activity at almost the same location as the fully active enzyme coded by SUC2.

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2. Jäger-Magiera, C. Mutation in a new gene prevents the formation of a fully active phosphofructokinase in Saccharomyces cerevisiae.

Mutants were isolated which blocked glucose fermentation in mutants with a deletion in one of the two phosphofructokinase structural genes PFK1 or PRK2. Our phosphofructokinase assays are now done in the presence of the activator fructose-2,6-bisphosphate instead of AMP. This results in higher specific activities of wild type cells from 500-700 mU instead of the usual 300-400 mU. These activities are reduced to 90-140 mU in the presence of the newly isolated mutant as shown in 4 tetrads from a mutant/wild type heterozygote. Samples of crude extracts with the same total phosphofructokinase activity were titrated with a polyclonal antiserum (J. Heinisch: Curr. Genet. 11:227, 1986). Inactivation of a given activity in the mutant crude extract (specific activity 116 mU/mg) was achieved with a serum dilution of 1:64 whereas only 1:256 was needed for the same amount of activity of the wild type extract (specific activity 618 mU/mg). This suggests that the mutant allele did not affect the total amount of phosphofructokinase produced but it prevented a maturation step required for an activation of a less active pre-phosphofructokinase to a fully functional form. The new gene is called PFA1 for phosphofructokinase activator.

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3. Schaaff, I. The effect of an overproduction of glycolytic enzyme on the rate of ethanol formation.

We reported previously (I. Schaaff and J. Heinisch: Yeast Newsletter, June 1987, p. 21) that a 4-8 fold increase in the specific activities in phosphoglucose isomerase, phosphofructokinase, phosphoglycerate kinase, phosphoglyceromutase and pyruvate kinase had no influence on the rate of ethanol formation from glucose. S. Sharma and P. Tauro (Biotechnol. Letters 8:735, 1986) reported that a mutant obtained from P.K. Maitra with increased activity of both pyruvate decarboxylase and alcohol dehydrogenase showed increased levels of ethanol production. A multicopy plasmid was constructed which carried both PDC1 and ADH1. The specific activities of these two enzymes were determined in the untransformed recipient strain at 1.51 U for pyruvate decarboxylase and 4.01 for alcohol dehydrogenase and in the transformed strain at 4.40 and 23.84 respectively in cells growing on a glucose medium. The rates of ethanol production in the same cultures were 15.8 for the recipient and 14.1 mol/mg dry weight per h in the transformed strain. This suggested that the activities of most of the glycolytic enzymes are not rate limiting not even in the case of the last two reactions from pyruvate to ethanol.

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4. Zimmermann, F.K. Induction of chromosomal malsegregation in Saccharomyces cerevisiae with mixtures of organic solvents.

Several aprotic polar solvents were shown to be strong inducers of chromosomal malsegregation while others appeared to be inactive in this test. Subacute concentrations of propionitrile and ethyl acetate, both extremely strong inducers at higher concentrations, elicited strong responses in combination with other solvents which when tested alone were inactive or only weakly active. However, such combination effects could not be observed with all solvents such as benzyl acetate or amyl propionate, two prominent flavoring esters. However, l-(-)- and d-(+)-carvone, the main components of caraway seed oil were strong inducers of chromosomal malsegregation. Similar combination effects have been observed over the years in respect to neurotoxicity of aprotic polar solvents as a consequence of industrial exposures or voluntary inhalation in solvent "sniffers". This indicates that the toxic potential of a given chemical may not only be determined by intrinsic properties of the pure compound but can be drastically influenced when combined with other agents.

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XI. Department of Food Science and Technology, University of California, Davis, CA 95616. Communicated by H.J. Phaff.

The following is an abstract of a recently presented paper.

Phaff, H.J., Starmer, W.T.[1], Tredick-Kline, J. [1]Department of Biology, Syracuse University, N.Y. 13244, U.S.A. Pichia kluyveri sensu lato - a proposal for two new varieties and a new anamorph. CBS Symposia Series No. 1, p. 403-414.

During our ecological studies of yeasts inhabiting necrotic tissue of various cactus species, we have recovered a number of heterothallic strains that are close relatives of Pichia kluyveri. This strongly fermenting species is not cactus specific, but is recovered occasionally from necrotic cactus tissue and periodically from Opuntia fruit. One group of related strains that is cactus specific originated from rotting stems of Opuntia spp. in Arizona and Texas. These strains are essentially non-fermentative or ferment very weakly. Their mating types are interfertile with those of P. kluyveri and their DNA relatedness to P. kluyveri is about 66%. These strains are described as P. kluyveri var. eremophila var. nov. An anamorph of that variety is described as Candida eremophila. A second group of slowly fermenting strains came for rotting arms of the columnar cactus Cephalocereus royenii on the island of Montserrat. These strains were also interfertile with P. kluyveri and showed about 72% DNA relatedness with that species, but were different from the variety eremophila to which they showed about 69% DNA relatedness. This group is described as P. kluyveri var. cephalocereana var. nov. Single-spore viabilities of the parental varieties varied from about 50 to nearly 100%. Intervarietal hybrids generally showed reduced spore viability. Fermentation velocity in P. kluyveri appears to be controlled by more than a single gene. The evolutionary divergence of the different varieties may have been promoted by host plant chemistry, as well as allopatry.

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XII. Department of Microbiology, Alko Laboratory, P.L. 350, 00101 Helsinki, Finland. Communicated by M. Korhola.

The following are some recently published works.

1. Korhola, M. Nevalainen, H. (Editors). 1987. Industrial Yeast Genetics. Proceedings of The Alko Symposium on Industrial Yeast Genetics, Helsinki, Finland, June 9-10.

Contents:

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2. Liljeström-Suominen, P.L., Joutsjoki, V., Korhola, M. Stable α-galactosidase producing derivatives of commercial baker's yeast. In: Industrial Yeast Genetics. Proceedings of The Alko Symposium on Industrial Yeast Genetics 9, 127-138, Helsinki, Finland, June 1987.

Beet molasses is widely used as a substrate for commercial yeast production. Complete hydrolysis of raffinose, present in beet molasses, by Saccharomyces strains requires secretion of α-galactosidase, in addition to invertase. Raffinose is not completely utilized by commercially-available baking strains which are Mel$^-$. The isolated MEL1 gene encoding secreted α-galactosidase from S. cerevisiae var. uvarum was either (1) integrated into chromosomal DNA or (2) introduced via an autonomously replicating 2μ based plasmid vector into the cells of a commercial mel^0 baker's yeast strain. The two strains were tested for their ability to utilize raffinose in molasses under conditions chosen to mimic baker's yeast production. α-Galactosidase was produced by these strains and all melibiose normally accumulating in the growth media was hydrolyzed. As a result, more carbohydrate was available for yeast growth and a potential savings in effluent treatment cost was achieved due to the reduced biological oxygen demand (BOD) load. The dough raising ability of this new strain was the same as that observed for the original baker's yeast.

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3. Korhola, M, Liljeström, P.L., Kopu, H., Ruohola, H., Torkkeli, T. Structure and expression of Saccharomyces meli gene. In: Stewart, G.G. et al. (eds.) Biological Research on Industrial Yeasts. Vol. III. Boca Raton: CRC Press, Inc., 1987, p. 119-124.

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4. Liljeström-Souominen, P.L., Joutsjoki, V., Korhola, M. 1988. Construction of a stable α-galactosidase-producing baker's yeast strain. Appl. Env. Microbiol. 54:245-249.

