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Y E A S T

A Newsletter for Persons Interested in Yeast

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Herman J. Phaff, Editor
University of California, Davis, California 95616

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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.

Herman J. Phaff
Editor

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

The strains listed below have been added to the ATCC since October 31, 1983. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

Saccharomyces cerevisiae ATCC 52679-52680	G.A. Farris University Sassari Italy
Sarcinomyces crustaceus ATCC 52681	G. Criseo University Messina Italy
Pityrosporum pachydermatis ATCC 52682	W. Nicklas Heidelberg Germany
Saccharomyces cerevisiae ATCC 52683	J. Carbon University of California Santa Barbara, CA
Saccharomyces rouxii ATCC 52698	M. Tamita Tokushima Pref. Ind. Res. Inst. Japan

Candida vini, ATCC 52706; Kluyveromyces fragilis, ATCC 52707; Kluyveromyces lactis, ATCC 52708; Kluyveromyces veronae, ATCC 52709; Metschnikowia pulcherrima, ATCC 52710; Saccharomyces bailii var. osmophilus, ATCC 52711; Saccharomyces chevalieri, ATCC 52712; Saccharomyces fermentati, ATCC 52713; Saccharomyces rosei, ATCC 52714; Saccharomyces rouxii, ATCC 52715; Saccharomyces uvarum, ATCC 52716; Saccharomycodes ludwigii, ATCC 52717

	I. Benda Bayerische Landesanstalt W. Germany
Kluyveromyces lactis ATCC 52732-52735	N. Gunge Mitsubishi-Kasei Institute of Life Sciences Tokyo, Japan
Cryptococcus neoformans ATCC 52815-52817	E.S. Jacobson Veterans Administration Med. Ctr. Richmond, VA

Candida agrestis, ATCC 52818; Candida montana, ATCC 52819; Candida parapsilosis, ATCC 52820; Cryptococcus laurentii var. flavescens, ATCC 52821; Pichia ohmeri, ATCC 52822; Rhodotorula fujisanensis, ATCC 52823; Saccharomyces rosei, ATCC 52824; Torulopsis colliculosa, ATCC 52825; Torulopsis stellata, ATCC 52826; Torulopsis dattila, ATCC 52827; Torulopsis kruisii, ATCC 52828

	S. Goto Yamanashi University Japan
Candida pulcherrima ATCC 52897	T. Okuda Nippon Roche Research Center Japan

Bullera armeniaca, ATCC 52900; Bullera crocea, ATCC 52901; Cryptococcus hinnuleus, ATCC 52902; Cryptococcus phylloplanus, ATCC 52903; Dekkera intermedia, ATCC 52904-52905; Pachytichospora transvalensis, ATCC 52906; Rhodotorula armeniaca, ATCC 52907; Sporobolomyces elongatus, ATCC 52908; Sporobolomyces foliicola, ATCC 52909

	D. Yarrow CBS The Netherlands
Saccharomyces cerevisiae ATCC 52910	T. Placido Gulbenkian Institute Portugal

Endomycopsis burtonii, ATCC 52919-52920; Endomycopsis fibuligera, ATCC 52921; Saccharomyces uvarum, ATCC 52922

	H. Sakai University of Philippines Philippines
Kluyveromyces lactis ATCC 52934	L. de Louvencourt Institut Nat'l Agronomique Paris, France
Saccharomyces cerevisiae ATCC 56009	H.M. Fried University of North Carolina Chapel Hill, NC
Saccharomyces chevalieri ATCC 56069	F.R. Uyenco University of Philippines Philippines
Saccharomyces diastaticus ATCC 56072	H. Tamaki Doshisha Women's College Kyoto, Japan

Saccharomyces bailii, ATCC 56074-56075; Saccharomyces bisporus var. mellis, ATCC 56076; Saccharomyces rouxii, ATCC 57077

	I. Banno Institute for Fermentation, Osaka Japan
Saccharomyces cerevisiae ATCC 56107	M.F. Tuite University Kent England

Saccharomyces cerevisiae ATCC 56108-56116	Bun-ichiro Ono Okayama University Okayama, Japan
Candida albicans ATCC 56118-56120	M. Casel Roman Cordoba University Spain
Saccharomyces cerevisiae ATCC 56121-56122	N. Pearsen University of Maryland Catonsville, MD
Pichia humboldtii ATCC 56128	D. Yarrow CBS The Netherlands
Saccharomyces cerevisiae ATCC 56183	R. Eschenbruck DSIR Te Kawwhata, New Zealand
Debaryomyces vanriji ATCC 56221	N. Kato Tottori University Japan
Pichia antillensis ATCC 56265-56267	M. Miranda University of California Davis, CA
Pichia sp. ATCC 56274	J.J. Ellis NRRL Peoria, IL
Saccharomcyes cerevisiae ATCC 56287	A. Wiemken Institut Allg. Botanik Zurich, Switzerland

Candida methylica, ATCC 56294; Cryptococcus aerius, ATCC 56295-56296, 56298; Cryptococcus albidus, ATCC 56297; Lipomyces lipofer, ATCC 56302-56303; Lipomyces starkeyi, ATCC 56304-56305; Lipomyces tetrasporus, ATCC 56306; Torulopsis wickerhamii, ATCC 56307

W.I. Golubev
Inst. Biochem. Physiol. Microorg.
Moscow, USSR

Kluyveromyces lactis ATCC 56309	R.C. Dickson University of Kentucky Lexington, KY
Saccharomyces cerevisiae ATCC 56395-56401	H. Roman University of Washington Seattle, WA

Ambrosiozyma monospora, ATCC 56460; Brettanomyces bruxellensis, ATCC 56461;

Candida homilentoma, ATCC 56462; Candida magnoliae, ATCC 56463; Candida milleri, ATCC 56464; Candida naeodendra, ATCC 56465; Candida parasilosis var. quercus, ATCC 56466; Citeromyces matritensis, ATCC 56467-56468; Cryptococcus amylolentus, ATCC 56469; Debaryomyces nepalensis, ATCC 56470; Debaryozyma yamadae, ATCC 56471; Kluyveromyces thermotolerans, ATCC 56472

C.P. Kurtzman
NRRL
Peoria, IL

Trichosporon inulinus
ATCC 56475

H. Sakai
University of Philippines
Philippines

Saccharomyces capensis, ATCC 56476; Saccharomyces cerevisiae, ATCC 56477; Saccharomyces oviformis, ATCC 56478; Saccharomyces rosei, ATCC 56479

E. Minarik
Bratislava
Czechoslovakia

Saccharomyces cerevisiae
ATCC 56489

M.J. Fletcher
DSIR
Auckland, New Zealand

Kluyveromyces aestuarii, ATCC 56491; Kluyveromyces africanus, ATCC 56492; Kluyveromyces blattae, ATCC 56493; Kluyveromyces bulgaricus, ATCC 56494; Kluyveromyces cicerisporus, ATCC 56495; Kluyveromyces drosphilarum, ATCC 56496; Kluyveromyces fragilis, ATCC 56497; Kluyveromyces lactis, ATCC 56498; Kluyveromyces polyporus, ATCC 56499; Kluyveromyces waltii, ATCC 56500; Kluyveromyces wikenii, ATCC 56501

H.J. Phaff
University of California
Davis, CA

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II. National Collection of Yeast Cultures, Colney Lane, Norwich NR4 7UA, UK. Communicated by Barbara Kirsop.

Recent Publications:

Barbara Krisop, 1983, "Culture Collections - their services to Biotechnology," Trends in Biotechnology, 1(No. 1), 4-8.

The services available from the major culture collections are described, generally using the UK service collections as examples. The names and addresses of the main collections are listed.

* * *

Kevin Painting & Barbara Kirsop. 1984. A note on the presence of novel DNA species in the spoilage yeasts Zygosaccharomyces bailii and Pichia membranaefaciens. Journal of Applied Bacteriology, 56, 331-336.

Two novel covalently closed circular DNA species of 5.4 and 6.0 kilobases were detected in strains of Zygosaccharomyces bailii with a rapid small scale isolation procedure. The 5.4 kb species was found in four strains and both species were found in three strains. A novel, covalently-closed circular DNA species of 6.9 kb was detected in four of 12 strains of Pichia membranaefaciens. Plasmid DNA (2 μm) (that is CCC DNA of approximately 6 kb in Saccharomyces cerevisiae) was detected in 38 of 40 strains of Sacch. cerevisiae confirming reports of the widespread distribution of this plasmid.

* * *

Barbara Kirsop & Jane Henry, 1984. Development of a miniaturised cryopreservation method for the maintenance of a wide range of yeasts. Cryoletters, in press.

Using aerobically grown, stationary phase cells and 5% glycerol as a cryoprotectant, a 2-step cooling method has been established for the cryopreservation of a wide range of yeasts. Survival levels are high and strains appear stable. Respiratory deficient mutants were not detected and plasmids were not lost during freezing and thawing. A miniaturised method using polypropylene straws has been developed.

* * *

NCYC Databank

As part of the UK Department of Trade & Industry's support for biotechnology, a scheme is under active review to establish a databank of the information held in the UK national collections. This is expected to be an extension of the NCYC databank already in operation. The NCYC databank includes between 50 to 100 characters on about 2000 yeast strains. The information on each strain is morphological, physiological and industrial and has been obtained using the methods prescribed in the standard work on yeast taxonomy, "The yeasts. A taxonomic study" edited by J. Lodder (1970, North Holland Publishing Co.). The databank may be searched for specific characters or combinations of characters and printouts may be obtained of all available data for a particular strain.

NCYC Computer Identification

The NCYC has prepared a probabilistic record of selected characterisitics of yeast taxa for use in the identification of unknown strains. The system is based on that described by Lapage et al. (Automation, Mechanisation and Data Handling in Microbiology, Society of Applied Bacteriology Technical Series No. 4, 1970, Academic Press) in which identification scores are calculated on the taxa most like the unknown strain. Comparing this system with the method used routinely by the NCYC, very high correlation has been obtained. Of the first 50 strains compared, 98% were identified as the same taxon. Of these, however, 10% obtained low scores and were found to represent ill-defined taxa with a high level of variable characters. The 2% of strains not identified as the same taxon could not be identified with any certainty by the standard method. It is felt that the method represents advantages to inexperienced users over keys, since an identification depends upon consideration of all tested characteristics - in effect simultaneously - rather than sequential

consideration of those tests occurring in the parts of the key in use.

* * *

III. Czechoslovak Collection of Yeasts, Centre of Chemical Research of the Slovak Academy of Sciences, Institute of Chemistry, Dubravska cesta 9, 84238 Bratislava, CSSR. Communicated by A. Kockova-Kratochvilová.

Below follows a list of publications from our group since 1980. Summaries are given for recent papers.

1. Kocková-Kratochvílová A., Černáková M., Slávíková E. (1980): Morphological changes during the life cycle of Aureobasidium pullulans (de Bary) Arnaud. Folia microbiol. 25:56-67.

2. Černáková M., Kocková-Kratochvílová A., Šuty L., Zemek J., Kuniak L, (1980): Biochemical similarities among strains of Aureobasidium pullulans (de Bary) Arnaud. Folia microbiol. 25:68-73.

3. Slávíková E., Kocková-Kratochvílová A. (1980): The yeasts of the genus Aureobasidium transferred by insects on the Lowlands of Zahorie (Slovakia, CSSR). Česká mykologie 34:199-207.

4. Slávíková E., Kocková-Kratochvílová A. (1980): The yeasts of the genus Debaryomyces transferred by insects on the Lowlands of Zahorie. Česká mykologie 34:21-28.

5. Rybarova J., Stros F., Kockova-Kratochvilova A. (1980): Candida ethanolica n.sp. Ztschr. allg. Microbiol. 20:579-581.

6. Slávíková E., Kocková-Kratochvílová A. (1981): The yeasts of different genera transferred by insects on the Lowlands of Zahorie. Česká mykologie 35:192-195.

7. Rybářová J., Štros F., Kocková-Kratochvílová A. (1981): Torulopsis ethanolitolerans n. sp. and T. ethanolitolerans var. minor n. var. Ztschr. allg. Microbiol. 21:739-742.

8. Kocková-Kratochvílová A., Slávíková E., Zemek J., Augustin J., Kuniak L., Dercová K. (1981): Numerical taxonomy of the genus Schwanniomyces Klocker. Biologia (Bratislava) 36:693-701.

9. Kocková-Kratochvílová A., Markovič O., Slávíková E. (1981): Phylogenetic significance of protein and nucleotide content in yeasts. Folia microbiol. 26:221-227.

10. Kocková-Kratochvílová A. (1982): Urease and extracellular nucleases of yeasts. Folia microbiol. 27:404-412.

11. Kocková-Kratochvílová A., Slávíková E., Zemek, J., Kadlečíková B., Kuniak L. (1983): Utilization of the hydrolytic activity in taxonomy of hyphal yeast Ascomycetes. Folia microbiol. 28:301-308.

Species producing pseudohyphae and true hyphae and forming asci with endospores were included in the studied group of yeast-like microorganisms.

These species belong taxonomically to various genera, i.e., Saccharomycopsis, Endomycopsis, Guilliermondiella, Arthroascus, Hormoascus, a.s. The group Candida lipolytica was treated separately (Blagodatskaja and Kockova-kratochvilova, Biologia 28:709, 1973). The verified phenotypes were compared using the numerical method and according to their hydrolytic activity.

12. Kocková-Kratochvílová A., Hubálek Z. (1983): Liquid nitrogen storage of yeast cultures. II. Stability of characteristics of studied strains. Antonie v. Leeuwenhoek 49:571-578.

Nineteen strains of yeasts possessing different characteristics were stored in liquid nitrogen and after 5 years phenotypic characters were evaluated and compared with equivalent strains preserved under paraffin oil. All qualitative characters tested remained stable, and quantitative characters varied only within the range of natural variability.

13. Kocková-Kratochvílová A., Švorcová L., Breierová E., Delgado R. (1984): Taxonomic characteristics of yeasts isolated from bath facilities. Česká mykologie 38:11-20.

This paper deals with the taxonomy an identification of yeasts and yeast-like microorganisms isolated from West-Bohemian bath facilities. Twenty eight strains were isolated representing 11 genera grouped into seven groups: Bullera, Cryptococcus, Candida and Torulopsis, Trichosporon and Geotrichum, Hyphozyma, Rhodotorula, sporogenic yeasts.

14. Cimerman A., Kocková-Kratochvílová A. (1984): Pichia labacensis n. sp. Ztschr. allg. Microbiol. 24: in press.

A new species of the yeast genus Pichia isolated in a Yugoslav factory is described. It differs from other Pichia species mainly in fermentation and assimilation abilities of various carbon substances. It is suitable for the production of fodder yeast from ultrafiltered spent sulphite liquor.

* * *

IV. Centraalbureau Voor Schimmelcultures, Yeast Division, Julianalaan 67a, 2628 BC Delft, Netherlands. Communicated by M.Th. Smith.

1. Recent publications:
   a. Smith, M.Th. & Batenburg-van der Vegte. 1984
   Ascospore morphology and Ultrastructure of species assigned to the genus Lipomyces Lodder et Kreger-van Rij. Int. J. Syst. Bact. 34, 80-86.

   b. Weijman, A.C.M. & Rodrigues de Miranda, L. 1983
   Xylose distribution within and taxonomy of the genera Bullera and Sporobolomyces. Antonie van Leeuwenhoek 49, 559-562.

2. Accepted for publication:

   a. Saëz, H. & Rodrigues de Miranda, L. 1984
   Candida sequanensis, a new yeast species. Antonie van Leeuwenhoek 50.

   b. Smith, M.Th. & Van Grinsven, A.M. 1984

Dekkera anomala sp. nov., the teleomorph of Brettanomyces anomalus, from spoiled soft drinks. Antonie van Leeuwenhoek 50.

3. Submitted for publication in Antonie van Leeuwenhoek:

   a. De Hoog, G.S., Rantio-Lehtimaki, A.H. & Smith, M.Th. 1984 Blastobotrys, Sporothrix and Trichosporiella: generic delimiation, new species and teleomorphs.

   b. Smith, M. Th. & Batenburg-van der Vegte, W.H. 1984 Ultrastructure of septa in Blastobotrys and Sporothrix.

   c. Weijman, A.C.M. & De Hoog, G.S. 1984 Carbohydrate patterns and taxonomy of Sporothrix and Blastobotrys.

* * *

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

Yeasts: Characteristics and Identification. J.A. Barnett, R.W. Payne and D. Yarrow (Cambridge University Press), published October 1983, has now more than half sold out, so we must consider a revision in a few years' time. We would be grateful for any criticisms or comments, directed towards improvements (some have been received already).

Other recent publications include the following.

R.W. Payne, D. Yarrow & J.A. Barnett (1982). The construction by computer of a diagnostic key to the genera of yeasts and other such groups of taxa. Journal of General Microbiology 128, 1265-1277.

J.A. Barnett & A.P. Sims (1982). The requirement of oxygen for the active transport of sugars into yeasts. Journal of General Microbiology 128, 2303-2312.

