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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

Associate Editors

Anna Kockova-Kratochvilova
Slovak Academy of Sciences
Bratislava, Czechoslovakia

Tadashi Hirano
Central Research Laboratory
The Jikei Univ. School of Medicine
3-25-8 Nishi-Shinbashi, Minato-ku
Tokyo 105, Japan

Richard Snow
Dept. of Genetics, Univ. of California
Davis, California 95616

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

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

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

Herman J. Phaff
Editor

NOTICE TO OUR READERS

The office of the Editor has been informed that invoice payments for the Yeast Newsletter by subscribers in foreign countries are subject to high service charges by their banks if payment is made directly to the Yeast Newsletter, Dept. of Food Science & Technology, University of California, Davis.

We are exploring with the University of California the possibility of direct transfer of the subscription fee on the bank account of the University of California. Unfortunately, it was too late to implement this change at this billing period, but we expect to have the system in operation for 1986. In the meantime, subscribers may wish to purchase dollars and pay cash in order to save the high service charge.

H.J. Phaff
Editor

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

1. Recent new yeasts available from CBS.

Candida agrestis Goto & Oguri 1983. J. Gen. Appl. Microbiol. 29:85-90. CBS 8055, from wild grapes Vitis coignetiae, = Pichia species. Type forms hat-shaped ascospores.

Candida bimundalis Wickerham & Santa Maria var. chlamydospora Nowakowska-Waszczuk & Pietka 1983. A new yeast producing chlamydospores. Program and Abstracts of IXth International Specialized Symposium on Yeasts, Yeasts in Human Environment, Smolenice 1983. p. 72. CBS 8250, from molasses, Poland = Candida tropicalis.

Candida euphorbiae van der Walt & Opperman 1983. Antonie van Leeuwenhoek 49:51-59. CBS 8033, from insect infested Euphorbia ingens. Anamorph of Pichia euphorbiae.

Candida lignophila Dill et al. 1984. Antonie van Leeuwenhoek 50:219-225. CBS 7109, from rotting wood of Drimys winteri.

Candida melinii Diddens & Lodder var. melibiosica Nowakowska-Waszczuk & Pietka 1983. Acta Microbiologica Polonica 32:73-85. CBS 8249, from fermentation vessels of citric-acid factory, Poland, = Candida krusei.

Candida montana Goto & Oguri 1983. J. Gen. Appl. Microbiol. 29:85-90. CBS 8057, from wild grapes Vitis coignetiae.

Candida silvicola Shifrine & Phaff var. melibiosica Nowakowska-Waszczuk & Pietka 1983. Acta Microbiologica Polonica 32:73-85. CBS 8251, from soil, = Candida boidinii.

Cryptococcus hinnuleus Shivas & Rodrigues de Miranda 1983. CBS 8079, from leaves of Banksia collina, Australia.

Cryptococcus phylloplanus Shivas & Rodrigues de Miranda 1983. Antonie van Leeuwenhoek 49:153-158. CBS 8073, from leaves of Banksia collina, Australia.

Dekkera anomala Smith & van Grinsven 1984. Antonie van Leeuwenhoek 50:143-148. CBS 8139 from spoiled soft drink. Sexual state of Brettanomyces anomalus.

Geotrichum robustum Fang et al. 1966. Acta Microbiologica Sinica 12:64-73 (in Chinese, English summary) CBS 8261, = Trichosporon beigelii.

Hansenula populi Phaff et al. 1983. Int. J. Syst. Bacteriol. 33:375-380. CBS 8094, from exudate of Populus trichocarpi.

Nematospora sinecauda Halley et al. 1985. Antonie van Leeuwenhoek 51(1). CBS 8199, from oriental mustard Brassica juncea.

Pichia antillensis Starmer et al. 1984. Int. J. Syst. Bacteriol. 34:350-354. CBS 7111, from cactus Cephalocereus royenii.

Pichia euphorbiae van der Walt & Opperman 1983. Antonie van Leeuwenhoek 49:51-59. CBS 8033, from insect infested Euphorbia ingens. Sexual state of Candida euphorbiae.

Pichia labacensis Cimerman & Kockova-Kratochvilova 1984. Z. Allg. Mikrobiol. 24:219-222. CCY 39-43-1, from cellulose factory, Yugoslavia. Culture of this strain sent to CBS was mixture of Saccharomyces cerevisiae and Pichia membranaefaciens.

Pichia pseudocactophila Holzschu et al. 1983. Can. J. Microbiol. 29:1314-1322. CBS 6929, from cactus Pachycereus pectin-aboriginum.

Rhodotorula armeniaca Shivas & Rodrigues de Miranda 1983. Antonie van Leeuwenhoek 49:159-166. CBS 8076, from leaves of Callistemon viminalis, Australia.

Rhodotorula hasegawae Yamada & Komagata 1983. J. Gen. Appl. Microbiol. 29:323-326. CBS 8253, from beer yeast.