Molasses is widely used as a substrate for commercial yeast production. The complete hydrolysis of raffinose, which is present in beet molasses, by Saccharomyces strain requires the secretion of α-galactosidase, in addition to the secretion of invertase. Raffinose is not completely utilized by commercially available yeast strains used for baking, which are Mel^-. In this study we integrated the yeast MEL1 gene, which codes for α-galactosidase, into a commercial mel^0 baker's yeast strain. The Mel^+ phenotype of the new strain was stable. The MEL1 gene was expressed when the new Mel^+ baker's yeast was grown in molasses medium under conditions similar to those used for baker's yeast production at commercial factories. The α-galactosidase produced by this novel baker's yeast hydrolyzed all the melibiose that normally accumulates in the growth medium. As a consequence, additional carbohydrate was available to the yeasts for growth. The new strain also produced considerably more α-galactosidase than did a wild-type Mel^+ strain and may prove useful for commercial production of α-galactosidase.

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XIII. Département de Biologie Végétale, Université Lyon 1, Bât. 405 - 2è étage 43, Boulevard du 11 novembre 1918, F-69622 Villeurbanne Cedex, France. Communicated by M. C. Pignal.

The following are abstracts of recently published or submitted papers.

1. Michel, A., Jacob, F., Perrier, J., Poncet, S. 1987. Yeast production from crude sweet whey. Biotechnol. Bioeng. 30:780-783.

The present work is concerned with the yeast culture from crude whey towards the complete lactose use and the maximum biomass production. The continuous culture of the strain Candida LY496 at dilution rate of 0.20 h^{-1} seemed to be the best way to obtain a complete purification. A patent was taken out for this process.

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2. Pignal, M.C., Chararas, C., Bourgeay-Causse. Yeasts from Ips sexdentatus (Scolytidae) - Enzymatic activity and vitamin excretion. In press in Mycopathologia.

Yeasts from the digestive tract of Ips sexdentatus was isolated. Four strains, representing the different identified yeast species, were chosen. These yeasts show osidasic activities on several oligosaccharides and some polysaccharides (starch and pectin), but no activity on cellulose or hemicellulose. These activities are very different from one strain to another. In some cases, we observe differences between the sugar assimilations of a living yeast and the enzymatic activities of the cell extract; we try to explain these differences. The osidases of these four strains could be complementary to those of the insect digestive tract. These yeasts secrete B group vitamins whose natural and amount are different following the strain. It is possible that the vitamins excreted by these yeasts play an important role in the physiology of the insect which is not able to synthesize these metabolites and is dependent on the supply of vitamins by symbiotic microorganisms.

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R. Montrocher, J.B. Fiol and G. Billon-Grand will be attending the 7th International Symposium on Yeasts in Perugia, August 1988.

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XIV. Departamento de Bioquimica, Universidade Federal do Rio de Janeiro, Instituto de Quimica, UFRJ - Bloco A - Sala 547 - Ilha do Fundao, 21941 Rio de Janeiro - RJ, Brazil. Communicated by A.D. Panek.

The following papers have recently been submitted for publication.

1. Panek, A.D., Trajano Silva, J., Ferreira, R., Panek, A.C. Fructose 2,6-bisphosphate and trehalose metabolism in Saccharomyces cerevisiae.

A regulatory mutant of Saccharomyces (fdp) unable to activate fructose 1,6-bisphosphatase was shown to have a normal response to the glucose and fructose signals as measured by the activation of trehalase activity, indicating that the inability of the strain to grow on these sugars is caused by a defect located beyond membrane interactions. In vivo experiments with a mutant strain deleted in the phosphoglucoisomerase gene (pgil-δ) showed that activation of trehalase and de-activation of the trehalose-6-phosphate synthase complex occurred at the same extent whether glucose or fructose were used as signals. These results suggest that $Fru2,6P_2$ is not involved in the interconversion of forms of the enzymes of trehalose metabolism. Furthermore, $Fru2,6P_2$ was assayed on the trehalose synthesizing activity, using cell free extracts and partially purified preparations of the complex, and no effect could be observed. It would seem, therefore, that regulation of camp fulfills the requirements for control of trehalose levels in Saccharomyces.

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2. Araujo, P.S., Panek, A.C., Ferreira, R., Panek, A.D. A simple method for the preparation of active and stable yeast trehalase.

A three step purification procedure for trehalase from Saccharomyces cerevisiae with a recovery of 76% of the original activity is presented. The enzyme was activated by a heat shock treatment prior to homogenization of the cells. A mutant strain deleted in SUC genes was used to avoid contamination by invertase. The lyophylized enzyme was stable for, at least, four months and could be used to determine trehalose in the range of 25 to 500 nmoles. The preparation was free of nonspecific phosphatases allowing for trehalose determinations in cell free extracts.

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3. Panek, A.D., Ferreira, R., Panek, A.C. Comparative studies between the glucose-induced phosphorylation signal and the heat shock treatment in mutants of Saccharomyces cerevisiae.

Addition to glucose to derepressed yeast cells, as well as a heat shock treatment, trigger the phosphorylation of trehalase and of trehalose-6-phosphate synthase. In the present paper evidence is provided for the requirement of the p21 protein in the transduction of the glucose signal. On the other hand, a heat shock at 52°C for 2 min was able to produce the same phosphorylating effect even in mutant strains deficient in the GTP binding protein. Moreover, it was shown that this treatment does not directly affect the cAMP-dependent protein kinase. The use of a series of mutant strains demonstrated that low levels of cAMP favour thermotolerance and the role of trehalose in viability is discussed.

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XV. Institut fur Mikrobiologie und Weinforschung, Johannes Gutenberg-Universitat Mainz, Postfach 3980, D-6500 Mainz, W.Germany. Communicated by F. Radler.

The following are abstracts of recently published papers.

1. Schmitt, M., Radler, F. 1987. Mannoprotein of the yeast cell wall as primary receptor for the killer toxin of Saccharomyces cerevisiae strain 28. J. Gen. Microbiol. 133:3347-3354.

The killer toxin KT28 of Saccharomyces cerevisiae strain 28 is primarily bound to the mannoprotein of the cell wall of sensitive yeasts. The mannoprotein of S. cerevisiae X2180 was purified; gel filtration and SDS-PAGE indicated an estimated M_r of 185,000. The ability to bind killer toxin KT28 increased during purification of the mannoprotein. Removing the protein part of the mannoprotein by enzymic digestion or removing the alkali-labile oligosaccharide chains of β-elimination did not destroy the ability to bind killer toxin KT28. However, binding activity was lost when the 1.6-α-linkages of the outer carbohydrate backbone were hydrolysed by acetolysis. The separated oligomannosides of the side chains also failed to bind toxin, indicating that the main mannoside chains were essential for the receptor activity. The reversible adsorption of killer toxin to mannoprotein was demonstrated by linking it covalently to Sepharose and using this material for affinity chromatography. A 90-fold increase in the specific activity of a preparation of killer toxin KT28 was achieved in this way.

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2. Zorg, J., Kilian, S., Radler, F. 1988. Killer toxin producing strains of the yeasts Hanseniaspora uvarum and Pichia kluyveri. Arch. Microbiol. 149:261-267.