R.W.M. Buhagiar, D. Yarrow & J.A. Barnett (1983). Bullera crocea and Bullera armeniaca, two new yeasts from fruit and vegetables. Journal of General Microbiology 129, 3149-3155.

A.P. Sims, E. Kopetzki, B. Schulz & J.A. Barnett (1984). The use of phenolic glycosides for studying the aerobic or anaerobic transport of disaccharides into yeasts. Journal of General Microbiology (in the press).

* * *

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

W.T. Starmer, M.A. Lachance, and H.J. Phaff participated in an expedition on the National Science Foundation research vessel "Cape Florida" during November 1983 in the Caribbean Sea. Our purpose was to extend our research project started in May 1982 (see Yeast Newsletter, June 1983) on the yeasts occurring in fruits and in rotting tissue of columnar and Opuntia cacti as well as Drosophila spp. utilizing these cacti on various Caribbean islands. On our

trip we collected about 700 yeast strains on Little Conception Island, Great Inagua (both in the Bahamas), Navassa Island, Jamaica, Cayman Brac, Little Cayman, and Grand Cayman Islands. The identification of the yeasts is nearing completion.

Below follow abstracts of recent publications.

1. William T. Starmer[1], Herman J. Phaff, Joanne Tredick, Mary Miranda, and Virginia Aberdeen[1], 1984. Pichia antillensis, a new species of yeast associated with necrotic stems of cactus in the Lesser Antilles. Int. J. Syst. Bacteriol. 34:(3) July issue.

[1]Department of Biology, Syracuse University, Syracuse, New York 13210.

Abstract

A description is given of Pichia antillensis, a new species of yeast which is closely related to Pichia opuntiae. Pichia antillensis, of which 20 strains were isolated, is heterothallic and occurs in nature both in the haploid and diploid state. It produces asci with four hat-shaped spores, which are rapidly released upon maturity. The guanine+cytosine content of its nuclear deoxyribunucleic acid is 33.4-33.7 mol%. The deoxyribonucleic acids of Pichia antillensis and Pichia opuntiae var. opuntiae show approximately 50% homology but the two species are not interfertile. Moreover, the two species are separated geographically and by host plant. Pichia antillensis occurs principally in necrotic tissue of the columnar cactus Cephalocereus royenii in the West Indies and Pichia opuntiae var. opuntiae in Opuntia rots in Australia. The type strain of P. antillensis is UCD-FST 82-651A (=ATCC 56267=CBS 7111).

* * *

2. J.S.F. Barker[1], P.D. East[1], H.J. Phaff and M. Miranda, 1984. The ecology of the yeast flora in necrotic Opuntia cacti and of associated Drosophila in Australia. Microbial Ecology (in press).

[1]Department of Animal Science, University of New England, Armidale, N.S.W. 2351, Australia.

Abstract

A survey was made of the yeast communities isolated from necrotic tissue of four species of prickly-pear cacti (Opuntia stricta, O. tomentosa, O. monacantha and O. streptacantha) which have colonized in Australia. Yeast communities were sampled from a number of localities and at different times. Cactus specific yeast accounted for 80% of the total isolates, and the three most common species contributed 63% of the total. Comparisons of the species compositions of the yeast communities indicated that the differences among communities were greater between Opuntia species than between different localities within a single cactus species, and also that differences between years were greater than average differences between localities within years. Multivariate statistical tests of association between yeast community and physical features of rots indicated that temperature, pH and age of rot all exerted some influence on the structure of the yeast community. Similar analyses involving Drosophila species inhabiting these cactus rots suggested

the existence of complex associations between Drosophila community, yeast community and physical and chemical attributes of the cactus necroses.

* * *

3. E. Guého, J. Tredick and H.J. Phaff, 1984. DNA base composition and DNA relatedness among species of Trichosporon Behrend. Antonie van Leeuwenhoek 50:17-32 (For abstract, see Yeast Newsletter 32(1), 5, 1983).

* * *

4. W.T. Starmer and H.J. Phaff, 1983. Analysis of the community structure of yeasts associated with the decaying stems of cactus. II. Opuntia species. Microb. Ecol. 9:247-259 (For abstract, see Yeast Newsletter 32(1), 5, 1983).

* * *

5. C.P. Kurtzman, H.J. Phaff, and S.A. Meyer, 1983. Nucleic acid relatedness among yeasts. In J.F.T. Spencer, D.M. Spencer, and A.R.W. Smith, eds. Yeast Genetics - Fundamental and Applied Aspects, p. 139-166. Springer Verlag, New York.

* * *

VII. Institute for Fermentation, Osaka, 17-85, Juso-honmachi 2-chome, Yodogawa-ku, Osaka 532, Japan. Communicated by Isao Banno.

The following paper has been recently published:

Banno, I., Mikata, K., and Kodama, K., 1983. Ascomycetous yeasts isolated from galleries of ambrosia beetles in Japan. Transactions of the Mycological Society of Japan 24:441-450.

Abstract

An introductory study of the yeasts recovered from tunnels bored in wood by ambrosia beetles has been carried out. A total of 385 yeast strains was isolated from 162 samples, which were collected in the form of cut wood or timber from various localities from Hokkaido to Yaku island in Japan. Among these, 188 strains were found to form ascospores and were identified as representatives of Debaryomyces hansenii, Hansenula anomala, H. bimundalis, H. californica, H. capsulata, H. saturnus, H. wingei, Hormoascus platypodis, Kluyveromyces thermotolerans, Pichia acaciae, P. membranaefaciens, P. nakazawae var. akitaensis, P. pinus, P. rhodanensis, and P. veronae. Three yeasts (AM 13, AM 72, and AM105), representative of three undescribed Pichia species were also isolated. Although no specific and exclusive relationship was found between kinds of yeasts and beetles or trees, tendencies were nevertheless noted: Hansenula anomala was isolated most frequently from wood samples infested by beetles of the genus Xyleborus; Hormoascus platypodis was prominently associated with invasions of Crossotarsus nipponicus in the tree Fagus crenata; The Pichia species coded AM72 was associated mainly with infestations of Scolytoplatypus shogun in the tree F. crenata; and the predominant yeast recovered from the tree Machilus thunbergii was Pichia acaciae. Most of the isolates of Hormoascus platypodis were found in the area

north of Yamagata, in contrast to the majority of Pichia acaciae being found in the area south of Gifu.

* * *

VIII. Department of Food Science and Technology, University of California, Davis, California 95616. Communicated by Ahmed Elbaggari.

Below follows the summary of my M.A. thesis in Microbiology, University of California, Davis.

Two hundred and two yeast cultures were isolated from six tanks of normally fermenting Spanish-style olives during various stages of the fermentation. These 202 cultures were identified and the results are as follows:

56 cultures of sporulating yeasts:

4 Debaryomyces hansenii
4 Hansenula anomala var. anomala
3 Kluyveromyces vanudenii
43 Pichia membranaefaciens
2 Saccharomyces rosei

146 cultures of non-sporulating yeasts:

40 Candida boidinii
1 Candida butyri
2 Candida diddensii
3 Candida famata
71 Candida krusei
2 Candida ohmeri
1 Candida pelliculosa
7 Candida stellata
4 Candida tropicalis
5 Candida valida
1 Kloeckera javanica
9 Rhodotorula rubra

The major species are C. krusei, C. boidinii and P. membranaefaciens. These three species grow on oleuropein and can use lactic acid as the sole source of carbon. C. krusei and C. boidinii utilized lactic acid very rapidly, while P. membranaefaciens utilized it weakly.

Only a few of the species found were acid forming yeasts.

* * *

IX. Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow[1] and Department of Biophysics and Medical Physics, University of California, Berkeley[2]. Communicated by J.R. Johnston[1] and R.K. Mortimer[2].

Pedigree of the Yeast Genetics Stock Center "Wild-Type" Strains S288C and X2180 of S. cerevisiae.

The rapid expansion of the molecular genetics of yeast leads us to suggest that greater attention is due to the interrelationships of the strains used in studies such as gene cloning and sequencing. Various heterogeneities in laboratory strains have recently been reported, including those pertaining to the distribution of restriction sites and the numbers and positions of transposable elements and repeated gene families.

To allow better comparison of these heterogeneities relative to the strains in which they have been observed, we have constructed the pedigree of one of the most common progenitor haploid strains, S288C and its diploid derivative, X2180. The former was used to isolate almost all of the mutants of the arginine, histidine, leucine, lysine, methionine, threonine, tryptophan and uracil biosynthetic pathways. Combinations of these mutants provide most of the original strains for the Yeast Genetics Stock Center which has been in operation since 1960. We have determined which strains were used by such early yeast biologists and geneticists as Lindegren; Reaume and Tatum; Pomper and Burkholder; Ephrussi; Roman, Douglas and Hawthorne; and Zirkle, Tobias and Mortimer.

The pedigree of S288C reveals six ancestral strains. These are S. cerevisiae 93 and S. carlsbergensis 126 (isolated by E.M. Mrak, University of California, Davis, in 1938); baker's yeasts (S. cerevisiae) FLD, LK (Lindegren, The Yeast Cell, 1949) and Yeast Foam (Ephrussi et al., Ann. Inst. Pasteur 76 : 419, 1949); and S. microellipsoideus NRRL-210. We have found that the contribution of the heterothallic diploid strain 93 is paramount and have estimated that 80-90% of the genome of S288C derives from this ancestral strain via its segregants 93-1C and 93-3B.

Cultures of strains 93 and 126 are still available at the University of California, Davis (H.J. Phaff) and now from the Yeast Genetics Stock Center. The former strain also was recently recovered by us from lypholyzed cultures made in 1951 at the University of California, Berkeley (RKM). Tetrad analysis of the latter culture shows that, in addition to mating type, strain 93 segregates for gal2, a gene for copper resistance (probably CUP1) and a gene for flocculation which is currently under investigation.

It is our opinion that strain S288C and hence ancestral strain 93 is a major source of the gene pool of a majority of strains currently in use in yeast genetics and molecular biology.

* * *

X. Biochemisch Laboratorium, Vrije Universiteit, de Boelelaan 1085, 1081 HV Amsterdam, The Netherlands. Communicated by J. Klootwijk.

The following manuscripts from our groups have been accepted for publication:

1. Martin Ph. Verbeet, Harm van Heerikhuizen, Jacobus Klootwijk, Ruud D. Fontijn and Rudi J. Planta. Evolution of yeast ribosomal DNA: molecular cloning of the rDNA units of Kluyveromyces lactis and Hansenula wingei and their comparison with the rDNA units of other Saccharomycetoideae. Mol. Gen. Genet., in press.

Summary

We have studied the evolution of the yeast ribosomal DNA unit to search for regions outside the rRNA genes that exhibit evolutionary constraints and therefore might be involved in control of ribosome biosynthesis. We have cloned one complete rDNA unit of Kluyveromyces lactis and Hansenula wingei and established the physical and genetic organisation of both units. Both species belong to the subfamily of the Saccharomycetoideae. The lengths of the rDNA units of K. lactis and H. wingei are 8.6 and 11.1 kb, respectively, and both

comprise the 5S rRNA gene in addition to the large rRNA operon. Sequence conservation was monitored by restriction enzyme mapping as well as heteroduplex analysis of the two cloned rDNA units with S. carlsbergensis rDNA. These analyses showed that, phylogenetically, K. lactis is closer to S. carlsbergensis than H. wingei. The non-transcribed spacers (NTS) of both K. lactis and H. wingei have diverged completely from S. carlsbergensis; moreover in H. wingei the NTS are about double the length of these in the other two species. The transcribed spacers of both K. lactis and H. wingei contain conserved tracts. A homologous sequence of about 60 bp was found in the middle of the external transcribed spacer of H. wingei upon heteroduplexing with S. carlsbergensis rDNA, whereas the sequence at the transcription initiation site itself was insufficiently homologous to form a duplex. The sequence of the homologous region was determined both in H. wingei and K. lactis and compared with that of S. carlsbergensis. The function of this conserved element within the external transcribed spacer is discussed.

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2. Annemarie E. Kempers-Veenstra, Harm van Heerikhuizen, Wouter Musters, Jacobus Klootwijk and Rudi J. Planta. Transcription of an artificial ribosomal RNA gene in yeast. The EMBO Journal, in press.

Abstract

We constructed an artificial yeast ribosomal RNA gene and studied its transcription after introduction into a recipient yeast strain. The artificial gene was constructed out of a fragment containing the sequence from position -207 to +128 relative to the site of initiation of Saccharomyces carlsbergensis 37S pre-rRNA, followed by a marker fragment from Spirodela oligorhiza chloroplast DNA and finally a fragment containing the sequence from position -36 to +101 relative to the 3'-end of the 26S rRNA gene. The resulting construct was cloned into the yeast-Escherichia coli shuttle vector pJDB207. Both Northern blot hybridization and R-loop analysis of RNA from transformed Saccharomyces cerevisiae cells revealed a discrete transcript of the expected length. S1 nuclease mapping as well as primer extension analysis showed that the major proportion of the transcripts was initiated at exactly the same site as 37S pre-rRNA. These results show that the respective rDNA fragments contain the information for correct initiation of transcription and formation of the 3'-end. A minor proportion of the transcripts was initiated at a number of sites between positions -1 and -100 upstream of the predominant start. The proportion and the pattern of these upstream starts is affected by the vector context of the artificial rRNA gene.

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3. Martin Ph. Verbeet, Jacobus Klootwijk, Harm van Heerikhuizen, Ruud D. Fontijn, Erno Vreugdenhil and Rudi J. Planta. 1984. A conserved sequence element is present aroung the transcription initiation site for RNA polymerase A in Saccharomycetoideae. Nucleic Acids Research 12:1137-1148.

Abstract

To identify DNA elements involved in the initiation of rRNA transcription in yeast we located the start site of the rRNA operon of Kluyveromyces lactis

and Hansenula wingei, both members of the Saccharomycetoideae, by S1 nuclease analysis and determined the surrounding nucleotide sequences.

Comparison of these sequences with those of Saccharomyces carlsbergensis, S. cerevisiae and S. rosei (all belonging to the same yeast subfamily) reveals an identical sequence at the site of transcription initiation from position +1 to +7 which is part of a larger conserved region extending from position -9 to +23; the conserved heptanucleotide sequence is supposed to constitute an important part of the promoter for yeast RNA polymerase A. The non-transcribed spacers (NTS) upstream of position -9 have diverged strongly with the exception of two short elements around positions -75 and -135. The external transcribed spacer (ETS) downstream of position +23 is largely conserved between K. lactis, S. rosei and S. carlsbergensis except for a divergent region around position +75. On the other hand, the ETS of H. wingei has diverged significantly.

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4. Jacobus Klootwijk, Martin Ph. Verbeet, Geertruida M. Veldman, Victoria C.H.F. de Regt, Harm van Heerikhuizen, Jan Bogerd and Rudi J. Planta. 1984. The in vivo and in vitro initiation site for transcription of the rRNA operon of Saccharomyces carlsbergensis. Nucleic Acids Research 12:1377-1390.

Abstract

We have performed a detailed analysis of the transcription initiation of the rRNA operon in the yeast Saccharomyces carlsbergensis. Electron microscopic analysis of R-looped pre-rRNA molecules together with a very sensitive S1-nuclease mapping showed the use of only a single transcription start at about 700 bp upstream of the 17S rRNA gene and not of the minor start sites proposed for the very closely related species S. cerevisiae by others [Bayev et al. (5), Swanson and Holland (6)]. The sequence of 730 bp of the initiating region is presented. In vitro transcription in concentrated lysates of yeast spheroplasts in the presence of (γ-SH)ATP or (γ-SH)GTP, followed by purification of the in vitro initiated RNA via Hg-agarose, revealed that on the endogenous template exactly the same site is used for transcription initiation as in vivo.

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5. K.G. Stryabin, M.A. Eldarov, V.L. Larionov, A.A. Bayev, J. Klootwijk, V.C.H.F. de Regt, G.M. Veldman, R.J. Planta, O.I. Georgiev and A.A. Hadjiolov. 1984. Structure and function of the nontranscribed spacer regions of yeast rDNA. Nucleic Acids Resarch 12:2955-2968.

Abstract

The sequences of the nontranscribed spacers (NTS) of cloned ribosomal DNA (rDNA) units from both Saccharomyces cerevisiae and Saccharomyces carlsbergensis were determined. The NTS sequences of both species were found to be 93% homologous. The major disparities comprise different frequencies of reiteration of short tracts of six to sixteen basepairs. Most of these reiterations are found within the 1100 basepairs long NTS between the 3'-ends of 26S and 5S rRNA (NTS1). The NTS between the starts of 5S rRNA and 37S pre-

rRNA (NTS2) comprises about 1250 basepairs. The first 800 basepairs of NTS NTS2 (adjacent to the 5S rRNA gene) are virtually identical in both strains whereas a variable region is present at about 250 basepairs upstream of the RNA polymerase A transcription start. In contrast to the situation in Drosophila and Xenopus no reiterations of the putative RNA polymerase A promoter are present within the yeast NTS. The strands of the yeast NTS reveal a remarkable bias of G and C-residues. Yeast rDNA was previously shown to contain a sequence capable of autonomous replication (ARS) (Szostak, J.W. and Wu, R. (1979), Plasmid 2, 536-554). This ARS, which may correspond to a chromosomal origin of replication, was located on a fragment of 570 basepairs within NTS2.