Saccharomyces boulardii Seguela et al. 1984. Saccharomyces boulardii: critères d'identification. VIth Symposium International on Yeasts, Montpellier 1984, p. XIV-II-P. CBS 5926, from fruit in IndoChina, = Saccharomyces cerevisiae.

Sporobolomyces elongatus Shivas & Rodrigues de Miranda 1983. CBS 8080, from leaves of Callistemon viminalis, Australia.

Sporobolomyces foliicola Shivas & Rodrigues de Miranda 1983. CBS 8075, from leaves of Banksia collina, Australia. Antonie van Leeuwenhoek 49:159-166.

Sterigmatomyces fuzhouensis Yue 1982. Acta Mycologia Sinica 1, 79-87. CBS 8243, from mango flower.

Torulopsis candida (Saito) Lodder var. nitratophila Nowakowska-Waszczuk & Pietka 1983. Acta Microbiologica Polonica 32:73-85. CBS 8252, from fermentation vessels in citric-acid factory, Poland, = Candida tropicalis.

Trichosporon adeninovorans Middelhoven et al. 1985. Antonie van Leeuwenhoek 51 (1). CBS 8244, from soil.

Trichosporon sporotrichoides (van Oorschot) van Oorschot & de Hoog 1981. Antonie van Leeuwenhoek 47:353-366. Basionym: Trichosporiella sporotrichoides 1980. CBS 8245, 8246.

2. Accepted for publication in Antonie van Leeuwenhoek (51) 1:

Smith, M.Th. & Poot, G.A. 1985. Conspecificity of Hanseniaspora nodinigri and Hanseniaspora vineae: Comparison by DNA reassociation.

* * *

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

The strains listed below have been added to the ATCC since April 30, 1984. Complete information on these strains may be obtained upon request from the Mycology Department of ATCC.

Candida boidinii ATCC 56507	T. Urakami Mitsubishi Gas Chemical Co. Japan
Pichia methanolica ATCC 56508-56510	"
Torulopsis sonorensis ATCC 56511-56512	"
Saccharomyces bailii ATCC 56574-56577	F. Radler Johannes Gutenburg Federal Republic Germany
Saccharomyces cerevisiae ATCC 56574-56577	W. Siede GSF-Stralenbiologie W. Germany
Yeast ATCC 56578	R. Eschenbruch Te Kawhata New Zealand
Ambrosiozyma monospora ATCC 56618	C.P. Kurtzman NRRL Peoria, IL
Saccharomyces cerevisiae ATCC 56619-56623	A.J. Klar Cold Spring Harbor Lab Cold Spring Harbor, NY
Kluyveromyces drosophilarum ATCC 56627	Marc-Andre Lachance University Western Ontario Canada
Saccharomyces dairensis ATCC 56628	"
Pichia kodamae ATCC 56666	D. Yarrow CBS, the Netherlands
Cryptococcus socialis ATCC 56685	H.S. Vishniac Oklahoma State University Stillwater, OK
Cryptococcus consortionis ATCC 56686	"

Cryptococcus friedmannii ATCC 56687	"
Cryptococcus neoformans ATCC 56693-56696	G.S. Bulmer University Oklahoma Oklahoma City, OK
Saccharomyces cerevisiae ATCC 56736-56743	D.M. Spencer University of London London, UK
Kluyveromyces fragilis ATCC 56752	C.P. Kurtzman NRRL Peoria, IL
Saccharomyces cerevisiae ATCC 56792-56805	J.O. Lampen Waksman Institute of Microbiology Piscataway, NJ
Candida albicans ATCC 56811	H. Murato Tokyo Univ. School of Medicine Tokyo, Japan
Candida guilliermondii ATCC 56822	A. Budak Medical Academy Krokaw, Poland
Saccharomyces cerevisiae ATCC 56823-56825	J. Kurjan Columbia University New York, NY
Saccharomyces cerevisiae ATCC 56826-56832	Bun-ichiro Ono Okayama University Okayama, Japan
Dekkera bruxellensis ATCC 56863-56864	D. Yarrow CBS, the Netherlands
Dekkera intermedia ATCC 56865	"
Brettanomyces intermedius ATCC 56866	"
Sterigmatomyces fuzhouensis ATCC 56867	"
Brettanomyces claussenii ATCC 56868	"
Brettanomyces intermedius ATCC 56869	"