By heat treatment killer strains of the type K1 of Saccharomyces cerevisiae that are known to harbour dsRNA plasmids were completely cured, whereas only a small fraction of the clones of the killer type K2 had lost the dsRNA dependent killer character. The K2 killers but not the strains of killer type K2 were easily cured by cycloheximide. Killer strains of Hanseniaspora uvarum were not curable by heat treatment. Curing was successful with cycloheximide or 5-fluorouracil. Two double-stranded RNA plasmids were detected in the killer strains of H. uvarum. The smaller dsRNA plasmid was absent in the strains that were cured of their killer character by 5-fluorouracil. The killer character of H. uvarum was transferred to S. cerevisiae by spheroplast fusion. The fusion products showing the killer character contained both dsRNA plasmids, obviously the smaller plasmid (M-dsRNA) carries the genes for killer toxin formation. Killer strains of Pichia kluyveri were not curable of their killer character, in these strains no dsRNA plasmids were detected.

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3. Schwartz, H., Radler, F. 1988. Formation of L(-)malate by Saccharomyces cerevisiae during fermentation. Appl. Microbiol. Biotechnol. 27:553-560.

When grown in a synthetic medium most of the 51 strains of the genera Saccharomyces, Saccharomycodes, Zygosaccharomyces and Schizosaccharomyces investigated formed L-malate during fermentation. The quantity varied between 0.1 and 2.6 g malate per liter. Two strains of Saccharomyces cerevisiae synthesized malate at a rate of about 1.5 g/l. Malate was liberated during the growth phase and not metabolized during the stationary phase. Optimum malate formation was observed at a sugar concentration of about 20% (w/v), at pH 5 and at suboptimal nitrogen concentrations of less than 300 mg N/liter. Of the amino acids aspartate and glutamate were most favourable. If ammonium salts were used as the nitrogen source, significant amounts of malate were formed when the pH was kept constant by buffering. Trace metals had no or only little influence on malate synthesis. Biotin and pantothenate were essential for growth. Added $^{14}CO_2$ led to the formation of approximately equal quantities of labelled malate and succinate by S. cerevisiae strain 52, whereas about ten times more malate than succinate was formed by Saccharomyces uvarum. Avidin strongly inhibited the formation of malate while the inhibition of succinate synthesis and of growth was comparatively much less. Malate is obviously formed by reduction of oxalacetate, the synthesis of which is catalysed by a biotin-dependent pyruvate carboxylase.

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4. Pfeiffer, P., Radler, F., Caspritz, G.,[1] Hänel, H,[2] 1988. Effect of a killer toxin of yeast on eucaryotic systems. Appl. Env. Microbiol. 54:1068-1069. Abteilungen [1]Pharmakologie und [2]Chemotherapie, Hoeschst AG, Postfach 80 03 20, D-6230, Frankfurt 80, Federal Republic of Germany.

The Saccharomyces cerevisiae killer toxin KT28, which inhibits sensitive yeasts, was shown to have no effect on several pathogenic fungi or on the protozoan Trichomonas vaginalis. At concentration of about 0.1 mg/ml, a partial inhibition of the skin pathogenic fungi Trichophyton rubrum and Microsporum canis was observed at pH 6.5. No pharmacological activity was detected in various tests with several animal organs.

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XVI. Department of Physics, Biophysics Laboratory, Rikkyo (St. Paul's) University, Nishi-Ikebukuro 3, Toshima, Tokyo 171, Japan. Communicated by D. Keszenman-Pereyra and K. Hieda.

The following are abstracts of recently published papers.

1. Keszenman-Pereyra, D., Hieda, K. 1988. A colony procedure of transformation of Saccharomyces cerevisiae. Curr. Genet. 13:21-23.

A rapid and simple yeast transformation procedure has been developed using colonies on agar plates. Saccharomyces cerevisiae SHY3 cells were picked up from colonies on YPD plates grown freshly or stored at 4°C and incubated with M13RK9-T DNA at 30°C for 1-2 h in a solution of Li^+, Ca^{2+}, Mg^{2+}, triacetin and polyethylene glycol. About 3,500 transformants were obtained per μg of double stranded M13RK9-T DNA. Unlike the existing spheroplast techniques, single stranded M13RK9-T DNA transformed intact cells below one-hundredth frequency of the duplex form.

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2. Keszenman-Pereyra, D., Hieda, K. 1988. Transformation of yeast cells by UV-irradiated DNA II. Comparison of damage repair pathways between plasmids and cells. J. Rad. Res. 29:25. (Abstracts of the Thirtieth Annual Meeting of the Japan Radiation Research Society, Tokyo, November 30 - December 2, 1987)

Repair-proficient and various repair-deficient strains of Saccharomyces cerevisiae carrying a non-reverting ura3-52 mutation, were either irradiated with 254 nm UV light or transformed by treatment with alkali cations with UV-irradiated vectors M13RK9-T single- and double-stranded forms) and YCp19. Results are summarized as follows: (1) The excision (RAD2) and recombinational (RAD52) repair systems are the principal pathways involved in the removal of UV-lesions from incoming ds-DNA. (2) A high fluences in a rad2-1 rad52-1 background, RAD6 pathway seems to be necessary for the repair of UV damaged ds-DNA. (3) ss-DNA is repaired only when more than one pyrimidine dimer is in the substrate and a RAD2 product is functional. (4) ss- and ds-DNAs are substrates for the yeast photolyase. (5) Prior irradiation of the host increased the survival of the UV-irradiated ds-DNA in the RAD^+, rad2-1 and rad2-1, rad52-1 strains and decreased the survival of the UV-irradiated ss-DNA in the RAD^+ strain. (6) The triple mutant rad2-1, rad6-1, rad52-1 exhibited a F_{37} of 0.36 J m^{-2} indicating that a minor chromosomal repair pathway is still present. These results indicate that there are differences in the removal of UV-damage from chromosomal and transforming DNA.

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XVII. University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, New Jersey. Communicated by M.J. Leibowitz.

The following book has been published recently.

Y. Koltin and M.J. Leibowitz, Editors. Viruses of Fungi and Simple Eukaryotes. Marcel Dekker, Inc. ISBN: 0-8247-7890-1.

List of selected chapter titles dealing with yeast:

Discovery of a mycovirus: an indirect route - W.J. Kleinshcmidt
Yeast Ty elements as retroviruses - J.D. Boeke, D.J. Garfinkel
Transcriptional regulation of ty elements in Saccharomyces cerevisiae - F. Winston
Recombination associated with yeast retrotransposons - S.W. Liebman, S. Picologlou
Double-stranded RNA replication in Saccharomyces cerevisiae: genetic control and in vitro replication - T. Fujimura, R. Esteban, R.B. Wickner
Structure, transcription, and replication of killer virus dsRNAs - J.A. Bruenn, M.E. Nemeroff, M. Lee, D.F. Pietras, J.J. Dowhanick, L.J. Field
Transcription and translation of the yeast killer virus genome - M.J. Leibowitz, I. Hussain, T.L. Williams
Secretion and action of yeast K1 killer toxin - H. Bussey, C. Boone, A. Dmochowska, D. Greene, H. Zhu, S.J. Lolle, T. Vernet, D. Dignard, D.Y. Thomas
Acquisition and expression of the killer character in yeast - S.L. Sturley, M. El-Sherbeini, S.-H. Kho, J.L. LeVitre, K.A. Bostian
The killer system of Ustilago maydis: secreted polypeptides encoded by viruses - Y. Koltin
Killer systems and pathogenic yeast - J.S. Kandel
Kluyveromyces linear DNA plasmids - N. Gunge.

For further information contact: Promotion Department, Marcel Dekker, Inc., 270 Madison Avenue, New York, N.Y. 10016 or phone 212-696-9000.

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XVIII. Groupe de Physiologie et Pathologie Végétales, Laboratoire de Biologie et Cytophysiologie Végétales, Faculté des Sciences et des Techniques, Université de Nantes. Communicated by L. Simon.