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XI. Department of Genetics, University of California, Berkeley, California 94720. Communicated by Seymour Fogel.

Below follow summaries of yeast genetic studies going on in our Department.

1. Seymour Fogel and Juliet W. Welch. 1982. Tandem gene amplification mediates copper resistance in yeast. Proc. Natl. Acad. Sci. USA 79:5342-5346.

Abstract

Resistance to copper's toxicity in yeast is controlled by the $CUP1^r$ locus. This gene was cloned by transforming sensitive recipients ($cup1^s$) with a collection of hybrid DNA molecules, consisting of random yeast DNA fragments inserted into the vector YRp7. Four resistant transformants were studied in detail. Autonomously replicating or integrated by homologous recombination into chromosomal sites, the corresponding plasmids and several subclones confer resistance on sensitive recipients carrying the natural variant allele, $cup1^s$. Tetrad analysis and genetic mapping established that integration occurs typically at the $cup1^s$ site located 28 centimorgans distal to thr1, a chromosome VIII marker. Restriction endonuclease cleavage and electrophoretic mobility studies revealed that the $CUP1^r$ locus consists of a tandem array of repetitive units. Each unit is 1.95 kilobases in length and contains single sites for Kpn I and Xba I and two Sau3A sites. The sensitive allele represents one repeat and the resistant allele embraces 15 tandemly arrayed repeat units. Progressive selections in higher copper concentrations establish strains with markedly enhanced resistance. Resistance, we propose, is mediated by the gene amplification mechanism based on unequal sister chromatide exchange.

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2. Juliet W. Welch, Seymour Fogel, Guy Cathala and Michael Karin. 1983. Industrial Yeasts Display Tandem Gene Iteration at the CUP1 Region. Molecular and Cellular Biology, 3:1353-1361.

Abstract

The gene copy number at the CUP1 locus and the resistance level to external copper was directly correlated in five wild-type commercial Saccharomyces strains. An increased copy number of the CUP1 gene leads to

increased accumulation of chelatin mRNA, which codes for a low-molecular-weight, copper-binding protein. The enhanced production of this rapidly inducible protein mediates resistance of the cell to copper. Industrial yeasts exhibit homologies to the amplified copper resistance repeat unit found in laboratory strains. However, the extent of tandem iteration is strain dependent, and the repetitious unit is either 1.7 or 1.5 kilobases in length compared with the 2.0-kilobase unit in laboratory strains. Strain 522 (Montrachet) contains two chromosome VIII segments distinguishable by their numbers of repeat units (2 and 11) and the size of the units (1.5 and 1.7 kilobases). Distillers yeast 513 carries a 1.5-kilobase repeat unit on each homologous chromosome, although they contain nine and five iterations, respectively.

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3. Seymour Fogel, Robert K. Mortimer, and Karin Lusnak. 1981. Mechanisms of Meiotic Gene Conversion, or "Wanderings on a Foreign Strand". Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance, Cold Spring Harbor Laboratory.

Contents:

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4. S. Fogel, T. Choi, D. Kilgore, K. Lusnak and M. Williamson. 1982. The Molecular Genetics of Non-Tandem duplications at ADE8 in Yeast. Rec. Adv. Yeast Mol. Biol. 1:269-288.

Summary

The ADE8 locus of chromosome IV has been cloned. Various subclones, principally those with fragment lengths of 2.5 and 4.0 kb, confer ADE8 function on recipient strains carrying the unique allele ade8-18. A visual screen for detecting integrations of the autonomously replicating vector is described along with diagnostic genetic tests that identify the genomic integration sites. Most integrants generate non-tandem duplications at the

ade8-18 site, though some also occur at trp1. None were found at ura3. The frequency of integration via homologous recombination into a genomic site is proportional to the physical length of the corresponding DNA segment, carried in the YRp17 vector. Similarly, overall plasmid excision rates are proportional to the total length of the integrated segment and the distribution of events for a plasmid with a given DNA insert is determined by the position of the mutant site within the genetic fine structure map. Meiotic gene conversion, intrachromosomal conversion, and postmeiotic segregation were studied in several hybrids containing two, three or four ADE8 sequences within conventional chromosomes or non-tandem duplications that are either isosequential or heterosequential.

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5. Juliet W. Welch and Seymour Fogel. A Recombinant DNA Strategy for Characterizing Industrial Yeast Strains, Submitted for publication in the Proceedings of the XVth International Congress of Genetics, New Delhi, 1983.

Remarkable for their enzymatic versatility in brewing, baking, wine making and single cell protein production, industrial yeasts are selected and maintained on a largely empirical basis. Accordingly, they comprise an uncharted and unharnessed reservoir of genetic variability with respect to ploidy, aneuploidy, structural heterozygosities, adaptive gene complexes and sequence polymorphisms. Despite their enormous diversity, they are essentially uncharacterized wild type strains that have not benefitted substantially from improvements either from classical approaches involving sexual breeding and selection cycles, or the more recent strategies opened by protoplast fusion, molecular cloning and recombinant DNA transformation. However, modest progress along these lines has been reported. The high level of empiricism in industrial processes based on yeast-catalyzed conversions is emphasized by the fact that commercial yeasts often represent complicated population mixtures rather than pure strains as their numerical or varietal designations would seem to imply. Clearly, such mixtures serve some predetermined end purposes beneficially. But, serious attempts centered on attaining stability, reproducibility control or genotypic improvement are effectively precluded.

Yeasts, in general, and especially the vast interfertile, or potentially interfertile species subsumed within the genus Saccharomyces, are organisms admirably adapted to dramatic improvement thru the application of conventional and modern genetic strategies. Yeast is a widely recognized excellent genetic tool and even more so as a cloning vehicle of choice. Increasingly, it is also viewed as a primary candidate for producing important pharmaceutical proteins. Our recent studies on the molecular mechanism of heavy metal resistance in yeast may be taken as a paradigm that illustrates how fundamental understanding at the molecular-genetical levels might be exploited to generate improved strains possessing novel and extraordinary enzymatic capabilities.

Resistance to copper is mediated by a low molecular weight, cystein-rich, copper-binding protein coded by the CUP1 gene located on chromosome VIII. Recently, we cloned this gene and discovered that copper resistant laboratory strains contain ten tandemly iterated copies of the gene while sensitive strains have only one copy. Each repeat unit contains a KpnI, an XbaI, two

Sau3A, and three DdeI sites. When the entire repeat unit was sequenced, we identified an open reading frame coding for a low molecular weight, cysteine-rich protein.

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6. Seymour Fogel and Juliet W. Welch. The Molecular Genetics of Copper Resistance in Yeast: Gene Amplification via Gene Conversion. Submitted for publication in the Proceedings of the XVth International Congress of Genetics, New Delhi, 1983.

Our knowledge concerning molecular events in recombination has been limited by the unavailability of specific homologies, non-homologies or heterologies defined by known base pair mismatches. However, recently developed recombinant DNA strategies readily provide these critical reagents.

Cloned genes, especially those organized as tandemly iterated DNA segments, provide novel experimental opportunities to the study of molecular recombination. Where restriction maps and DNA sequences are known, it is possible to frame rather precise questions with the expectation that clear insights will be forthcoming. This paper reports on the recombinatorial behavior of the *CUP1*^{r} locus in yeast.

The *CUP1*^{r} locus was recently isolated. By molecular cloning and restriction analysis, we demonstrated that the mendelizing genetic locus which conferred resistance to high exogenous copper levels represented 10 tandemly arrayed copies of a 2.0 kb sequence. The same sequence was found to occur only once as a single copy locus in the naturally occurring sensitive strain. In general, phenotypic resistance level and the specific mRNA level exhibited by the different strains was directly proportional to the gene copy number. The molecular basis for copper resistance was shown to be associated in various industrial strains with overproduction of a low molecular weight, copper-binding cysteine-rich protein designated copper-chelatin. A study concerning the primary structure and transcription of the amplified *CUP1*^{r} locus revealed that the nucleotide sequence of the basic amplification unit contains a copper regulated transcription unit. It resembles the metal-lothionein proteins responsible for protecting mammalian cells against the toxic effects of heavy metal ions such as cadmium. Finally, we could establish by a combination of Southern blots and meiotic tetrad analysis that repeated cycles of selection in progressively higher copper concentration lead to an expansion of the amplified locus. In one instance, the copy number increased from 10 in the haploid parent to eleven and fourteen copies respectively in the homologues of the disomic haploid derivitive. Such strains display an enhanced copper resistance about 2.5-fold greater than their progenitor.

In earlier publications and prior to establishing the molecular structure of *CUP1* as a tandemly iterated unit, we reported that *CUP1*^{r} behaved as a typical mendelian unit in extensive gene conversion studies involving some thirty miscellaneous heterozygous sites widely distributed over the yeast genome. Our findings were based on scoring the four ascosporal colonies of a meiotic tetrad as either sensitive or resistant on a single synthetic complete medium plate containing 1.0 mM copper. Accordingly, the significance and validity of these findings concerning the CUP1 locus are limited. We propose to supplement them by the more critical studies reported here. Primarily, the

new data are based on a direct molecular examination of the CUP1 locus in unselected meiotic tetrads and secondarily on the segregation patterns for copper resistance-sensitivity levels scored on a graded series of synthetic complete agar plates with copper concentrations ranging from 0.01 mM to 1.2 mM.

In effect, the present meiotic study addresses the question of gene copy number constancy at a genetic locus composed of tandemly organized units each coding for the same protective metal-binding protein. We inquire: "Is copy number conserved in meiosis?" Or, can copy numbers be repartioned between homologues through reciprocal unequal crossing over with overall numerical conservation? Alternatively, we may ask--: is gene copy number, or amplification and deamplification, attributable to non-reciprocal recombination, i.e., gene conversion characterized by yeast's usual features of parity, fidelity, polarity, coconversion, flanking marker exchange and postmeiotic segregation. Our investigations also bear on issues that relate to the modal length of meiotic heteroduplex DNA sequence and the physical length of the coconverted segment that is transferred non-reciprocally to a recipient sequence during genetic recombination.

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7. S. Fogel, R.K. Mortimer, and Karin Lusnak. 1983. Meiotic gene conversion in yeast: molecular and experimental perspectives. In Yeast Genetics-Fundamental and Applied Aspects, J.F.T Spencer, D.M. Spencer, and A.R.W. Smith, eds. 65-107. Springer Verlag, New York.

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8. S. Fogel, J.W. Welch and E. Louis. Meiotic Gene Conversion Mediates Gene Amplification in Yeast. Submitted to: 49th Symposium on Quantitative Biology Recombination at the DNA Level, June 1984, Cold Spring Harbor, New York.

Our present strategies focus on meiotic genetic analyses complemented by physical, recombinant DNA studies concerning relevant chromosome sequences. The four ascosporal colonies from unselected tetrads were assayed for meiotic changes in amplification or deamplification of the DNA sequences that confer resistance to ionic copper. Composed of a basic 2.0 kb unit containing two Sau3A, one Xba and one Kpn site, but no EcoR1 sites, the $\underline{CUP1^r}$ locus may embrace up to fourteen tandemly iterated repeat units. This chromosome VIII locus is flanked by external EcoR1 sites. Thus, the molecular and genetic stability of the locus can be monitored reliably by assessing the physical length of genomic EcoR1 fragments that hybridize to ^{32}P labelled probes specific for the 2.0 kb repeat unit. Copy number changes were analyzed in complete tetrads from two hybrids. One was homozygous for a nine copy iteration at the $\underline{CUP1^r}$ locus; the other carried 10 and 5 copies, respectively, in each homologue. To date, among 69 tetrads examined by Southern blot analysis, 13 or 19% displayed a copy number change. With a few exceptions, the copy number increases/decreases, are typically non-reciprocal in character. However, a few reciprocal exchanges and unequal crossovers were also identified. Formally equivalent to gene conversions, the informational transfers may involve DNA segments, up to 20 kb in a single event, a length tenfold greater than our earlier estimates based on studies with single copy genes. We propose a generalized molecular model to account for

amplification/deamplification changes mediated by meiotic gene conversion. The model considers the consequences of oblique (offset) synaptic pairings between homologous segments and generates quantitative predictions concerning the basic gene conversion frequency as a function of copy number. Additionally, it describes the qualitative and quantitative distribution of copy number shifts among unselected tetrads derived from a wide array of hybrids. Predicted and observed distributions will be compared.

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9. Beth Rockmill. 1984. Meiotic Nondisjunction Mutants in Yeast. Ph.D. dissertation, University of California, Berkeley.

Abstract

Mutants defective in meiotic chromosome disjunction were identified by employing a screen to detect colonies generating high frequencies of aneuploid spores. Chromosome VIII disomic spores were distinguished by enhanced copper resistance attributed to a cup1 gene dosage effect. Two partially dominant mutants, dis1-1 and dis1-2, were chosen for further study. These are allelic and are located distal to the centromere. Dis-1 mutants generate disomes at rates of about 10^{-4} disomes per ascus, or one hundred times more frequently than wild type strains, and exhibit a temperature effect.

A recombination deficiency is not the cause of the nondisjunction as found by a half-tetrad analysis among disomes in an interval on chromosome VIII. The disomes are generated early in meiosis, i.e., mostly prior to meiosis I disjunction, although more often in dis1-1 than in dis1-2 strains. This suggests that the dis1-1 allele may function earlier in meiosis. Only one disome is found in aberrant asci. This is consistent with an early separation of chromatids at meiosis I and implies that the dis-1 defect is in meiosis II disjunction. The DIS-1 mutants are not chromosome VIII specific. 5% of the selected chromosome VIII disomes are additionally disomic for chromosome III. The high frequency of multiple disomy indicates that rare nuclei undergo high levels of nondisjunction. The hypothesis for the mechanism of disomy generation in the DIS-1 mutants is that centromeres often separate precociously. This may reflect a defect in the regulation of centromere replication or cohesion.

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XII. Department of Biochemistry, Purdue University, West Lafayette, IN 47907. Communicated by G.B. Kohlhaw.

Below follow abstracts of three recent papers from our laboratory.

1. Vijay R. Baichwal, Thomas S. Cunningham, Paula R. Gatzek, and Gunter B. Kohlhaw. 1983. Leucine Biosynthesis in Yeast. Identification of Two Genes (LEU4, LEU5) that Affect α-Isopropylmalate Synthase Activity and Evidence that LEU1 and LEU2 Gene Expression is controlled by α-Isopropylmalate and the Product of a Regulatory Gene. Current Genetics 7:369-377.

Summary

Tetrad analysis indicates that α-isopropylmalate synthase activity of yeast is determined by two separate genes, designated LEU4 and LEU5. LEU4 is identified as a structural gene. LEU5 either encodes another α-isopropylmalate synthase activity by itself or provides some function needed for the expression of a second structural gene. The properties of mutants affecting the biosynthesis of leucine and its regulation suggest that the expression of LEU1 and LEU2 (structural genes encoding isopropylmalate isomerase and β-isopropylmalate dehydrogenase, respectively) is controlled by a complex of α-isopropylmalate and a regulatory element (the LEU3 gene product). Similarities and differences between yeast and Neurospora crassa with respect to leucine biosynthesis are discussed.

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2. Li-Fen L. Chang, Thomas S. Cunningham, Paula R. Gatzek, Wen-Ji Chen, and Gunter B. Kohlhaw. 1984. Cloning and Characterization of Yeast LEU4, One of Two Genes Responsible for α-Isopropylmalate Synthesis. Genetics, in press.

Abstract

By complementation of an α-isopropylmalate synthase-negative mutant of Saccharomyces cerevisiae (leu4 leu5), a plasmid was isolated that carried a structural gene for α-isopropylmalate synthase. Restriction mapping and subcloning showed that sequences sufficient for complementation of the leu4 leu5 strain were located within a 2.2 kilo-base SalI - PvuII segment. Southern transfer hybridization indicated that the cloned DNA was derived intact from the yeast genome. The cloned gene was identified as LEU4 by integrative transformation that caused gene disruption at the LEU4 locus. When this transformation was performed with a $LEU4^{fbr}$ LEU5 strain, the resulting transformants had lost the 5', 5', 5'-trifluoro-D,L-leucine resistance of the recipient strain, but were still Leu^+. When it was performed with a LEU4 leu5 recipient, the resulting transformants were Leu^-. The α-isopropylmalate synthase of a transformant that carried the LEU4 gene on a multicopy plasmid (in a leu5 background) was characterized biochemically. The transformant contained about 20 times as much α-isopropylmalate synthase as wild type. The enzyme was sensitive to inhibition by leucine and coenzyme A, was inactivated by antibody generated against α-isopropylmalate synthase purified from wild type, and was largely confined to the mitochondria. The subunit molecular weight was 65-67,000. Limited proteolysis generated two fragments with molecular weights of about 45,000 and 23,000. Northern transfer hybridization showed that the transformant produced large amounts of LEU4-specific RNA with a length of about 2.1 kilonucleotides. The properties of the plasmid-encoded enzyme resemble those of a previously characterized α-isopropylmalate synthase that is predominant in wild type cells. The existence in yeast of a second α-isopropylmalate synthase activity that depends on the presence of an intact LEU5 gene is discussed.