Brettanomyces naardenensis ATCC 56870	"
Brettanomyces anomalus ATCC 56871-56872	"
Saccharomyces uvarum ATCC 56878	K. Yoshioka Kirin Brewery Gunman, Japan
Candida albicans ATCC 56879-56886	D.A. Stevens Santa Clara Valley Med. Ctr. San Jose, CA
Candida boidinii ATCC 56897	P.J. Large University Hull England
Kluyveromyces fragilis ATCC 56898	R.R. Zall Cornell University Ithaca, NY
Saccharomyces cerevisiae ATCC 56899-56901	M.J. Leibowitz UMDNJ-Rutgers Medical School Piscataway, NJ
Saccharomyces cerevisiae ATCC 56913	J. Matoon University Colorado Colorado Springs, CO
Saccharomyces cerevisiae ATCC 56957-56960	S. Tabata Nara Medical University Nara, Japan
Cryptococcus neoformans ATCC 56989-56992	D. Swinne Institute of Tropical Medicine Antwerp, Belgium
Saccharomyces cerevisiae ATCC 56993-58006	J. Polaina Carlsberg Lab Copenhagen, Denmark
Rhodotorula mucilaginosa ATCC 58014	R.L. Blanton Culture Centre Algae & Protozoa Cambridge, England
Schwanniomyces alluvius ATCC 58015	W.N. Ingledew University Saskatchewan, Saskatoon Canada
Schwanniomyces castellii ATCC 58016-58017	"

Hansenula anomala ATCC 58044	C.P. Kurtzman NRRL Peoria, IL
Hansenula fabianii ATCC 58045-58046	"
Hansenula holstii ATCC 58047-58048	"
Hansenula subpelliculosa ATCC 58049	C.P. Kurtzman NRRL Peoria, IL
Wangiella dermatitidis ATCC 58058-58060	P.J. Szaniszlo University of Texas Austin, TX
Issatchenkia occidentalis ATCC 58064-58065	C.P. Kurtzman NRRL Peoria, IL
Issatchenkia scutulata ATCC 58066-58067	"
Issatchenkia terricola ATCC 58068-58069	"
Pichia guilliermondii ATCC 58070	"
Pichia membranaefaciens ATCC 58071-58072	"
Pichia rhodanensis ATCC 58073-58074	"
Pichia veronae ATCC 58075-58076	"
Candida deserticola ATCC 58088, ATCC 58090	M. Miranda University of California Davis, CA
Pichia deserticola ATCC 58089, ATCC 58091	"
Trichosporon cutaneum ATCC 58094	S. Dagley University of Minnesota St. Paul, MN

* * *

III. Microbiology Research Group, C.S.I.R., P.O. Box 395, Pretoria, 0001 Republic of South Africa. Communicated by J.P. van der Walt.

The following paper has been published recently:

J.P. van der Walt and D. Yarrow (1984). The genus Arxiozyma gen. nov. (Saccharomycetaceae). S. Afr. J. Bot. 3:340-342.

Abstract

The concurrence of the diploid condition of the vegetative phase, the verrucose ascospores with protuberances projecting from the electron-opaque outer layer of the ascosporal wall and the Coenzyme Q6 system in the yeast species described as Saccharomyces telluris, excludes it from all recognized genera of the Endomycetes. It is consequently proposed that this species be assigned to the new genus Arxiozyma. The diagnosis of the new genus is given.

* * *

IV. Institute for genetics of microorganisms, Moscow 113545, USSR. Communicated by G.I. Naumov.

G. Naumov, M. Vustin, I. Babjeva. Two biological species in the genus Arthroascus von Arx.

Summary of a manuscript to be submitted soon for publication.

A species of yeast-like fungi, Endomyces schoeni, was described by Nadson and Krassilnikov (1932)**. The authors pointed to the similarity of this fungus with Endomyces javanensis. The latter was later included in Endomycopsis (Lodder, Kreger-van Rij, 1952; Lodder, 1970) and then was considered to be a new genus Arthroascus (von Arx, 1972). The Dutch researchers did not mention the species E. schoeni Nad. et Kras. in these publications. The strain E. schoeni BKM Y-1073 which originated from Nadson and Krassilnikov, is physiologically very similar to the type culture of A. javanensis (BKM Y-1069). But there are morphological differences between these species: strain 1069 has asci with only 1-2 large rough spores, but strains 1073 and 2695 (the latter was recently isolated)-with 3-4 small smooth spores. With the aid of hybridization and recombination we have found two biological species in the genus Arthroascus. For hybridization we used only monosporic cultures with abundant sporulation, high self-fertility (80-90%) and with single or double auxotrophic mutations. The hybrid prototrophic colonies were selected on minimal medium. Intra-strain hybrids have high fertility (72-96%) and normal meiotic recombination of auxotrophic markers. The interstrain hybrids 1073 x 2695 have the same properties. The possibility of hybridization of A. javanensis 1069 with the strains of E. schoeni 1073 and 2695 shows that these yeasts belong to the genus Arthroascus, in accordance to the genetic concept of the genus (Naumov, 1978). The interspecific hybrids A. javanensis x E. schoeni (1069 x 1073; 1069 x 2695) differed from inter- and intrastrain hybrids by the absence of recombination for genetic markers and by sterility (0-6%), which are the properties of hybrids of biological species (Naumov et al., 1981). The description of A. schoeni (Nads. et Krass.) comb. nov. is being prepared for publication.