The following is an abstract of a recently submitted paper.

Simon, L. Aureobasidium pullulans: morphogenetic, metabolic and energetic assessment.

Five various composition culture media (with or without phosphorus and organic nitrogen) are described inducing different morphogenetic development in Aureobasidium pullulans (L.C.P.87.43). Energetic phenomenons (as measured by intracellular ATP and polyphosphate contents), pH values correlated to organic or inorganic nitrogen uptake, and glucose consumption are factors that influence either cellular or filamentous development. Cellular forms (chlamydospores and swollen cells), induced by a rapid acidification of the culture medium and by an extracellular glucose excess, are ATP and polyphosphate energy consumer for the first days culture during growth and ammonium nitrogen consumption. Hyphal forms, induced by organic nitrogen source and alkalinization of the culture medium, are high energy consumers for the first twenty-fourth hours culture of the exponential growth, but energy producers (ATP) over the next days. Possible types of glucose phosphorylation are discussed.

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

Since the previous issue of the Yeast Newsletter the following papers have appeared.

1. Noshiro, A., Purwin, C., Laux, M., Nicolay, K., Scheffers, W.A. and Holzer, H. 1987. Mechanism of stimulation of endogenous fermentation in yeast by carbonyl cyanide m-chlorophenylhydrazone. J. Biol. Chem. 262:14154-14157.

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2. Scheffers, W.A. 1988. Alcoholic fermentation. In: G.S. de Hoog, M.Th. Smith and A.C.M. Weijman (eds.) The Expanding Realm of Yeast-like Fungi. Elsevier Science Publishers, Amsterdam, pp. 321-332.

In the characterization of yeasts, physiological traits play an essential role. Likewise, in the realm of yeast-like fungi physiological features will be indispensable in characterizing these organisms. Moreover knowledge of their physiology may provide important clues for fundamental and applied research on this group of organisms. Starting from current procedures in yeast research, some physiological aspects will be discussed. In particular, factors determining the capacity for alcoholic fermentation of various sugars will be considered. The standard fermentation test in the Durham tube, based on inspection for visible gas formation, is an arbitrary criterion. By testing for ethanol production, fermentative ability may be demonstrated in many yeasts as well as in some yeast-like fungi.

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The following papers have been accepted for publication.

3. Postma, E., Scheffers, W.A., van Dijken, J.P. Adaptation of the kinetics of glucose transport to environmental conditions in the yeast Candida utilis CBS 621: a continuous-culture study. Journal of General Microbiology: in press.

The relation between the kinetic parameters of glucose transport and the physiology of Candida utilis CBS 621 was studied in chemostat cultures. In glucose-limited cultures the transport parameters were dependent on the growth rate of the yeast. Three different transport systems were encountered which differed by an order of magnitude in their affinity constants, namely a high-affinity (K_m 25 μM), a medium-affinity (K_m 190 μM), and a low-affinity uptake system (K_m 2000 μM). At dilution rates below 0.52 h^{-1} cells possessed the high- and medium-affinity uptake system. At higher dilution rates the high-affinity system was absent and both the medium- and low-affinity system were present. At a dilution rate close to μ_{max} (0.57 h^{-1}) only the low-affinity system was detected. The in situ contribution of each of the transport systems to glucose consumption in glucose-limited cultures was estimated on the basis of their kinetic parameters (K_m and V_{max}) and the residual glucose concentration in these cultures. The sum of the calculated rates of transport corresponded to the in situ rate of glucose consumption by the cultures as determined from the yield constant and the dilution rate. The dependence of the transport parameters on the growth rate and hence on the environmental sugar concentration was also evident in cells grown under nitrogen limitation. In contrast to carbon-limited cells, nitrogen-limited cultures growing at D = 0.15 h^{-1} did not exhibit the high-affinity glucose uptake, whereas the medium- and low-affinity systems were present.

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4. Pronk, J.T., Bakker, A.W., van Dam, H.E., Straathof, A.J.J., Scheffers, W.A., van Dijken, J.P. Preparation of D-xylulose from D-xylose. Enzyme & Microbial. Technology: in press.

A simple method is described for the preparation of D-xylulose. It comprises the isomerisation of D-xylose with xylose isomerase (EC 5.3.1.5) yielding an equilibrium mixture of D-xylulose and D-xylose. This is followed by the quantitative oxidation of residual D-xylose to D-xylonic acid with immobilised A. calcoaceticus cells. A combination of methanol precipitation and ion exchange is used for the removal of xylonic acid. This procedure offers many advantages over existing methods for the preparation of D-xylulose. The purity of the final product compares favourably to that of a commercial D-xylulose preparation.

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5. Rouwenhorst, R.J., Visser, L.E., van der Baan, A.A., Scheffers, W.A., van Dijken, J.P. Production, distribution, and kinetic properties of inulinase in continuous cultures of Kluyveromyces marxianus CBS 6556. Appl. Env. Microbiol. (in press).

From a screening of several Kluyveromyces strains, the yeast Kluyveromyces marxianus CBS 6556 was selected for a study of the parameters relevant to the commercial production of inulinase (EC 3.2.1..7). This yeast exhibited superior properties with respect to growth at elevated temperatures (40-45°C), substrate specificity and inulinase production. In sucrose-limited chemostat cultures growing on mineral medium the amount of enzyme decreased from 52 [mg cell dry wt]$^{-1}$ at a dilution rate of 0.1 h^{-1} to 2 U [mg cell dry wt]$^{-1}$ at D=0.8 h^{-1}. Experiments with nitrogen-limited cultures further confirmed that synthesis of the enzyme is negatively controlled by the residual sugar concentrations in the culture. High enzyme activities were observed during growth on non-sugar substrates and indicate that synthesis of the enzyme is the result of a derepression/repression mechanism instead of by induction as hitherto supposed. A substantial part of the inulinase produced by K. marxianus was associated with the cell wall. The enzyme could be released from the cell wall via a simple chemical treatment of cells. Results are presented on the effect of cultivation conditions on the location of the enzyme. Inulinase was active with sucrose, raffinose, stachyose and inulin as substrates and exhibited an S/I ratio of 15 at standard assay conditions. The enzyme activity decreased with increasing chain length of the substrate.

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6. Verduyn, C., Breedveld, G.J., Schreuder, H., Scheffers, W.A., van Dijken, M.P. Properties of enzymes which reduce dihydroxyacetone and related compounds in Hansenula polymorpha CBS 4732. Yeast (in press).

Hansenula polymorpha CBS 4732 grown on a variety of substrates contained very high activities of enzymes catalyzing the NADH-linked reduction of dihydroxyacetone, acetoin, diacetyl, acetol, methylglyoxal and acetone. The enzymes catalyzing these reductions have been purified and their kinetic properties are described. Three different enzymes were found responsible for the above mentioned activities, namely: 1) dihydroxyacetone reductase; 2) acetone reductase and 3) alcohol dehydrogenase. So far, the physiological function of dihydroxyacetone reductase and acetone reductase in obscure. The kinetic properties of dihydroxyacetone reductase and the regulation of the synthesis of this enzyme suggest that it does not function as a glycerol dehydrogenase.

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7. Verduyn, C., Breedveld, G.J., Scheffers, W.A., van Dijken, J.P. Purification and properties of dihydroxyacetone reductase and 2,3-butanediol dehydrogenase from Candida utilis CBS 621. Yeast (in press).