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3. Athena Andreadis, Yun-Pung Hsu, Mark Hermodson, Gunter Kohlhaw, and Paul Schimmel. 1984. Yeast LEU2: Repression of mRNA Levels by Leucine and Primary Structure of the Gene Product. J. Biol. Chem., in press.

Summary

Enzyme activity associated with the yeast LEU1 and the LEU2 gene product (β-isopropylmalate dehydrogenase) drops sharply when yeast is grown in the presence of leucine. Northern hybridizations with LEU2-specific probes establish that this is accompanied by a 5-fold repression in LEU2 mRNA levels. A similar repression was noted recently for LEU1 mRNA levels (Y.-P. Hsu and P. Schimmel, J. Biol. Chem., in press). S1 nuclease mapping of the 5'-end of the LEU2 mRNA shows a major start at approximately 16 nucleotides upstream of the AUG initiation codon. This initiation site in the gene is retained in an extensive LEU2 5'-noncoding region deletion which still expresses the LEU2 gene product (Erhart, E. and Hollenberg, C.D. (1983) J. Bacteriol. 156, 625-635).

The primary structure of the LEU2 gene product was established from the nucleotide sequence of the gene coding region and from fitting amino acid sequences of scattered internal peptides to the nucleotide sequence. The 364 amino acid protein has a 13 amino acid stretch which is highly homologous to the partially sequenced yeast LEU1 gene product (isopropylmalate isomerase). The homology occurs about 290 amino acids from the respective NH_2-termini of the two proteins. The homology may represent residues which interact with β-isopropylmalate, a common ligand for the enzymes.

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XIII. Institute of Molecular Biology, Bulgarian Academy of Science, 1113 Sofia, Bulgaria. Communicated by P.V. Venkov and L.I. Stateva.

Below follow summaries of some recent publications of our laboratory.

1. Liliana Waltschewa, Oleg Georgiev, and Pencho Venkov; (1983); Relaxed Mutant of Saccharomyces cerevisiae: Proper Maturation of Ribosomal RNA in Absence of Protein Synthesis; Cell 33: 221-230.

   We have characterized a relaxed yeast mutant, S. cerevisiae SY15, isolated by mutagenesis with ethylmethanesulfonate of strain A364A. Starvation for a required amino acid or treatment with cycloheximide blocks protein synthesis in both parental and mutant strains, while the synthesis of total RNA is inhibited by 72% in A364A and 23% in SY15 cells. In the absence of protein synthesis, the transcription of 37S primary precursor to rRNA is not inhibited in the SY15 mutant, and the rRNA transcripts are correctly processed, although at a reduced rate, and are almost free of ribosomal proteins. The relaxed phenotype in yeast is accompanied by alteration in the regulation of rRNA biosynthesis at transcriptional and posttranscriptional levels.

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2. Lubomira Stateva and Pencho Venkov; (1984), Genetic analysis of Saccharomyces cerevisiae SY15 Relaxed Mutant; Mol. Gen. Genet. (accepted for publication).

   Evidence is presented showing that the relaxed phenotype of the SY15 mutant is determined by one nuclear recessive mutation. The most characteristic patterns of the relaxed phenotype in yeast - rRNA accumulation and rRNA processing in the absence of protein synthesis - were found to segregate in first and second generation crosses.

Therefore, the interruption of rRNA processing that occurs after starvation for a required amino acid is a pleiotropic manifestation of the stringent control itself. It is suggested that the locus for the stringent response in Saccharomyces cerevisiae (designated STR) coordinates the synthesis of rRNA on transcriptional and post-transcriptional levels.

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3. L.W. Waltschewa and P.V. Venkov, (1984); A Phosphorylated Compound is Involved in the Regulation of Ribosomal RNA Transcription in Saccharomyces cerevisiae; (to appear in Metabolism and Enzymology of Nucleic Acids Including Gene Manipulations., vol. 5, eds. J. Zelinka and J. Balan, Publ. House, Slovak Acad. of Sci., Bratislava.

A phosphorylated compound (PC) has been shown to accumulate in relaxed (but not in stringent) segregants after starvation for a required amino acid. The observed co-segregation of PC accumulation with the relaxed phenotype evidences the participation of PC in the regulation of rRNA synthesis in yeast. The kinetics of PC labeling and transcription of rRNA in stringent and relaxed strains, starved for a required amino acid have also been studied. The inhibition of PC labeling occurs a short time before the inhibition of rRNa transcription in stringent strains. However, in relaxed strains there is no significant inhibition of rRNA transcription, the labeling of PC is increased several times until it reaches a plateau.

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4. Liliana W. Waltschewa, Karl-Heinz Scheit* and Pencho Venkov, Transcription of Ribosomal RNA Genes in Yeast Nuclei is Stimulated by an Acid Soluble Compound (manuscript in preparation).

*Max - Plank Institute fuer Biophysicalische Chemie, Abteilung Molekulare Biologie, Goettingen D-3400, BRD.

We report the existence in Saccharomyces cerevisiae of effector molecules which stimulate the in vitro transcription of yeast ribosomal RNA genes. The effector was found in acid extracts of growing stringent strains and in a relaxed mutant, starved or not starved for a required amino acid. Amino acid deprivation leads to disappearance of the effector molecules in the stringent cells. These results suggest that the biosynthesis of ribosomal RNA in yeast might be under positive control mediated by acid soluble effector molecules.

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XIV. Department of Biological Sciences, Goldsmiths' College, University of London, London SE14 6NW, England. Communicated by J.F.T. Spencer.

Below follow three abstracts of work from our laboratory which will be presented at the International Yeast Symposium in Montpellier, France, in July.

1. J.F.T. Spencer, Karen Hearn, Dorothy M. Spencer and C. Bizeau.

Characteristics of some intergeneric hybrids, obtained by protoplast fusion, having S. diastaticus as a parent. II. S. diastaticus x Candida pseudotropicalis, and S. diastaticus x Hansenula wingei.

Abstract

Candida pseudotropicalis and Hansenula wingei were fused with a petite mutant of S. diastaticus, and the hybrids obtained were characterised. Unlike the hybrids previously obtained between S. diastaticus and C. pseudotropicalis, the hybrids from the most recent fusion resembled S. diastaticus more closely than C. pseudotropicalis. They metabolised starch relatively slowly and showed little growth on lactose. The hybrids sporulated, though spore viability was at best extremely low, and no segregants were obtained. The hybrids of H. wingei also sporulated, though too few spores were obtained for genetic analysis. Other characteristics of the hybrids are described.

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2. C. Bizeau, Stephanie Boucher, Dorothy M. Spencer and J.F.T. Spencer. Characteristics of some intergeneric hybrids, obtained by protoplast fusion having S. diastaticus as a parent. I. S. diastaticus x H. capsulata and S. diastaticus x T. glabrata.

Abstract

T. glabrata and H. capsulata were fused with petite strains of S. diastaticus and the hybrids were characterised. As was previously reported the H. capsulata x S. diastaticus hybrids were relatively stable, and karyogamy occurred, since electron micrographs showed only one nucleus per cell. Unlike the hybrids previously described, these sporulated less freely or not at all, and only a few viable spores were obtained. The T. glabrata x S. diastaticus hybrid was also uninucleate, and its fermentation and assimilation patterns were intermediate between those of the parents. Electron microscopy revealed some bizarre forms in the cell morphology.

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3. Dorothy M. Spencer, Tone Bjaaland, C. Bizeau and J.F.T. Spencer. Performance of some hybrids of baking yeast with Saccharomcyes diastaticus obtained by protoplast fusion.

Abstract

Some non-sporulating or weakly-sporulating baking yeast strains, of different bread-raising abilities, were hybridised with two petite strains of S. diastaticus by protoplast fusion. The hybrids obtained were tested for dough-raising ability by a cylinder test. The hybrids sporulated weakly or moderately, but produced only a few viable spores. One segregant was obtained, which grew well on a starch-containing medium. A baking strain which sporulated well and produced numerous viable spores was also fused with a petite strain of S. diastaticus. This hybrid utilised starch and sporulated well. The single-spore clones obtained by dissection of asci from the hybrid all metabolised starch. Results of cylinder tests from these segregants are presented and compared with those of the parent strain.

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XV. Research Institute of Seed Production and Trading Company, H-6601 Szentes, P.O. Box 41, Hungary. Communicated by Miklos Pesti.

The following papers have been published or are in press:

M. Pesti, E.K. Novák, A. Svoboda and L. Ferenczy, 1980. Protoplast fusion between polyene-resistant and sensitive mutants of Candida albicans. Acta Microbiol. Acad. Sci. Hung. 27:249.

M. Pesti, E.K. Novák, L. Ferenczy and A. Svoboda, 1981. Freeze fracture electron microscopical investigation of Candida albicans cells sensitive and resistant to nystatin. Sabouraudia 19:17-26.

M. Pesti, J. McA. Cambell and J.F. Peberdy, 1981. Alteration of ergosterol content and chitin synthase activity in Candida albicans. Current Microbiol. 5:187-190.

M. Pesti, S. Paku and E.K. Novák, 1982. Some characteristics of nystatin-resistant sterol mutants of Candida albicans. Acta Microbiol. Acad. Sci. Hung. 29:55-66.

M. Pesti and L. Ferenczy, 1982. Protoplast fusion hybrids of Candida albicans sterol mutants differing in nystatin resistance. J. gen. Microbiol. 128:123-128.

M. Pesti, D. Becher and G. Bartsch, 1983. The effect of miconazole on ergosterol-less mutant of Candida albicans. Acta Microbiol. Hung. 30:25-29.

The following papers have been submitted to Acta Microbiologica Hungarica.

M. Pesti and R. Novák, 1984. Alteration of glycerol uptake and utilization in a Candida albicans sterol mutant.

M. Pesti, L. Horváth, L. Vigh and T. Farkas, 1984. Lipid content and ESR determination of plasma membrane order parameter in Candida albicans sterol mutants.

Three nystatin-resistant, auxotrophic sterol mutants have been deposited in the ATCC with the following accession numbers: ATCC 44829, ATCC 44830, ATCC 44831. Strains and complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

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XVI. Facultad de Medicina, Departamento de Biofisica, General Flores 2125, Montevideo, Uruguay and Universidade de São Paulo*, Instituto de Quimica, Departamento de Bioquimica, Caixa Postal 20780 Sao Paulo, SP Brazil. Communicated by Ana Clara Schenberg Frascino*.

The following is an abstract of a paper recently accepted for publication in International Journal of Radiation Biology: Nunes, E., Brum, G., Candreva, E.C., and A.C. Schenberg Frascino, Common repair pathways acting upon U.V. and

X-ray induced damage in diploid cells of Saccharomyces cerevisiae.

Abstract

Studies on X-ray sensitive mutants of Saccharomyces cerevisiae (Benathen, A. and C.A. Beam, 1977, Radiat. Res. 69:99) show that the XS6, XS8 and XS9 genes are not only involved in the repair of X-ray-induced damage but also in the repair of UV-induced damage. Analysis of the UV sensitivity of multiple xs mutants indicates the participation of three repair pathways which differ from excision repair.

Under conditions which can influence repair, such as plating of the UV-irradiated cells in the presence of caffeine, followed or not by hyperthermic incubation, the wild type strain shows a diphasic survival curve, consisting of an exponential component for low doses and a sigmoidal one for higher doses. Comparison with the survival curves obtained for the sensitive mutants suggests that the first component of the wild type survival curve corresponds to the inhibition of the XS6 and XS8 gene products while the appearance of a radio-resistant fraction in the population relies on the induction of another repair pathway.

A sequential model of repair with two branching points is proposed to explain the results.

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XVII. Mitsubishi-Kasei Institute of Life Science, 11, Minamiooya, Machida-shi, Tokyo, Japan. Communicated by Norio Gunge.

The following papers have been published recently.

1. Norio Gunge, 1983. Yeast DNA plasmids. Annu. Rev. Microbiol. 37:253-276.

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2. Yuji Sugisaki, Norio Gunge, Kenji Sakaguchi, Makari Yamasaki & Gakuzo Tamura, 1983. Kluyveromyces lactis killer toxin inhibits adenylate cyclase of sensitive yeast cells. Nature 304:464-466.

Abstract

K_1 killer toxin secreted by the K_1^+ strain of Saccharomyces cerevisiae, has been well characterized. It is a simple protein of molecular weight (MW) 11,470 (ref. 3), encoded by a double-stranded, linear RNA plasmid, called M RNA, of MW 1.1-1.7 x 10^6 (refs 4-6). It is lethal to sensitive Saccharomyces cerevisiae which does not carry M RNA. Leakage of K^+ and ATP is the first distinct response in sensitive cells, and the toxic action is thought to be due to its action as a protonophore or K^+ ionophore. Recently, a further killer toxin has been found in Kluyveromyces lactis IFO 1267, and it is associated with the presence of the double-stranded linear DNA plasmids, pGK1-1 (MW 5.4 x 10^6) and pGK1-2 (MW 8.4 x 10^6)[12]. It has been shown, by curing pGK1-1 or deletion mapping, that the structural gene for the killer toxin and immunity-determining gene reside on the smaller plasmid. Moreover, the plasmids could be transferred from K. lactis to S. cerevisiae by protoplast

fusion and protoplast transformation. As the K. lactis toxin is encoded by a DNA plasmid and has a relatively wider action spectrum than K_1 killer toxin, the mode of action of the toxin is highly interesting. Here we report that K. lactis toxin inhibits adenylate cyclase in sensitive yeast cells and brings about arrest of the cells at the G_1 stage.

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Norio Gunge, Kousaku Murata, and Kenji Sakaguchi, 1982. Transformation of Saccharomyces cerevisiae with Linear DNA Killer Plasmids from Kluyveromyces lactis. J. of Bact., 151:462-464.

Abstract

Protoplasts of Saccharomyces cerevisiae were mixed with linear DNA plasmids, pGK1-1 and pGK1-2, isolated from a Kluyveromyces lactis killer strain and treated with polyethylene glycol. Out of 2,000 colonies regenerated on a nonselective medium, two killer transformants were obtained. The pGK1 plasmids and the killer character were stably maintained in one (Pdh-1) of them. Another transformant, Pdl-1, was a weak killer, and the subclones consisted of a mixture of weak and nonkiller cells. The weak killers were characterized by the presence of pGK1-1 in a decreased amount, and nonkillers were characterized by the absence of pGK1-1. The occurrence of two new plasmids which migrated faster than pGK1-1 in an agarose gel was observed in Pdl-1 and its subclones, whether weak or nonkillers. Staining with 4',6-diamidino-2-phenylindole revealed that the pGK1 plasmids exist in the cytosol of transformant cells with numerous copy numbers.

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

Below follow abstracts of four recent papers from our laboratory.

1. Michael J. Leibowitz and Regina W. Wang. 1984. Visualization and Elution of Unstained Proteins from Polyacrylamide Gels. Analytical Biochemistry 137, 161-163.

Abstract

Proteins fractionated by electrophoresis on 18% polyacrylamide gels with low crosslinking can be directly visualized by ultraviolet light-induced fluorescence and can be recovered by electroelution.

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2. Dennis J. Thiele, Ernest M. Hannig, and Michael J. Leibowitz. 1984. Multiple L Double-Stranded RNA Species of Saccharomyces cerevisiae: Evidence for Separate Encapsidation. Molecular and Cellular Biology, 4:92-100.

Abstract

The L double-stranded (ds) RNA component of Saccharomyces cerevisiae may contain up to three dsRNA species, each with a distinct sequence but with identical molecular weights. These dsRNAs have been separated from each other by denaturation and polyacrylamide gel electrophoresis. The 3' terminal sequences of the major species, L_A dsRNA, were determined. Secondary structural analysis supported the presence of two stem and loop structures at the 3' terminus of the L_A positive strand. In strain T132B NK-3, both the L_A and L_C species are virion encapsidated. Two distinct classes of virions were purified from this strain, each with a different RNA polymerase activity and with distinct protein components. The heavy virions harbored L_A dsRNA, whereas the L_C dsRNA species copurified with the light virion peak. Thus, L_A and L_C dsRNAs, when present in the same cell, may be separately encapsidated.

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3. Ernest M. Hannig, Dennis J. Thiele, and Michael J. Leibowitz. 1984. Saccharomyces cerevisiae Killer Virus Transcripts Contain Template-Coded Polyadenylate Tracts. Molecular and Cellular Biology, 4:101-109.