**G. Nadson, N. Krassilnikov (1932): Dokl. Akad. Nauk USSR, N13A, 323-327.

* * *

V. Commonwealth Mycological Institute, Culture Collection & Industrial Services, Ferry Lane, Kew, Surrey TW9 3AF, United Kingdom. Communicated by P.D. Bridge.

Below follows the abstract of a recent paper on numerical classification of yeast. Additional papers are in preparation.

Paul D. Bridge and John W. May (1984). A Numerical Classification of Fission Yeasts of the Genus Schizosaccharomyces Lindner. J. Gen. Microbiology 130:1921-1932.

A numerical classification of the yeast genus Schizosaccharomyces was undertaken using 60 strains and 100 characters. Three distinct clusters were observed, corresponding to S. pombe (S_{SM} = 83%), both varieties of S. japonicus (S_{SM} = 78%), and S. octosporus (S_{SM} = 75%). Schizosaccharomyces malidevorans and S. slooffiae, each of which is available only as the type, could not be differentiated from S. pombe and S. octosporus, respectively.

* * *

VI. University of London, Goldsmiths' College, Department of Biology, New Cross London SE14 6NW, England. Communicated by J.F.T. Spencer.

The following is an abstract of a paper soon to be submitted for publication.

The Use of Mitochondrial Mutants in Hybridization of Industrial Yeast Strains. V. Relative Parental Contributions to the Genomes of Interspecific and Intergeneric Yeast Hybrids Obtained by Protoplast Fusion, as Determined by DNA Reassociation.

J.F.T. Spencer[1,2], D.M. Spencer[2], C. Bizeau[3], Ann Vaughan Martini[4] and A. Martini[4]

[1]Department of Biology, Thames Polytechnic, Wellington Street , London SE18.
[2]Department of Biological Sciences, Goldsmiths' College, University of London.
[3]Institut National de la Recherche Agronomique, 34060 Montpellier Cedex.
[4]Istituto di Biologia Vegetale, Sexione di Microbiologia, Universita di Perugia, Italy.

Summary

The contributions of each of the parental strains to the genomes of the sporulation and non-sporulating hybrids, Saccharomyces diastaticus x Hansenula capsulata, S. diastaticus x Hansenula wingei, S. diastaticus x Torulopsis glabrata, S. diastaticus x Candida pseudotropicalis, S. diastaticus x Saccharomyces rouxii, S. diastaticus x Saccharomyces kluyveri, and S. diastaticus x Saccharomyces bayanus, obtained by protoplast fusion, were determined by the method of DNA reassociation. Petite mutants of S. diastaticus NCYC 625, and respiratory-competent strains of the other species, were used. In all of the hybrids but one, the DNA from the S. diastaticus parent showed 93.3 to 109.3% homology with the DNA from the hybrids, and with the other parents from -7.7% (S. kluyveri) to 20.0% (S. bayanus).

Reassociation between the DNA from S. diastaticus and the DNA from the other parental strains ranged from 4.7 to 19.4%. Reassociation between DNAs from S. diastaticus and T. glabrata and that of the S. diastaticus x T. glabrata fusion hybrid were 15.2 and 18.9%, respectively. Further investigation of this hybrid is desirable. The fusion products were relatively stable as compared to some fusion hybrids selected by use of nuclear markers, and could be maintained on normal media, with little or no selection pressure, but use of an appropriate carbon source. In most of the hybrids, except for S. diastaticus x T. glabrata, the S. diastaticus parent contributed most of the genome, and only a single chromosome or a fragment of a chromosome appeared to be transferred to the Saccharomyces nucleus to form the genome of the fusion product.

* * *

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

1. A floppy disk for identifying yeasts, by J.A. Barnett (University of East Anglia), R.W. Payne (Rothamsted Experimental Station) & D. Yarrow (Centraalbureau voor Schimmelcultures) is being marketed by Cambridge University Press. This disk is based on material from Yeasts: Characteristics and Identification (Barnett, Payne & Yarrow, Cambridge University Press, 1983).

The program on the disk is designed for use by those with little experience of computing. With this program, the user can either (i) get the names of yeasts which give the results of tests that he has obtained, or (ii) get a list of those yeasts which have a particular set of characteristics that he is interested in. Identifications can be obtained within a few minutes.

Should the names of more than one yeast fit the results obtained, the program can be made to select further tests which would distinguish between those yeasts. The program can also allow for mistakes.

Cambridge University Press hope to have this disk on sale at the beginning of 1985, and its estimated price is about £75.

2. Two recent publications from this laboratory are as follows.

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 130, 1933-1940.

A.K. McCann & J.A. Barnett (1984). Starch utilization by yeasts: mutants resistant to carbon catabolite repression. Current Genetics 8, 525-530.

* * *

VIII. Department of Biological Chemistry, California College of Medicine, University of California, Irvine, California 92717 USA. Communicated by Anita M. Newman.

Below follows a summary of a computer-based yeast strain management program which we developed for use in our own lab. We thought that others might find the program useful as well.