Candida utilis CBS 621 contained four different enzymes capable of reducing carbonyl compounds such as dihydroxyacetone, acetoin, diacetyl, acetol, methylglyoxal and acetone, namely alcohol dehydrogenase, acetone reductase, dihydroxyacetone reductase and 2,3-butanediol dehydrogenase. The dihydroxyacetone reductase of C. utilis did not oxidize glycerol, thus providing evidence that this enzyme cannot function as a glycerol-2-dehydrogenase during growth of the yeast on glycerol. This enzyme may, however, play a role in the assimilation of 2,3-butanediol by C. utilis. The organism also contained a separate 2,3-butanediol dehydrogenase which was unable to reduce dihydroxyacetone. Both dihydroxyacetone reductase and 2,3-butanediol dehydrogenase were present at a very high activities during growth of C. utilis on a variety of substrates, including 2-3-butanediol.

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8. Verduyn, C., Breedveld, G.J., Scheffers, W.A., van Dijken, J.P. Metabolism of 2,3-butanediol in yeasts. Yeast: in press.

The biochemistry and physiology of 2,3-butanediol metabolism has been studied in a number of selected yeast species. Candida utilis CBS 621 exhibited diauxic growth of 2,3-butanediol. The first phase was characterized by the utilization of the two optically active stereoisomers and associated accumulation of acetoin. In the second phase of growth the meso-form of 2,3-butanediol was utilized together with acetoin. An attempt is made to explain these phenomena on the basis of the substrate specificity of the two enzymes which oxidize 2,3-butanediol in C. utilis. Although whole cells oxidized acetoin and diacetyl at high rates, attempts to identify the enzymes responsible for these oxidations were unsuccessful. In C. utilis and other yeasts metabolism of 2,3-butanediol probably involves a cleavage of the substrate into C_2-units which are assimilated by the glyoxylate cycle. In the few yeasts which have been found to grow on 2,3-butanediol differences may be encountered with respect to the substrate specificity for the three stereoisomers of 2,3-butanediol. For example, Candida salmanticensis CBS 5121 showed no diauxic growth and utilized only two of three stereoisomers.

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9. Verduyn, C., Breedveld, G.J., Scheffers, W.A., van Dijken, J.P. Substrate specificity of alcohol dehydrogenase from the yeasts Hansenula polymorpha CBS 4732 and Candida utilis CBS 621. Yeast (in press).

The substrate specificity of alcohol dehydrogenase (ADH) from Hansenula polymorpha and Candida utilis has been compared with that of the classical ADH from baker's yeast. Cell-free extracts of H. polymorpha and C. utilis exhibited a much higher ratio of butanol to ethanol oxidation from baker's yeast ADH. This was also observed with the purified enzymes. The ratio of activities with ethanol and butanol was pH-dependent. With the baker's yeast enzyme the activity strongly decreased with increasing chain length, whereas the enzymes from H. polymorpha and C. utilis showed a high reactivity with long-chain alcohols. In addition, the affinity constant for ethanol was more than ten-fold lower than that of the baker's yeast enzyme. The purified preparations yielded several protein bands on polyacrylamide slab gels, each of which showed activity with both ethanol and butanol.

* * *

Finally, the following patent was granted:

10. van Dijken, J.P., Scheffers, W.A. A method for producing ethanol from xylose-containing substance. U.S. Patent No. 4, 701, 414 (to Alfa-Laval AB, Tumba, Sweden), d.d. October 20, 1987.

XX. Instituto de Investigaciones Biomedicas, CSIC, Facultad de Medicina de la UAM, Arzobispo Morcillo, 4, 28029 Madrid, Spain. Communicated by R. Lagunas.

The following are abstracts of recent publications or communications.

1. Lagunas, R. 1986. Misconceptions about the energy metabolism of Saccharomyces cerevisiae. Yeast 2: 221-228.

Contents

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2. Cid, A., Gancedo, C., Lagunas, R. 1987. Inactivation of the glucose transport system in sporulating yeast. FEMS Microbiology Letters 41:59-61.

The glucose transport system of Saccharomyces cerevisiae was inactivated during sporulation. The glucose uptake capacity of a culture (measured with xylose as substrate) decreased to 50% when about 15% of the population was transformed into asci. At the end of sporulation the residual transport capacity was about 20% of the initial one. No changes in glucose transport were observed in a non-sporulating diploid treated in the same conditions. Reappearance of the transport did not occur during germination if this was inhibited by cycloheximide.

* * *

3. Lagunas, R. The Pasteur effect today. International Conference on Regulation in Bioenergetics and Nonlinear Phenomena in Biosciences. Max-Planck-Institut für Ernährungsphysiologie. March 16-18, 1987.

The Pasteur effect is defined as an inhibition of the rate of sugar consumption by respiration. It is generally accepted that his phenomenon is important in most facultative anaerobes and also that it was discovered by Pasteur in Saccharomyces cerevisiae. However, there are serious reasons to think that these ideas are erroneous. Actually, it seems that the Pasteur effect is irrelevant in many facultative anaerobic cells and also that it was not discovered by Pasteur in yeast. The explanation given for the occurrence of the Pasteur effect is based on the greater energetic efficiency of respiration (aerobiosis) versus fermentation (anaerobiosis). If a facultative anaerobe shifts from sugar fermentation to complete oxidation it might produce up to 18 times more ATP per mol of catabolized sugar. This value is calculated assuming a P/O=3 in the respiratory chain. Therefore, to maintain the same ATP production rate, a shift from anaerobiosis to aerobiosis might decrease up to 18 times the rate of sugar consumption (Pasteur effect). However, this maximal value is never reached and a Pasteur effect equal or greater than 2 is rarely observed (1-4). One reason for this apparent paradox is that most facultative cells remain fermenting even in aerobiosis, whereas complete oxidation of sugars only occurs exceptionally (1-3). S. cerevisiae is one of the many facultative anaerobes that does not show Pasteur effect unless very special experimental conditions are used (4-6). The reason why it is widely accepted that the Pasteur effect was discovered in this organism is based on misinterpretations by modern biochemists of Pasteur's data (7-8). These misinterpretations, that were reasonable when formulated, have remained unchanged in scientist's mind although long time ago they have been shown to be erroneous. Unfortunately very often, as Francis Bacon already stated in 1620, "the logic in use serves rather to fix and give stability to the errors, which have their foundation in commonly received notions, than to help the search after truth".

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XX. Department of Plant Sciences, University of Western Ontario, London, Ontario. Communicated by M.A. Lachance.

Summary of a communication presented at the February 1988 meeting of the Ontario Pesticide Advisory Committee by P.G. Kevan, Queen's Park, Toronto.

1. Kevan, P.G.[1], Eisikowitch, D.[1,2], Lachance, M.A. Yeasts and milkweeds: using yeasts to suppress fruit and seed-set in milkweeds. [1]Department of Environmental Biology, University of Guelph, Guelph, Ontario. [2]On leave from Department of Botany, Tel Aviv University, Israel.

Field milkweed, Asclepias syriaca, is a noxious weed and difficult to control. We discovered that the yeast, Metschnikowia reukaufii, is potentially a biocontrol agent which may affect the fruiting capabilities of the plant. During pollination, which requires insects, the pollinia (special packages of pollen as produced by the plant) are moved from flower to flower as the insects forage for nectar. When the pollinium is inserted into the stigmatic cavity, its pollen may germinate and grow in the sugary nectar (ca. 20% sucrose), into the stigmatic tissue to eventually bring about fertilization of the ovules, seed-set, and fruit development. The yeast is also readily transported by pollinators such as bumblebees, honeybees, flies, and moths. Pollinia in sugar solutions and in nectar from flowers that opened in the laboratory always germinate. However, if the yeast is inoculated into the sugar solutions or clean nectar the pollinia fail to germinate. Thus, M. reukaufii suggests itself as an easily vectored floral epiphyte of milkweed and which has potential as an augmentative biocontrol agent for reducing seed-set by milkweed. Studies involving the application of different yeast biotypes in the field and in boxed plants are underway to determine the applicability of using M. reukaufii as a biological control agent.