Abstract

The M double-stranded RNA component of type 1 killer strains of the yeast Saccharomyces cerevisiae contains an internal 200-base pair adenine- and uracil-rich region. The plus strands of this viral genomic RNA contain an internal adenine-rich region which allows these strands to bind to polyuridylate-Sepharose as tightly as do polyadenylated RNAs with 3'-terminal polyadenylated tracts of 70 to 100 residues. Internal template coding of an adenine-rich tract in positive polarity in vivo and in vitro transcripts of M double-stranded RNA may serve as an alternate method of transcript polyadenylation. The 3'-terminal residue of the in vitro m transcript is a non-template-encoded purine residue. The 5' terminus of this transcript is involved in a stem-and-loop structure which includes an AUG initiation codon, along with potential 18S and 5.8S rRNA binding sites. Except for the 3'-terminal residue, transcription in vitro shows complete fidelity.

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4. Dennis J. Thiele, Ernest M. Hannig, and Michael J. Leibowitz. 1984. Genome Structure and Expression of a Defective Interfering Mutant of the Killer Virus of Yeast. Virology, in press.

Abstract

A large internal deletion in M_1 double-stranded (ds)RNA from the killer virus of Saccharomyces cerevisiae generates a suppressive (S3) dsRNA molecule. Strains which harbor S3 dsRNA are defective in toxin production and immunity to the toxin. The biochemical defect in expression has been investigated, and is apparently due to truncation of the protoxin polypeptide translation reading frame on S3 dsRNA. Transcription in vivo, and in isolated virions in vitro, results in the synthesis of a full-length positive polarity messenger RNA, denoted s. The s transcript contains no long poly(A) tracts as determined by its lack of affinity for oligo(dT)-cellulose, and as inferred by sequence analysis of approximately 87% of the S3 dsRNA genome. The data support a model for template-coding of polyadenylate in transcripts derived from the wild-type M_1 dsRNA. The orientation of the sequences conserved on S3

dsRNA with respect to M_1 dsRNA has been determined. Some of the conserved sequences are likely to be required for the maintenance and replication of these viral dsRNA genomes in S. cerevisiae.

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XIX. Institut für Mikrobiologie und Weinforschung der Johannes Gutenberg-Universität Mainz, Saarstrasse 21, D-6500 Mainz, Federal Republic of Germany. Communicated by F. Radler.

The following article was recently published.

P. Pfeiffer and F. Radler. 1984. Comparison of the killer toxin of several yeasts and the purification of a toxin of type K_2. Arch Microbiol. 137:357-361.

Abstract

A total of 13 killer toxin producing strains belonging to the genera Saccharomyces, Candida and Pichia were tested against each other and against a sensitive yeast strain. Based on the activity of the toxins 4 different toxins of Saccharomyces cerevisiae, 2 different toxins of Pichia and one toxin of Candida were recognized. The culture filtrate of Pichia and Candida showed a much smaller activity than the strains of Saccharomyces. Extracellular killer toxins of 3 types of Saccharomyces were concentrated and partially purified. The pH optimum and the isoelectric point were determined. The killer toxins of S. cerevisiae strain NCYC 738, strain 399 and strain 28 were glycoproteins and had a molecular weight of M_r = 16,000. The amino acid composition of the toxin type K_2 of S. cerevisiae strain 399 was determined and compared with the composition of two other toxins.

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XX. The Research Laboratory, Arthur Guinness Son & Co. (Great Britain) Ltd., Park Royal Brewery, London NW10 7RR, England. Communicated by J.R.M. Hammond.

The following is an abstract of a paper that will appear in the May/June 1984 issue of the Journal of the Institute of Brewing.

J.R.M. Hammond and K.W. Eckersley. 1984. Fermentation Properties of Brewing Yeast with Killer Character.

Summary

The cytoplasmically-inherited killer character of a laboratory strain of Saccharomyces cerevisiae has been transferred to three different commercially-used brewing yeasts; two ale strains and one lager strain. The ease with which the character can be transferred is very strain dependent. In addition to killer character, mitochondria from the brewing strain have been transferred into the new "killer" brewing strains. Fermentations carried out with the manipulated strains produced beers which were very similar to those produced by the control brewing strains. The beers produced by killer brewing strains containing brewing yeast mitochondria were most like the control beers and could not be distinguished from them in three glass taste tests. In

addition to producing good beers the genetically manipulated yeasts killed a range of contaminant yeasts and were themselves immune to the action of Kil-k_1 killer yeasts.

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

Below follow abstracts of our work published since December 1983.

1. John Londesborough and Kaija Varimo. 1984. Characterization of Two Trehalases in Baker's Yeast. Biochemical Journal 219: (in press).

   Trehalase activities at pH 5 (not inhibited by EDTA) and pH 7 (inhibited by EDTA) were present in the soluble fraction of disintegrated commercial baker's yeast. The pH 5 activity binds strongly to concanavalin A, is only partially salted out by saturated $(NH_4)_2SO_4$, has an apparent M_r of 215000 (by gel filtration) and is an acidic protein. It has a K_m of 1.4 mM, a broad pH optimum (at 40 mM-trehalose) between pH 4 and 5, and is activated by about 30% by 20-300 mM neutral salts such as KCl, $NaNO_3$ and $MnCl_2$. The enzyme is strongly inhibited by acetic acid/acetate buffers, with a K_1 of about 15 mM-acetic acid. The pH 7 activity does not bind to concanavalin A, is salted out at 20-32% (w/v) $(NH_4)_2SO_4$ and has an M_r of 170000 (by gel filtration). It is absolutely dependent on Ca^{2+} or Mn^{2+} ions (Mg^{2+} is ineffective) and strongly inhibited by neutral salts in the 20-100 mM range. It can be activated by treatment with MgATP in the presence of cyclic AMP. Activation decreases, but does not abolish, the Ca^{2+} requirement, and does not change the K_m for trehalose (5.7 mM) or shift the sharp pH optimum at pH 6.7 (at 40 mM-trehalose).

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2. Kari Suoranta and John Londesborough. 1984. Purification of Intact and Nicked Forms of a Zinc-Containing, Mg^{2+}-Dependent, Low K_m Cyclic AMP Phosphodiesterase From Bakers' Yeast. The Journal of Biological Chemistry (in press).

   A low K_m cyclic AMP phosphodiesterase was purified to homogeneity from microsomes of bakers' yeast. "Intact" enzyme, purified from microsomes prepared in the presence of the protease inhibitor phenylmethyl-sulfonyl fluoride, had a specific activity of 0.6 µmol/min/mg of protein (30°C, pH 8.0, 1 µM cyclic AMP), a pI of 6.65 ± 0.15, and a molecular weight of 61,000 determined by gel electrophoresis in the presence of sodium dodecyl sulfate. Gel filtration of native enzyme suggested it is a monomer. When phenylmethylsulfonyl fluoride was omitted, a product ("nicked" enzyme) was obtained with a specific activity of 1.2 µ mol/min/mg of protein, the same pI, and a similar amino acid composition; but gel electrophoresis now showed two bands, with molecular weights of 45,000 and about 17,000, together with a small amount of the 61,000 band. Apart from the higher specific activity of the nicked enzyme, no difference was found between the catalytic properties of the two enzyme forms. Between 40 nM and 1 µ M cyclic AMP, an apparent K_m of 170 nM was observed at pH 8.0, but at higher cyclic AMP concentrations (2-30 µ M), Hofstee plots curved upwards. Cyclic deoxy-AMP was a

substrate, but cyclic GMP was not and did not affect the activity towards cyclic AMP. Both enzyme forms contained tightly bound zinc. The metal chelators, 8-hydroxyquinoline and ortho-phenanthroline, caused progressive partial inactivation of the enzyme and a decrease in its affinity for cyclic AMP. Dialysis against Zn^{2+}, Cu^{2+}, Co^{2+}, or Mn^{2+} (but not Mg^{2+} or Ni^{2+}) reversed these changes.

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3. Kaija Varimo, Kai Harju, Kari Edelmann and Matti Korhola. 1983. Selenium Enriched Yeast. Kemia-Kemi 10:1049-1050.

Selenium is an analog of sulfur and behaves biochemically in such a way that it tends to replace sulfur. If yeast is grown in the absence of a source of inorganic sulfur, such as sulfate or sulfite, and instead is given selenite (or selenium dioxide) or selenate, selenium is incorporated into analogs of the sulfur-containing amino acids. Thus, selenocysteine, selenocystine, selenomethionine, selenoglutathione and some other sulfur analog compounds are formed.
Selenium-rich yeast was prepared by growing baker's yeast by standard procedures with SeO_2 as a source of selenium. The resulting yeast was extensively washed and drum-dried. Disintegration and fractionation of selenium-rich yeast gave the following results: 58% of the incorporated selenium was found in the fraction of soluble proteins and nucleic acids, 15% in the cell wall fraction, and likewise 9% in the mitochondrial and microsomal fractions. Free amino acids, small peptides, other small molecular weight compounds and salts (non-acid precipitable fraction) contained 19% of the selenium.

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XXII. Laboratory of Industrial Biochemistry, Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606, Japan. Communicated by T. Kamihara.

The following article has been published.

T. Kamihara and I. Nakamura. Regulation of respiration and its related metabolism by vitamin B_1 and vitamin B_6 in Saccharomyces yeasts. Advances in Biochemical Engineering/Biotechnology, 29, 35-82 (1984).

Abstract

Thiamine added to a vitamin B_6-free medium was accumulated in the free form in Saccharomyces yeast cells, and caused growth inhibition and respiratory deficiency under aerobic conditions. The thiamine-induced respiratory deficiency occurred as the result of a sequence of events as follows: (1) a decrease in vitamin B_6 content, (2) a reduction in the activity of δ-aminolevulinate synthase, (3) heme deficiency, (4) cytochrome deficiency, and (5) respiratory deficiency. However, the growth inhibition was shown to be partially due to the respiratory deficiency, and the participation of some other events caused by the thiamin-induced vitamin B_6 deficiency was suggested. The cytochrome deficiency also caused alteration in lipid composition; unsaturated fatty acid content was decreased and sterol composition was changed. Associated with the respiratory deficiency, the

activity of NAD-linked glutamate dehydrogenase was decreased. In contrast, the NADP-linked enzyme activity was markedly increased. It was suggested that this increase was not caused by the respiratory deficiency but by a thiamine-enhanced glucose effect. The thiamine-grown cells showed an altered amino acid pool. Reflecting the respiratory deficiency, the thiamine-grown cells showed elevated activities of glycolysis and ethanol production. It was, however, suggested that the glycolytic rate was not determined by phosphofructokinase activity, and ethanol production was controlled independently of glycolytic activity in the thiamine-supplemented culture.

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XXIII. Miami University, Department of Microbiology, Oxford, Ohio, 45056. Communicated by J.K. Bhattacharjee.

The following papers have been published recently from my laboratory:

1. Kinzel, Jerome J., and J.K. Bhattacharjee. 1982. Lysine biosynthesis in Rhodotorula glutinis: Properties of pipecolic acid oxidase. J. Bacteriol. 151:1073-1077.

2. Winston, Melissa K., and J.K. Bhattacharjee. 1982. Growth inhibition by aminoadipate and reversal of the effect by specific amino acid supplements in Saccharomyces cerevisiae. J. Bacteriol. 152:874-879.

3. Bhattacharjee, J.K. 1983. Lysine biosynthesis in eukaryotes. In Amino acid biosynthesis and genetic regulation, K.M. Herrmann and R.L. Somerville (eds.), Ch. 13, Addison-Wesley.

4. Kinzel, Jerome J., Melissa K. Winston, and J.K. Bhattacharjee, 1983. Role of Lysine-α-Ketoglutarate Aminotransferase in Catabolism of Lysine as a Nitrogen Source for Rhodotorula glutinis. J. Bacteriol. p. 417-419.

5. Borell, C., L.A. Urrestarazu, and J.K. Bhattacharjee, 1984. Multiple genes (LYS9 and LYS14) for Saccharopine reductase. J. Bacteriol. 159 (in press).

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XXIV. Centro de Investigaciones en Fisiologia Celular, Universidad Nacional Autonoma de Mexico, Apartado postal 70-600, 04510 Mexico, D.F. Communicated by Antonio Peña.

The following is a recent publication from our laboratory.

A. Peña, S. Uribe, J.P. Pardo and M. Borbolla. 1984. The use of a cyanine dye in measuring membrane potential in yeast. Arch. Biochem. Biophys. In press.

Summary

3,3'dipropyl-thiacarbocyanine was used as membrane potential probe in yeast by following both its fluorescence changes and its uptake by the cells under different conditions. Results indicate that the uptake of the dye into the cytoplasmic compartment is translated into an increased fluorescence, and the uptake by the mitochondria produces a quenching of the fluorescence. The

experiments to measure uptake showed that a large amount of the dye is taken up by the cells under "deenergized" conditions. The uptake of the dye, however, is significantly reduced by the omission of the substrate, or by deenergization of the mitochondria, or by the addition of K^+ but not of Na^+. This cyanine seems to be a good qualitative indicator of the potential of the plasma membrane and the mitochondria of the cells, with a faster response than those probes used before in yeast.

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XXV. Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309. Communicated by Robert O. Poyton.

The following three papers deal with recent work from our laboratory on The Nuclear-coded Subunits of Yeast Cytochrom c Oxidase.

1. Scott D. Power, Michael A. Lochrie, Kevin A. Sevarino, Thomas E. Patterson, and Robert O. Poyton. 1984. I. Fractionation of the Holoenzyme into Chemically Pure Polypeptides and the Identification of Two New Subunits Using Solvent Extraction and Reversed Phase High Performance Liquid Chromatography. The Journal of Biological Chemistry 259 (in press).

Previously, cytochrome c oxidase from the yeast Saccharomyces cerevisiae has been thought to be composed of seven different polypeptide subunits. Four of these are small polypeptides (4,000-15,000 daltons), subunits IV-VII, which are encoded by nuclear DNA. Studies described here reveal the presence of two new polypeptides in this size range. These polypeptides, designated as subunits VIIa and VIII, co-migrate with subunit VII (R.O. Poyton and G. Schatz (1975) J. Biol. Chem. 250, 752-761) on low resolution sodium dodecyl sulfate (SDS) polyacrylamide gels, can be partially resolved on high resolution SDS polyacrylamide gels, and can be completely separated from one another by reversed phase high performance liquid chromatography.

In order to determine the sequences of each of these six nuclear-coded polypeptides (subunits IV, V, VI, VII, VIIa, and VIII), we have developed new methods for the large scale purification of the holoenzyme and have employed a new strategy for the isolation of each polypeptide. By using octyl-Sepharose chromatography to isolate holocytochrome c oxidase and by extracting the holoenzyme with aprotic organic solvents and fractionating these extracts by reversed phase high performance liquid chromatography, it is possible to isolate several milligrams of each of these subunits. Each subunit preparation gives a single peak during reversed phase high performance liquid chromatography, a single band during SDS-polyacrylamide gel electrophoresis, a single NH_2-terminal sequence, and a unique amino acid composition and tryptic peptide map. Since each purified subunit preparation gives close to a 100% yield of its NH_2-terminal amino acid during quantitative Edman degradation, we conclude that no subunit has a blocked NH_2 terminus and that no subunit preparation contains either blocked or unblocked contaminating polypeptides. Thus, each consists of a single unique polypeptide species.

Together, these results demonstrate that yeast cytochrome c oxidase contains six, rather than four, small subunit polypeptides. Thus, it appears that these polypeptides, in combination with the three polypeptides encoded by

mitochondrial DNA, constitute a holoenzyme which contains nine subunits, instead of seven as proposed earlier.

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2. Scott D. Power, Michael A. Lochrie, Thomas E. Patterson, and Robert O. Poyton. 1984. II. The Amino Acid Sequence of Subunit VIII and a Model for its Disposition in the Inner Mitochondrial Membrane. J. Biol. Chem. 259 (in press).

The amino acid sequence of subunit VIII from yeast cytochrome c oxidase is reported. This 47-residue (M_r = 5364) amphiphilic polypeptide has a polar NH_2 terminus, a hydrophobic central section, and a dilysine COOH terminus. An analysis of local hydrophobicity and predicted secondary structure along the peptide chain predicts that the hydrophobic central region is likely to be transmembranous. Subunit VIII from yeast cytochrome c oxidase exhibits 40.4% homology to bovine heart cytochrome c oxidase subunit VIIc, at the level of primary structure. Secondary structures and hydrophobic domains predicted from the sequences of both polypeptides are also highly conserved. From the location of hydrophobic domains and the positions of charged amino acid residues we have formulated a topological model for subunit VIII in the inner mitochondrial membrane.

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3. Scott D. Power, Michael A. Lochrie, and Robert O. Poyton. 1984. III: Identification of Homologous Subunits in Yeast, Bovine Heart, and Neurospora Crassa Cytochrome c Oxidases. J. Biol. Chem. 259 (in press).