Anita M. Newman and Calvin S. McLaughlin, 1984. A Yeast Strain Management program to be used with Lotus 123.

Summary

We have designed a strain management program to be used in conjunction with the commercially available Lotus 123 program on an IBM PC computer or compatible machine. It is intended to be easy to understand for the person with only limited familiarity with Lotus 123. Menus are included at each step, from which appropriate alternatives may be chosen. However, the full capabilities of Lotus are always present for those who wish to use them.

Information on up to 700[1] strains may be stored in any one file. This may include:

1. Strain name
2. A genus species designation
3. Storage location (or a strain identification number)
4. Mating type
5. Up to 10 markers entered in any order
6. Three lines of comments of up to 72 characters each.

The records stored may be searched by:

1. Strain name
2. Storage location (or identification number)
3. Up to 4 markers and mating type

A further search may be performed on the strains retrieved in an initial search. A search among 700 strains for 4 markers plus mating type requires 2 minutes. A search only for strain name requires 15 seconds.

The strains are stored in alphabetical order. A printout may be made of the entire strain list in any one file, including all the characteristics of each strain[2]. This may be either in alphabetical order or by storage location. Similarly, a printout may be made of the characteristics of any or all strains retrieved during a search.

We would be happy to provide a copy of this program to anyone with Lotus 123 who is interested in using it. (Instructions for its use are contained on the disk). To obtain a copy please send a disk with a self-addressed mailer with postage to:

Dr. Anita Newman
Dept. of Biological Chemistry
Med. Sci. I
University of California, Irvine
Irvine, CA 92717

[1]This assumes 640K of memory (with 256 K, 150 strains may be entered per file).

[2]The printing aspects of the program have been set up to work with an Epson FX80 printer. If this printer or a similar printer were not used, changes might have to be made at 3 specific locations within the program.

* * *

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

1. The following two papers have been published recently.

   Snow, R. (1983). Genetic improvement of wine yeast. In Yeast Genetics: Fundamental and Applied Aspects (J.F.T. Spencer, D.M. Spencer and A.R.W. Smith, eds.), 439-459. Springer-Verlag, New York.

   Williams, S.A., R.A. Hodges, T.L. Strike, R. Snow and R.E. Kunkee (1984). Cloning the gene for the malolactic fermentation of wine from Lactobacillus delbrueckii in Escherichia coli and yeasts. Appl. Environm. Microbiol. 47:288-293.

2. I have written two programs for the Apple II computer that may be of use to yeast researchers. Both are written in Pascal and require at least one disk drive and a printer.

   The first is a program to calculate maximum likelihood estimates of map distanes (R. Snow, Genetics 92:231 (1979)). The program will utilize data from two-point crosses as well as first-division segregation and ranked tetrad data. Output can be displayed on the screen or be printed out.

   The second is a program to maintain a stock list. The entries in the list have the following form:

   ```
   2389    a arg6 his7 lys2
           α trp5 ade6 cyh2
           2373-1c / 2232-39c
   ```

   The number at left is the stock number. The genotypes of the two strains that were mated to give diploid 2389 are on the first and second lines, while their own stock numbers are on the third line. For haploid strains, only one genotype and strain number is used.

   The program has the capability of adding, deleting, or changing data, printing out all or specified parts of the list, and of searching the list for strains with specified genotypes or strain numbers. Up to 10 search parameters can be specified. Each 5-1/4" floppy disk can store the data for 500 strains. If your llist is longer than this it can be divided up among several disks, with the strains numbered consecutively.

   I will be happy to supply these programs to anyone interested if they will send me a blank 5-1/4" floppy disk.

* * *

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

The following paper represents an ecological study from our laboratory.

Jane M. Bowles and M.A. Lachance (1983) Patterns of variation in the yeast florae of exudates in an oak community. Can. J. Bot. 61:2984-2995.

Yeasts associated with exudates of 16 red oak trees (Quercus rubra) were sampled repeatedly over a 2-year period. The 210 yeasts isolated were assigned to 28 species whose frequencies were characteristic of each habitat. Significant variation among the habitats was detected in the species diversity, composition, nutritional breadth, and physiological specificity of their yeasts. Some exudates were recognized as "typical" sap fluxes by their physical characteristics and their similar yeast florae. Others differed to various degrees in their yeast species composition, or in the physiological structure of their yeast communities. Among the factors linked to the observed variation were features of the adjacent vegetation, colonization by ants, or growth in open parts of the study area.

* * *

XI. Departamento de Patologia Tropical, Instituto Nacional de Pesquisas da Amazonia, 69000 Manaus, Brazil. Communicated by Wai Yin Mok.

The following paper has appeared recently:

Wai Yin Mok, Regina C.C. Luizao, Maria do Socorro Barreto da Silva, Maria Francisca S. Teixeira and Emiro G. Muniz (1984). Ecology of pathogenic yeasts in Amazonian soil. Appl. Env. Microbiol. 47:390-394.