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The following chapter has been published recently.

2. Approaches to yeast identification. 1987. Lachance, M.A. In Berry, D.R., Russell, I., and Stewart, G.G., Editors. Yeast Biotechnology. London, Allen & Unwin. pp. 33-51.

The concluding remarks, given here, may be relevant to Dr. Barnett's contribution.

Yeast systematics may be approached from many directions, and for several different purposes. Substantial efforts are now channelled towards creating a classification system which would be an image, as faithful as possible, of the paths followed by yeasts to evolve into the present array of species. Natural yeast taxa do not necessarily lend themselves well to convenient classification, because of one major component of the biological reality, variation. The use of recently developed molecular techniques by yeast taxonomists has not solved the problem of identification, and the development of rapid and clear-cut identification systems by brewers and other practically minded researchers has been of little use in evolutionary systematics. Nevertheless, research in this area should benefit from enhanced communication between the many researchers involved.

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The following review paper has been recently accepted for publication.

3. Lachance, M.A., Starmer, W.T.,[1], and Phaff, H.J.[2] Identification of yeasts found in decaying cactus tissue. Can. J. Microbiol. 34 (In press). [1]Department of Biology, Syracuse University, Syracuse, New York 13244, and [2]Department of Food Science and Technology, University of California, Davis, California 95616.

Contents

Summary

The nomenclature and principal diagnostic properties of yeasts hitherto isolated from cactus necroses have been reviewed. The yeasts associated with cactus fruits tend to be the same as those in fruits of other plants, in contrast with the yeasts found in somatic tissue necroses, many of which are strictly cactophilic and not found elsewhere. The majority of cactus-inhabiting yeasts are members of the subfamily Saccharomycetoideae, with the genus Pichia being the best represented. Many cactophilic yeasts are nutritionally specialized, and thus difficult to classify on the basis of their expressed phenotypes.

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The following are abstracts to be presented at the VIIth ISY in Perugia.

4. The yeast community of morning glory and associated drosophilids in a Hawaiian kipuka. Lachance, M.A., Starmer, W.T.,[1] and Bowles, J.M.[2] [1]Department of Biology, Syracuse University, Syracuse NY 13244, U.S.A. [2]R.R.#3, Thorndale, Ontario, Canada N0M 2P0.

We have examined the yeast community of morning glory [Ipomoea acuminata (Vahl) R & S] and its associated drosophilids in a kipuka, a characteristic island (literally "hole") of vegetation located amidst recent lava flows on the slopes of volcanos on the island of Hawaii. Soon after opening, the short-lived flowers are visited by a number of insects. Of special interest are 2 drosophilid species, Scaptomyza calliginosa Hardy and Drosophila floricola Sturtevant, which colonize the corolla, ultimately ovipositing and leaving. A larva develops in the closed corolla, which eventually becomes senescent and drops off. To see if a dynamic interaction similar to that found in cacti exists between yeasts, the plant, and the insects, we have examined the yeast flora of the various components. In order to ascertain the role of the flies as vectors, yeasts were isolated periodically from tagged or bagged flowers by direct streak plating of inner corolla scrapings on acidified YM, and from insects by allowing single flies to walk on plates for 12 hours. The yeasts shared by morning glory and drosophilids consisted of 4 major species and a few minor ones. The dominant forms were (1) Candida azyma (van der Walt, Johannsen et Yarrow) Meyer et Yarrow, (2) a (new?) Candida species physiologically similar to Metschnikowia reukaufii Pitt et Miller but with a distinct morphology, (3) a (new) Candida species exhibiting generally very poor growth but strong extracellular lipase activity, and (4) a new heterothallic species of Metschnikowia. Most of these yeasts are short-lived on normal culture media. The 4 dominant yeasts are vectored by drosophilids and probably serve as food for the growing larvae. A succession takes place in the flower, such that the vectored yeasts are eventually replaced by other species, mostly basidiomycetous anamorphs that must be dispersed by other means. Jointly with a yellow-pigmented, Gram-variable, rod-shaped bacterium, the minor species are ostensibly involved in flower decay. When compared with preliminary surveys of the same habitat conducted in 1976 and 1979, this detailed 1987 study suggests that the structure of the yeast community associated with the kipuka morning glory is stable over time.

5. Lachance, M.A. Restriction mapping of rDNA and the Taxonomy of Kluyveromyces van der Walt emend van der Walt.

The construction of restriction endonuclease maps of ribosomal DNA (rDNA) is an accessible means of extracting information from a molecular phenotype that contains both rapidly and slowly evolving sequences. The approach is convenient because of the repeated nature of yeast rDNA, which usually allows visualization of DNA fragments without recourse to labelled probes. The genus Kluyveromyces having been used as a model in the appraisal of new taxonomic methods, it was of interest to see how much variation exists in its rDNA restriction was of interest to see how much variation exists in its rDNA restriction patterns. Whole DNA was extracted from the type strains of the 13 nomenspecies recognized by Sidenberg and Lachance (Int. J. Syst. Bacteriol. 36:94 [1986]) and from other strains as well. The purified DNAs were treated with the 12 endonucleases ApaI (A), BamHI (B), SacI (C), EcoRI (E), BglI (G), HindIII (H), KpnI (K), SmaI (M), ScaI (S), XhoI (X), and XbaI (Y), singly or in pairs, the digests were subjected to agarose gel electrophoresis, and the gels were stained with ethidium bromide. The length of the repeating unit ranged from ca. 8.4 kb (in K.aestuarii) to ca. 10.9 kb (in K.phaffii). The length variation resided as expected in the nontranscribed spacer. The patterns confirmed some of the inferences articulated by various students of the genus. The closely related species K.marxianus and K.lactis constituted a core to which could be linked first K.wickerhamii, and then K.dobzhanskii and K.aestuarii. This last observation is all the more exciting in that K.aestuarii's remote kinship with those other species had been deduced on the basis of morphology and physiology, but never confirmed by approaches involving DNA reassociation, prototroph selection, or isoenzyme electrophoresis. The presumed relatedness between K.waltii and K.thermotolerans was endorsed by rDNA mapping as well, but evidence linking these two species to the rest of the genus is wanting. Homologies in the restriction patterns suggest that the multispored species together with K.delphensis form a loose assemblage acting as a bridge between the "core" species and the species K.phaffii and K.lodderi. Mapping of rDNA was not in defense of restricting the name Kluyveromyces to the multispored species and reestablishing Zygofabospora to accomodate the 4-spored species, as suggested recently by Naumov (Mycol. Phytopathol. 21:2 [1987]).

* * *

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

The following correspondence regarding yeast nomenclature will no doubt be of interest to many readers of the Yeast Newsletter.

March 22, 1988

Dear Professor J.A. Barnett:

The yeasts of the genus Candida are being more widely used in the biotechnological processes now. It is known that there are pathogenic species among the representatives of this genus. So, some non-microbiologists believe that all strains of the Candida species should be considered pathogenic to a certain extent and as a result, must not be used in biotechnology. This point of view seems erroneous to me, and I intend to discuss this question in the relevant international scientific and organizing committees on microbiology and biotechnology.

