Y E A S T

A Newsletter for Persons Interested in Yeast

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Foreign Subscribers: It has come to our attention that mailing of the Yeast Newsletter by printed matter involves a 2-3 month delay in your receiving it. If you are not receiving the Yeast Newsletter by airmail (which takes approximately 2 weeks) and would like to, please let us know. An additional $4 per year is required to cover postage and handling for this service.

I wish all readers of the Yeast Newsletter a prosperous and scientifically rewarding New Year.

Herman J. Phaff
Editor

I. Centraalbureau Voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, Netherlands. Communicated by G.S. de Hoog.

Below follows some recent news from the CBS Baarn.

The next issue of Studies in Mycology, to be published by the end of 1983, is entitled, "Character analyses of selected red yeasts". It is edited by G.S. de Hoog and contains 8 short articles. The contents are:

Introduction
G.S. de Hoog: Morphology of anamorphs, I. Rhodosporidium
G.S. de Hoog: Morphology of anamorphs, II. Sporidiobolus and Sporobolomyces
G.S. de Hoog & T. Boekhout: Teliospores, teliospore-mimics and chlamydospores
T. Boekhout & W.A.M. Linnemans: Ultrastructure of mitosis in Rhodosporidium toruloides
G.W. van Eijk, H.J. Roeymans & A.C.M. Weijman: Biochemical characteristics: volatiles, carotenoids, sterols and fatty acids
A.C.M. Weijman, I.J.A. Vlug & G.W. van Eijk: Carbohydrate patterns
W. Windig & J. Haverkamp: Pyrolysis mass spectrometry, I. Rhodosporidium
W. Windig & G.S. de Hoog: Pyrolysis mass spectrometry, II. Sporidiobolus and related taxa
Summary and conclusions
References

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II. Microbiology Research Group of the South African Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, South Africa. Communicated by J.P. van der Walt.

Three publications have recently appeared from our Research Group.

1. J.P. van der Walt, A.C.M. Weijman[1] and J.A. von Arx[1]. The anamorphic yeast genus Myxozyma gen. nov. Sydowia, Annales Mycologici Ser. II, 34: 191-198 (1981).

   [1]Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

2. J.P. van der Walt, D. Yarrow[1], A. Opperman and L. Halland. Pichia kodamae sp. nov., a new homothallic yeast species. The Journal of General and Applied Microbiology 28: 155-160 (1982).

   [1]Yeast Division, Centraalbureau voor Schimmelcultures, Delft, The Netherlands.

3. J.A. von Arx, J.P. van der Walt and N.V.D.M. Liebenberg. The classification of Taphrina and other fungi with yeast-like cultural states. Mycologia 74(2) 285-296 (1982).

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III. Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pushchino, Moscow region, 142292, USSR. Communicated by V.M. Blagodatskaya.

Ascospores in Candida salmanticensis (Santa Maria) van Uden et Buckley.

Ascospore formation was observed on nutrient agar (Difco 0001) and Gorodkova agar after 3 days at 18-20°C in the type strain CBS #5121. Vegetative cells were directly transformed into asci. Ascospores (1-4 per ascus) are smooth and sphaerical. This yeast belongs to the genus Saccharomyces because of its morphological and physiological characteristics. There is a resemblance of this yeast to Sacch. kluyveri. We have found that Sacch. kluyveri differs in the following properties: lack of fermentation of trehalose, melezitose and cycloheximide resistance.

We find it difficult to decide now how significant these differences are and whether Sacch. salmanticensis should be considered as a separate species or as a synonym of Sacch. kluyveri.

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IV. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852. Communicated by S.C. Jong.

The strains listed have been added to the ATCC since May 12, 1982. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

Saccharomyces cerevisiae ATCC 46572-46573	Dr. Breck Byers University of Washington Seattle, Washington
Hansenula fabianii ATCC 46574	Dr. J.S. McDonald NADC Ames, Iowa
Candida lusitaniae ATCC 46575	"
Saccharomyces cerevisiae ATCC 46589	Dr. Maria de van Broock Avda. Belgrano y Pje Caseros Argentina
Saccharomyces cerevisiae ATCC 46590	Dr. Breck Byers University of Washington Seattle, Washington
Saccharomyces cerevisiae ATCC 46605	"
Saccharomyces cerevisiae ATCC 46696-46697	Dr. Bun-ichiro Ono Okayama University Japan
Saccharomyces cerevisiae ATCC 46739-46742	Dr. B. Sprinson Roosevelt Hospital New York, New York

Saccharomyces cerevisiae ATCC 46747-46748	Dr. F. Fatichenti Instituto de Microbiologia Agraria Italy
Candida pseudotropicalis ATCC 46764	Dr. J.H. Jorgensen University of Texas San Antonio, Texas
Saccharomyces uvarum ATCC 46776	Dr. J.J. Ellis NRRL Peoria, Illinois
Saccharomyces cerevisiae ATCC 46785-45786	Mrs. Barbara Kirsop NCYC England
Saccharomyces carlsbergensis ATCC 46787-46788	Dr. C.A. Michels Queens College of New York Flushing, New York
Saccharomyces cerevisiae ATCC 46789-46793	Dr. V.L. Seligy National Research Council Ottawa, Canada
Kluyveromyces lactis ATCC 46794	Dr. N. Gunge Mitsubishi Chemical Industry Japan
Saccharomyces cerevisiae ATCC 46795-46818	Dr. A.D. Panek Institute Quimica Rio de Janeiro, Brazil
Saccharomyces cidri ATCC 46819	Dr. R.K. Latta National Research Council Ottawa, Canada
Saccharomyces kluyveri ATCC 46820	"
Saccharomyces mrakii ATCC 46821	"
Saccharomyces cerevisiae ATCC 46822-46827	Dr. Friederike Eckhardt York University Canada
Torulopsis ethanolitolerans ATCC 46859	Dr. A. Kockova Kratochvilova Slovak Academy of Sciences Czechoslovakia
Torulopsis ethanolitolerans var. minor ATCC 46860	"
Candida curiosa ATCC 46861	Dr. M. Vidal Leiria Gulbenkian Institute of Science Portugal

Saccharomyces cerevisiae ATCC 46862-46877	Dr. Peter Niederberger Mikrobiologisches Institut Zürich, Switzerland
Saccharomyces cerevisiae ATCC 46894-46897	Dr. Gonzalez Munoz University of Granada Spain
Saccharomyces cerevisiae ATCC 46901	Dr. Peter Niederberger Mikrobiologisches Institut Zürich, Switzerland
Kluyveromyces lactis ATCC 46909	Dr. R.K. Latta National Research Council Ottawa, Canada
Saccharomyces cerevisiae ATCC 46910-46914	Dr. Antonio Jimenez University Autonoma de Madrid Madrid, Spain
Torulaspora fermentati ATCC 46935	Dr. R.E. Kunkee University of California Davis, California
Schizosaccharomyces pombe ATCC 46944-46946	Dr. A.M. Grossenbacher-Grunder University of Bern Bern, Switzerland
Endomycopsis fibuligera ATCC 46949	Linda Bengston ATCC
Schizosaccharomyces japonicus var. *versatilis* ATCC 46952-56953	Dr. E. Johannsen Microbiology Research Group Pretoria, South Africa
Schizosaccharomyces malidevorans ATCC 46954	"
Schizosaccharomyces pombe ATCC 46955-46959	"
Candida albicans ATCC 46977	Dr. A. Datta Jawaharlal Nehre University India
Saccharomyces uvarum ATCC 46991	Dr. T. Kamihara Kyoto University Kyoto, Japan
Hanseniaspora valbyensis ATCC 48013	Dr. M.T. Smith CBS The Netherlands
Eeniella nana ATCC 48014-48016	"

Schwanniomyces alluvius
ATCC 48086

Dr. W.M. Ingledew
University Saskatchewan
Saskatoon, Canada

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

The following are recent papers from our laboratory:

1. S. Goto. Reproduction by multilateral budding of two strains of Kloeckera corticis. J. Gen. Appl. Microbiol., 27, 349-351 (1981).

Abstract

Reproduction by multilateral budding was observed in two strains belonging to Kloeckera corticis. The two strains reproduced not only by bipolar budding but also by multilateral budding. In addition to the usual bipolar budding, several buds, viz., three, four and five buds, developed at different places on single yeast cells.

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2. S. Goto. Multilateral budding in the apiculate yeast genera Hanseniaspora, Nadsonia, Saccharomycodes, Wickerhamia, and Kloeckera. J. Gen. Appl. Microbiol., 28, 303-310 (1982).

Abstract

In addition to the usual bipolar budding, the multilateral budding that developed at different lateral sites on a single cell was observed in 25 strains belonging to the apiculate yeast genera Hanseniaspora, Nadsonia, Saccharomycodes, Wickerhamia, and Kloeckera. Effects of several culture conditions on the formation of this multilateral budding were also examined. The rates of this multilateral budding were about 0.001 to 4.0%, but the rates varied with different kinds of media and yeast species, and several yeasts showed multilateral budding at pH 6.0-6.5. Conidiogenesis on lateral sites, as well as bipolar budding, was of the percurrent type by basipetal succession. In addition to lemon-shaped cells, cells of various shapes resulted from this multilateral budding. Thus this multilateral budding can be supplemented as one of the diagnostic characters of the above apiculate yeast genera.

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3. S. Goto, R. Aono, J. Sugiyama and K. Horikoshi. Exophiala alcalophila, a new black yeast-like hyphomycete with an accompanying Phaeococcomyces alcalophilus morph, and its physiological characteristics. Trans. mycol. Soc. Japan., 22, 429-439 (1981).

Abstract

Two strains of a previously undescribed pleomorphic species assignable to the black, yeast-like hyphomycetes which grow even at pH 10.4 have been isolated from soil in Japan. The name Exophiala alcalophila Goto et

Sugiyama sp. nov., with an accompanying new yeast morph Phaeococcomyces alcalophilus Goto et Sugiyama sp. nov., is proposed here.

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4. M. Yamazaki, S. Goto and K. Komagata. Comparison of enzymes from strains of wine yeast and related yeasts on electrophoresis. J. Institute of Enol. & Viticult., Yamanashi Univ., 17 (1982) in press.

Abstract

Electrophoretic patterns of five enzymes in seventeen strains of wine and related yeasts were studied with the use of polyacrylamide slab gel electrophoresis with specific staining. The strains used were eight strains belonging to Saccharomyces cerevisiae, two strains belonging to S. bayanus, two strains belonging to S. uvarum, one strain of S. fermentati, one strain of S. bailii, and three strains of Saccharomyces sp.. The strains of S. cerevisiae, S. bayanus, and S. uvarum showed rather similar electrophoretic patterns of five enzymes; however, their patterns were quite distinguishable from those in the strains of S. fermentati and S. bailii. Of eight S. cerevisiae strains tested, one distiller's yeast previously identified as one belonging to S. formosensis was different from the other seven strains in their electrophoretic patterns of aldolase and alcohol dehydrogenase. This strain was similar to strains of S. bayanus in electrophoretic patterns of five enzymes. Two strains of S. uvarum showed quite different electophoretic patterns of five enzymes from each other, and one strain previously identified as S. carlsbergensis was similar to S. cerevisiae in its electrophoretic patterns of five enzymes. Two unidentified strains of wine yeast showed the same electrophoretic patterns of five enzymes as those of S. cerevisiae. The strain Jerez-5 of Saccharomyces sp. was thought to belong to S. bayanus, since their electrophoretic patterns of five enzymes and physiological properties were similar.

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

Below follows the abstract of a recent publication.

1. Marc-Andre Lachance, Benjamin J. Metcalf,[1] and William T. Starmer[1]. 1982. Yeasts from Exudates of Quercus, Ulmus, Populus, and Pseudotsuga: New Isolations and Elucidation of Some Factors Affecting Ecological Specificity. Microb. Ecol. 8:191-198.

[1]Department of Biology, Syracuse University, Syracuse NY, 13210, USA.

Abstract

The yeast flora associated with exudates of Quercus, Ulmus, Populus, and Pseudotsuga was examined in the light of new isolations in geographic areas different from those in previous reports. Application of multivariate analytic methods indicated that geographic distance, although a meaningful ecological factor, is largely overshadowed by host tree specificity, provided that yeast community physiological profiles and not yeast taxa, are used as ecological descriptors. Some physiological attributes used in classifying yeasts were identified as particularly important in shaping the

yeast communities of those trees. The possible divergence between these attributes and those generally considered taxonomically useful is discussed.

2. The publication "Sporopachydermia quercuum, a new yeast species found in exudates of Quercus rubra" (see Yeast Newsletter XXXI No. 1, p. 7 for abstract) has now been published in Can. J. Microbiol. 28:567-571 (1982).

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VII. Department of Animal Science, Genetics Research Laboratories, University of New England, Armidale 2351, N.S.W., Australia. Communicated by D.C. Vacek.

The following are summaries of recent papers from this laboratory.

1. Barker, J.S.F., G.L. Toll[1], P.D. East, M. Miranda[2], and H.J. Phaff[2]. Heterogeneity of the yeast flora in the breeding sites of cactophilic Drosophila. Can. J. Microbiol. (in press).

[1]Uncle Bens of Australia, P.O. Box 153, Wodonga, Vic., 3690, Australia.

[2]Department of Food Science and Technology, University of California, Davis, California, 95616, U.S.A.

Summary

Yeasts were isolated from 278 rots in cladodes and basal stems of Opuntia stricta sampled over 15 months at one locality. The most common yeast species among 370 nonidentical isolates representing 20 species were Candida sonorensis (27% of isolates) and Pichia cactophila (24%). These species generally were present in all months and throughout the area sampled. Other relatively common species (3-9% of all isolates) had much more restricted distributions in both time and space. The mean number of yeast species per rot was 1.27 ± 0.082, and no yeasts were isolated from 20% of the rots sampled. Significant differences among months or seasons in average number of yeast species per rot were due primarily to lower numbers in the summer and winter months, while differences among rot types resulted mainly from a higher number in the longer persisting basal stem rots. Both hierarchical diversity analysis and analysis of the distributions of the 10 most common yeast species showed temporal effects (month or season) to be the major component contributing to heterogeneity, while microgeographic effects and rot type also were significant. The seasonal and microgeographic heterogeneity are interpreted in terms of longer persisting rots in part of the locality providing a reservoir for yeast species through the winter, and are discussed in relation to the maintenance of genetic variation in cactophilic Drosophila that utilize the rots as feeding and breeding sites.

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2. Vacek, Don C. 1982. Interactions between microorganisms and cactophilic Drosophila in Australia. In Ecological Genetics and Evolution: The Cactus-Yeast-Drosophila Model System (J.S.F. Barker and W.T. Starmer, eds.), pp. 175-190, Academic Press, Australia.

Summary

Cactophilic Drosophila in Australia develop in decaying Opuntia spp. (Platyopuntia), a habitat which is a complex result of the metabolism of host plant, microorganism, and insect. What is the role of microorganisms as important descriptors of habitat in the context of habitat choice? Investigations were made at the following three levels: (1) microorganism effects at the species level, i.e., adult feeding and oviposition preferences, larval feeding preferences, and nutritional sufficiency of microorganisms for larval development; (2) microorganism effects at the genotype level, i.e., association among adult genotypes and their feeding and oviposition preferences; and (3) heterogeneity of yeast species in the environment.