Abstract

In an investigation of Amazonian soil as a natural reservoir for pathogenic fungi, 1,949 soil samples collected from diverse geographical and ecological settings of the Brazilian Amazon Basin were analyzed for the presence of non-keratinophilic fungi by the indirect mouse inoculation procedure and for the presence of keratinophilic fungi by the hair bait technique. All soil samples were acidic with low pH values. From 12% of the soil samples, 241 yeast and yeastlike isolates pertaining to six genera and 82 species were recovered, of which 63% were Torulopsis and 26% were Candida species. Nine fungi with known pathogenic potentials were encountered among 43% (104) of the isolates: T. glabrata, C. guilliermondii, C. albicans, C. pseudotropicalis, C. stellatoidea, C. tropicalis, Rhodotroula rubra and Wangiella dermatitidis. The yeast flora was marked by species diversity, low frequency of each species, random geographical distribution and an apparent lack of species clustering. The composition and distribution of the yeast flora in soil differed from those of the yeast flora harbored by bats, suggesting that the Amazonian external environment and internal bat organs act as independent natural habitats for yeasts.

* * *

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

Below follow abstracts of recent papers.

1. Shoji Goto and Hiroyuki Oguri, 1983. Yeast flora in wild grapes from mountainous places around the Kofu Basin of Central Japan. Trans. mycol. Soc. Japan, 24:151-157.

Abstract

Qualitative and quantitative studies were made of the yeast flora in wild grapes (Vitis coignetiae Pulliat) collected at harvest time from various mountainous places around the Kofu Basin in Central Japan. Of the 14 samples obtained from five localities in 1976, 1978 and 1979, yeasts were isolated from 11 samples taken from three localities. The yeast populations in these 11 samples ranged from 1.2×10^1 to 8.1×10^3 colonies per milliter. One hundred and twenty-five isolates were assignable to 17 species belonging to 8 genera. Kloeckera apiculata, Aureobasidium pullulans, and Cryptococcus laurentii var. flavescens predominated in the majority of samples and they were isolated at high frequency. In some samples, however, these yeasts were not isolated.

* * *

2. Shoji Goto and Hiroyuki Oguri, 1983. Identification of the yeasts isolated from wild grapes. J. Inst. Enol. Vitic. Yamanashi Univ., 18:27-32.

Abstract

One hundred and six isolates of yeasts and yeast-like fungi obtained from wild grapes (Vitis coagnitiae) were studied taxonomically and were identified with 17 species belonging to 8 different genera. The dominant groups were Kloeckera apiculata, Aureobasidium pullulans, and Cryptococcus laurentii var. flavescens and the minor yeasts were Rhodotorula fujisanensis, R. rubra, Saccharomyces cerevisiae, S. rosei, Pichia ohmeri, Candida agrestis, C. krusei, C. montana, C. parapsilosis, Torulopsis cantarellii, T. colliculosa, T. dattila, T. kruisii, and T. stellata.

* * *

3. Masatake Imai[1] and Shoji Goto, 1984. Aging of "Nukadoko": Changes in Yeast Flora and identification of the isolates. Nippon Nogeikagaku Kaishi, 58:(6) 545-551.

[1]Research Institute, Morinaga & Co., 2-1-1, Shimosueyoshi, Tsurumi-ku, Yokohama 230.

Abstract

Changes in yeast flora of "Nukadoko" (mixture of rice bran and salt used for vegetable pickling) were followed during 120 days, and the isolated yeasts were identified. The aging response at 2 years was also examined. Yeasts were not detected in fresh "Nukadoko", but after 30 days the total count of yeast reached 1.2×10^6 colonies/g and after 60 days, 5.9×10^7/g. After reaching a maximum count, the yeast showed a noticeable decrease and reached a

stable level of 1 x 10^6 colonies/g, almost the same level as 2 year-aged "Nukadoko".

Candida krusei, Torulopsis etchellsii, Candida lipolytica, Rhodotorula minuta and Candida sp. were detected in the representative 64 isolated strains of "Nukadoko" at the tested periods. At all aging periods, C. krusei was dominant and reached 50-67% of flora, and T. etchellsii was second with 20-30%. Candida sp. was detected at 10% only before aging for 90 days. C. lipolytica was detected only after aging for 90 days. Thus, 90 days seem near the alternation point between these species. The rate of the main yeast flora at the bottom of "Nukadoko" aged for 120 days was almost the same as that of 2 year-aged "Nukadoko". The flavors produced by pure cultures of isolated C. krusei, C. lipolytica and T. etchellsii resembled flavors in aged "Nukadoko"; Candida sp. produced a "Malted rice" flavor.

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4. Masashi Yamazaki and Kazuo Komagata[1], 1982. Asporogenous Yeasts and their supposed ascosporogenous states: An electrophoretic comparison of enzymes. J. Gen. Appl. Microbiol., 28:119-138.