In connection with this, I am forwarding to you the opinion of Dr. V. Golubev, an expert on the yeasts. The material goes under the title "Heterogeneity of the genus Candida". Would you be so kind as to give your remarks concerning the suggested material or express your point of view on this score.

With the best regards.

Sincerely yours,

(signed)
Professor V. Eroshin
Institute of Biochemistry and
Physiology of Microorganisms
USSR Academy of Sciences
Pushchino, Moscow Region 142292
USSR

Enclosure in Prof. Eroshin's letter:

Heterogeneity of the Genus Candida. Golubev, W.I., Curator of Yeasts, All-Union Culture Collection, Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, USSR.

The yeasts are higher fungi growing primarily in a one-cell phase. From the taxonomic point of view this group is heterogeneous even at the higher taxonomic levels; approximately 500 yeast species known till now include ascomycetes, basidiomycetes and deuteromycetes. The features of life cycles and sexual reproduction serve as the basis for the classification of yeasts. If sexual characteristics are unknown (as in the case of imperfect yeasts), the classification is restricted mainly to their listing and proposing keys for their identification. These imperfect organisms are grouped in form-taxa, and more detailed studies reveal, as a rule, their diversity. The patent example is the genus Candida Berkhout. At present the genus Candida includes about 200 species. Its heterogeneity is reflected by a very wide range of such chemotaxonomic characteristics as the GC-content (from 30 to 66 mol%) and coenzyme Q systems (from Q-6 to Q-10). On the basis of these data as well as the types of cell wall ultrastructure and budding, it was established that Candida species are imperfect forms (anamorphs) of both ascomycetous and basidiomycetous organisms. The evident heterogeneity of the genus Candida stimulated numerous proposals to revise this taxon though not always were they well grounded. To avoid confusion and provide stability of nomenclature it was suggested to conserve this generic name. Nevertheless, recently, a number of Candida species were transferred to other genera (Cryptococcus, Rhodotorula) or new genera were proposed for them (Apiotrichum, Golubevia, Vanrija). In some cases there is ample evidence that certain Candida species are anamorphs of the perfect ascosporogenous yeast genera Arxiozyma, Citeromyces, Clavispora, Debaryomyces, Issatchenkia, Kluyveromyces, Metschnikowia, Pichia, Saccharomyces, Stephanoascus, Torulaspora, Wickerhamiella and Yarrowia, and the basidiosporogenous yeast genera Filobasidium and Leucosporidium. Thus, in total the species of the genus Candida can be distributed into no less than 20 yeast genera. The given data show that generalized conclusions about the species of the genus Candida on the whole (except the general characteristics applicable to all higher fungi or all yeasts) are illegitimate. They have no sound taxonomic reason.

Reply:

April 27, 1988

Dear Professor Eroshin:

Thank you for your letter of 22 March about Dr. Golubev's proposals for Candida species. Clearly, Dr. Golubev is absolutely right and the genus Candida is, as he says, very heterogeneous indeed. He has a strong case for revising it. However, I believe that his proposals involve important, wider issues.

He has touched on one of the problems that such revisions produced, namely, the confusion produced by changing names. Generally, yeast taxonomists have been highly irresponsible and ignored this problem and I think Dr. Golubev should be congratulated on giving it due consideration.

May I illustrate the kind of difficulty that comes of extensive name-changing with the following example? I give an annual course to students on yeast physiology. I expect the students to read reviews and research papers. However, names have changed so fast that it is exceedingly difficult for students (non-taxonomists) to know whether they are reading about the same or different yeasts. For instance, Saccharomyces carlsbergensis, S. uvarum and S. cerevisiae may, or may not, refer to the same kind of yeast. The same applies to Kluyveromyces fragilis, K. marxianus, Saccharomyces fragilis and Fabospora fragilis: these names may, or may not, be synonymous.

As I see it, the difficulty arises from a conflict of interest, which I believe, given a sense of responsibility, could well be mostly resolved. The ordinary (non-taxonomist) worker is like a man going into a large shop. He wants butter to be labelled butter ALWAYS! Not sometimes fruit-juice and at other times soap. Very many taxonomists want to classify according to some measure of ancestral relationship, or of genetic difference. They are prepared, each time they find out just a little more, to change the names to-and-fro to accord with their latest findings. Unfortunately, too many are uninterested in the effects on non-taxonomists, for whom they should be providing a service.

This conflict of interest could surely be resolved by a change in the rules of nomenclature, at least for the yeasts. Some change is already necessary, because the use of dried type specimens, in place of living cultures, is ludicrous for yeasts.

I propose that taxonomists should continue to publish their findings, as now, but an international standing commission should review the publications and, at intervals of several years, issue of list of accepted species. This list would then stand until the next list is issued. Such a procedure should avoid some of the changes in nomenclature that are then reversed or altered after only a year or two.

My proposals assume that the taxonomists wish primarily to give a service to the scientific community. Those who have no such wish will doubtless reject the proposals out of hand.

I hope I have answered your letter adequately.

Every good wish.

Yours sincerely,

(signed) James A. Barnett

c.c. Professor Golubev
Professor Lachance
Dr. Yarrow
Dr. Kurtzman

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XXII. MEETINGS

1. XVIIIth Annual Conference on Yeasts, held in Smolenice Castle, 10th to 12th February, 1988. Communicated by Dr. Anna Kocková-Kratochvilová, Institute of Chemistry of Slov. Acad. Sci., Bratislava 84238, Dúbravská cesta 9, Czechoslovakia.

1st section: Immunology and pathogenicity

Šandula J.: Biologic activity of polysaccharides of the yeast cell wall.
Kogan, G., Masler L.: The structure of cellular polysaccharides of pathogenic Candida yeasts.
Pavliak, J., Šandula J.: Immunological properties of cellular mannans of pathogenic Candida yeasts.
Tomšiková, A.: Practical application of biotypization in epidemiology of vaginal candidoses.
Tomšiková, A., Pavliak, V.: The device of anti-Candida compounds by modern serological methods.
Kopecká, M.: Electron/optical microscopy of yeast cell walls and of regenerating protoplast walls.
Masler, L., Kogan, G.: Structural and physico-chemical characteristics of β-1,3-glucans with immunological properties.
Navarová, J., Trnovec, T., Ďurišová, M.: Antiinfection efficiency of β-1,3-glucans.
Breierová, E., Kocková-Kratochvilová, A.: The application of yeast polysaccharides in the preservation of yeasts in liquid nitrogen.

2nd section: Minisymposium "Genetic Manipulations"

Kováč, L.: Gene manipulations in yeasts: from dreams to the reality.
Šilhánková, L.: The organization of yeast nuclear genome and its genetic analyses.
Vondrejs, V.: Induced fusion of protoplasts in yeasts, perspectives and limitation.
Turňa, J.: Enzymes used in techniques of DNA recombination, their application and availability.
Palková, Z.: Cloning vectors and the building of gene banks in yeasts.
Šubik, J., Zigová, M., Karas, D.: Isolation and molecular cloning of yeast genes.
Hostinová, A., Godány, A., Kuntzel, H.: Complementation of cdc25 mutation of the life cycle of Saccharomyces cerevisiae with extragenic suppressor plasmids and its delated forms.
Kolarov, J.: Yeasts as models for the study of the function of oncogenes in the cell division.
Brozmanová, J., Černáková, L., Kleibl, K., Škorvaga, N.: The study of repair processes in yeasts by interspecies translocation of respiratory genes.
Timko, J.: The DNA sequencing and the determination of the specificity of restrictive endonucleases.
Šimúth, J., Klondiny, J.: Some problems of heterological expressions.