Sequences for the NH_2-terminal halves of subunits IV, V, VI, VII and VIIa from yeast cytochrome c oxidase have been determined and used to identify homologous subunits in bovine heart and Neurospora crassa cytochrome c oxidases. In conjunction with the complete sequence of subunit VIII (S.D. Power, M.A. Lochrie, T.F. Patterson, and R.O. Poyton (1984) J. Biol. Chem. 259), we have been able to identify counterparts to yeast subunits IV, V, VI, and VIII in bovine heart cytochrome c oxidase and counterparts to yeast subunits IV and V in Neurospora crassa cytochrome c oxidase. The sequences of these nuclear-coded subunits are conserved between species at a level of 30-50%. Thus, they are conserved to the same extent as the three mitochondrially coded subunits (I, II, and III). The similar degree of homology between species for both the nuclear and mitochondrially coded subunits of cytochrome c oxidase suggests that both sets of polypeptides are conserved coordinately and are, therefore, important components of the functional holoenzyme.

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XXVI. Botanisches Institut der Universität Düsseldorf, Universitätsstrasse 1, D-4000 Düsseldorf, West Germany. Communicated by Georg Michaelis.

Change of Address:

I have recently accepted a position at the Botanisches Institut der Universität Düsseldorf, Universitätsstrasse 1, D-4000 Düsseldorf, Federal Republic of Germany. Tel 0211/3114801 or 3112881 (in the morning). My former address was Fakultät für Biologie der Universität Bielefeld, Postfach 8640, D-

4800 Bielefeld, Federal Republic of Germany.

Below follows the abstract of our recent work concerning the precursor form and signal peptide of a mitochondrially synthesized protein.

Elke Pratje, Gertrud Mannhaupt, Georg Michaelis and Konrad Beyreuther (1983). A nuclear mutation prevent processing of a mitochondrially encoded membrane protein in Saccharomyces cerevisiae. The EMBO Journal 2:1049-1054.

Subunit II of cytochrome oxidase is encoded by the mitochondrial OXI1 gene in Saccharomyces cerevisiae. The temperature-sensitive nuclear pet mutant ts2858 has an apparent higher mol. wt. subunit II when analyzed on lithium dodecylsulfate (LiDS) polyacrylamide gels. However, on LiDS-6M urea gels the apparent mol. wt. of the wild-type protein exceeds that of the mutant. Partial revertants of mutant ts2858 that produce both the wild-type and mutant form of subunit II were isolated. The two forms of subunit II differ at the N-terminal part of the molecule as shown by constructing and analyzing nuclear ts2858 and mitochondrial chain termination double mutants. The presence of the primary translation product in the mutant and of the processed form in the wild-type lacking 15 amino-terminal residues was demonstrated by radiolabel protein sequencing. Comparison of the known DNA sequence with the partial protein sequence obtained reveals that six of the 15 residues are hydrophilic and, unlike most signal sequences, this transient sequence does not contain extended hydrophobic parts. The nuclear mutation ts2858 preventing post-translational processing of cytochrome oxidase subunit II lies either in the gene for a protease or an enzyme regulating a protease.

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XXVII. Albert-Ludwigs Universität, Biochemisches Institut, Hermann-Herder-Str. 7, D-7800 Freiburg I. Br., West Germany. Communicated by Dieter H. Wolf.

1. The following paper has recently been published in FEBS-Letters 166, 321-325 (1984):

   Vacuoles are not the Sole Compartment of Proteolytic Enzymes in Yeast

   O. Emter and D.H. Wolf

   Localization in vacuoles, the lysosome-like organelle of yeast, was checked for several newly detected proteolytic enzymes of the yeast Saccharomyces cerevisiae. While aminopeptidase Co (Achstetter, T., Ehmann, C., and Wolf, D.H. (1982) Biochem. Biophys. Res. Commun. 109, 341-347) and carboxypeptidase S (Wolf, D.H. and Weiser, U. (1977) Eur. J. Biochem. 73, 553-556) were found in vacuoles, proteinase yscD (in preparation) and proteinase yscE (Achstetter, T., Ehmann, C., Osaki, A., and Wolf, D.H. (1984) J. Biol. Chem., in press) as well as a variety of other proteolytic activities detectable with the aid of chromogenic peptide substrates do not reside in this cell compartment. The results presented show that the vacuole is not the sole locus of proteolytic enzymes. The question remains to be answered whether the proteolytic enzymes found outside the vacuole are actually digestive enzymes responsible for protein degradation or whether these proteinases serve highly specific functions.

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The following two papers are in press in the Journal of Biological Chemistry.

2. Identification of New Proteolytic Enzymes of Yeast.

T. Achstetter, O. Emter, C. Ehmann, and D.H. Wolf.

A previous study led to the discovery of new proteinases in the yeast Saccharomyces cerevisiae (Achstetter, T., Ehmann, C., and Wolf, D.H. (1981) Arch. Biochem. Biophys. 207, 445-454). The search for proteolytic enzymes active in the neutral pH range has been extended. Studies were done on a mutant lacking four well known proteinases involved in protein degradation - the two endoproteinases A and B and the carboxypeptidases Y and S. When ^{3}H methylcasein was used as substrate, absence of these four enzymes resulted in a reduction of proteolytic acitivity in cell extracts of about 99.7%. The residual 0.3% of the ^{3}H methylcasein splitting activity found in mutant extracts could be separated by gel filtration into several distinct activity peaks. In the search for new proteolytic enzymes, besides ^{3}H methylcasein twenty-nine chromogenic peptides (aminoterminally blocked peptidyl-4-nitroanilides) were used as substrates. For the detection of endoproteolytic activity using chromogenic peptide substrates, two versions of the assay were used. In one system the direct cleavage of the 4-nitroanilide bond by proteinases was measured. In the second version the cleavage of the chromogenic peptide at some site other than the 4-nitroanilide bond was measured. Both variations, which finally make use of the same detection principle, - the liberation of free 4-nitroaniline - led to the discovery of multiple proteinase activities in the soluble cell extract and in a purified membrane fraction. Regulation of a variety of proteolytic activities under different growth conditions of cells was observed. Ion exchange chromatography was used as tool for the reproducible separation of the soluble multiple proteolytic activities found in these cells. The multiplicity of the new proteinases found leads to the assumption that proteolysis plays a central role in the regulation of cellular functions in yeast.

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3. Proteinase yscE, A New Yeast Peptidase

T. Achstetter, C. Ehmann, A. Osaki, and D.H. Wolf. J. Biol. Chem. (in press).

A new peptidase, which we call proteinase yscE, was purified from the yeast Saccharomyces cerevisiae. The enzyme cleaves the synthetic substrates Cbz-Gly-Gly-Leu-4-nitroanilide, Cbz-Ala-Ala-Leu-4-nitroanilide and Suc-Phe-Leu-Phe-4-nitro-anilide at the 4-nitroanilide bond and exhibits a small activity against ^{3}H methylcasein. Optimum pH for cleavage of the chromogenic substrates is found to be in the range of 8.2 to 8.6. The purified enzyme has an apparent Stokes radius of R_S = 75.2 Å (M_r = 600,000) as judged by gel chromatography and is composed of subunits. Mercurials were found to be strong inhibitors of the enzyme activity.

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XXVIII. Kirin Brewery Co., Ltd., Brewing Science Laboratory, 3, Miyahara-cho, Takasaki-shi, GUNMA 370-12, Japan. Communicated by Kazuo Yoshioka.

1. Kazuo Yoshioka and Naoki Hashimoto. 1983. Cellular Fatty Acid and Ester Formation by Brewers' Yeast. Agric. Biol. Chem., 47(10):2287-2294.

Abstract

The activity of alcohol acetyltransferase, bound to the cell membrane and responsible for the formation of acetate esters, was affected by the fatty acid composition of the cell membrane. When saturated fatty acids, which only slightly inhibit alcohol acetyltransferase activity, were incorporated into the cell membrane, the enzyme activity and ester formation were only slightly affected. On the other hand, when unsaturated fatty acids, which strongly inhibit the enzyme activity, accumulated in the cell membrane, ester formation was suppressed with inhibition of the enzyme activity. The mechanism of formation of acetate esters by brewers' yeast was explained by the alcohol acetyltransferase activity under the influence of the fatty acid composition of the cell membrane.

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2. Kazuo Yoshioka and Naoki Hashimoto. 1984. Acetyl-CoA of Brewers' Yeast and Formation of Acetate Esters. Agric. Biol. Chem., 48(1):207-209.

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3. Kazuo Yoshioka and Naoki Hashimoto. 1984. Ester Formation by Brewers' Yeast during Sugar Fermentation. Agric. Biol. Chem., 48(2):333-340.

Formation of acetate esters by brewers' yeast during sugar fermentation was investigated in relation to alcohol acetyltransferase activity influenced by the fatty acid composition of the yeast cell membrane. Glucose gave more acetate esters with a higher activity of alcohol acetyltransferase than the other carbohydrates. When maltose was fermented, the activity of alcohol acetyltransferase bound to the cell membrane was suppressed by unsaturated fatty acids accumulated in the cell membrane and the formation of acetate esters was greatly reduced without insufficient fermentation. On the other hand, when fructose was fermented, the ester formation was reduced with a decrease in the enzyme activity and the formation of higher alcohols through insufficient fermentation.

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Two earlier papers on this subject are:

4. Kazuo Yoshioka and Naoki Hashimoto. 1981. Ester Formation by Alcohol Acetyltransferase from Brewers' Yeast. Agric. Biol. Chem., 45(10):2183-2190.

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5. Kazuo Yoshioka and Naoki Hashimoto. 1982. A New Micromethod for Determination of Acetyl-CoA with Alcohol Acetyltransferase. Agric. Biol. Chem., 46(8), 2155-2156.

* * *

XXIX. Department of Biotechnology, Swiss Federal Institute of Technology, Hoenggerberg, 8093 Zürich, Switzerland. Communicated by O. Käppeli.

At the Department two research projects involving yeasts are in progress: (i) Regulation of glucose metabolism in Saccharomyces cerevisiae and (ii) Cytochrome P450 of yeasts: Induction, isolation and catalytic activity.

Ad(i): Glucose metabolism is investigated by means of the continuous culture technique. Below are the abstracts of two recent papers which report on our main findings so far.

1. Magdalena Petrik, Othmar Kappeli and Armin Fiechter. 1983. An Expanded Concept for the Glucose Effect in the Yeast Saccharomyces uvarum: Involvement of Short- and Long-term Regulation. Journal of General Microbiology, 129:43-49.

Summary

When Saccharomyces uvarum was cultivated in continuous culture, it exhibited the typical growth behaviour of a glucose-sensitive yeast. Metabolic changes related to glucose-repressed growth were assessed by an analysis of overall culture parameters (biomass formation, ethanol and acetate production and gas exchange rates) and by measuring the mitochondrial cytochrome content. These functions were mainly affected by the glucose effect; the steady state values of these variables were first established in the chemostat as a function of dilution rate.

The short- and long-term regulation taking place when the cells were submitted to repression was assessed by administering glucose pulses and by shifts in the dilution rate. The primary response of the cells to the initiation of repressed growth was the formation of ethanol and acetate. Since there was no repression of oxygen uptake rate and of cytochrome content prior to this response, it was concluded that ethanol and acetate formation was not the consequence of repression of respiratory activity, but resulted from the regulation of pyruvate dehydrogenase and pyruvate decarboxylase activities. Long-term adaptation of the cells occurred within 24 to 48 h of the initiation of repressed growth as manifested by a decrease of mitochondrial cytochrome content to the steady state value corresponding to that of repressed growth.

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2. Martin Rieger, Othmar Käppeli and Armin Fiechter. 1983. The Role of Limited Respiration in the Incomplete Oxidation of Glucose by Saccharomyces cerevisiae. Journal of General Microbiology, 129:653-661.

Summary

The respiratory capacity of Saccharomyces cerevisiae growing in

continuous culture on glucose and on mixtures of glucose and ethanol was investigated. An oxygen uptake rate of 8 mmol $g^{-1}h^{-1}$ was found to limit the ability of the organism to degrade a substrate purely oxidatively. On glucose as sole energy and carbon source, this respiration rate was invariably achieved at an identical growth rate and thus at an identical substrate uptake rate when the inlet glucose concentration was varied. The rate of ethanol co-consumption together with glucose was strictly governed by this limiting maximum respiratory capacity and no repression of respiration was observed at dilution rates where ethanol was excreted by the cells. Hence, a limitation in some step in the oxidative branch of catabolism is likely to be responsible for incomplete oxidation of glucose at high growth rates rather than an undefined action of glucose repression.

* * *

Ad(ii): For yeast of the main metabolic types (glucose-sensitive Saccharomyces sp.; oxygen-sensitive Candida tropicalis with glucose and hydrocarbon as the substrate, and oxidative Trichosporon cutaneum) we have shown the presence of cytochrome P450 in the microsomal fraction. It seems that this cytochrome is generally present in yeasts although not visible in the reduced CO-difference spectrum of whole cells. The culture conditions, for cells with high cytochrome P450 content were elaborated in continuous culture and a method for a fast isolation of the microsomal fraction was developed. Analysis of cytochrome P450 from different yeasts and from such cultivated at different conditions revealed that at least two cytochrome P450 species are present in yeasts. Below are the summaries of the relevant papers.

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3. Maria Trinn, Othmar Käppeli and Armin Fiechter. 1982. Occurrence of Cytochrome P450 in Continuous Culture of Saccharomyces cerevisiae. European J. Appl. Microbiol. Biotechnol., 15:64-68.

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4. Othmar Käppeli, Marija Sauer and Armin Fiechter. 1982. Convenient Procedure for the Isolation of Highly Enriched, Cytochrome P450-Containing Microsomal Fraction from Candida tropicalis.

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5. Dominique Sanglard, Othmar Käppeli and Armin Fiechter. 1984. Metabolic Conditions Determining the Composition and Catalytic Activity of Cytochrome P450 Monooxygenases in Candida tropicalis. J. Bacteriol., 157:297-302.

Summary

In the microsomal fraction of Candida tropicalis cells, two distinct monooxygenases were detected, depending on the growth conditions. The distinction of the two monooxygenases was evident from: (i) the absorption maxima in the reduced CO difference spectrums of the terminal oxidases (cytochromes P450 and P448); (ii) the contents of the monooxygenase components (cytochromes P450/P448, NADPH-cytochrome c (P450) reductase and cytochrome b_5) and (iii) the catalytic activity of the complete system (aliphatic

hydroxylation and N-demethylation activity). The occurrence of the respective monooxygenases could be related to the carbon source (n-alkanes or glucose). Oxygen limitation led to a significant increase of cytochrome P450/P448 content, independent of the carbon source utilized by the cells. An improved method for the isolation of microsomes enabled us to demonstrate the presence of cytochrome P448 in glucose-grown cells.

* * *

XXX. National Research Council Canada, Prairie Regional Laboratory, 110 Gymnasium Road, University Campus, Saskatoon S7N 0W9, Sask., Canada. Communicated by J.W.D. GrootWassink.

The following paper will soon appear in Enzyme & Microbial Technology.

G.M. Hewitt and J.W.D. GrootWassink. 1984. Simultaneous production of inulase and lactase in batch and continuous cultures of Kluyveromyces fragilis.

Abstract

Under different induction conditions, the industrial yeast Kluyveromyces fragilis is an excellent producer of the enzymes inulase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) and lactase (β-D-galactoside galactohydrolase, EC 3.2.1.23), producing 27 and 1.6 U mg^{-1} dry cell weight, respectively. In order to improve overall enzyme yields, conditions for the simultaneous production of both enzymes in a one-stage fermentation have been examined. Techniques employed include carbon-limited batch and continuous culture, single and mixed carbon substrates, and the use of a mutant semi-constitutive for inulase production. Synthesis of both enzymes suffered strongly from carbon catabolite repression in batch cultures grown on single and mixed inducing substrates. Only glycerol and DL-malate did not repress either enzyme. The non-metabolizable analogues of lactose, isopropyl-β-D-thiogalactoside and methyl-β-D-thiogalactoside induced lactase in glycerol grown batch cultures, but were ineffective in sucrose grown continuous cultures. They also depressed the normally high levels of inulase in such continuous cultures. The highest simultaneous inulase and lactase activities in the wild-type yeast were obtained in continuous culture on an equal mixture of D-fructose and D-galactose; 25 and 0.78 U mg^{-1} dry cell weight, respectively. In this fermentation the combined yield per unit carbon substrate of the two enzymes was 141%, compared to a reference value of 100% for the highest yield of each enzyme in separate fermentations. On the same mixture of D-fructose and D-galactose, the mutant produced ~60 and 0.70 U mg^{-1} dry cell weight, respectively. The combined enzyme yield per unit of carbon substrate was 172%.

* * *

XXXI. Institut D'Oenologie Station Agronomique et Oenologique, 351, Cours de la Libération, 33405 Talence Cedex, France. Communicated by Suzanne Lafon-Lafourcade.

Below are listed a number of recent publications from our Institute.

1. S. Lafon-Lafourcade, E. Carre, and P. Ribéreau-Gayon. 1983. Occurrence of Lactic Acid Bacteria During the Different Stages of Vinification and Conservation of Wines. Applied and Environmental Microbiology, 46:874-880.