Larvae prefer to consume those yeast species on which they have shortest developmental time and these yeasts also are preferred by adults for feeding and oviposition. The environment is heterogeneous with respect to yeast species; temporal effects, rot type, and microclimate are important components contributing to this heterogeneity. There is some evidence that genotype affects the yeast preferred for oviposition, and therefore, there is the possibility that habitat choice plays a role in the maintenance of genetic variation in these species.

Furthermore, adults sometimes emerge from rot pockets without detectable yeasts and axenic larvae placed on a live bacteria-cactus homogenate medium are able to develop to adults. Therefore, yeasts are not absolutely necessary for larval nutrition but cactus-yeast induced metabolites are probably an important component in host plant or breeding site recognition by these Drosophila.

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

The following papers have been published or are in press.

1. D.L. Holzschu and H.J. Phaff. 1982. Taxonomy and Evolution of some ascomycetous cactophilic yeasts. In "Ecological Genetics and Evolution", J.S.F. Barker and W.T. Starmer, eds. 127-141. Academic Press Australia, Sydney (reprints not available).

2. W.T. Starmer, H.J. Phaff, M. Miranda, M.W. Miller and W.B. Heed. 1982. The yeast flora associated with the decaying stems of columnar cacti and Drosophila in North America. In "Evolutionary Biology" Vol. 14. M.K. Hecht, B. Wallace, and G.T. Prance, eds. 269-295. Plenum Publ. Corp.

3. H.J. Phaff, Yuzo Yamada*, Joanne Tredick and Mary Miranda. Hansenula populi, a New Homothallic Species of Yeast from Exudates of Cottonwood Trees. Int. J. Syst. Bacteriol. 1983 (April issue).

*Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Shizuoka University, Shizuoka 422, Japan.

Abstract

The evolutionary affinities among several morphologically and physiologically similar species of the ascogenous yeast genus Hansenula were studied by a technique of genome comparison in combination with nuclear DNA base composition determinations. The results revealed a novel member of the genus Hansenula that was recovered five times in 1968 from slime exudates of Populus trichocarpa (cottonwood trees) in the province of British Columbia and in the state of Alaska. The new species is named Hansenula populi because of its specific habitat in exudates of cottonwood trees. This species is homothallic and occurs naturally in the haploid state. Conjugation between vegetative cells precedes sporulation and up to four hat-shaped spores are produced in zygotes. The spores are liberated from the asci soon after formation. H. populi resembles H. nonfermentans and H. dryadoides but differs from these species in habitat, mol% guanine & cytosine of their nuclear deoxyribonucleic acid, maximum growth temperature and in the assimilatory ability of several carbon compounds.

4. G.H. Fleet and H.J. Phaff. 1981. Fungal Glucans - Structure and Metabolism. In "Encyclopedia of Plant Physiology, New Series, Vol. 13B. Plant Carbohydrates II. - Extracellular Carbohydrates". W. Tanner and F.A. Loewus, eds. pp. 416-432. Springer Verlag, Berlin (reprints not available).

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IX. Istituto di Biologia Vegetale, Sezione Microbiologia Agraria, University of Perugia, 06100 Perugia, Italy. Communicated by A. Martini.

As of November 1982, the Institutes of Botany and Agricultural Microbiology ("Istituto di Botanica" and "Istituto di Microbiologia Agraria e Tecnica") of the University of Perugia merged into a single Institute which took the new name of "Istituo di Biologia Vegetale". The former "Istituto di Microbiologia Agraria e Tecnica" to which we belong, will be a section of the above Institute ("Sezione di Microbiologia Agraria").

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The following publications have appeared since the abstracts given in the Yeast Newsletter 30(1981): (2) 94-95:

F. Federici. Extracellular Enzymatic Activities in Aureobasidium pullulans. Mycologia 74: 738-743 (1982).

G. Rosini, F. Federici & A. Martini. Yeast Flora of Grape Berries during Ripening. Microb. Ecol. 8: 83-89 (1982).

F. Federici. Effects of Ultrasonic Treatment on Yeast Budding. J. Gen. Appl. Microbiol. 27: 195-197 (1981).

G. Rosini, F. Federici, A.E. Vaughan & A. Martini. Systematics of the Species of the Yeast Genus Saccharomyces Associated with the Fermentation Industry. European J. Appl. Microbiol. Biotechnol. 15: 188-193 (1982).

F. Federici. A note on milk clotting ability in the yeast genera Cryptococcus and Rhodotorula. J. Appl. Bacteriol. 52: 293-296 (1982).

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The following papers have been submitted or accepted for publication:

G. Rosini. The Occurrence of Killer Characters in Yeasts Associated with Fermenting Natural Substrates (submitted to Canadian Journal of Microbiology).

Abstract

Yeast strains of the ascoporogenous genera Saccharomyces, Saccharomycodes, Schizosaccharomyces, Hanseniaspora, Hansenula, Kluyveromyces, Pichia and of the anascosporogenous genera Kloeckera and Torulopsis were examined for killer, sensitive, neutral, and killer/sensitive characters against a killer strain (NCYC738) and a sensitive strain (NCYC1006) of Saccharomyces cerevisiae. Thirty-one Saccharomyces cerevisiae strains out of 782 screened were able to kill the sensitive test strain, 707 appeared to be sensitive and 44 were neutral. A high frequency of killer phenotypes (71.4%) was found in Hansenula anomala var. anomala. No killer cultures were present in the remaining 17 species considered.

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F. Federici & Marina D'Elia. Growth and Amylase Production by the Yeast-like Fungus Aureobasidium pullulans in Starch-limited Culture (accepted by Enzyme and Microbial Technology).

Abstract

One strain of the yeast-like fungus Aureobasidium pullulans converts starch into biomass with high yield (Y_{starch}=0.590) and releases the amylolytic enzyme into the cultural medium (ca 2.2-2.3 U/ml). The rate of starch hydrolysis seems to be high enough so as not to limit the specific growth rate.

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X. Department of Biology, Japan Women's University, 2-8-1, Mejirodai, Bunkyo-ku, Tokyo 112, Japan. Communicated by Masako Osumi.

The following papers have been published recently:

1. J. Hosoi and M. Osumi, 1981. Alternate arrangement of large and small particles in crystalloid of Kloeckera yeast microbody of halite structure type. J. Electron Microsc. 30, 158-160.

2. J. Hosoi and M. Osumi, 1981. Confirmation of composite crystal model of the crystalloid of Kloeckera microbody by optical transforms. J. Electron Microsc. 30, 321-326.

3. M. Osumi, M. Sato and M. Nagano, 1981. Structure of the microbody crystalloid in methanol grown yeasts as a composite crystal of catalase and alcohol oxidase. In "Current Developments in Yeast Research". G.G. Stewart and I. Russel, eds. pp. 69-74. Pergamon Press, Toronto.

4. M. Osumi, M. Nagano, N. Yamada, J. Hosoi, and M. Yanagida. 1982. Three-dimensional structure of the crystalloid of the microbody of Kloeckera sp.: Composite crystal model. J. Bacteriol. 151, 376-383.

[Editor's note: the yeast referred to in these publications was originally misidentified as Kloeckera sp. and later reidentified as Candida boidinii].

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XI. Department of Botany and Genetics, University of Guelph, Guelph, Ontario, Canada, N1G 2W1. Communicated by Alexander Lautenbach.

The following is an abstract of recent work in the laboratory of Dr. R.E. Subden (manuscript is in preparation).

More than 500 yeast strains (about 200 species) were screened for their ability to grow on malic acid with the ultimate goal of selecting gene donors for construction of deacidifying wine yeasts. A strain of S. rouxii was found that possessed superior ability to utilize malate aerobically and anerobically and was selected for protoplast fusion with Saccharomyces cerevisiae. S. rouxii was mutagenized and a lysine auxotroph was selected with a reversion frequency of $<10^{-7}$. This auxotroph was then fused with a tryp$^-$ura$^-$ strain of S. cerevisiae. Most of the prototrophic malate utilizing fusion products initially obtained were unstable in their malate assimilation, but two stable products were isolated. The API 20C identification profiles differ from those of the two parental strains. Current investigations focus on the regulation of malate utilization and ploidy levels of the fusion products.

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XII. University of Edinburgh, Department of Zoology, West Mains Road, Edinburgh EH9 3JT, Scotland. Communicated by J.M. Mitchison.

Below follow the titles and summaries of five papers from our group. All are in press.

1. J. Creanor and J.M. Mitchison, with a statistical appendix by D.A. Williams (Department of Statistics, University of Edinburgh), Patterns of Protein Synthesis During The Cell Cycle of the Fission Yeast Schizosaccharomyces pombe. J. Cell Sci. (1982) in press.

Summary

The rate of protein synthesis through the cell cycle of Schizosaccharomyces pombe has been determined from the incorporation of pulses of [^{3}H]tryptophan in synchronous cultures prepared by selection in an elutriating rotor. This selection procedure caused minimal perturbations as judged by asynchronous control cultures, which had also been put through the rotor. The rate of synthesis showed a periodic pattern rather than a smooth exponential increase. There was a sharp increase in the rate at an

'acceleration point' at about 0.9 of the cycle. Model-fitting by a novel procedure suggests that the average single cell has an increasing rate of protein synthesis for the first 60% of the cycle and a constant rate for the remaining 40%.

The same pattern was shown in less extensive experiments with [^{3}H]leucine and [^{3}H]phenylalanine. It was also shown in a series of size mutants, which indicates that the pattern is not size-related, in contrast to earlier work on the rates of synthesis of messenger RNA. However, one large mutant (cdc 2.M35r20) had a significantly earlier acceleration point.

Care was taken to justifiy the assumption that the rate of incorporation of tryptophan was a valid measure of the rate of protein synthesis. A tryptophan auxotroph was used to eliminate the problem of endogenous supply and the size of the metabolic pool was measured through the cycle. This pool did not show cell cycle related fluctuations. An operational model of the pools is presented.

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2. J. Creanor, S.G. Elliott, Y.C. Bisset and J.M. Mitchison. Absence of Step Changes in Activity of Certain Enzymes During the Cell Cycle of Budding and Fission Yeasts in Synchronous Cultures. J. Cell Sci. (1983) in press.

Summary

Synchronous cultures prepared by selection from an elutriating rotor were used to measure activity changes during the cell cycle of the following enzymes: - acid phosphatase in Schizosaccharomyces pombe and Saccharomyces cerevisiae, α-glucosidase in S. cerevisiae and β-galactosidase in Kluyveromyces lactis. There was no sign of step rises in activity in acid phosphatase but there were indications in S. cerevisiae of the linear pattern with rate doublings once per cycle which had been found previously in S. pombe. There was also no sign of step rises in the other two enzymes, in contrast to earlier results using different techniques. Asynchronous control cultures showed little or no perturbations after the first hour.

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3. J. Richard Dickinson. Nucleoside Diphosphokinase and Cell Cycle Control in the Fission Yeast Schizosaccharomyces pombe. J. Cell Sci. (1983) in press.

Summary

Centrifugal elutriation was used to prepare synchronous cultures of Schizosaccharomyces pombe. Nucleoside diphospokinase activity was measured throughout the cell cycle. In the wild-type strain (972) nucleoside diphosphokinase activity doubled in a stepwise fashion. The midpoint of the rise in enzyme activity was at 0.65 of a cycle, 0.29 of a cycle before the next S-phase. Synchronous cultures of the mutant weel-6 were also prepared. In this strain S-phase is delayed, occurring about 0.3 cycle later than in the wild-type. In weel-6 the midpoint of the stepwise doubling in nucleoside diphosphokinase activity occurred at 0.084; showing that the rise in enzyme activity is also delayed.

Addition of cycloheximide to an exponentially-growing culture caused an immediate inhibition of protein synthesis, yet nucleoside diphosphokinase activity continued to increase exponentially for a further 300 minutes. This indicates that the stepwise doubling of nucleoside diphosphokinase activity during the cell cycle is not achieved by a simple control of protein synthesis.

The temperature-sensitive cdc⁻ mutants were also used: cdc2-33, a mutant whose single genetic lesion results in the twin defects of a loss of mitotic control, and a loss of commitment to the cell cycle; and cdc10-129, which has a defect in DNA replication. In both mutants a temperature shift-up of an asynchronously-growing culture from the permissive (25°C) to the restrictive temperature (36.5°C) results in a rapid inhibition of DNA replication. In both mutants nucleoside diphosphokinase continues to increase exponentially. Therefore, although nucleoside diphosphokinase is required for DNA replication, apparently DNA replication is not required for an increase in nucleoside diphosphokinase activity.

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4. Douglas Roy and Peter A. Fantes. Benomyl Resistant Mutants of Schizosaccharomyces pombe Cold-Sensitive for Mitosis. Current Genetics (1982) in press.

Summary

We have isolated 150 benomyl resistant mutants of the fission yeast Schizosaccharomyces pombe. Seven of these mutants were found to be cold sensitive for mitosis. These mutants were the subject of physiological, cytological and genetical characterisation. Growth and division of the seven mutants were similar to the wild type strain at 35°C. After shift from the permissive (35°C) to the restrictive temperature (20°C) the mutants became blocked in mitosis whilst cellular growth continued. Consequently, elongate cells were formed. Six of the seven benomyl resistant mutants became blocked in mitosis at 20°C with a single aberrant nucleus. In every case the benomyl resistant and cold sensitive phenotype was due to a mutation in a single nuclear gene. These mutants were found to comprise a single genetic linkage group (ben4) and were unlinked to existing TBZ/MBC resistant mutants of S. pombe. Whilst no cross resistance was found in our mutants to TBZ, six of the seven mutants were super-sensitive to the spindle poison CIPC. We believe that the phenotype exhibited by these mutants is consistent with a defective tubulin subunit.

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5. J.M. Mitchison. Dissociation of Cell Cycle Events. In "Cell Cycle Clocks" Ed L. Edmunds, 1983 in press. Marcel Dekker Inc: New York.

Summary

It is convenient to separate most of the cell cycle into two dissociable sets, growth and the DNA-division cycle. The latter cycle has three main events; DNA synthesis, mitosis and cell division, and is a dependent sequence in the sense that an event will not normally occur unless the preceding one has been completed. There are, however, several cases in which these dependency relations break down. The DNA-division cycle also

includes the periodic synthesis of a number of proteins associated with the main events, e.g. histones and enzymes of DNA synthesis. Most enzymes, however, do not appear to be synthesised periodically during growth, but there is evidence in fission yeast that most of the bulk components of growth follow linear or partially linear patterns with rate changes at characteristic but differing points of the cycle. Controls that may operate between growth and the DNA-division cycle include size controls that can trigger DNA synthesis or mitosis, and modulating effects of mitosis on growth. As well as these controls, however, there are some dissociation experiments which indicate the existence of independent timers which can control periodic events when the DNA-division cycle has been blocked or perturbed.

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XIII. Biology Group, Alberta Research Council, Edmonton, Alberta, Canada. Communicated by Toshi Kaneda.

Hasegawa, Y., Higuchi, K., Obata, H., and Tokuyama, T. (Kansai University, Osaka, Japan) and Kaneda, T. A cyclohexanecarboxylic acid-utilizing yeast: Isolation, identification, and nutritional characteristics. Can. J. Microbiol., 28: 942-944 (1982).

Abstract

A yeast capable of utilizing cyclohexanecarboxylic acid as sole carbon and energy source, strain KUY-6A, was isolated from soil by the enrichment technique. Taxonomical studies indicated that strain KUY-6A was Trichosporon cutaneum.