[1]The Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

Abstract

The relationship of nine Candida species, five Torulopsis species, and five species and two varieties of Kloeckera to the ascosporogenous yeast species considered to be their teleomorphs was studied by electrophoretic comparison of enzymes. Close relationships were seen between fourteen teleomorphs and their anamorphic counterparts, Candida guilliermondii and Pichia guilliermondii, Candida krusei and Issatchenkia orientalis (Pichia kudriavzevii), Candida lambica and Pichia fermentans, Candida melinii and Hansenula wingei, Candida utilis and Hansenula jadinii, Candida macedoniensis and Kluyveromyces marxianus, Candida pseudotropicalis and Kluyveromyces fragilis, Candida parapsilosis (group 2) and Lodderomyces elongisporus, Torulopsis holmii and Saccharomyces exiguus, Torulopsis colliculosa and Torulaspora delbrueckii (Saccharomyces fermentati), Torulopsis stellata and Torulaspora delbrueckii (Saccharomyces rosei), Kloeckera africana and Hanseniaspora vinea, Kloeckera apiculata and Hanseniaspora uvarum and Kloeckera japonica and Hanseniaspora valbyensis. There was no apparent relation between the four pairs: Candida valida and Pichia membranaefaciens, Candida parapsilosis (group 1) and Lodderomyces elongisporus, Torulopsis molischiana and Hansenula capsulata, and Torulopsis candida and Debaryomyces hansenii and D. marama. From these results, this zymographic technique is considered to be a useful tool for the study of relationships between the different states of a yeast species.

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5. Masahi Yamazaki and Kazuo Komagata, 1983. An electrophoretic comparison of enzymes of ballistosporogenous yeasts. J. Gen. Appl. Microbiol., 29:115-143.

Abstract

The patterns produced by nine enzymes of ballistosporogenous yeasts and supposedly related yeasts were studied by electrophoresis on polyacrylamide gel. Forty-two strains belonged to the genera Sporobolomyces, Sporidiobolus, Aessosporon and Bullera and twenty-four strains to Rhodotorula, Rhodosporidium, Cryptococcus, unidentified ballistosporogenous yeasts, and Candida edax. Four Sporobolomyces salmonicolor strains, two Sp. holsaticus strains, two Sp. odorus strains, five Sporidiobolus salmonicolor strains, two Aessosporon salmonicolor strains, and one strain of Aessosporon dendrophilum produced similar electrophoretic patterns. Moreover, mating was observed between some of these strains, Sporobolomyces roseus and Sp. shibatanus differed from Sp. salmonicolor in their glucose-6-phosphate dehydrogenase (EC 1.1.1.49), but the patterns of the other enzymes were similar; all three species differed clearly from Sporobolomyces singularis, Sp. gracilis, Sp. puniceus, and Sp. antarcticus. Sporobolomyces albo-rubescens showed a peculiar 6-phosphogluconate dehydrogenase (EC 1.1.1.41) pattern and was similar to two Rhodotorula rubra strains in the patterns of their enzymes. Close relationships were also seen between Sporidiobolus ruinenii and Rhodotorula graminis, and between Bullera alba and a strain in Cryptococcus albidus var. albidus in the electrophoretic patterns of their enzymes. Four unidentified strains which had lost the ability to produce ballistospores had patterns similar to those of Bullera alba, Rhodotorula glutinis, Cryptococcus laurentii var. flavescens, and Cr. macerans. Three colorless strains, putatively derived from a strain of Sporobolomyces roseus, showed the same electrophoretic patterns as the strains from which they originated.

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6. Masashi Yamazaki, Shoji Goto, and Kazuo Komagata, 1983. An electrophoretic comparison of the enzymes of Saccharomyces yeasts. J. Gen Appl. Microbiol., 29:305-318.

Abstract

The relationships among 36 strains of 17 Saccharomyces species were studied by comparing ten enzymes electrophoretically. Twelve of the 13 strains of S. cerevisiae tested produced rather similar patterns, but one distiller's yeast previously classified as S. formosensis differed in fructose-1,6-bisphosphate aldolase (EC 4.1.2.13). Three S. uvarum strains split into two categories with quite different enzyme patterns. One included strains previously named S. carlsbergensis and was similar to S. cerevisiae in its enzyme pattern. The patterns of S. cerevisiae, S. bayanus, S. chevalieri, S. diastaticus, and S. italicus were nearly identical. Strains of S. delbrueckii, S. fermentati, S. rosei, and S. saitoanus, belonging to the so-called Torulaspora group, had similar patterns which were readily distinguishable from those of S. cerevisiae. The patterns of S. bailii, S. bisporus, S. florentinus, and S. rouxii, belonging to the so-called Zygosaccharomyces group, differed from each other and from the S. cerevisiae and Torulaspora strains.

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XIII. Yamaguchi University, Biological Institute, Faculty of Science, Yamaguchi 753, Japan. Communicated by Nobundo Sando.

Below follow abstracts of three recent papers from our laboratory.