Abstract

We showed that the growth of lactic acid bacteria during alcoholic fermentation depends on the composition of the must. We illustrated how the addition of sulfur dioxide to the must before fermentation and the temperature of storage both affect the growth of these bacteria in the wine. Whereas species of Lactobacillus and Leuconostoc mesenteroides were isolated from grapes and must, Leuconostoc oenos was the only species isolated after alcoholic fermentation. This organism was responsible for the malolactic fermentation. Isolates of this species varied in their ability to ferment pentoses and hexoses. The survival of Leuconostoc oenos in wines after malolactic fermentation depended on wine pH, alcohol concentration, SO_2 concentration, and temperature of storage.

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2. Catherine Geneix, Suzanne Lafon-Lafourcade and Pascal Ribéreau-Gayon. 1983. The Effect of Fatty Acids on the Viability of Saccharomyces cerevisiae. Comptes Rendues Acad. Sc. Paris 296:Serie III, 943-947.

Abstract

Ethanol is not the only factor that inhibits the growth of Sacch. cerevisiae during fermentation. Nutritional deficiencies have little effect. Fatty acids of the C6, C8, C10 groups formed during fermentation seem to be major causes for the decrease of the yeast population and arrest of the fermentation.

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3. E. Carre, Suzanne Lafon-Lafourcade and A. Bertrand. 1983. Desacidification Biologique Des Vins Blancs Secs Par Fermentation De L'Acide Malique Par Les Levures. Connaissance Vigue Vin 17:43-53.

Abstract

A considerable malolactic fermentation can be obtained by adding to musts some commercial active dry yeasts. An addition of thiamine may increase this phenomenon. When addition of Schizoaccharomyces pombe takes place at the end of alcoholic fermentation consequences of the metabolism of this yeast strain about organoleptic properties of wine are considerably decreased. With a well-advised utilization of these yeasts it is possible to obtain a considerable deacidification of wines.

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4. Suzanne Lafon-Lafourcade, E. Carre, Aline Lonvaud-Funel and P. Ribéreau-Gayon. 1983. Induction De La Fermentation Malolactique Des Vins Par Inoculation D'Une Biomasse Industrielle Congelee De L. Oenos Apres Reactivation. Connaissance Vigne Vin 17:55-71.

Abstract

Stimulation of malolactic fermentation can be obtained by adding to wine an industrial biomas of L. oenos. Before use, deepfreeze bacteria need to be reactivated according to some special conditions which have been studied. Yeasts concerned in alcoholic fermentation have an influence on malolactic fermentation capacity of wines; this fact is in connection with some differences observed in wine technology. Among the strains of commercial dry yeasts, the influence of S. cerevisiae is lower than that of S. bayanus.

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5. Catherine Geneix, Suzanne Lafon-Lafourcade and P. Ribéreau-Gayon. 1983. Les Causes, La Prevention et le Traitement Des Arrets de la Fermentation Alcoolique. Connaissance Vigne Vin 17:205-217.

Abstract

In complete nutritive medium rich in sugar, such as grape must, the inhibition of alcoholic fermentation is caused by substances produced by the yeast which, acting synergistically with ethanol, are toxic to the yeasts themselves. Among these are decanoic and octanoic acids and their corresponding ethyl esters; their adsorption by yeast cell walls permits the prevention and treatment of fermentation stoppages (Patent n° 8309215).

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6. S. Lafon-Lafourcade, A. Lonvaud-Funel and E. Carre. 1983. Lactic acid bacteria of wines: stimulation of growth and malolactic fermentation. Antonie van Leeuwenhoek 49:349-352.

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XXXII. Université de Nantes, Laboratoire de Biologie et Cytophysiologie Végétales, 2, rue de la Houssinière, F. 44072 NANTES Cedex, France. Communicated by L. Simon.

1. A. Poulard, C. Cuinier and L. Simon, 1984. A Few Remarks Concerning Several Factors that Influence the Composition of the Yeast Microflora in Fermentations During Dry White Wines Vinifications. Communication to be presented at the VIth International Symposium on Yeast, Montpellier, France, July 1984.

A good growth of the autochthonous yeast microflora in dry white fermentations seems to be necessary to produce typical aromas and bring about the entire breakdown of the saccharides that are present in musts. For that, fermentation temperature is important for it can change the yeast metabolism. The few data on that topic led us to study the implication of the thermic factor on the musts' microflora over a 4-year period. Musts of 3 white grape varieties (2 Muscadets and 1 Gros Plant) which were collected on different soils of the Nantes vineyeard were regularly observed.

For each sample, the decanted amd homogenized must inside very large vats is divided into 3 series where the fermentations are carried out by

autochthonous yeasts. The temperatures are regulated in the experimental series at 12-14° C, 18-20° C and 26-28° C. Some must samplings at the main stages of the alcoholic fermentation allowed isolation and identification of 996 yeast strains. The study of the principal identified groups and species show: (i) the highest temperatures promote the development of yeasts such as Saccharomyces cerevisiae whose fermentative characteristic is dominant; in the case of grapes infested in the vineyard with Botrytis cinerea the amount of oxidative yeasts grows parallel with the increase of must temperature, (ii) when Saccharomyces cerevisiae coexists with Saccharomyces uvarum in a must, cool temperatures (12-14° C) exert an inhibitory effect on Saccharomyces cerevisiae development and favour those of Saccharomyces uvarum; inversely, at 26-28° C Saccharomyces cerevisiae strains development is promoted while the density of Saccharomyces uvarum decreases. Such a competitive phenomenon is also observed when significant cellular amount of Saccharomyces bayanus cohabit with Saccharomyces cerevisiae.

Experimental vinifications carried out in large vats permitted 3 other observations: (i) during alcoholic fermentation yeast intervention is more dependent on species that colonize grape berries than on a specific cellar microflora, (ii) bioclimatic factors of soils have a determining action on the microflora character, (iii) there is no risk of wine organoleptic nature modifications, or of changing the character of aromas by working at low fermentation temperatures. In fact, average temperatures (18-20° C) enable one to obtain harmonious wines that are characterized by a low level of higher alcohols and a large amount of ethyl esters of fatty acids.

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2. L. Simon, B. L. Zhang, and G. J. Martin, 1984. Application of natural abundance deuterium NMR spectroscopy to the study of isotopic fractionation and metabolic activity of yeasts during alcoholic fermentation. Communication to be presented at the VIth international symposium on yeast, Montpellier, France, July 1984.

^2H NMR at the natural abundance level has been applied recently to the quantitative determination of Site Specific Isotopic Fractionation (SSIF) of hydrogen in synthetic and natural molecules (Martin 1981, 1982 and 1983). SSIF was extensively studied in the case of alcoholic fermentation and it has been shown that the deuterium contents of water and sugars in the fermentation medium govern the distribution of deuterium atoms in CH_2DCH_2OH (I), CH_3CHDOH (II), CH_3CH_2OD (III) and HOD (IV) (Martin, 1983). The measurement of the ratio R defined as $R = 3\ N_{II}/N_I$ - where N represents the number of molecules I and II - offers a precise way to determine SSIF.

We have used this technique to quantitatively study the influence of yeast species on SSIF observed during alcoholic fermentation. Different strains of yeasts (lyophilized, commercial yeasts and living yeasts growth in the laboratory, POULARD, 1981) were used to ferment synthetic and natural mixtures containing sugars and water, the deuterium content of which being carefully determined beforehand.

For each kind of yeast, 2 to 4 fermentation runs were performed with sugars of different natural origins and the R ratios of the ethanols extracted from the mixture using standard techniques were determined (grape sugars, (D/H) water = 148 ppm).

YEAST	INITIAL CONCENTRATION (g or cell/ml)	FERMENTATION (days)	YIELD (g%)	R(NMR)
Baker's yeast - Fala	(3 g/400 ml)	16	4,8	2.560
- Springer	(3 g/400 ml)	16	4,9	2.566
Saccharomyces cerevisiae "brocades"	$0.190.10^6$/ml	20	4,8	2.522
Sacch. bayanus	$0.800.10^5$/ml	20	4,9	2.518
Sacch. bayanus "brocades"	$0.650.10^5$/ml	20	4,7	2.529
Sacch. uvarum	$0.109.10^6$/ml	20	4,5	2.459
Sacch. rosei	$0.107.10^6$/ml	20	4,4	2.664
Saccharomycodes ludwigii	$0.115.10^6$/ml	25	3,3	2.717
Zygosaccharomyces baillii	not measurable	25	2,3	2.723
Hanseniaspora uvarum	$0.920.10^5$/ml	25	1,1	2.621

The R values decrease with the alcohol forming ability of the yeast, but not with its origin. It appears that the whole population of the yeasts investigated here show important enzymatic fractionation factor of hydrogen isotopes arising from water and from sugars. This behaviour may offer a new and promising way for classifying yeasts on the basis of a special enzymatic activity.

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3. Liliane Simon, Jean Pierre Bossy, and Jean Pierre Garrec, 1984. Association of P and Ca in metachromatic granules of the hyphae in Aureobasidium pullulans. Ultrastructural and x-ray microanalytical studies. Submitted to Physiologie Végétale.

Abstract

The repartition of metachromatic granules in the hyphae of a strain of Aureobasidium pullulans cultured in saprophytic conditions was examined by TEM. P and CA accumulation was evidenced in these granules by X-ray microanalysis. Traces of S were also detected. These results are compared with those obtained on blastospores of the same species.

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XXXIII. Research Institute for Viticulture and Enology, Matuškova 25, 833 11 Bratislava, Czechoslovakia. Communicated by E. Minarik.

The following papers were published or are in press (summaries):

1. E. Minarik: Pure, mixed and associated wine yeast cultures in wine making (in Slovak). Vinohrad (Bratislava) 22, 1984 (in press).

   Mixed or associated wine yeast starters Saccharomyces rosei - S. cerevisiae or S. rosei - S. oviformis enable a more intensive fermentation of musts compared with pure yeast culture fermented grape juice. Resulting wines show substantial lower volatile acid

content. The optimal ratio of S. rosei : S. cerevisiae or S. rosei : S. oviformis should be 9:1, the total volume of yeast starter being 5%. Favourable results could be achieved first of all in musts with higher sugar content.

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2. E. Minarik: Contaminating yeast species and their significance in biological stability of wines (in Slovak). In: Progress in Viticultural and Enological Research, pp. 242-256, Príroda, Bratislava 1984.

In young wines prior to second racking Saccharomyces cerevisiae, S. oviformis and film-forming Candida vini predominate. In bottled wines with residual sugar a fructo- and osmophilic yeast, S. bailii var. bailii, is the predominating yeast variety causing wine turbidity and refermentations. Basic physiological, biochemical and technological properties of this harmful yeast variety are briefly discussed. Their origin may be suggested in the filling line and in concentrated must used for adjusting the sugar level in sweet wines.

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XXXIV. Ecole Nationale Supérieure Agronomique de Montpellier, Chaire de Génétique et de Microbiologie, 34060 Montpellier Cedex, France. Communicated by P. Galzy.

Below are listed articles recently published by workers of my laboratory.

1. Gonde P., Blondin B., Ratomahenina R., Arnaud A. and Galzy P. 1982. Selection of yeast strains for cellobiose alcoholic fermentation. J. Ferment. Technol. 60:579-584.

2. Blondin B., Ratomahenina R., Arnaud A. and Galzy P. 1983. Purification and properties of the β-glucosidase of the yeast capable of fermenting cellobiose to ethanol: Dekkera intermedia Van der Walt. European J. Appl. Microbiol. Biotechnol. 17:1-6.

3. Leclerc M., Gonde P., Ratomahenina R., Arnaud A. et Galzy P. 1984. Essais de fermentation du xylose et du cellobiose par deux souches de levure. Rev. Ferment. Ind. Aliment. 38:165-169.

4. Appaire V., Guiraud J.P. et Galzy P. 1983. Selection of yeast for single cell protein production on media based on Jerusalem artichoke extracts. Zeitsch. Allg. Microbiol. 23:211.

5. Chabbert N., Braun P., Guiraud J.P., Arnoux M. et Galzy P. 1983. Productivity and fermentescibility of Jerusalem artichoke according to harvesting date. Biomass 3:209.

6. Guiraud J.P., Bajon A., Chautard P. et Galzy P. 1983. Inulin hydrolysis by an immobilized yeast-cells reactor. Enzyme Microb. Technol. 5:185.

7. Moulin G., Malige B. and Galzy P. 1983. Balanced flora of an industrial fermentor-Production of yeast from whey. J. of Dairy Science 66:21-28.

8. Moulin G., Legrand M. and Galzy P. 1983. The importance of residual aerobic fermentation in aerated medium for the production of yeast from glucidic substrates. Process Biochemistry October 1983, 5-8.

9. Novotny C., Fuentes J.L., Carre Ph., Boze H., Moulin G. and Galzy P. 1983. Homothallism expression in Kluyveromyces lactis. Heredity 51:549-559.

10. Louis-Eugene S., Ratomahenina R., Galzy P. 1984. Reduction enzymatique du diacetyle et de l'acetoine par une souche de Saccharomyces uvarum Beijerinck. Zeitschrift für allgemeine Mikrobiologie 24:151-159.

11. Montet D., Ratomahenina R., Ba A., Pina M., J. Graille and Galzy P. 1983. Production of single G11 protein from vegetable oils. J. Ferment. Technol. 61:417-420.

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XXXV. Technical Research Centre of Finland (VTT), Biotechnical Laboratory, Tietotie 2, 02150 Espoo, Finland. Communicated by Veijo Mäkinen.

The following paper has been published:

M.-L. Suihko and V. Mäkinen. Tolerance to acetate, propionate and sorbate by Saccharomyces cerevisiae and Torulopsis holmii. Food Microbiology 1 Nr. 2 (1984).

Summary

Several strains of Saccharomyces cerevisiae and one of Torulopsis holmii were investigated with regard to their tolerance to acetate, propionate and sorbate. The S. cerevisiae strains had poor acetic acid tolerance in sour dough pH 4.3, the dough raising power decreasing on average by 67% in the presence of 0.34% sodium acetate, while T. holmii was not affected by this concentration. Propionate (0.16%) and sorbate (0.08%) were more inhibitory than acetate, inhibiting the dough raising power even in normal dough at pH 5.9. Torulopsis holmii also had a good tolerance to propionate and sorbate and seems suitable for raising sour doughs.

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XXXVI. Culture Collection Information

1. Catalogue - Yeast Genetic Stock Center - Fifth Edition 04/01/84. Principal Investigator: Robert Mortimer; Curator: Rebecca Contopoulou; Address: Department of Biophysics and Medical Physics, University of California, Berkeley, CA 94720; Phone: (415) 642-0815.

   The Yeast Genetic Stock Center maintains a collection of approximately

650 genetically defined strains of the yeast Saccharomyces cerevisiae. These strains are stored at 4°C in "milk-paper replicas" for ease of dissemination and at -75°C in 20% glycerol as a back-up supply. The Center is located in Donner Laboratory in the Department of Biophysics and Medical Physics at the University of California, Berkeley. The YGSC collects, maintains, monitors and provides these strains for basic and applied research and for teaching in the biological sciences. Thus the Stock Center acts as a depository for yeast mutants of general interest, making them available to a large number of investigators and educators; approximately 1000 samples are sent annually to scientists all over the world. At intervals of two to three years the YGSC publishes an updated catalogue of strains listing new acquisitions and revisions. Since 1982, at the suggestion of NSF, grant funding has been supplemented by charging users of the Stock Center a fee for strains.

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2. Centraalbureau voor Schimmelcultures, List of Cultures, 30th edition.

An up to date 30th edition of the CBS List of Cultures is now available at Hfl. 35,-- (microfiche edition: Hfl. 10,--). The new version has been extended with approximately 3000 cultures and now lists a total of 28000 strains, including 21500 filamentous fungi, 5000 yeasts and 1500 actinomycetes. About 3500 of these are type strains. Each strain is listed with its source and date of isolation, depositor, possible mating type and any known biochemical properties.

The List of Cultures may be ordered by writing to the Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, the Netherlands. Cultures are supplied on request at Hfl. 85,-- (dutch florins) per culture for commercial organizations and Hfl. 40,-- per culture for non-profit organizations. Reductions are given for orders of 10 or more, or subscriptions of 10 cultures per year, viz. Hfl. 65,-- and Hfl. 30,-- per culture respectively.

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3. IFO List of Cultures, 7th edition 1984.

The seventh edition of List of Cultures held by IFO appeared in May, 1984. The new catalogue lists a total of 8,100 strains, including 1,318 yeasts, 4,848 filamentous fungi, 806 bacteria, 1,086 actinomycets, and 46 bacteriophages. The strains are listed with depositor, history, their source, mating type, references, and accession number in other organizations. Numerical index with media and growth temperatures is also given in the catalogue.

The List of Cultures may be ordered by writing to the Business Center for Academic Societies Japan, 16-3, Hongo 6-chome, Bunkyo-ku, Tokyo 113. Price for the 7th edition is ¥2,500 plus handling charge. Cultures are supplied on request at ¥3.000 per culture for non-profit organizations, and ¥6.000 for commercial firms (extra charge is added to all orders shipped outside Japan). Orders and other correspondence should be addressed to the Institute for Fermentation-Osaka.