Strain KUY-6A grew on a number of carboxylic acids. Among the cyclic compounds tested, cyclohexanecarboxylic acid was the best substrate. Cyclopentanecarboxylic acid, cycloheptanecarboxylic acid, cyclopentanone, cyclohexanone, and cyclopentanol also supported growth. In addition, the organism used the monocarboxylic acids, butyric, valeric and caproic; the dicarboxylic acids, succinic, glutaric, adipic, pimelic, suberic; and the aromatic acids, benzoic and o-, m-, and p-hydroxybenzoic. The yeast did not require any vitamins for growth, although thiamine gave slight stimulation. The cell dry weight yield was 0.75 g from 1 g of cyclohexanecarboxylic acid used.

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XIV. National Research Council Canada, Prairie Regional Laboratory, University Campus, Saskatoon S7N 0W9, Sask. Canada. Communicated by J.W.D. GrootWassink.

The abstract below is of work done in our laboratory and will appear in the January issue of the Journal of General Microbiology.

Inducible and Constitutite Formation of β-Fructofuranosidase (Inulase) in Batch and Continuous Cultures of the Yeast Kluyeromyces fragilis.

By J.W.D. GrootWassink and G.M. Hewitt

Inulase production by Kluyveromyces fragilis on various fermentable and non-fermentable carbon sources was examined in carbon-limited continuous culture. Fructose and sucrose supported superior inulase yields [above 24 µmol sucrose hydrolysed min^{-1} (mg cell dry wt)$^{-1}$ at pH 5.0, 50°C], while some other carbon sources, including lactose, galactose, ethanol and lactate, did not stimulate inulase formation beyond basal levels. Thus fructose was identified as the primary physiological inducer. Isolation of a constitutive mutant also provided genetic evidence for the inducible nature of inulase in the wild-type. The mutant was generated spontaneously and selected in continuous culture. It produced high inulase activities in continuous culture irrespective of the carbon source. Inulase formation in the wild-type and mutant strain was further controlled by general carbon catabolite repression as suggested by enzyme yield patterns in batch and continuous culture.

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XV. Biochemisches Institut der Universitat Freiburg, Hermann-Herder-Strasse 7, D-7800 Freiburg im Breisgau, West Germany. Communicated by Dieter H. Wolf.

The following paper will be published soon in Biochem. Biophys. Res. Comm.

Aminopeptidase Co, A New Yeast Peptidase

Tilman Achstetter, Claudia Ehmann and Dieter H. Wolf

Peptide bond hydrolysis represents an essential mechanism in the regulation of cell metabolism at the posttranslational level. Complete understanding of this regulatory mechanism depends on a knowledge of the intracellular peptidases and their characteristics, and of the cellular processes which they catalyze. Eight peptidases - two endoproteinases, two carboxypeptidases, one dipeptidase, and three aminopeptidases have been so far characterized in the yeast Saccharomyces cerevisiae. In addition mutants lacking the two endoproteinase activities and the two carboxypeptidase activities led to the detection of a variety of new endoproteinases and carboxypeptidases (for review see Wolf, D.H. (1982) Trends Biochem. Sci. 7, 35-37).

Here we describe the discovery of a new aminopeptidase - aminopeptidase Co. The enzyme is only active in the presence of Co^{2+} -ions. Zn^{2+} - and Mn^{2+} -ions are inhibitory. The enzyme activity is also inhibited by EDTA. Of the p-nitroanilide derivatives tested only those containing basic amino acids are cleaved. Optimum pH for activity of aminopeptidase Co was found to be around 8.5. In the absence of Co^{2+} -ions gel filtration indicated the molecular weight of the enzyme to be about 100,000.

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XVI. Centre National de la Recherche Scientifique, Laboratoire D'enzymologie, 91190 Gif Sur Yvette, France. Communicated by J. Schwencke.

Recently published papers from our laboratory are the following:

1. J. Schwencke: Measurement of proteinase B activity in crude yeast extracts. A novel procedure of activation using peptin. Analytical Biochemistry, 118: 315-321 (1981).

* * *

In collaboration with Dr. E. Moustacchi (Institut Curie, Section Biologie, Centre Universitaire, F-91405 Orsay, France) we have recently published the following papers:

2. J. Schwencke and E. Moustacchi: Proteolytic activities in yeast after UV irradiation. I. Variation in proteinase levels in repair proficient Rad$^+$ strains. Molecular and General Genetics 185: 290-295 (1982).

Summary

Specific proteolytic activities are known to be induced in Escherichia coli following irradiation. Consequently it seemed of interest to investigate whether variations in proteinase activities occur in yeast.

Among the five best known proteinases of Saccharomyces cerevisiae, we have found that proteinase B activity increases up to three fold in wild-type RAD$^+$ yeast cells after a dose of 50 Jm^{-2} of 254 nm ultraviolet light (40% survival). Carboxypeptidase Y and aminopeptidase I (leucine aminopeptidase) activities were only moderately increased. Proteinase A activity was only slightly enhanced, while aminopeptidase II (lysine aminopeptidase) was unaffected in both RAD$^+$ strains studied.

The observed post-UV-increase in proteinase B activity was inhibited by cycloheximide and was dose dependent. Increases in proteinase B levels were independent of the activation method used to destroy the proteinase B-inhibitor complex present in the crude yeast extracts.

A standard method for comparison of the postirradiation levels among different proteinases, strains and methods of activation is presented.

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3. J. Schwencke and E. Moustacchi: Proteolytic activities in yeast after UV irradiation. II. Variation in proteinase levels in mutants blocked in DNA-repair pathways. Molecular and General Genetics 185: 296-301 (1982).

Summary

When the levels of three common yeast proteinases in exponentially growing cells of mutants blocked in different repair pathways are compared to that of isogenic wild-type cells, it can be seen that the level of proteinase B is enhanced in the mutants whereas the levels of leucine aminopeptidase (Leu.AP) and lysine aminopeptidase (Lys.AP) are similar in all strains.

As in its corresponding wild type, the level of proteinase B activity is further enhanced after UV-irradiation in a mutant blocked in excision-repair (rad1-3). In contrast, following the same treatment the level of proteinase B remains almost constant in a mutant blocked in a general error-

prone repair system (rad6-1) and in a mutant defective in a more specific mutagenic repair pathway (pso2-1). Cycloheximide, an inhibitor of protein synthesis, blocks the post-UV enhancement in proteinase B activity observed in rad1-3 indicating that, as in the wild-type cells, an inducible process is involved.

The levels of Lys.AP and Leu.AP are, respectively, either unaffected or only moderately increased following UV-treatment of the repair defective mutants, as in wild-type strains.

It is obvious that the induction of protease B activity following UV-treatment in Saccharomyces cannot be equated to the induction of the RecA protein in Escherichia coli. However the correlation found between the block in mutagenic repair and the lack of UV-induction of protease B activity leads to questions on the possible role of certain protease activities in mutagenic repair in eucaryotic cells.

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XVII. Technische Hogeschool Delft, Laboratorium voor Microbiologie, Julianalaan 67a, 2628 BC Delft, the Netherlands. Communicated by W.A. Scheffers.

Below follow six abstracts of recent work from this laboratory that have been accepted for publication in the journals indicated.

1. Peter M. Bruinenberg, Johannes P. van Dijken and W. Alexander Scheffers, A Theoretical Analysis of NADPH Production and Consumption in Yeasts, Journal of General Microbiology, in press.

Summary

Theoretical calculations of the NADPH requirement for yeast biomass formation reveal that this parameter is strongly dependent on the carbon and nitrogen source. The data obtained have been used to estimate the carbon flow over the NADPH pathways in these organisms, namely the hexose monophosphate pathway and the $NADP^+$-linked isocitrate dehydrogenase reaction. It was calculated that during growth of yeasts on glucose with ammonium as the nitrogen source at least 2% of the glucose metabolized has to be completely oxidized via the hexose monophosphate pathway for the purpose of NADPH synthesis. This figure increases to approximately 20% in the presence of nitrate as the nitrogen source. Not only during growth on glucose but also on other substrates such as xylose, methanol, or acetate the operation of the hexose monophosphate pathway as a source of NADPH is essential, since the $NADP^+$-isocitrate dehydrogenase reaction alone cannot meet the NADPH demand for anabolism. NADPH production via these pathways requires an expenditure of ATP. Therefore, the general assumption made in calculations of the ATP demand for biomass formation that generation of NADPH does not require energy is, at least in yeasts, not valid.

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2. Peter M. Bruinenberg, Johannes P. van Dijken and W. Alexander Scheffers, An enzymatic analysis of NADPH production and consumption in Candida utilis, Journal of General Microbiology, in press.

Summary

Candida utilis CBS 621 was grown in chemostat cultures at D = 0.1 h^{-1} on glucose, xylose, gluconate, acetate, or ethanol as the growth-limiting substrate with ammonia or nitrate as the nitrogen source and analyzed for NADPH-producing and -consuming enzyme activities.

Nitrate and nitrite reductases were strictly NADPH-dependent. For all carbon sources, growth with nitrate resulted in elevated levels of HMP pathway enzymes.

$NADP^+$-linked isocitrate dehydrogenase did not vary significantly with the NADPH requirement for biosynthesis. Growth on ethanol strongly enhanced activity of $NADP^+$-linked aldehyde dehydrogenase. Neither $NADP^+$-linked malic enzyme nor transhydrogenase activities were detectable under any of the growth conditions. The absence of transhydrogenase was confirmed by the enzyme profiles of cells grown on mixtures of glucose and formate.

It is concluded that the HMP pathway and possibly $NADP^+$-linked isocitrate dehydrogenase are the major sources of NADPH in Candida utilis.

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3. K. Nicolay[1], W.A. Scheffers[2], P.M. Bruinenberg[2], and R. Kaptein[1]. Phosphorus-31 Nuclear Magnetic Resonance Studies of Intracellular pH, Phosphate Compartmentation and Phosphate Transport in Yeasts. Archives of Microbiology, in press.

[1]Department of Physical Chemistry, University of Groningen, Nijenborgh 16, 9747 AG GRONINGEN, the Netherlands.

[2]Technische Hogeschool Delft, Laboratorium voor Microbiologie, Julianalaan 67a, 2628 BC Delft, the Netherlands.

Abstract

^{31}P NMR spectra were obtained from suspensions of Candida utilis, Saccharomyces cerevisiae and Zygosaccharomyces bailii grown aerobically on glucose. Direct introduction of substrate into the cell suspension, without interruption of the measurements, revealed rapid changes in pH upon addition of the energy source. All ^{31}P NMR spectra of the yeasts studied indicated the presence of two major intracellular inorganic phosphate pools at different pH environments. The pool at the higher pH was assigned to cytoplasmic phosphate from its response to glucose addition and iodoacetate inhibition of glycolysis. After addition of substrate the pH in the compartment containing the second phosphate pool decreased. A parallel response was observed for a significant fraction of the terminal and penultimate phosphates of the polyphosphate observed by ^{31}P NMR. This suggested that the inorganic phosphate fraction at the lower pH and the polyphosphates originated from the same intracellular compartment, most probably the vacuole. In this vacuolar compartment, pH is sensitive to metabolic conditions. In the presence of energy source a pH gradient as large as 0.8 to 1.5 units could be generated across the vacuolar membrane. Under certain conditions net transport of inorganic phosphate across the vacuolar membrane was observed during glycolysis: to the cytoplasm when the cytoplasmic phosphate concentration had become very low due to sugar

phosphorylation, and into the vacuole when the former concentration had become high again after glucose exhaustion.

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4. C. Verduyn, J.P. van Dijken and W.A. Scheffers. A simple, sensitive and accurate alcohol electrode. Biotechnology and Bioengineering, in press.

Abstract

The construction and performance of an enzyme electrode is described which specifically detects lower primary aliphatic alcohols in aqueous solutions. The electrode consists of a commercial Clark-type oxygen electrode on which alcohol oxidase (E.C. 1.1.3.13; from Hansenula polymorpha) and catalase were immobilized. The decrease in electrode current is linearly proportional to ethanol concentrations between 1 and 25 ppm. The response of the electrode remained constant during 400 assays over a period of two weeks. The response time was between one and two minutes. Assembly of the electrode took less than one hour.

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5. W.A. Scheffers, J.P. van Dijken, Guniz Kaytan, M. Kloosterman, M.R. Wijsman and B.H.A. van Kleeff. Effect of Oxygen on Growth, Alcohol, Acetic Acid, and Glycerol Production by the Yeasts Brettanomyces intermedius and Zygosaccharomyces bailii. In: H. Dellweg (ed.) - Energie durch Biotechnologie. 5. Symposium Technische Mikrobiologie. Institut für Gärungsgewerbe und Biotechnologie, Berlin. 1982 in press.

Summary

In contrast to the well-known Pasteur effect (inhibition of fermentation by oxygen), an inhibition of alcoholic fermentation in a variety of yeasts may be provoked by the absence of oxygen. This so-called Custers effect is explained as a disturbance of the redox balance of the yeast cell upon transfer from aerobic to anaerobic conditions. It is demonstrated that the Custers effect in Brettanomyces intermedius depends on a combination of two characteristics: a strong capacity to produce acetic acid, and an incapacity to restore the redox balance via the production of reduced metabolites such as glycerol. An analogous explanation may apply to other cases where fermentation is inhibited by the absence of oxygen.

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6. P.M. Bruinenberg, J.P. van Dijken and W.A. Scheffers. Production and Consumption of Redox Equivalents during Growth of Candida utilis CBS 621 on Xylose. In: H. Dellweg (ed.) - Energie durch Biotechnologie. 5. Symposium Technische Mikrobiologie. Institut für Gärungsgewerbe und Biotechnologie, Berlin 1982 - in press.

Summary

Aerobic metabolism of xylose in Candida utilis proceeds via xylitol and xylulose. The first step is catalyzed by a reductase which is strictly dependent on NADPH. Xylitol is oxidized by an NAD^+-linked xylitol

dehydrogenase. Since transhydrogenase activity ($NADH + NADP^+ \rightarrow NADPH + NAD^+$) could not be detected in Candida utilis, the metabolism of D-xylose to hexose phosphates results in a net production of NADH. This explains the inability of the yeast to ferment D-xylose to ethanol under strictly anaerobic conditions.

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XVIII. Instituto de Quimica, Universidade Federal do Rio de Janeiro, Cidade Universitaria, Ilha do Fundao, Caixa Postal 1573, Rio de Janeiro, Brazil. Communicated by Anita D. Panek.

Below follow abstracts of three recent publications from our Institute.

1. M.S. Operti, D.E. Oliveira, A.B. Freitas-Valle, E.G. Oestreicher, J.R. Mattoon, and A.D. Panek (1982). Relationships between Trehalose Metabolism and Maltose Utilization in Saccharomyces cerevisiae. III. Evidence for Alternative Pathways of Trehalose Synthesis. Current Genetics 5:69-76.