3rd section: Biotechnology

Votruba, J.: Mathematical model of anaerobic production of ethanol by Saccharomyces cerevisiae.
Vraná, D., Sobotka, N., Votruba, J., Havlík, I.: The use of transitive states to the monitoring of the physiology of yeast Candida utilis.
Hronček, J.: The elaboration of output yeast biomass by the method of desolvatation.
Šturdík, E.: The complete fractionation of yeast biomass.

Posters

[1]Kothera M., Vondrejs, V., Palková, Z.: The use of dielectrophorese at the following of zymocine effect.
[2]Pavliček, I., Kothera, M., Vondrejs, V., Palková, Z.: The attempt of the use of dielectrophorese for controlled protoplast fuse of Schizosaccharomyces pombe.
[3]Palková, Z., Vondrejs, V., Zandražil, S.: The gene expression of calf pro-chymosine in Saccharomyces cerevisiae.
[4]Rysavá, D., Šilhánková, L.: The tolerance of Yarrowia lipolytica to inhibitors of mitochondrial functions and its changes by mutations.
[5]Michalčaková, S., Sulo, P., Minarík, E.: The study of genetical properties of vine yeasts.
[6]Svoboda, A.: The conjugation in Schizosaccharomyces japonicus.
[7]Večerek, B., Janderová, S.: The influencing of the ratio of hybrids and cybrids produced by induced protoplast fusion.
[8]Rutkay-Nedecky, B., Šubik, J.: The use of induced loss of chromosomes in genetical mapping in Saccharomyces cerevisiae.
[9]Krupová, E.: Partial purification of 2-oxoglutarate dehydrogenase of Saccharomyces cerevisiae.
[10]Forrová, H., Kolarov, J.: The disorder and deletion of the genome DNA in Saccharomyces cerevisiae by LEU-2 gene.
[11]Šipicky, M.: Affl-: suppressor of the conjugation and of haploid sporulation in Schizosaccharomyces pombe.
[12]Paulovicová, E., Blanáriková, Z.: Device of phagocytosis preceding to the stimulation of granulocytes in Saccharomyces cerevisiae and PMA.
[13]Běhalová, B., Voříšek, J.: The dynamics and cytology of the accumulation of sterols in Saccharomyces cerevisiae.
[14]Vojteková, G., Hanusková, A.: The regulation of the fermentation process in cider by physical and biological way.
[15]Dercová, K., Augustin, J., Mokrá, J.: Formaldehyde metabolism in methanolotrophic and non-methanolotrophic yeasts.
[16]Kocková-Kratochvilová, A., Slávíková, E., Kovačovská, R.: Isolation and identification of yeasts and yeast-like organisms from the sea of Zahorie.
[17]Kocková-Kratochvilová, A., Slávíková, E.: Candida mucifera n. sp.
[18]Šmogrovičová, D., Augustin, J., Kosáková, Z.: The production of amylolytic enzymes by yeasts.
[19]Přikrylová, D.: The mathematical modelling of cell cycle of Saccharomyces cerevisiae.
[20]Kossachzka, Z., Vojtková-Lepšiková, A., Machová, E.: Autolytic methods for the nucleic acid content decreasing in yeasts.
[21]Omelková, J.: The effect of saponins on the yeast growth.
[22]Vancová, A.: Cellobiase activity of the cell wall glucosidase of Cryptococcus albidus.
[23]Vršanska, M., Biely, P., Defaye, J.: The induction of xylanase system in Cryptococcus albidus by thioanalogues of xylobiose and methyl-β-D-xyloside.
[24]Longauerová, D., Halama, D.: The growth of Candida tropicalis in liquid media obtained after bacterial fermentation of lignocellulosic materials.
[25]Havlik, I.: The elaboration of fermentation data by the computer IBM PC.
[26]Biely, P., Lee, H., To, R. J. B., Latta, R. K., Schneider, M.: Acylxylan esterase of Rhodotorula rubra.
[27]Breierová, E., Kocková-Kratochvilová, A.: Storage of Candida utilis, C. guilliermondii and related species in liquid nitrogen.

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2. The Sixth International Congress of Culture Collections (ICCC-VI) sponsored by the World Federation of Culture Collections (WFCC) will be held from October 30, 1988 to November 5, 1988 at Maryland University, U.S.A.

The theme of the congress, "The microbial gene pool", will be emphasized through symposia, round-tables, and posters. The poster session on three days will allow a variety of topics pertaining to activities of culture collections, preservation of microorganisms, computer usage in culture collections, microbial taxonomy, etc. We have received more than 50 poster abstracts and would like to encourage more. All those interested should apply for the poster session of ICCC-VI and send poster abstracts as soon as possible so that they may be included in the publicity.

Contact: Dr. K. Komagata, Chairman of Scientific Program, ICCC-VI
Japan Collection of Microorganisms
RIKEN
Wako-shi, Saitama
351-01 JAPAN

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3. Molecular and Cellular Biology of Yeasts and Filamentous Fungi. Organizers: W.E. Timberlake and M.J. Holland, April 3-9, 1989. Steamboat Springs, Colorado.

The yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe and the filamentous fungi Neurospora crassa and Aspergillus nidulans have been used extensively to advance our understanding of numerous cellular processes such as gene regulation, mitosis, meiosis, and organelle movement. Much of the technology used for analysis of these organisms, most notably traditional and reverse genetics, has begun to be applied to related species that are of particular importance because of their impact on humans. These include both beneficial fungi and fungi pathogenic to humans, animals, and plants. Although work with these humans, animals, and plants. Although work with these other fungal species proceeds more slowly than that with well-studied species, breakthroughs are being made. Major advances continue with the well-studied species because of the sophistication with which their genomes can be manipulated. This meeting will integrate the concerns of individuals doing cutting-edge research in the areas of molecular and cellular biology of the yeasts and filamentous fungi. It will feature the latest and most relevant results from laboratories working with the classical genetic models and also from those working with industrial and pathogenic fungi.

Session topics include: Chromosome Structure and Function, Reproductive Strategies, Control of Cell Proliferation, Transcriptional Control Mechanisms Post-Transcriptional Control Mechanisms, Mechanisms of Pathogenesis of Human and Plant Pathogens.

Contact: University of California, Los Angeles, UCLA Symposia
103 Molecular Biology Institute
Los Angeles, CA 90024-1378
BA61

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XXIII. Brief News Items.

1. The new membership of the Executive Committee of the MYCOLOGY DIVISION, International Union of Microbiological Societies is now as follows:

Chairman: Dr. A. Stenderup, Institute of Medical Microbiology, University of Aarhus, DK-8000 Aarhus, Denmark
Past Chairman: Dr. N. L. Goodman, Department of Pathology, College of Medicine, University of Kentucky, Lexington, Kentucky 40536, U.S.A.
Vice-Chairman: Dr. H. Jean Shadomy, Medical College of Virginia, Virginia Commonwealth Univ., MCV Station, Richmond
Secretary: Mrs. Inge Russell, Labatt Brewing Company Ltd., 150 Simcoe Street, London, Ontario, Canada N6A 4M3

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2. Position available.

Director of the French National Yeast Collection, to be recruited in 1988. The Director tasks will be: 1) To set up the Collection with his collaborators, and 2) To conduct research on Yeast Molecular Taxonomy. The collection will be established on the Campus of the Institut National Agronomique Paris-Grignon, close to the laboratory of yeast genetics. Information can be obtained by writing to H. Heslot or C. Gaillardin, Centre de Biotechnologies Agro-Industrielles, INAPG - 78850 Thiverval Grignon (France). Tel: (1) 30.54.45.10

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