Isao Banno, Ph.D.
Curator, Yeast Collection
Principal microbiologist
Institute for Fermentation, Osaka
17-85, Juso-honmachi 2-chome
Yodogawa-ku
Osaka 532, Japan

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4. NCYC Catalogue

The 1984 edition of the NCYC catalogue is now available, price £4. It includes new strains, increased information on culture applications and details of the NCYC computer databank. In addition, there are greater number of references, and cross-referencing with other collection strain numbers is now complete. This and the previous edition (June 1981) were produced by computer type-setting from tapes provided by the NCYC. This system enables more frequent updates than hitherto.

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XXXVII. Books

1. The Yeasts - A Taxonomic Study, Third Completely Revised Edition, edited by N.J.W. Kreger-van Rij.

Since the last edition was published in 1970, there have been many new developments in yeast taxonomy: new species have been described, mating experiments have yielded perfect states of ascomycetous and basidomycetous yeasts, and new characters as well as changed evaluation of old characters have led to reclassifications. The 1970 edition included 39 genera and 349 species while the present edition includes 60 genera and 500 species. The rapid developments in yeast taxonomy requires continuous critical reviews. The taxonomic system presented in this edition may be considered as a stage in the evolution towards a satisfactory classification. The volume comprises of five main chapters (with various sub-sections) and the contents are as follows:

Chapter 1 - General Classification of the yeasts
Chapter II - Methods for the isolation, maintenance, classification and identification of yeasts
Chapter III - Discussion of the genera belonging to the ascosporogenous yeasts
Chapter IV - Discussion of the genera belonging to the basidiosporogenous yeasts.
Chapter V - Discussion of the genera belonging to the imperfect yeasts.
Chapter VI - Keys

The contributors to this volume have been chosen from the leading experts in the field and are as follows:

D.G. Ahearn, Atlanta, Georgia, U.S.A.
R.J. Bandoni, Vancouver, Canada
J.W. Fell, Miami, Florida, U.S.A.
E. Johannsen, Pretoria, South Africa
N.J.W. Kreger-van Rij, Groningen, The Netherlands
C.P. Kurtzman, Peoria, Illinois, U.S.A.
K.J. Kwon-Chung, Bethesda, Maryland, U.S.A.
Sally A. Meyer, Atlanta, Georgia, U.S.A.
M.W. Miller, Davis, California, U.S.A.
H.J. Phaff, Davis, California, U.S.A.
L. Rodrigues, Delft, The Netherlands
R.L. Schlitzer, Atlanta, Georgia, U.S.A.
M.Th. Smith, Delft, The Netherlands
A. Statzell Tallman, Florida, U.S.A.
J.P. van der Walt, Pretoria, South Africa
D. Yarrow, Delft, The Netherlands

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2. Maintenance of Microorganisms. A Manual of Laboratory Methods, 1984. B.E. Kirsop and J.J.S. Snell, eds. 208 pp. £11.60 (UK only)/$20.00.

Information on culture maintenance is sparse and scattered widely through the literature. This volume fills an immense gap by bringing together methods for the preservation of a wide range of microorganisms which have been developed and tested by recognised authorities.

To ensure that the manual is easy to use, each chapter is devoted to a particular group of microorganisms rather than to a method. An assessment of shelf life and suitability for different organisms is provided for each method. General information on the service culture collection has been included, together with comprehensive lists of suppliers, useful addresses, and over 1000 references.

Contents
Introduction, J.J.S. Snell and B.E. Kirsop
Service Collections: their Functions, B.E. Kirsop
General Introduction to Maintenance Methods, J.J.S. Snell
Maintenance of Bacteria by Freeze-drying, R.H. Rudge
Maintenance of Bacteria on Glass Beads at -60°C to -76°C, D. Jones, P.A. Pell and P.H.A. Sneath
Maintenance of Bacteria in Gelatin Discs, J.J.S. Snell
Maintenance of Anaerobic Bacteria, C.S. Impey and B.A. Phillips
Maintenance of Leptospira, S.A. Waitkins
Maintenance of Industrial and Marine Bacteria and Bacteriophages, I.J. Bousfield
Maintenance of Methanogenic Bacteria, H. Hippe
Maintenance of Fungi, D. Smith
Maintenance of Yeasts, B.E. Kirsop
Maintenance of Algae and Protozoa, E.A. Leeson, J.P. Cann and G.J. Morris
Maintenance of Parasitic Protozoa by Cryptopreservation, E.R. James

References. Appendix I: Documentation. Appendix II: Culture Collections and Federations. Appendix III: List of Suppliers. Genus Index. Subject Index.

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XXXVIII. Meetings

1. The X International Specialized Symposium on Yeast Molecular Biology and Genetics will be held in Bulgaria, Autumn 1985. For information contact P.V. Venkov, Institute of Molecular Biology, Bulgarian Academy of Science, 1113 Sofia, Bulgaria.

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2. A workshop on fungal Dimorphism will be held June 26-30, 1984, at the Conference Centre Corsendonk, Oud-Turnhout, Belgium.

   It was organized by Hugo Vanden Bossche and the Janssen Research Foundation.

   Address for information:
   Hugo Vanden Bossche
   Lab. Comparative Biochemistry
   Dept. Life Sciences
   Janssen Pharmaceutica
   B-2340 Beerse, Belgium

Scientific Programme

Session 1 CELL WALL AND MEMBRANE COMPONENTS - Molecular, ultrastructural, and biochemical approaches
Chairpersons: Jack Sobel (USA) and Marcel Borgers (Belgium)

(1) David Kerridge (UK): Membrane function and dimorphism (2) Enrico Cabib (USA): Regulation of the biosynthesis of β(1-3)-glucan, a structural component of the fungal cell wall (3) Gioconda San-Blas (Venezuela): Proteinases as regulating factors in the synthesis of glucan in Paracoccidioides brasiliensis (4) J. Sietsma (The Netherlands): Differences in the cell wall assembly during protoplast regeneration and hyphal tip extension (5) C.A. Vermeulen (The Netherlands): Biochemical and biophysical properties of chitin newly synthesized in vivo at the hyphal apex and in vitro (6) Patrick Marichal (Belgium): N-acetylglucosamine: a substrate contributing to the yeast/mycelium transition in Candida albicans (7) Marc Horisberger (Switzerland): Architecture of Schizosaccharomyces pombe and Saccharomyces rouxii cell walls (8) Yoshinori Nozawa (Japan): Ultrastructural and biochemical characteristics of Candida albicans membranes (9) Cesar Nombela (Spain): A genetic approach towards the study of dimorphism in Candida albicans (10) Jim Cutler (USA): Molecular approaches to studies on dimorphism of Candida abicans (11) Peter Russell (USA): Molecular events in the spore-to-mycelium transition in Neurospora crassa

Discussion
Moderator: Jack Sobel

Session 2 FACTORS REGULATING DIMORPHISM - Morphological, ultrastructural and biochemical approaches
Chairpersons: David Kerridge (UK) and George Kobayashi (USA)

(12) Felipe San-Blas (Venezuela): New morphological model of dimorphism in Paracoccidioides brasiliensis (13) Neil Gow (UK): Cytology of yeast/mould dimorphism in Candida albicans (14) Maxwell G. Shepherd (New Zealand): Regulation of morphogenesis in Candida albicans (15) Paul Szaniszlo (USA): Aspects of cellular development in the black yeast Wangiella dermatitidis (16) Todayo Hashimoto (USA): Ethanol-induced germ-tube formation in Candida albicans (17) Antonio Cassone (Italy): Protein metabolism during growth and differentiation in Candida albicans (18) Andre Van Laere (Belgium): Regulation of trehalose breakdown and glycolysis in Mucor rouxii (19) George Kobayashi (USA): Morphogenesis in Histoplasma capsulatum: The effect of reducing compounds on the phase transition

Discussion
Moderator: David Kerridge

Session 3 EFFECTS OF ANTIFUNGAL AGENTS ON DIMORPHISM
Chairpersons: Frank Odds (UK) and Doris Rast (Switzerland)

(20) Frank Odds (UK): Influence of antifungal agents on Candida albicans morphogenesis (21) Marcel Borgers (Belgium): Ultrastructural correlates of antifungal activities (22) Hugo Vanden Bossche (Belgium): Hypothesis on the molecular basis for the antifungal activity of azole derivatives. Focus on Candida albicans and Aspergillus fumigatus (23) Jef Van Gestel (Belgium): Studies of the aerial conidation of Penicillium italicum and the effects thereon of the fungicide imazalil (24) Rolf Furter (Switzerland): A comparison of the chitin synthetase-inhibitory and antifungal efficacies of nikkomycins and polyoxins: structure activity relationships

Discussion
Moderator: Frank Odds

Session 4 IMMUNOLOGICAL, ENDOCRINOLOGICAL AND PATHOLOGICAL ASPECTS OF DIMORPHISM
Chairpersons: Heinz Seeliger (FRG) and Jan Van Cutsem (Belgium)

(25) E. Drouhet (France): Some immunological and pathological aspects of dimorphism in Candida albicans and Histoplasma (26) Helen Buckley (USA): Some physiological and immunological aspects of dimorphism in Candida albicans (27) David Stevens (USA): Hormonal effects of fungal dimorphism (28) Anthony Hilger (USA): Factors contributing to pathogenesis of Candida albicans (29) Jack Sobel (USA): Virulence factors of Candida albicans in the pathogenesis of experimental vulvovaginal candidiasis

Discussion
Moderator: Heinz Seeliger

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3. XVth Annual Conference on Yeasts, held in Smolenice Castle (CSSR) from 22 to 24 February 1984. Communicated by A. Kocková-Kratochvílová.

Minisymposium "Secretion by yeast-like organisms" (O. Nečas)

Farkaš, V.: Biochemical viewpoint of secretion of the material of the cell wall.
Svoboda, A.: Secretional mutations in yeasts.
Streiblová, E.: Disorder in secretion in cytokinetical mutants.
Voříšek, J. and Schwencke, J.: Ultracytochemical staining of dipeptidyl-aminopeptidase in Saccharomyces cerevisiae (development of new methods).

Section: Immunology, pathogenicity and ecology

Tomšíková, A.: Yeast-like organisms and immunity.
Paulovičová, E. and Šandula, J.: Immunological studies of antigens in some pathogenic species of the genus Torulopsis.
Stollárová, V.: composition of natural communities of yeasts in Mochovce (Slovakia).

Minisymposium "Regulation in metabolism" (A. Kotyk)

Horák, J.: Ways of secretion of proteins in yeasts.
Šubík, J.: Utilization of maltose in yeasts and its regulation.
Biely, P.: Checking of the production of a xylan-degradating system in yeasts on the level of transport of inducers.
Vraná, D.: Influence of substrate limitation on the cell cycle of budding yeasts.
Vršanská, M., Hrnová, M., Biely, P.: Enzyme production of selected microorganisms lysing yeasts.

Minisymposium: Enzyme engineering (J. Zemek)

Zemek, J.: Methods for the immobilization of yeast enzymes and cells.
Poledníková, M., Šedová, H., Kahler, M: Immobilization of brewing and wine yeasts.
Kučera, J.: Chinone polymers applied for the immobilization of proteins.
Matusová, A.: Features of yeast amylases and their affinity to modified α-glucans.
Augustin, J.: Blastobotrys aristata and B. proliferans, two new species of yeast-like microorganisms useful in the preparation of maltotriose, maltotetraose and cellotriose.

Section: Genetics (V. Kováčová)

Vondrejs, V., Janderová, B., Bendová, O.: Utilization of zymocin in the improvement of industrial yeasts.
Kováčová, V., Frčová, D., Kopilcová, T.: Problems in the hybridization of wine yeasts.
Vlčková, V., Kováčová, V.: Comparison of the reversibility of some loci after the action of UV radiation in standard and UV-sensitive strains of S. cerevisiae (rad1-2, rad18).
Palková, Z.: Construction of a gene bank of S. diastaticus in E. coli.

Section: Technology (E. Minarik)

Malík, F.: Characteristics of active dry wine yeast preparations.
Minárik, E., Navara, A.: Influence of yeast starter amount for the fermentation of musts with high sugar content.
Farkaš, J.: quick method for the estimation of presence of yeasts and bacteria in wine.
Farkaš, J.: Influence of vitamin B_1 and H_2S for the intensification of sulphur dioxide activity in wine.

Posters:

Stollárová, V.: Composition of natural yeast communities in Mochovce.
Hašek, J., Svobodová, J., Streiblová, E.: Immunofluorescence staining of microtubuli in yeasts.
Šubík, J., Obernauerová, M.: Method of fluorescence staining of living and dead yeast cells.
Svobodová, J., Hašek, J., Streiblová, E.: Influence of antitubular substances in S. uvarum.
Ruml, T., Šilhánková, L.: Gene mapping responsible for thiamine transport in S. cerevisiae.
Pikálek, P.: Testing of the mutagenicity of chemical substances in yeast systems.
Bendová, O., Špaček, R.: Characteristics of zymocin produced by Hansenula sp.
Davaasurengijn, T., Janderová, B., Vondrejs, V., Bendová, O.: Brewing yeast S. uvarum capable of splitting dextrins.
Kotyk, A., Alonso, A.: Transport of ethanol in yeasts.
Michaljaničová, D.: Transport of ethanol in S. cerevisiae.
Opekarová, M., Sigler, K., Kotyk, A.: Endogenous energetic sources and excretion of K^+ from S. cerevisiae.
Kotal, P., Vernerová, J., Jirsa, M., Kordač, V.: Metabolism of porphyrins in yeasts.
Hrmová, M., Biely, P., Vršanská, M., Petráková, E.: Enzyme complexes decomposing cellulose and xylans in the yeast Trichosporon cutaneum.
Zemek, J.: Preparation of affinity hydrolases of modified yeast cells.
Augustin, J.: Chromolytic substrates of chitin metabolism.
Gemeiner, P., Breier, A., Marko, V., Mislovičová, D., Hrmová, M., Angyal, R., Šturdík, E.: The evolution and application of chemoselective and biospecific sorbents.
Kadlečíková, B.: Investigation of yeast-like organism interaction with ionogenic chromolytic biopolymers.
Krajňáková, V.: The preparation of enzyme control standards from yeasts.
Zámocký, J.: Yeast esterase and lipase affinity to modified anhydrosaccharides
Vernerová, J., Kurzová, V.: Sensitivity of brewing yeast S. uvarum to changes in the composition of wort.
Šmogrovičová, D., Augustin, J., Šajbidor, J.: Characterization of metabolic an physiologic properties of yeasts producing increased lipid amounts.
Šajbidor, J., Augustin, J., Šmogrovičová, D.: The study of lipid composition of yeasts.
Šusta, J., Hodaň, J., Opekarová, M., Siegler, K.: Acidification ability of brewing yeasts.
Vojteková, G.: Ecology of yeasts in part of the wine-region of Little

Carpathians - application of newly isolated yeast strains in practice. Vojtková-Lepsíková, A., Machová, E., Koszacka, Z.: Utilization of pentoses by yeasts.

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XXXIX. Brief News Items

1. On July 1, 1984, I retire after 37 years on the staff of McMaster University. My retirement plans are to continue for a year with laboratory research and publishing on the subjects of vacuolar polyphosphate and apomixis in Saccharomyces cerevisiae. Following that I hope to continue contributing to the yeast field with publications of a more general nature and reviews. I thank the many yeast workers who have aided my research efforts for three and one half decades through supplying cultures, reprints, inspiration and good advice. The assistance, technical and intellectual, of the students and co-workers who have shared my interest in yeast sporulation over these years is acknowledged with much appreciation.

J.J. Miller
Department of Biology
McMaster University
Hamilton, Ontario, Canada L8S 4K1

2. Availability of a postdoctoral position

Research Associateship in Molecular Yeast Taxonomy

A research associateship is available to study the evolutionary affinities of the ascomycetous yeasts. Taxa will be compared through extent of divergence of ribosomal RNA and other highly conserved molecular sequences. The position is available for one year with probability of renewal for a second year. Salary is $25,365 per year. Assistance is provided with relocation expenses. Ph.D. or equivalent training and U.S. citizenship are required. For further information, contact Dr. Cletus P. Kurtzman, Northern Regional Research Center, 1815 North University Street, Peoria, IL 61604. Tel (309) 685-4011. The USDA is an Equal Opportunity Affirmative Action Employer.

3. Change of Address:

Old Address	New Address
Dr. E. Moustacchi	Dr. E. Moustacchi
Institut Du Radium-Biologie	Institut Curie-Biologie
Bâtiment 110	26 Rue D'Ulm
Orsay 91, France	75231 Paris Cedex 05, France

4. Dr. Mario Luzzati moved from Yeast genetics to Streptomyces genetics and from Laboratoire de Biologie Générale to Laboratoire de Biologie Génétique et Moleculaire, at the same address, Bât. 400, Université Paris-Sud, 91405 Orsay-Cedex, France. Dr. Claude Gerbaud, Laboratoire de Biologie Générale will continue studies on yeast genetics.