Summary

A specific deficiency in UDPG-linked trehalose-6-phosphate synthase in the yeast, Saccharomyces cerevisiae has been associated with a single nuclear gene, sst1. Strains bearing this abnormal allele lacked the capacity to accumulate trehalose during growth on glucose or galactose medium or when incubated with glucose in nonproliferating conditions. However, sst1 strains still exhibited trehalose accumulation during growth on maltose medium, provided they contained a gene for maltose fermentation (MAL gene). Introduction of a constitutive MAL^c gene into an sst1 strain rendered the strain capable of accumulating trehalose during growth on glucose medium, but did not restore the normal capacity to convert glucose to trehalose in nonproliferating conditions. Different systems, I and II, of trehalose accumulation are proposed. System I would require the UPDG-linked synthase, whereas system II, which is normally specific for maltose, would utilize a different enzyme. It is unlikely that system II produces trehalose by transglucosylation, since it converted glucose to trehalose in MAL^c sst1 strains. The results indicate that maltose specifically induces the production of the MAL gene-product, which, in turn, would stimulate the formation (or activation) of system II.

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2. M.H.M. Rocha-Leao, A.D. Panek, and V.L.A. Costa-Carvalho (1982). Glycogen Accumulation by Saccharomyces cerevisiae: Influence of Carbon Source. IRCS Medical Science: Biochemistry; Cell and Membrane Biology; Metabolism and Nutrition; Microbiology, Parasitology and Infectious Diseases. 10: 544-545.

Glycogen metabolism was studied under conditions in which catabolite repression effects caused by glucose are reduced by using galactose as substrate for growth. During aerobic growth on glucose as limiting nutrient, little accumulation of glycogen occurs. On galactose, however, growth is slowed down, glycogen accumulation is continuous and cells exhibit greater capacity for glycogen synthesis. It is known that when yeast cells grow on glucose, glucose-6-phosphate (G6P) levels are low and the D to I

conversion of glycogen synthase becomes decisive for glycogen accumulation. During growth on galactose G6P levels are even lower; therefore, the high glycogen accumulation observed under these conditions suggests that: I. The ratio between the D and I forms of glycogen synthase is lower during growth on galactose than on glucose. II. Cells growing on galactose show higher total activity of both forms of synthase, a fact which could be related to catabolite repression.

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3. Ortiz, C.H., Maia, J.C.C., Tenan, M.N., Braz-Padrao, G.R., Mattoon, J.R.* and Panek, A.D. Regulation of Yeast Trehalase by a Monocyclic, 3'5'-Cyclic AMP-Dependent Phosphorylation-Dephosphorylation, Cascade System. Journal of Bacteriology in press, Febr., 1983.

*Department of Biology, University of Colorado, Colorado Springs, USA.

Summary

Mutation at the GLC1 locus in Saccharomyces cerevisiae results in simultaneous deficiencies in glycogen and trehalose accumulation. Extracts of yeast cells containing the glc1 mutation exhibit an abnormally high trehalase activity. This elevated activity is associated with defective 3', 5'-cyclic AMP-dependent monocyclic cascade which, in normal cells, regulates trehalase activity by means of protein phosphorylation and dephosphorylation. Trehalase in extracts of normal cells is largely in a cryptic form which can be activated in vitro by ATP.Mg in the presence of 3'5'-cyclic AMP. Normal extracts also exhibit a correlated cAMP-dependent protein kinase which catalyzes incorporation of label from $\gamma^{-32}P$ adenosine triphosphate into protamine. In contrast, cAMP has little or no additional activating effect on trehalase or on protamine phosphorylation in extracts of glc1 cells. Similar, unregulated activation of cryptic trehalase is also found in glycogen-deficient strains bearing a second, independently isolated mutant allele, glc1-2. Since trehalase activity is not directly affected by cAMP, the results indicate that the glc1 mutation results in an abnormally active protein kinase which has lost its normal dependence on 3',5'-cyclic AMP. Trehalase in extracts of either normal or mutant cells undergoes conversion to a cryptic form in a Mg^{++} -dependent, fluoride-sensitive reaction. Rates of this reversible reduction of activity are similar in extracts of mutant and normal cells. This same, unregulated protein kinase would act on glycogen synthase, maintaining it in the phosphorylated low activity D-form. The glc1 mutants provide a novel model system for investigating the in vivo metabolic functions of a specific, cAMP-dependent protein kinase.

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XIX. Department of Comparative Biochemistry, Janssen Pharmaceutica Research Laboratories, B-2340 Beerse, Belgium. Communicated by Hugo van den Bossche.

Below follows the abstract of a presentation at the 121th meeting of the Belgian Biochemical Society, November 6, 1982. Abstract published in: Archives Internationales de Physiologie et de Biochemie 90 (1982) B218-B219.

H. van den Bossche and G. Willemsens. Effects of the Antimycotics, Miconazole and Ketoconazole, on Cytochrome P-450 in Yeast- and Rat Liver Microsomes.

The antimycotic activity of miconazole and ketoconazole may be due, at least partly, to an accumulation of C-14 methylated sterols such as lanosterol, 24-methylenedihydrolanosterol, obtusifoliol and 14-methyl-fecosterol (van den Bossche et al., 1978, 1980). C-14 methylated sterols are known to disturb membrane functions (Yeagle et al., 1977) and cell growth (Bloch, 1979).

The presence of the 4,14 dimethyl- and 14-methyl-sterols in the miconazole and ketoconazole treated Candida albicans indicates that the 14-demethylation is sensitive to both imidazole derivatives whereas the 4-demethylation is not. The 4-demethylation of lanosterol is a cyanide-sensitive and carbon monoxide-insensitive reaction whereas the 14-demethylation is CN-insensitive and CO-sensitive (Gibbons et al., 1979; AOYAMA et al., 1981). The latter property indicates that cytochrome P-450 is a component of the enzyme system required to initiate oxidation of the 14 α-methyl group of lanosterol in yeast microsomes.

In this paper we report the effects of both miconazole and ketoconazole on the yeast and rat liver microsomal cytochrome P-450.

Yeast microsomes were obtained from Saccharomyces cerevisiae grown in a polypeptone - yeast extract - glucose medium (10:10:40 g/l). The liver microsomal fraction was obtained from female Wistar rats.

The content of cytochrome P-450 was determined by measuring the reduced carbon monoxide difference spectrum (Omura & Sato, 1964). Miconazole and ketoconazole, dissolved in DMSO, were added to the dithionite-reduced microsomal suspension 5 min before bubbling with CO and measurement of the absorption spectrum. Control spectra were determined in the presence of DMSO (final concentration: 0.3%). Control values of 0.18 ± 0.04 and 0.032 ± 0.013 nmoles/mg protein were found in rat liver- and S. cerevisiae microsomes, respectively. In the presence of 2.5×10^{-8} M miconazole a 25% decrease was found in the cytochrome P-450 content of S. cerevisiae microsomes. A 50% decrease was observed at 8×10^{-8} M miconazole or at 5.5×10^{-8} M ketoconazole.

The cytochrome P-450 of rat liver microsomes was much less sensitive to both miconazole and ketoconazole; at 2×10^{-4} M a 41 and 48% decrease was observed, respectively. This difference in sensitivity coincides extremely well with the much higher effects of both antimycotics on ergosterol synthesis in yeast as compared with cholesterol synthesis in e.g. rat liver or human fibroblasts (van den Bossche et al., 1980; Willemsens et al., 1980).

It is tempting to speculate that the miconazole and ketoconazole induced inhibition of the 14-demethylation of sterols in yeast cells results from an effect on the microsomal cytochrome P-450.

Supported in part by IWONL.

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Gibbons, G.F., Pullinger, C.R. & Mitropoulos, K.A. (1979) Biochem. J. 183, 309-315.
Aoyama, Y., Yoshida, Y., Sato, R., Susani, M. & Ruis, H. (1981) Biochim. Biophys. Acta 663, 194-202.
Omura, T. & Sato, R. (1964) J. Biol. Chem. 239, 2370-2378.
Willemsens, G., Cools, W., van den Bossche, H. (1980) in The Host Invader Interplay (van den Bossche, H. ed.) pp. 691-694, Elsevier/North-Holland, Amsterdam.

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XX. Department of Microbiology, Faculté de Pharmacie - Avenue Ch. Flahault, 34060 Montpellier Cedex, France. Communicated by J.-M. Bastide.

Below follow abstracts of recent papers from our laboratory:

1. M. Bastide, S. Jouvert, J.-M. Bastide (1982).
Etude cytologique de l'action des imidazoles sur Candida albicans.
Path. Biol., 30: (6) 458-462.

2. M. Bastide, S. Jouvert, J.-M. Bastide (1982).
A comparison of the effects of several antifungal imidazole derivatives and polyenes on Candida albicans: an ultrastructural study by scanning electron microscopy.
Can. J. Microbiol., 28: 1119-1126.

Abstract

The early events in the interaction of two polyene (amphotericin B and nystatin) and five imidazole (clotrimazole, ketoconazole, miconazole, isoconazole and econazole) antimycotics used at fungicidal concentrations with the surface of Candida albicans was studied by scanning electron microscopic examination of treated intact young yeast cells, treated spheroplasts, and spheroplasts liberated from treated young yeast cells. In all cases, treatment lasted two hours. The polyenes passed through the yeast cell wall and interacted with the cytoplasmic membrane causing the spheroplasts to lose their characteristic spheric form and to liberate the contents. Clotrimazole caused the formation of numerous circular openings in the cytoplasmic membrane, but only when the agent was used to treat spheroplasts directly. Ketoconazole, miconazole, isoconazole and econazole interacted with the cell wall causing formation of convolutions and wrinkles. The three imidazole derivatives that are structurally closely related (miconazole, isoconazole and econazole) inhibited the enzyme-catalyzed release of spheroplasts from young yeast cells.

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XXI. Division of Infectious Diseases, Department of Medicine, Medical College of Virginia, Richmond, Virginia 23298, U.S.A. and Research Service (151), McGuire Veterans Administration Medical Center, Richmond, Virginia 23224. Communicated by Eric S. Jacobson.

The following abstracts represent recent publications on Histoplasma capsulatum and Cryptococcus neoformans:

1. E.S. Jacobson and A.C. Harrell. A prototrophic yeast-strain of Histoplasma capsulatum. Mycopathologia 77, 65-68 (1982).

Abstract

A newly derived strain of Histoplasma capsulatum can be grown stably as yeast in a minimal medium containing glucose, biotin, tartrate and inorganic salts.

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2. E.S. Jacobson and A.C. Harrell. Cysteine-independent and cysteine-requiring yeast-strains of Histoplasma capsulatum. Mycopathologia 77, 69-73 (1982).

Abstract

Recently we described a strain of Histoplasma capsulatum, designated H-35, which is able to grow as yeast on a minimal medium consisting of inorganic salts, glucose and a trace of biotin. Using this strain as a prototrophic wild type we sought auxotrophic mutants. Mutagenized yeast-cells were starved for inorganic sulfate in sulfur-free minimal medium. Sulfate was then added, and growing prototrophic cells were killed by addition of amphotericin B. After 24 hours non-growing auxotrophs were 'rescued' by removal of amphotericin and addition of yeast extract. This 'mutant enrichment' cycle was repeated two additional times, after which the cells were plated on blood agar and 800 yeast-colonies were picked. Seventeen of these yeast-strains required cysteine for growth, as compared with strain H-35, which grew as yeast on minimal medium.

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3. E.S. Jacobson and D.J. Ayers. Auxotrophic Mutants of Cryptococcus neoformans. Journal of Bacteriology, July 1979, 318-319.

Abstract

Auxotrophic mutants of Cryptococcus neoformans have been obtained by using the methods of mutagenesis and replica-plating.

4. E.S. Jacobson, D.J. Ayers, A.C. Harrell and C.C. Nicholas. Genetic and Phenotypic Characterization of Capsule Mutants of Cryptococcus neoformans. Journal of Bacteriology, June 1982, 1292-1296.

Abstract

Stable mutants with reduced capacity to produce capsules were isolated from suspensions of Cryptococcus neoformans after treatment of the wild type with a mutagen. The mutants could be assigned one of two phenotypes, hypocapsular or acapsular. Hypocapsular mutants were immunochemically and physiochemically indistinguishable from the wild type, whereas acapsular mutants lacked a major capsular antigen and a negatively charged exterior. In genetic analysis, the mutant trait segregated as a Mendelian gene (1:1) when random basidiospores from an outcross were studied, and analysis of products of single meiotic events from outcrossed mutants was likewise consistent with meiotic segregation. Two-factor crosses yielded the expected four classes of progeny, with recombinants equal to parentals. We conclude that chromosomal genes are responsible for synthesis of the cryptococcal capsule and that random basidiospore analysis represents a useful technique for genetic analysis in this species.

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5. E.S. Jacobson and W.R. Payne. Uridine Diphosphate Glucuronate Decarboxylase and Synthesis of Capsular Polysaccharide in Cryptococcus neoformans, Journal of Bacteriology, 152:932-934 (1982).

Abstract

Uridine diphosphate (UDP) glucuronate decarboxylase activity is comparable in encapsulated and non-encapsulated strains of C. neoformans, requires nicotinamide adenine dinucleotide (NAD) (K_a = 0.2 mM) and is inhibited by NADH (K_i = 0.1 mM) and UDP xylose.

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6. C.C. Nicholas. Recombinational mapping of capsule mutants in Cryptococcus neoformans. M. Sc. thesis, Virginia Commonwealth University, Richmond, Virginia. December 1982.

Abstract

Seven capsule-negative mutants of Cryptococcus neoformans were classified by crossing them to each other and observing the rate of recombination between the two capsule negative genes in colonies derived from random basidiospores. The rate was obtained by enumeration of normally encapsulated colonies in the progeny by means of India ink staining. The loci of the seven capsule negative mutants were found to reside at three areas on a single chromosome, which we tentatively designated as linkage group 1. Random basidiospore analysis appears to be a very effective method for genetic mapping in C. neoformans.

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

The following papers were published recently or have been accepted for publication:

1. C. Cabeca-Silva, A. Madeira-Lopes and N. van Uden. Temperature relations of ethanol-enhanced petite mutation in Saccharomyces cerevisiae. FEMS Microbiology Letters 15, 149-151, 1982.

2. J. Anacleto and N. van Uden. Kinetics and activation energetics of death in Saccharomyces cerevisiae induced by sulfur dioxide. Biotechnology and Bioengineering 24, 2477-2486, 1982.

3. C. Leão and N. van Uden. Effects of ethanol on the glucose transport system of Saccharomyces cerevisiae. Biotechnology and Bioengineering 24, 000-000, 1982.

4. I. Spencer-Martins. Extracellular iso-amylase of the yeast Lipomyces kononenkoae. Applied and Environmental Microbiology 44, 000-000, 1982.

5. M. Lemos-Carolino, A. Madeira-Lopes and N. van Uden. The temperature profile of the pathogenic yeast Candida albicans. Zeitschrift für Allgemeine Mikrobiologie (accepted).

6. A. Madeira-Lopes. Glucose-less death in yeast at supraoptimal temperatures. Ciencia Biologica (Portugal) 7, 59-64, 1982.

7. I. Sá-Correia and N. van Uden. Effects of ethanol on thermal death and on the maximum temperature for growth of the yeast Kluyveromyces fragilis. Biotechnology Letters (accepted).

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XXXIII. Carlsberg Laboratory, Department of Physiology, Gl. Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark. Communicated by M.C. Kielland-Brandt.

The following is an abstract of a recent paper:

S. Holmberg: Genetic differences between Saccharomyces carlsbergensis and S. cerevisiae II. Restriction endonuclease analysis of genes in chromosome III. Carlsberg Res. Commun. 47, 233-244 (1982).

Chromosome III in a haploid Saccharomyces cerevisiae strain has been previously substituted for by its homologue from S. carlsbergensis. With this chromosome substitution line the two homologous chromosomes were shown to undergo crossing over only in a limited region and to differ in nucleotide sequence at the HIS4 locus. In the present study the S. carlsbergensis chromosome III was compared to its S. cerevisiae homologue at several additional loci.

Cloned DNA from the S. cerevisiae loci HML, HIS4, LEU2, MAT and SUP-RLI was used as hybridization probes in the analysis of nucleotide sequence homology at these loci and HMR. Virtually no differences were detected at SUP-RLI and HMR, located in the region where the two chromosomes recombine, wheras considerable differences were found in the non-recombining part. The data are consistent with the assumption that HML, MAT and HMR of the S. carlsbergensis chromosome are organized as in S. cerevisiae. Segments X and Z1, which are involved in mating type interconversion, were closely

homologous to their S. cerevisiae counterparts, whereas Yα as well as sequences outside the HML and MAT cassettes were substantially different.

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XXIV. University of Medicine and Dentistry of New Jersey, Rutgers Medical School, Busch Campus, Piscataway, New Jersey 08854. Communicated by Michael J. Leibowitz.

Below follow abstracts of two papers recently published by our laboratory.

Dennis J. Thiele and Michael J. Leibowitz, Structural and Functional Analysis of Separated Strands of Killer Double-Stranded RNA of Yeast, Nucleic Acids Res. 10: 6903-6918 (1982).

Abstract

The two strands of the M double-stranded RNA species from a killer strain of Saccharomyces cerevisiae have been separated, and the 3'-terminal sequences of these strands have been determined. The positive strand programs the synthesis of the putative killer toxin precursor (M-p32) in a rabbit reticulocyle in vitro translation system. Only the negative strand hybridizes to the positive polarity transcript (m) synthesized in vitro by the virion-associated transcriptase activity. Secondary structural analysis of the extreme 3'-terminus of the negative strand using S_1 nuclease is consistent with the presence of a large stem and loop structure previously proposed on the basis of RNA sequence data. This structure, and a similar structure at the corresponding 5'-terminus of the positive strand, may have functional significance in vivo.

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Michael J. Leibowitz, Role of Protein Synthesis in the Replication of the Killer Virus of Yeast, Current Genetics 5: 161-163 (1982).

Abstract

Growth of yeast in the presence of the protein synthesis inhibitors cycloheximide and cryptopleurine results in elimination ("curing") of the cytoplasmically-inherited killer virus. Yeast mutants with ribosomal subunits resistant to the translational effects of one of these antibiotics are specifically resistant to curing by that antibiotic, indicating that these substances act on the virus by their effect on protein synthesis.

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XXV. Kyoto University, The Research Institute for Food Science, Uji, Kyoto, Japan 611. Communicated by Akira Kimura.

Recently we have developed a new method of transformation of intact yeast cells without making protoplast. The paper will be published in J. Bacteriol. 153, January issue (1983) (in press). The method is very simple and time saving, therefore, I am sure it must be useful for research in yeasts. The new method is summarized as follows:

Intact yeast cells treated with alkali cations took up plasmid DNA. Li^+, Cs^+, Rb^+, K^+, and Na^+ were effective in inducing competence. Conditions for the transformation of Saccharomyces cerevisiae D13-1A with plasmid YRp7 were studied in detail with CsCl. The optimum incubation time was 1 h, and the optimum cell concentration was 5×10^7 cells per ml. The optimum concentration of Cs^+ was 1.0 M. Transformation efficiency increased with increasing concentrations of plasmid DNA. Polyethylene glycol was absolutely required. Heat pulse and various polyamines or basic proteins stimulated the uptake of plasmid DNA. Besides circular DNA, linear plasmid DNA was also taken up by Cs^+-treated yeast cells, although the uptake efficiency was considerably reduced. The transformation efficiency with Cs^+ or Li^+ was comparable with that of conventional protoplast methods for a plasmid containing arsl, although not for plasmids containing a 2 μm origin replication.

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Other papers are concerned with the control mechanism of fermentative production of sugar nucleotide and cytidine derivatives by yeast. Recent results were summarized and published at the "4th International Symposium on Genetics of Industrial Microorganisms (GIM-82)", which was held in Kyoto from June 6-11, 1982.

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The following papers on yeast have recently been published or will be published in the near future.

(1) A. Kimura, K. Murata and M. Shimosaka, "Application of plasmid to the production of Energy (ATP) and Glutathione". Abstract of 4th International Symposium on Genetics of Industrial Microorganisms, p. 25 (1982).

(2) I. Umemura, H. Fukuda, Y. Fukuda and A. Kimura: Controlling Mechanism of ATP Regeneration by Glycolytic Pathway in a Yeast: Hansenula jadinii, Eur. J. Appl. Microbiol. Biotechnol., 15, 133 (1982).

(3) H. Ito, Y, Fukuda, K. Murata and A. Kimura; Transformation of Intact Yeast Cells Treated with Alkali Cations, J. Bacteriol., 153 (1) (1983) in press.

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XXVI. Biological Institute, Faculty of Science, Nagoya University, Nagoya 464, Japan. Communicated by Naohiko Yanagishima.

The following are recent publications from our laboratory.

1. N. Yanagishima and K. Yoshida 1981. Sexual Interactions in Saccharomyces cerevisiae with special reference to the regulation of sexual agglutinability. In: Sexual Interactions in Eukaryotic Microbes, D.H. O'Day and P.A. Horgen, eds. Academic Press, New York, pp. 261-291.

2. M. Yamaguchi, K. Yoshida and N. Yanagishima 1982. Isolation and partial characterization of cytoplasmic α agglutination substance in the yeast Saccharomyces cerevisiae, FEBS Letters 139:125-129.

3. Y. Sato, A. Sakurai, N. Takahashi, Y-M. Hong, Y. Shimonishi, C. Kitada, M. Fujino, N. Yanagishima and I. Banno 1981. Amino acid sequence of α_K substance, a mating pheromone of Saccharomyces kluyveri, Agric. Biol. Chem. 45:1531-1533.

4. N. Yanagishima 1981. Mechanism and regulation of sexual agglutination in Saccharomyces cerevisiae. In: Current Developments in Yeast Research - Advances in Biotechnology, G.G. Stewart and I. Russell, eds., Pergamon Press, Toronto, pp. 245-250.

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XXVII. Department of Genetics, University of California, Davis, CA 95616. Communicated by S.R. Snow.

1. Below follows the abstract of the Ph.D. thesis of Steven Arthur Williams, completed August 1982 in Genetics (research supervisor S.R. Snow).

Cloning and Expression of the Malolactic Gene of Lactobacillus delbrueckii in E. coli and Yeast.

Abstract

The purpose of this research was to transfer a chromosomal gene from a gram-positive prokaryote into a lower eukaryote, yeast, and to achieve expression of this gene in the heterologous host. The gene coding for the malolactic enzyme (catalyzing the conversion of L-malate to L-lactate) was cloned from the lactic acid bacterium Lactobacillus delbrueckii and transformed into Escherichia coli and yeast (Saccharomyces cerevisiae). Neither of these organisms possess the malolactic fermenting capability. This particular gene was selected because its expression in yeast could be of considerable interest and value to wine makers. The fragment of L. delbrueckii DNA cloned on pBR322 carrying the malolactic gene is about five kilobases long. E. coli cells transformed with plasmid pBR322 carrying the gene converted L-malate to L-lactate at a moderate level.

The fragment was transferred to other plasmids, including yeast/E. coli hybrid shuttle vectors. In the best cases, E. coli cells harboring the malolactic gene converted about 10% of the malate to lactate when grown anaerobically in a medium containing 0.3% malate. In yeast, the best expression has been achieved by transferring the gene to a shuttle vector containing both a yeast 2μ plasmid and chromosomal origin of DNA replication. When yeast cells harboring this plasmid were grown anaerobically for five days, about 1.0% of the malate was converted to lactate.

When a laboratory yeast strain containing the malolactic gene was used to make wine in a trial fermentation, about 1.5% of the malate in the grape must was converted to lactate. Clearly, there exist barriers to expression of the prokaryotic malolactic gene in yeast and to a lesser extent in E. coli. These barriers must be overcome in order to obtain useful levels of malolactic function in yeast.

This work adds new knowledge to the little that is known about the expression of genes from gram-positive prokaryotes in gram-negative

prokaryotes, and is also the first example of expression of a chromosomal gene from a gram-positive prokaryote in a eukaryotic host. Thus, these results add new information to the study of heterologous gene expression. Such information will be valuable in future genetic engineering experiments involving the transfer of prokaryotic genes to eukaryotic hosts in order to broaden their biochemical capabilities.

2. The following is an abstract of a paper submitted to the Jour. Inst. Brew.

Rous, C.V., Snow, S.R., and Kunkee, R.E. Reduction of higher alcohols by fermentation with a leucine-auxotrophic mutant of wine yeast.

Abstract

Several auxotrophic mutants requiring branched chain amino acids (valine, leucine, or isoleucine) were isolated in a strain of Montrachet wine yeast. They were tested for their ability to produce lowered amounts of higher alcohols ("fusel oil": isobutyl, active amyl, and isoamyl alcohols) in grape juice fermentations. One strain which required leucine was especially good in this respect. This mutation is recessive and is the result of a deficiency for the enzyme α-isopropylamate dehydratase. In trial fermentations with this mutant, the resulting wines contained up to 20% less total fusel oil and 50% less isoamyl alcohol compared to the parent Montrachet strain. An experienced taste panel did not discern any gross degradation of taste quality in wine made with the mutant strain compared to that made with the parent strain. The mutant strain could be of commerical importance in preparation of distilling material for alcoholic beverages since the reduced fusel oil content would not require any special distillation procedures which are normally used to avoid the unpleasant flavor associated with concentrated higher alcohols. Reduction of the isoamyl alcohol content is particularly significant since this fusel oil component is usually present in the highest amount.

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XVIII. Department of Genetics, Microbiology and Biophysics, Faculty of Science Charles University, Prague, Czechoslovakia, PSC 128 44, Vinicna 5. Communicated by Olga Bendová.

Below follows the abstract of one of our recent papers.

Bendová, O., Kupcová, L., Janderová, B., Vondrejs, V., Vernerová, J.: "Ein Beitrag zur Brauerei Hefehybridisierung". Submitted for publication in Brauwissenschaft.

ABSTRACT

Hybrid clones of brewer's yeast were constructed by means of induced protoplast fusion of superkiller strain T 158 C (α, his^-) of Saccharomyces cerevisiae producing killer factor (K^+) and resistant to the factor (R^+), and of brewer's yeast Saccharomyces uvarum P 9 (polyploid, his^+) sensitive to the killer factor (R^-). Selection of hybrid strains of (R^+, K^+, his^+) character proceeded in minimal agar medium in the presence of killer factor. The rate of hybrid clone appearance was 10^{-5}.

Hybrid clone S. uvarum P 9-LK-12/1 was isolated after repeated cultivation of hybrid strain P9-LK-12 under industrial conditions. It has all of the important properties of the parental brewer's yeast strain and in addition it is able to eliminate contamination by killer sensitive strains of Saccharomyces spp.

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XXIX. Alko, Box 350, SF-00101, Helsinki 10, Finland. Communicated by Heikki Suomalainen.

Below follows a list of our work published since June 1982 (see Yeast Newsletter XXXI (1) 1982).

1. John Londesborough. Activity of Cyclic-AMP Phosphodiesterase in Permeabilised Cells of Bakers' Yeast. European Journal of Biochemistry 126: 631-637 (1982).

   Yeast cyclic-AMP phosphodiesterases were assayed in situ, using cells permeabilised with cytochrome c, to get information about the kinetics of these enzymes at the high concentrations of macromolecules occurring in vivo. Protamine treatment was not suitable, because it perturbed the intracellular localisations of both the (Mg-dependent) low K_m enzyme and the (EDTA-insensitive) high-K_m enzyme. The pH-dependence of K_m and V for EDTA-insensitive activity in situ agreed well with the behaviour of pure high-K_m enzyme, except that near pH 8 Hofstee plots were bent slightly upwards both for activity in situ and with crude broken-cell preparations. Hofstee plots of Mg-dependent activity in situ were distinctly concave, and could be resolved mathematically into two activities, one (accounting for about 30% of the Mg-dependent V) with a K_m close to the value in vitro of 0.2 µM, and the other with an apparent K_m of 3 µM. The 3 µM K_m activity probably represents a fraction of the low-K_m enzyme that is particle-bound at the high protein concentrations occurring in situ.

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2. Kaija Varimo and John Londesborough. Adenylate Cyclase Activity in Permeabilised Yeast. FEBS Letters 142: 285-288 (1982).

   Adenylate cyclase (AC) was assayed in bakers' yeast cells permeabilised by treatment with toluene/ethanol or protamine. The main properties of AC in permeabilised cells (apparent absolute dependence on Mn^{2+}, pH optimum of 6.2, K_m of 1.7 mM at pH 6.2) were the same as in broken cell preparations. AC of permeabilised cells was not inactivated by preincubation with glucose. An essential basic group with a pK of 5.5 in free enzyme and about 7.0 in the enzyme-substrate complex and an essential acidic group ($pK_2^E = 6.1$, $pK_2^{ES} \simeq 7.1$) were tentatively suggested to be imidazoles.

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3. John Londesborough. Cyclic Nucleotide-dependent Inactivation of Yeast Fructose 1,6-Bisphosphatase by ATP. FEBS Letters 144: 269-272 (1982).

The activity of fructose 1,6-bisphosphatase (EC 3.1.3.11) at 10 mM $MgCl_2$ was rapidly decreased by 60% when high speed supernatants of bakers' yeast were incubated with 2.8 mM $MgCl_2$, 1.2 mM ATP and 180 µM cyclic AMP at 30°C, pH 7.5. The activity at 2 mM $MnCl_2$ was not changed. Cyclic AMP could be replaced by cyclic GMP, cyclic IMP or, less effectively, cyclic UMP, and Mg^{2+} ions could be replaced by Ca^{2+} but not by Mn^{2+} nor Co^{2+}. The results suggest that the rapid and reversible phase of the inactivation of fructose 1,6-bisphosphatase by glucose is caused by a cyclic AMP-dependent protein kinase that is activated by the transient rise in cyclic AMP known to follow addition of glucose to bakers' yeast.

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4. M. Korhola, E. Oura and H. Suomalainen. Glucose/Molasses Effects on Enzyme Activities and Fermentative Activity of Fully Aerobic Continuous Cultures of Baker's Yeast. Folia Microbiologica (in press).

Supplementing the molasses medium with glucose was observed to have deleterious effects on the quality of industrially grown baker's yeast. The phenomenon was investigated in the laboratory using beet molasses and glucose in fed-batch fully aerobic continuous cultures of baker's yeast.
Molasses (sucrose) and glucose were used in the following concentrations: (I) molasses 50 g/l; (II) molasses 40 g/l and glucose 10 g/l; (III) molasses 10 g/l and glucose 40 g/l. The enzyme activity changes fell into three classes. With increasing concentration of glucose the activity of hexokinase, malate dehydrogenase and pyruvate decarboxylase increased while the activity of α-glucosidase (maltase) decreased. The activities of alcohol dehydrogenase, invertase, maltose permease and phosphofructokinase were indifferent to the changes in the relative amounts of glucose and sucrose in the growth medium.
Yeast harvested from these experiments was used to ferment 1% glucose, 1% sucrose, 1% maltose and 1% mixture of the three sugars. It was found that the maltose fermentation was most affected by high relative glucose concentrations in the growth medium.

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5. K. Edelmann and Liisa Penttila. Amylases in Yeast Production. Abstract of paper presented at the International Symposium on the Use of Enzymes in Food Technology, May 5-7, 1982, Versailles, France.

Major factors in the economy of the production of baker's yeast are the cost of the carbon source and the treatment of waste water. The modern grain processing industry offers a great variety of hydrolysates which can be competitive with conventional molasses. We have tested the suitability of some such syrups in yeast

propagations. The proportion of assimilable sugars in the total carbohydrate varied from 37% to 81%.
Amyloglucosidase was added to propagations in order to hydrolyze the oligosaccharides to sugars assimilable by yeast.
Amyloglucosidase improved the yeast growth considerably, but the yields were still 10-40% lower than with beet molasses. The fermentative activity of the yeast produced was not affected by the raw material used. The organic loads in spent medium were noticeably lower with treated syrups than with molasses. The BOD values can be as low as 2.4 g/l compared with 10 g/l for molasses propagations.

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6. Matti Korhola. Selection of Yeast Strains for Added Ethanol Tolerance. Abstract of paper presented at the 11th Conference of Yeast Genetics and Molecular Biology, September 13-17, 1982, Montpellier, France.

Continuous anaerobic cultivation in the chemostat in a defined medium was used to select strains of baker's yeast which showed increased tolerance to ethanol added to the culture medium. The maximum ethanol productivities were determined first. In the medium containing 10% glucose the maximum output of yeast was 1.0 g/l/h, the volumetric ethanol productivity 4.6 g/l/h, and the specific ethanol productivity 0.8 g/g/h. In the medium containing 5% glucose the corresponding values were 1.3, 6.3, and 2.0, respectively.
Ethanol tolerant stains were then selected by feeding in glucose medium with increasing concentration of ethanol. The selection was carried out over several months.
The resulting strains were characterized according to their growth rate, fermentation rate in a Warburg apparatus, DNA content per cell, DNA restriction pattern and activities of enzymes known to be inhibited by ethanol (hexokinase, 3-phosphoglycerate dehydrogenase) and activity of alcohol dehydrogenase.

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7. The following publications have appeared since the last communications. The abstracts of reports have been given in the Yeast Newsletter 30 (1981): 1, 45-46 and 31 (1982): 1, 37-38.

Erkki Oura, Heikki Suomalainen and Risto Viskari. Breadmaking, Economic Microbiology, vol. 7, Fermented Foods, ed. by A.H. Rose, Academic Press, Oxford 1982, pp. 87-146.

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H. Suomalainen. Yeast in alcohol production and bread making, Acta Alimentaria 11 (1982), 289-307.

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J. Londesborough and L. Jonkkari. Low K_m cyclic AMP phosphodiesterase of yeast may be bound to ribosomes associated with the nucleus, Molecular and Cellular Biochemistry 46 (1982), 65-71.

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Erkki Oura and Heikki Suomalainen. Biotin-active compounds, their existence in nature and the biotin requirements of yeasts, Journal of the Institute of Brewing 88 (1982), 299-308.

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XXX. Department of Botany, University of Baluchistan, Quetta, Pakistan, Communicated by S.R. Chughtai.

Little attention has been paid to the mutagenic and carcinogenic potential of food products to which the general human population is daily exposed. Several recent studies indicate the presence of mutagenic components, either as natural compounds, contaminants, or as a result of processing, in many food products, some of which have been in use for hundreds of years.

We have been interested in the mutagenicity testing of various food additives, particularly the food colours and flavours. Very recently, we have initiated assessing the mutagenic and recombinogenic potential of some commonly consumed condiments and spices. They are consumed to different extents in various parts of the world, and are essential dietary components in certain regions such as the South East Asian countries. The following is the summary of the paper which we intend to present at the 21st Pakistan Science Conference to be held at the Islamia University, Bhawalpur, Pakistan from December 31, 1982 to January 5, 1983.

"Mutagenicity Testing of Some Spices and Condiments in Diploid Yeast" by Sarah Batool, Masoma Qazalabash and S.R. Chughtai.

Very little information is available about the mutagenic and carcinogenic potential of most of the commonly used condiments and spices which form an important group of naturally occurring flavouring agents. We are testing some of these agents for their mutagenic and recombinogenic potential using the diploid strain D7 of the yeast Saccharomyces cerevisiae. The strain could be utilized for the simultaneous detection of mitotic gene conversion at the trp5, mitotic crossing over at the ade2 and reverse mutation at the ilv1 loci. It has been successfully employed for the genotoxicity testing of complex mixtures. The compounds are assayed by the simple test developed by Kunz et al. (Mutation Res., 73-215-220, 1980). The cells are grown on appropriate media and on aqueous extract of the test compound is added to the central well made on the petri dishes. The cytotoxicity of a compound is indicated by the appearance around the well of a killing or inhibitory zone, and colonies of comparatively smaller size. The genotoxicity is indicated by the increased frequency of convertants, revertants and aberrant colonies. The following condiments have been tested so far: Nutmeg, Mace, Cardamum small (Elletaria cardamoum), Cardamum large (Amomum subulatum), Cinnamon, Clove, Black Pepper, Onion, Green Chillies, and Curry Powder (a mixture of about 12 different spices and condiments). The results indicate that Cinnamon and Clove are weakly

mutagenic and recombinogenic and they also caused cell killing and inhibited cell division. All other tested compounds proved negative; they neither induced genetic damage nor had cytotoxic effects under the experimental conditions employed. However, one variety of Green Chillies, locally called Shimla, which is also used as a vegetable and generally thought to be less harmful than the other varieties, showed cytotoxic effects. These observations are of preliminary nature and further studies are in progress for their verifications; the testing of various other condiments and certain widely used plant products is also underway. Betel Nut extracts which we have already shown to be negative in this strain (Chughtai et al., Pak. J. Bot., 14:6, 1982) were also negative when tested by this method.

We are also testing some other chemicals, including food colours and laboratory stains etc., by the same method. In preliminary tests, Gentian Violet, Crystal Violet, Fast Green FCF, Janus Green B, Basic Fuchsin, and Vino (a commerical colour mixture for foods) all proved potent cytotoxins. Investigations are underway for assessing their mutagenic and recombinogenic potentials and the results will be published in due course.

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XXXI. Northern Regional Research Center, USDA, 1815 North University Street, Peoria, Illinois 61604. Communicated by C.P. Kurtzman.

The following are recent publications from our laboratory.

Kurtzman, Cletus P., Rodney J. Bothast and James E. VanCauwenberge. Conversion of D-Xylose to Ethanol by the Yeast Pachysolen tannophilus. U.S. Patent No. 4,359,534.

Abstract

A method has been discovered for converting D-Xylose to ethanol relying on the unique ability of the yeast Pachysolen tannophilus to ferment this five-carbon sugar without the use of added enzymes. This process will be particularly useful in the production of ethanolic fuel from plant biomass.

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P.J. Slininger, R.J. Bothast, J.E. VanCauwenberge, and C.P. Kurtzman. 1982. Conversion of D-Xylose to Ethanol by the Yeast Pachysolen tannophilus. Biotechnol. Bioeng. 24:371-384.

Abstract

The yeast Pachysolen tannophilus was found to be capable of converting D-Xylose to ethanol. Batch cultures initially containing 50 g/L D-xylose yielded 0.34 g of ethanol per gram of pentose consumed. Aerobic conditions were required for cell growth but not for ethanol production. Both alcohol formation and growth were optimum when incubation temperature was 32°C, when pH was near 2.5, and when D-xylose and ethanol concentrations did not exceed 50 and 20 g/L, respectively.

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XXXII. Department of Microbiology, Haryana Agricultural University, Hissar, India. Communicated by D.S. Dahiya.

Below follow titles and summaries of recently published research papers and our current research activities.

1. Sharma Suresh, Dhamija, S.S., Dhiya, D.S. and Bardiya, M.C. "Fermentation alcohol from cane molasses by fast fermenting yeast". Ind. J. microbiol. 20(No. 1) p 34 (1980).

2. Dahiya, D.S., Bardiya, M.C., Dhamija, S.S., Sharma Suresh and Tauro, P. "Ethanol production from cane molasses; Effect of fermentation conditions on yeast recycling". Int. Sugar J. pp. 203-206, July, 1980.

3. Dhamija, S.S., Dahiya, D.S. and Bardiya, M.C. (1980) "Yeast screening and fermentation studies on beet molasses". Proc. 44th S.T.A. India, G.65.

4. Dhamija, S.S., Dahiya, D.S., Bardiya, M.C. and Tauro, P. "Ethanol from unclarified cane molasses: I. Recycling of yeast using centrifugation". Int. Sugar J. pp. 39-41, Feb. 1982.

Summary

Studies conducted on laboratory scale indicate that the pretreatment steps of Melle-Boinot process of yeast recycling in molasses can be by-passed. By using unclarified molasses and without activation treatment of yeast, fermentation time could be as short as 12 h by recycling the yeast recovered from 75% of wash. The fermentation efficiencies were as high as 91-93% in all the six cycles tested.

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5. Yadav, B.S., Dahiya, D.S. and Tauro, P. (1981) "Screening of yeasts for fermentation of sugar cane juice and sugar cane (Preliminary Report)". Proc. S.T.A. India.

Summary

From a screening study involving a large number of yeast strains for direct fermentation of sugar cane juice, one strain, Saccharomyces cerevisiae Strain 39, which can effectively ferment sugar cane juice to ethanol has been obtained. Initial conditions for the fermentation of juice using this strain have been standardized and about 9% alcohol can be produced in 36 h. This strain has also been tested for fermenting sugar cane directly.

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6. Dahiya, D.S., Koshy, M., Dhamija, S.S., Yadav, B.S. and P. Tauro (1982) "Spent wash recycling for Molasses Fermentation" Int. Sugar J. (In Press).

Summary

Use of distillery spent wash as a diluent in molasses fermentation has been attempted. The screening studies with different yeast strains could reveal that strain 21 is capable of fermenting spent wash-diluted molasses solutions without any nutrient supplementation. Increasing concentration of spent-wash in the fermentation medium, however, decreased the rate of fermentation and the fermentation efficiency. It was further noted that the cell sedimentation rate decreases with increasing concentration of spent wash in the medium.

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7. B.S. Yadav, D.S. Dahiya and P. Tauro (1982) "Studies on Fermentation of Sugarcane Juice for the production of Ethanol". Proc. 46th S.T.A. India.

Mixed juice from sugar mills had sugar levels between 12 and 13% while 18.7% was the total reducing sugars in the juice obtained from HAU Farm.

The rate of fermentation and alcohol production was found higher when the mixed juice from mills was used as fermentation raw material than when diluted juice from HAU Farm was used. It was further observed that there is no effect of clarification of mixed juice before fermentation on the alcohol production. Addition of 200 ppm urea results in higher alcohol production and higher levels of urea (400-1000 ppm) in the fermentation medium reduces the fermentation time and increases the alcohol production as well, ultimately.

The optimum fermentation temperature for strain No. 39 was found to be around 35°C for efficient fermentation of cane juice. The fermentation time has been reduced to 18 h by reuse of yeast sludge and the increase in alcohol production was also observed.

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8. Current research interests are centered in the following areas:

1. Kinetics of unclarified molasses fermentation by S. cerevisiae.

2. Use of spent wash (Effluent) as mash water in cane molasses fermentation - a method to control pollution.

3. Improvement of Distillers' yeast through genetic manipulations.

4. Direct utilization of excess sugar cane/cane juice through fermentation for production of biofuel.

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XXXIII. Chaire de Génétique et Microbiologie, I.N.R.A. - E.N.S.A., Place Viala 34060, Montpellier Cedex, France. Communicated by P. Galzy.

The following are recent papers from our laboratory:

1. G. Moulin, Hélène Boze, P. Galzy
A comparative study of the inhibitory effect of ethanol and substrates on the fermentation rate of parent and respiratory deficient mutant.
Biotechnol. Letters 3, 351-356, 1981.

2. G. Moulin, B. Malige, P. Galzy
Etude physiologique de Kluyveromyces fragilis; consequence sur la production de levure sur lactoserum.
Le Lait 61, 323-332, 1981.

3. K. Oteng-Gyang, G. Moulin, P. Galzy
A study of the amylolytic system of Schwanniomyces castellii.
Zeitschrift für Allgemeine Mikrobiol. 21, 537-546, 1981.

4. G. Moulin, Hélène Boze, P. Galzy
Utilization of a respiratory-deficient mutant for alcohol production.
J. Ferment. Technol. 60, 25-29, 1982.

5. S. Demeulle, J.P. Guiraud, P. Galzy
Study of inulinase from Debaryomyces phaffii.
Zeitschrift für Allgemeine Mikrobiol. 21, 181-189, 1981.

6. J.P. Guiraud, J. Daurelles, P. Galzy
Alcohol production from Jerusalem artichoke using yeasts with inulinase activity.
Biotechnol. Bioeng. 23, 1461-1465, 1981.

7. A. Ba, R. Ratomahenina, J. Graille et P. Galzy
Etude de la croissance de quelques souches de levures sur les sous-produits d'huile d'arachide.
Oleagineux, 36, n° 8-9, p. 439-445, 1981.

8. F. Martinet, R. Ratomahenina, J. Graille et P. Galzy
Production of food yeasts from the solid fraction of palm oil.
Biotechnol. Letters, 4, n° 1, p. 9-12, 1982.

9. B. Blondin, R. Ratomahenina, A. Arnaud et P. Galzy
A study of cellobiose fermentation by Dekkera strain.
Biotechnol. Bioeng. (in press).

10. J.P. Guiraud, T. Deville-Duc, P. Galzy
Selection of yeast strains for ethanol production from inulin.
Folia Microbiol. 26, 147-160, 1981.

11. P. Chautard, J.P. Guiraud, P. Galzy
Inulinase activity of Pichia polymorpha.
Acta Microbiol. (A.S.H.) 28, 245-255, 1981.

12. J.P. Guiraud, C. Bernit, P. Galzy
Study of inulinase of Debaryomyces cantarellii.
Folia Microbiol. 27, 19-24, 1982.

13. J.P. Guiraud, J.M. Caillaud, P. Galzy
Optimization of alcohol production from Jerusalem artichoke.
Eur. J. Appl. Microbiol. Biotechnol. 14, 81-85.

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XXXIV. Research Institute for Viticulture and Enology, 833 11 Bratislava, Czechoslovakia. Communicated by E. Minarik.

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

1. E. Minarik: Microbiology. Research results in the years 1977-1980, (in German). Vitis (GFR) 1982 21: 145-176.

A world review of papers published in the last 4 years on wine yeasts, bacteria and mould research is given. Relevant papers on yeast systematics, taxonomy, ecology, physiology, biochemistry and genetics are discussed. Problems on SO_2- and H_2S-formation by wine yeasts, L-malic acid decomposition by Schizosaccharomyces, application of pure yeast starter, problems of yeast selection, active dry yeast in winemaking, action of wine fermentation inhibitors, secondary action of pesticides on wine yeasts, wine spoilage caused by various Saccharomyces spp., the biology of "Flor" - Sherry yeast and many other questions of wine microbiology are briefly summarized; 271 quotations are given.

2. E. Minarik, O. Sestinova: Influence of mixed and associated yeast cultures on fermentation of must and wine quality. Kvasny prumysl (Prague) 28, 1982 (accepted for publication) (in Slovak).

By the application of mixed or associated cultures of Saccharomyces rosei - S. cereivisae or S. rosei - S. oviformis in the fermentation of musts with high sugar content, a more profound sugar fermentation and lower volatile acid formation by yeasts compared to the fermentation of must by a single strain yeast starter (S. cerevisiae or S. oviformis) could be achieved. The optimal ratio of S. rosei : S. cerevisiae or S. oviformis should be 9:1 using 5% yeast starter. The results indicate the possibility of practical application in the fermentation of musts with high sugar content from botrytized grapes.

3. E. Minarik, Z. Silharova and O. Jungova: The yeast flora of bottled wines from South Moravia (in Slovak). Vinohrad (Bratislava) 20, 1982 (accepted for publication).

The most common yeast species occurring in finished bottled South Moravian wines is the osmophilic and fructophilic thermotolerant Saccharomyces bailii var. bailii accompanied by airborne Torulopsis sp. S. bailii var. bailii is thus the most widespread yeast in bottled wines. It is frequently causing spoilage of natural sweet table wines in many wine regions of Czechoslovakia. S. bailii var. bailii does not however occur in bulk wines, though it may be found in the course of bottling on the filling line. Physiological properties of this dangerous yeast in winemaking are briefly discussed.

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XXXV. Books

The second edition of The Yeasts (A.H. Rose and J.S. Harrison, editors) is now in preparation. Below follows the lists of contents of volumes 1 and 2.

The Yeasts - Second Edition - Volume 1

1.	Introduction	A.H. Rose and J.S. Harrison
2.	Classification	N.J.W. Kreger-van Rij
3.	Molecular Taxonomy	H.J. Phaff and Cletus P. Kurtzman
4.	The Typological Yeast Species and its Delimitation	J.P. van der Walt
5.	Yeasts Associated with Plants, Insects and Soils	H.J. Phaff
6.	Aquatic Yeasts	D.G. Ahearn and A. Hagler
7.	Yeasts Associated with Man and Animals	R. Hurley and J. de Louvois
8.	Division Cycles	A.E. Wheals
9.	Life Cycles	R. Davenport
10.	Reaction to the Chemical Environment	A.H. Rose
11.	Temperature Relations	K. Watson
12.	Response to Radiation	A.P. James and A. Nasim
13.	Batch and Continuous Culture	A. Fiechter and O. Kappeli
14.	Cell Aggregation	G.B. Calleja
15.	Adhesion to Non-Cellular Surfaces	L.J. Douglas
16.	Yeast Toxins	T.W. Young

The Yeasts - Second Edition - Volume 2 - Yeast Organelles

1.	Introduction	A.H. Rose and J.S. Harrison
2.	Cytology of yeasts; an overview	C.F. Robinow and B.F. Johnson
3.	Separation of yeast organelles	D. Lloyd and T.G. Cartledge
4.	Capsules and walls	G.H. Fleet

5.	Periplasm	W.N. Arnold
6.	Plasma membranes	A.H. Rose
7.	Nuclei and plasmids	D.H. Williamson
8.	Ribosomes	J.R. Warner
9.	Mitochondria	A. Tzagoloff
10.	Peroxisomes	W. Harder and M. Veenhuis
11.	Storage compounds	Anita D. Panek
12.	Internal membraneous systems and organelles	J. Schwencke

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The third edition of "The Yeasts - a taxonomic study", N.J.W. Kreger-van Rij, ed., North Holland: Amsterdam, has gone to press and will hopefully be available in the fall of 1983.

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XXXVI. Obituary

Frederick Stuart Michael Grylls

Zymologists throughout the world were saddened to learn of the sudden death, on the 22nd September, 1982, of Michael Grylls, Research Director of the Distillers Company (Yeast) Limited. Grylls was born on 29th September, 1927 and, after reading Natural Sciences and Chemical Microbiology at Cambridge University, he joined the Distillers Company Central Research Department at Epsom in Surrey. There he was one of a team of microbiologists, which included Dawson, Harrison and Trevelyan, and who were making outstanding contributions to our understanding of glycolysis in yeast. After seven years, he moved to the Patent Department of the Distillers Company, only to return to the Research Department as Director in 1968. One of his first tasks was to organize the transfer of the Research Department to the new Glenochil Technical Centre at Menstrie in Clackmannanshire, Scotland. At Glenochil, he had to rebuild the team devoted to yeast research, since several of the more senior workers had not moved to Scotland. The new team concentrated on problems of strain improvement with yeasts used for baking and distilling, particular efforts being directed towards active dried yeast.

Michael Grylls did not publish a great deal, particularly after he left the Research Department in Epsom. However, he was frequently seen at yeast meetings, both in the United Kingdom and in other countries throughout the world. He had an extremely lively mind and could rarely resist the temptation to indulge in discussions, heated or otherwise, always taking great care to respect the confidential nature of much of the research which

he was directing at Glenochil. As a friend, colleague and adversary in discussion, he is deeply missed.

Anthony H. Rose
University of Bath, England

* * *

Your Editor has recently learned of the tragic news that Dr. Siavash Baharaeen was killed in an automobile accident on November 24, 1982. He was doing postdoctoral research in the Department of Biochemical Sciences, University of Houston, Texas, after obtaining his Ph.D. degree with Dr. Helen Vishniac of the Department of Microbiology at Oklahoma State University. Dr. Baharaeen made an important contribution to the taxonomy of antarctic yeast species by the technique of DNA-DNA complementarity experiments. He was to have given a lecture on his research at the third International Mycological Congress in Tokyo in August 1983.

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XXXVII. Meetings

1. The Fifteenth Annual Meeting of the Yeast Genetics Society of Japan was held from October 19 to 21, 1982 at the Ohmori Plant of Asahi Breweries Ltd., Tokyo, Japan. The following topics were presented and discussed.

   Session 1: Gene Regulation, Recombination and Mapping (Chairpersons, H. Mori, H. Tamaki and T. Takahashi).

   T. Takahasi and K. Sakai (Centr. Res. Lab., Asahi Breweries Ltd.). Three genes controlling H_2S production in Saccharomyces cerevisiae.

   T. Yamasaki (Dept. Physics, Rikkyo Univ.). Increase in mitotic recombination frequencies in phosphate buffer in yeast cells.

   T. Yamazaki (Dept. Ferment. Technol., Yamanashi Univ.). Discrimination of auxotrophic mitotic recombinants in Saccharomycodes ludwigii by using dye plate.

   T. Tsuzaki, K. Matsumoto, I. Uno* and T. Ishikawa* (Dept. Ind. Chem., Tottori Univ., *Inst. Appl. Microbiol., Univ. Tokyo). Isolation of mutants bearing the nonsense cyr1 mutation in Saccharomyces cerevisiae.

   T. Yoshimatsu, K. Matsumoto, I Uno* and T. Ishikawa* (Dept. Ind. Chem., Tottori Univ., *Inst. Appl. Microbiol., Univ. Tokyo). Isolation and characterization of suppressor mutants (scy) from cyr1 strains.

   S. Takemura, K. Matsumoto, I Uno* and Y. Kaneko** (Dept. Ind. Chem., Tottori Univ., *Inst. Appl. Microbiol., Univ. Tokyo, **Dept. Ferment. Technol., Osaka Univ.). Isolation of processing mutants of nonspecific phosphatase from pep4 strain.

   H. Tamaki (Doshisha Women's Coll.). On the additional chromosome of abortive cells resulting from the protoplast fusion in yeasts.

J. Ishiguro (Dept. Biol., Konan Univ.). Aneuploid mapping of a structural gene coding for ribosomal protein L23 of the yeast S. cerevisiae.

S. Hayashi, Y. Nakaseko, T. Toda and M. Yanagida (Dept. Biophys., Kyoto Univ.). Isolation and characterization of S. pombe mutants supersensitive to thiabendazole.

Session 2: Sporulation and Life Cycles I (Chairpersons, N. Sando and M. Tsuboi).

O. Niwa (Dept. Biophys., Kyoto Univ.). Isolation of a S. pombe mutant which sporulates without nitrogen starvation.

C. Shimoda (Dept. Biol., Osaka City Univ.). Genetic analysis of meiosis-deficient mutants in the fission yeast Schizosaccharomyces pombe.

M. Tsuboi (Dept. Biol., Osaka City Univ.). Genetic analysis of nonsporulating mutants of Saccharomyces cerevisiae.

Y. Nakatomi (Oriental Yeast Co. Ltd.). Protoplast fusion between Saccharomyces rosei and Saccharomyces cerevisiae.

I. Miyakawa, H. Aoi, T. Eguchi, N. Sando, S. Nishibayashi* and T. Kuroiwa* (Biol. Inst., Yamaguchi Univ., *Dept. Cell Biol., Natl. Inst. Basic Biol.). Configurational changes in mitochondrial nucleoids during mating, meiosis and sporulation of Saccharomyces cerevisiae.

S. Okamot, M. Yamamoto and T. Iino* (Inst, Medical Sci., *Lab. Genetics, Univ. Tokyo). Spindle pole body morphogenesis in hfd1-1 and its suppressor, SPS mutants in Saccharomyces cerevisiae.

K. Matsumoto, I Uno* and T. Ishikawa* (Dept. Ind. Chem, Tottori Univ., *Inst. Appl. Microbiol., Univ. Tokyo). Control of cell division in Saccharomyces cerevisiae mutants defective in adenylate cyclase and cyclic AMP-dependent protein kinase.

I. Uno, K. Matsumoto* and T. Ishikawa (Inst. Appl. Microbiol., Univ. Tokyo, *Dept. Ind. Chem., Tottori Univ.). Initiation of meiosis in yeast mutants defective in adenylate cyclase and cyclic AMP-dependent protein kinase.

Session 3: Radiation Effects and Mutation (Chairpersons, T. Ito, S. Nakai and K. Hieda).

Y. Iwamoto, I. Mifuchi and K.L. Yielding* (Dept. Microbiol., Shizuoka Col. Pharm., *Dept. Anat., Univ. South Alabama). Cell inactivation and mutagenic effects by photodynamic action of 3-azide-10-methylacridinium chloride.

S. Nakai and I. Machida (Div. Genet., Natl. Inst. Rad. Sci.). Induction of sister chromatid recombination by radiations in Saccharomyces cerevisiae.

I. Machida and S. Nakai (Div. Genet., Natl. Inst. Rad. Sci.). Induction of mutations, intra- and intergenic recombination by near-UV light in Saccharomyces cerevisiae.

Y. Hayakawa and K. Hieda (Dept. Phys., Rikkyo Univ.). Effects of fluence fractionation on induction of UV damage in diploid yeast cells.

K. Hieda, K. Kobayashi*, A. Ito** and T. Ito** (Dept. Phys., Rikkyo Univ., *Inst. Biol. Sci., Univ. Tsukuba, **Inst. Phys., Col. Gen. Educ., Univ. Tokyo). Comparisons of far- and vacuum-UV radiation effects on dry yeast cells.

A. Ito and T. Ito (Inst. Phys., Col. Gen. Educ., Univ. Tokyo). Sensitivities to the membrane-attacking phototreatments in exponential and stationary yeast cells.

T. Watanabe and T. Ito (Inst. Phys., Col. of Gen. Educ., Univ. Tokyo). Photooxidation of lipids in yeast (Saccharomyces cerevisiae) cells: comparison of near-UV action with photo-sensitization by exogeneous sensitizers.

K. Kobayashi (Inst. Biol. Sci., Univ. Tsukuba). Gene conversion induction by ionizing radiation in repair deficient strains of yeast, Saccharomyces cerevisiae.

K. Adzuma, T. Ogawa and H. Ogawa (Dept. Biol., Osaka Univ.). Isolation and nucleotide sequence of RAD52 gene in Saccharomyces cerevisiae.

Session 4: Biochemistry (Chairpersons, T. Mizunaga, K. Suda, I. Uno and S. Harashima).

M. Sunairi, K. Watabe, M. Takagi and K. Yano (Dept. Agricul. Chem., Univ. Tokyo). n-Alkane induced microsomal proteins of Candida yeast.

H. Iida and I. Yahara (Tokyo Metrop. Inst. Med. Sci.). Durable synthesis of heat shock proteins in G_0 cells of yeast and higher eucaryotes.

R. Miyama, M. Ikeda, T. Miyakawa, E. Tsuchiya, I. Yamashita and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Inhibition of biosynthesis of lipopeptidyl mating hormone in Tremella mesenterica by compactin.

S. Murakami, J.W. Bodley* and D.M. Livingston* (Biophys. Div., Cancer Res. Inst., Kanazawa Univ., *Dept. Biochem., Medical School, Univ. Minnesota). Sensitivity of Saccharomyces cerevisiae spheroplasts to the action of diphtheria toxin.

M. Miyazaki and M. Uritani (Inst. Mol. Biol., Nagoya Univ.). Studies on the cycloheximide sensitivity factor (CH-SF) in the S-100 fraction from yeasts.

A. Tsuboshita, E. Tsuchiya, T. Miyakawa, I. Yamashita and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Purification and properties of a cytoplasmic factor which activates chromosomal DNA synthesis by isolated nuclei prepared from Saccharomyces cerevisiae.

E. Tsuchiya, T. Miyakawa, I. Yamashita and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Preparation of *in vitro* DNA-replicating-system from yeast nuclei.

T. Miyakawa, K. Imai, M. Nishihara, E. Tsuchiya, I. Yamashita and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Role of rhodotorucine A metabolism at the cell surface of the target cell and the properties of the metabolic enzyme.

K. Matsumoto, I Uno* and T. Ishikawa* (Dept. Ind. Chem, Tottori Univ., *Inst. Appl. Microbiol., Univ. Tokyo). Characterization of cyclic AMP-requiring yeast mutants (CYR3) altered in the regulatory subunit of protein kinase.

I Uno, K. Matsumoto* and T. Ishikawa (Inst. Appl. Microbiol., Univ. Tokyo, *Dept. Ind. Chem., Tottori Univ.). Characterization of cyclic nucleopeptide phosphodiesterase (PDE I) - deficient mutant in yeast.

K. Adachi, I. Uno, K. Matsumoto* and T. Ishikawa (Inst. Appl. Microbiol., Univ. Tokyo, *Dept. Ind. Chem., Tottori Univ.). Substrates for cyclic AMP-dependent protein kinase in yeast.

Session 5: Structure and Function of Cell Organelles (Chairpersons, M. Osumi, K. Tanaka and T. Hirano).

K. Takeo (Res. Inst. Chemobiodynamics, Chiba Univ.). Cytological studies on freezing damage of yeasts, especially *Saccharomyces cerevisiae* and *Candida albicans*.

T. Hirano, M. Yamaguchi and A. Tanaka* (Jikei Univ. Sch. Med., *Tokyo Metrop. Inst. Med. Sci.). Surface structure of yeast protoplasts.

T. Kamihara, Y. Uejima, I. Nakamura, Y. Yamada* and Y. Tani* (Dept. Indust. Chem., Kyoto Univ., *Seibo Women's Jr. Col.). Role of inositol in morphogenesis in *Candida tropicalis*.

T. Hatano, K. Hamada, Y. Okubo, E. Tsuchiya, T. Miyakawa, I. Yamashita and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Biochemical changes in cell surface during sexual differentiation in the heterobasidiomycete *Tremella mesenterica*.

K. Umesono, T. Toda and M. Yanagida (Dept. Biophys., Kyoto Univ.). Visualization of chromosomes in *S. pombe*.

K. Tanaka and T. Kanbe (Inst. Med. Mycol., Nagoya Univ. Sch. Med.). Mitochondrial topology during cell division in *Candida albicans*.

M. Nagano, M. Osumi, N. Baba* and K. Kanaya* (Dept. Biol., Japan Women's Univ., *Dept. Elec. Eng., Kogakuin Univ.). Analysis of filamentous structure in yeast mitochondria.

M. Osumi, M. Nagano, N. Baba* and K. Kanaya* (Dept. Biol., Japan Women's Univ., *Dept. Elec. Eng., Kogakuin Univ.). Structural analysis of the microbody crystalloid in yeast grown in methanol by digital processing method.

Session 6: Sporulation and Life Cycle II (Chairpersons, N. Yanagishima and E. Tsuchiya).

K. Tanaka, S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Further characterization of homothallic mutation, hmlα2, in Saccharomyces cerevisiae.

K. Tanaka, S. Harashima and Y. Oshima (Dept. Ferment. technol., Osaka Univ.). Directionality of mating type interconversion in Saccharomyces cerevisiae.

T. Suzuki, S. Nishibayashi*, T. Kuroiwa* and K. Tanaka (Inst. Med. Mycol., Nagoya Univ., *Dept. Cell Biol., Natl. Inst. Basic Biol.). Ploidy in Candida albicans.

H. Fujimura, K. Yohida and N. Yanagishima (Biol. Inst., Nagoya Univ.). α Pheromone-binding protein in a cells in Saccharomyces cerevisiae.

Y. Nakagawa and N. Yanagishima* (Biol. Lab., Col. Gen. Educ., *Biol. Inst., Nagoya Univ.). Do diploid cells produce sex pheromone?

Session 7: Cytoplasmic Inheritance (Chairperson, C. Shimoda).

A. Uchida, A. Takano* and K. Suda** (Biol. Div., Col. Gen. Educ., Kobe Univ., *Dept. Agri., Kyoto Univ., **Biol. Lab., Nara Univ. Educ.). Distribution of ultraviolet light irradiated mitochondrial genomes during meiosis in yeast.

K. Suda, T. Takegami, K. Sonoda, K. Ikai*, S. Nagai* and A. Uchida** (Biol. Lab., Nara Univ. Educ., *Dept. Biol., Nara Women's Univ., **Biol. Div., Col. Gen. Educ., Kobe Univ.). Stable state of rho⁻ mutants in Saccharomyces cerevisiae.

K. Ikai, S. Nagai, A. Morimoto*, K. Suda* and A. Uchida** (Biol. Lab., Nara Women's Univ., *Biol. Lab., Nara Univ. Educ., **Biol. Div., Col. Gen. Educ., Kobe Univ.). Suppressiveness and segregation of mitochondrial drug-resistance markers during sporulation in Saccharomyces cerevisiae.

Session 8: Plasmid and Cloning (Chairpersons, M. Yamamoto, N. Gunge, M. Yanagida, K. Matsumoto, T. Ikemura, M. Takagi, T. Fukasawa and Y. Oshima).

T. Ikemura (Dept. Biophys., Kyoto Univ.). Gene expressivity and codon choice pattern.

Y. Kikuchi (Lab. Mol. Genetics, Keio Univ. Sch. Med.). Stable maintenance of the 2μm-plasmid.

N. Gunge and C. Yamane (Mitsubishi-Kasei Inst. Life Sci.). Incompatibility of pGK1-killer plasmids with mitochondrial DNA from S. cerevisiae.

Y. Shimada, S. Harashima* and Y. Oshima* (Osaka Municipal Tech. Res. Inst., *Dept. Ferment. Technol., Osaka Univ.). Structural characterization of mini-plasmid originated from yeast plasmid, YRp7.

T. Sakurai, H. Araki and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Structural and functional analyses of the pSR1 plasmid isolated from Saccharomyces rouxii.

H. Tatsumi, K. Ushio* and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., *Higashimaru Shoyu Co. Ltd.). Replication and recombination functions associated with pSR1 plasmid of Saccharomyces rouxii.

K. Ushio, H. Tatsumi* and Y. Oshima* (Higashimaru Shoyu Co. Ltd., *Dept. Ferment. Technol., Osaka. Univ.). Studies on host-vector system of Saccharomyces rouxii.

J. Sakaguchi and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Construction of plasmids which polymerize in tandem and propagate autonomously in Schizosaccharomyces pombe.

K. Sakai and M. Yamamoto* (Centr. Res. Lab., Asahi Brew. Ltd., *Inst. Med. Sci., Univ. Tokyo). Expression of the resistance gene to a drug G418 in fission yeast.

K. Suzuki, I. Yamashita, E. Tsuchiya, T. Miyakawa, H. Sakamoto and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Transformation of Saccharomyces cerevisiae with linear deoxyribonucleic acids.

A. Takagi, S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Cell fusion-associated transformation in Saccharomyces cerevisiae.

K. Noguchi, O. Niwa* and M. Yanagida* (Katakurachikkarin Co., Ltd., *Dept. Biophys., Kyoto Univ.). Plasmid transfer between Schizosaccharomyces pombe and Saccharomyces cerevisiae by cell fusion.

M. Yamakawa, C. Yamane and N. Gunge (Mitsubishi-Kasei Inst. Life Sci.). Transformation of yeast cells by PEG treatment.

M. Tsuboi and M. Ohi (Dept. Biol., Fac. Sci., Osaka City Univ.). Selection of cells transformed with plasmid YRp7 by protoplast sporulation in Saccharomyces cerevisiae.

T. Toda and M. Yanagida (Dept. Biophys., Kyoto Univ.). Cloning of tubulin genes by complementation in fission yeast.

M. Yamamoto and J. Sakaguchi (Inst. Med. Sci., Tokyo Univ.). Detection of DNA segments in Schiz. pombe which have homology with Chlamydomonas tubulin cDNAs.

T. Fukasawa and Y. Nogi (Lab. Mol. Genetics, Keio Univ. Sch. Med.). Isolation of DNA fragment which suppresses a mutation in the regulatory gene GAL80 in Saccharomyces cerevisiae.

H. Hashimoto, Y. Kikuchi*, Y. Nogi* and T. Fukasawa* (Nikka Whisky Ltd., *Lab. Molec. Genetics, Keio Univ. Sch. Med.). Isolation and dosage effect of yeast regulatory gene GAL4.

S. Okamoto and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Characterization of methyl benzimidazol-2-yl-carbamate resistant mutants and cloning of the gene in Saccharomyces cerevisiae.

S. Irie, M. Yamadaki*, S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., *CALPIS Co. Ltd.). Increased expression of the yeast HIS5 gene in Escherichia coli.

T. Mizunaga, A. Takasaki, K. Arima* and T. Oshima* (Dept. Agr. Chem., Univ. Tokyo, *Suntory Inst. Biomed. Res.). Product of cloned yeast repressible acid phosphatase gene (PHO 5) in Saccharomyces cerevisiae and Escherichia coli.

N. Nakanishi and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Cloning of the complex locus aro 3 in Schizosaccharomyces pombe.

Y. Kaneko, A. Toh-e* and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., *Dept. Ferment. Technol., Hiroshima Univ.). Isolation of a DNA fragment which complements the pho8 mutation of the repressible alkaline phosphatase structural gene.

K. Ogawa, Y. Kaneko, A. Toh-e* and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., *Dept. Ferment. Technol., Hiroshima Univ.). Isolation of DNA fragments which complement the phosphatase regulatory gene mutations.

Y. Nogi, H. Hashimoto* and T. Fukasawa (Lab. Molec. Genetics, Keio Univ. Sch. Med., *Nikka Whisky Ltd.). The nucleotide sequence of the 5'-flanking region of yeast GAL7 gene.

K. Arima, N. Nakamura*, T. Oshima, I. Kubota, T. Mizunaga** and A. Toh-e*** (Suntory Inst. Biomed. Res. *Suntory Centr. Res. Inst., **Dept. Agr. Chem., Tokyo Univ., ***Dept. Ferment. Technol., Hiroshima Univ.). DNA sequence of yeast repressible acid phosphatase (PHO5) gene.

M. Takagi, M. Chang, N. Tanaka and K. Yano (Dept. Agricul. Chem., Univ. Tokyo). Construction and utilization of the gene library of Candida maltosa.

T. Oshima, T. Ashikari*, K. Matsubara, S. Tanaka and H. Nakazato (Suntory Inst. Biomed. Res., *Suntory Centr. Res. Inst.). High expression vector of Saccharomyces yeast.

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

An intensive international course on "Yeast Taxonomy and Yeast Identification" took place in our Institute from 20 September - 15 October 1982. The teachers were N.J.W. Kreger-van Rij (Groningen, Netherlands),

C.P. Kurtzman (Peoria, Illinois), S.A. Meyer (Atlanta, Georgia), J. Fell (Miami, Florida) and J. Barnett (Norwich, U.K.). The twenty students came from the following countries: Federal Republic of Germany, Czechoslovakia, Canada, The Netherlands, United Kingdom, Mexico, People's Republic of China, Brazil, Portugal, Japan, Spain, U.S.A., Philippines and the Soviet Union.

In the unanimous view of the students, the teachers and the host Institute the course had exceptional quality and was a full success both scientifically and socially. A second international course on the same subject is being planned for 1984.

The following three students of the course are staying in the Institute for a period of five months to do yeast work in a UNESCO-sponsored program:

Mrs. Michiko Kobatake, National Institute of Hygienic Sciences, Tokyo, Japan;
Mrs. Li-Ming-Xia, Institute of Microbiology, Academia Sinica, Beijing, China;
Miss Jovita Martinez-Cruz, Department of Biotechnology and Bioengineering, National Polytechnical Institute, Mexico City, Mexico.

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3. VIth International Symposium on Yeast, July 9-13, 1984, Montpellier, France.

First Circular

In accordance with the resolution of the fifth International Symposium on Yeasts in London in 1980, the 6th International Symposium on Yeast will be held on July 9-13, 1984 at Faculty of Pharmacy, 15 avenue Charles Flahault, 34060 Montpellier, France. The old city of Montpellier is a nice town standing at eight kilometers from the Mediterranean seaside and fifty kilometers from the Cevennes mountains. July is usually warm and sunny.

Scientific Programme

Suggested topics are as follows*:

1. Nutrition and growth
2. Oxydative and fermentative metabolism
3. Subcellular structure biochemistry
4. Biological cycle
5. Biosynthesis and regulation
6. Enzymology and mechanism of enzyme action
7. Technics for genetical and biochemical studies of yeast
8. Histochemistry and cytology
9. Genetics
10. Ecology
11. Pathology and immunology
12. Industrial and agricultural yeasts
13. Membrane and transport
14. Taxonomy

*Suggestions for further topics, free sections or panel discussions on special themes will be accepted.

Membership

Membership will be open to all persons interested in the scientific programme. In addition, accompanying members are also welcome.

Language

The language of the Symposium will be english.

Social Programme

Social programme will be organized both for active and accompanying members.

Accommodation

Possibilities will be indicated in a further circular.

Preliminary registration forms may be obtained from the Administrative Secretariat and should be returned before March 1, 1983. Further announcements will be sent to those who return this form.

Scientific Secretariat
Chaire de Genetique et Microbiologie
Ecole Nationale Superieure Agronomique
Place Viala
34060 Montpellier Cedex - France
Tel. (67) 63.02.02

Administrative Secretariat
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Le Capoulie
Rue Maguelone BP 1041
34006 Montpellier Cedex - France
Tel. (67) 92.42.72

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XXXVIII. Brief News Items

1. It is my great pleasure to announce to all of the readers of the Yeast NewsLetter that I have become the curator of the Mycology Division of the Japan Collection of Microorganisms (JCM) last September. JCM was newly established two years ago as the semi-governmental culture collections of microorganisms of Japan, and the Mycology Division has started this year.

   I would greatly appreciate receiving cultures for our Institute of yeasts and yeast-like fungi from all of the contributors of the Yeast Newsletter.

Dr. T. Nakase
Curator of Mycology
Japan Collection of Microorganisms
The Institute of Physical and
Chemical Research,
Hirosawa, Wako-Shi, Saitama, 351,
Japan

2. Below follows news from our laboratory.

Two of us attended the 11th International Conference on "Yeast Genetics and Molecular Biology", Montpellier, France, September 13-17, 1982 with the following communications:
- R. Montrocher & M.L. Claisse. Cytochrome spectra of some Candida and related yeasts, taxonomic implications.
- J.B. Fiol & M.L. Claisse. Cytochrome spectra of Kluyveromyces yeasts: taxonomic implications.

M.C. Pignal, Section Levures
Box 405, University of Lyon I
43 Blvd du 11 Nov. 1918
69622 Villeurbanne, Cedex France

3. B.N. Johri and K. Johri. Reaction of heterobasidiomycetous yeast genera Bullera and Sporobolomyces to Diazonium Blue B. Current Science 51:613-614. (Dept. of Microbiology, G.B. Pant University of Agriculture & Technology, Pantnagar 263 145, India).

4. M.A. Lachance. 1982. Reaction of an ascomycetous yeast with Diazonium Blue B. Can. J. Microbiol. 28:1194-1195. (Dept. of Plant Sciences, Univ. of Western Ontario, London, Ont., Canada, N6A 5B7).

5. B.F. Johnson, G.B. Calleja, B.Y. Yoo, M. Zucker, and I.J. McDonald. 1982. Cell Division: Key to cellular morphogenesis in the fission yeast, Schizosaccharomyces. International Review of Cytology, 75:167-208. (Division of Biological Sciences, National Research Council of Canada, Ottawa, Ont., Canada, K1A OR6).

6. The Mycology Unit in the National Institute of Amazonian Research (I.N.P.A.) is pursuing studies on the natural habitats of pathogenic fungi within the Amazon biota. In past and recent studies with soil samples, quiropterans and amphibians, we have encountered a diversified mycoflora composed of medical and non-medical yeasts. Our present efforts focus on the taxonomy of these isolates, and prospective research includes examination on possible host-parasite relationship and numerical systematics.

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The following paper has been published.

W.Y. Mok, F.P. Castelo and M.S. Barreto Da Silva, 1981. Occurrence of Exophiala werneckii on salted freshwater fish Osteoglossum bicirrhosum. J. Fd Technol. 16, 505-512.

Summary

Fungal contamination occurred on the salted Amazonian fish Osteoglossum bicirrhosum which were air dried in the shade. The human pathogenic fungus Exophiala werneckii was recovered as the sole contaminant from all salted

fish. It was implicated for the first time as a halophilic saprophyte causing food spoilage.

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7. International Journal of Systematic Bacteriology. The Editorial Board of the IJSB has been reorganized because of excessive delays in processing manuscripts. The new Editor-in-Chief has delegated to the Associate Editors full authority for the selection of reviewers and acceptance of manuscripts. It is our aim to shorten the time from receipt of manuscripts to the publication stage from the present 9-12 months to 3-6 months. The undersigned will handle all manuscripts dealing with yeast systematics. Instructions to authors are given in the January issue. There are no page charges. Manuscripts must be submitted to ASM Publication Office, 1913 I Street N.W., Washington, DC 20006, USA.

H.J. Phaff, Associate Editor