1. Katsuji Ueki, Masao Abe, Katsuro Tada and Nobundo Sando, 1983. Cell size dependency of the sporulation process in the yeast Saccharomyces cerevisiae. J. Gen. Microbiol., 129: 3619-3627.

Abstract

Physiological changes during the sporulation process were compared between large and small cells prepared from stationary phase cells of Saccharomyces cerevisiae. There were marked differences in the sporulation capacity between cells of different size. NH_4^+ and methylamine did not block sporulation in large cells, but did in small cells. Large cells could sporulate in water without exposure to acetate sporulation medium, but small cells could not. During sporulation, small cells became insensitive to NH_4^+ and methylamine and acquired the ability to sporulate in water at an early stage. After acquiring the ability to sporulate in water, sporulation in small cells proceeded through a series of physiological changes common to those occurring in large cells. These results suggested that the initiation point of sporulation varied with cell size.

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2. Isamu Miyakawa, Hiroyuki Aoi, Nobundo Sando and Tsuneyoshi Kuroiwa, 1984. Fluorescence microscopic studies of mitochondrial nucleoids during meiosis and sporulation in the yeast Saccharomyces cerevisiae. J. Cell Sci. 66: 21-38.

Abstract

Configurational changes of mitochondria and mitochondrial nucleoids (mt-nucleoids) during meiosis and sporulation in the yeast, Saccharomyces cerevisiae, were examined using the mitochondrial membrane-binding fluorescent dye, dimethyl aminostyrylmethylpyridiniumiodine (DASPMI) and the DNA-binding fluorescent dye, 4', 6-diamidino-2-phenylindole (DAPI). In zygotes just after mating, mt-nucleoids were observed as many small discrete light spots in the cytoplasm. During meiosis in zygotes, mt-nucleoids at first coalesced with each other into a long string and then separated into spherical nucleoids in four spores. These changes paralleled those in mitochondria observed using DASPMI. The use of spheroplasts allowed us to examine the behaviour of mt-nucleoids at higher resolution and to identify several distinct meiotic prophase stages of the cell nucleus during early sporulation. In diploid spheroplasts at the stationary phase, 50-70 of the mt-nucleoids were observed to be separated from each other and each spherical mitochondrion contained only one mt-nucleoid. At the later stage of premeiotic DNA synthesis, a single branched giant mitochondrion was formed as a result of complete mitochondrial fusion. All of the mt-nucleoids were arranged in an array on a giant mitochondrion and coalesced into a string-like network. Through meiosis I and II, strings of mt-nucleoids were observed close to the dividing nuclei. At late meiosis II, a ring of mt-nucleoids enclosing each daughter nucleus was formed. In ascospores, discrete small nucleoids were visible close to each spore nucleus with a "string-of-beads" appearance. Many mt-nucleoids were excluded from the ascospores and remained in the residual cytoplasm of the ascus.

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3. Tsuneyoshi Kuroiwa, Hisashi Kojima, Isamu Miyakawa and Nobundo Sando, 1984. Meiotic karyotype of the yeast Saccharomyces cerevisiae. Exp. Cell Res., 153: 259-265.

Abstract

A cytogenetic study of the meiotic chromosomes of the budding yeast S. cerevisiae was undertaken to high resolution epifluorescence microscopy. Condensation of chromatin into separate chromosomes takes place during prophase I. At metaphase I, there are 16 separate and distinct bivalents which are roughly classified into three groups by morphological differences and DNA content.

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XIV. Department of Microbiology, Shizuoka College of Pharmacy, 2-2-1 Oshika, Shizuoka, 422 Japan. Communicated by Tamotsu Morita.

The following papers previously listed as submitted to Chem. Pharm. Bull. have now been published:

1. Hamada, H., Yamashita, M., Kojima, M. and Morita, T. (1984). Effect of methanol on the induction of respiration deficient mutants by acriflavine in yeast. Chem. Pharm. Bull. 32(2): 623-627. Abstract: In this Letter, Vol. XXXII (2), p. 60.

2. Morita, T. and Mifuchi, I. (1984).: Ethanol enhancement of cytochrome P-450 contents in yeast, Saccharomyces cerevisiae D7. Chem. Pharm. Bull. 32(4): 1624-1627. Abstract: In this Letter, Vol. XXXII (2), p. 61.

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Below follows abstracts of two recent papers accepted for publication:

1. Morita, T. and Yanagihara, Y.: Osmotic sensitive mutants of Saccharomyces cerevisiae as screening organisms for promutagens and procarcinogens. Chem. Pharm. Bull. 33 (4), 1985.

Abstract

An attempt to improve a response of yeast cells to promutagens and procacinogens in mutagenicity assay was made by using osmotic sensitive mutants of yeast, Saccharomyces cerevisiae. Four osmotic sensitive mutants of yeast which showed increased sensitivity to 1.5M KCl were induced by ethylmethanesulfonate treatment. One of the mutants, strain C658-K42, was highly sensitive to antibiotics such as mitomycin C, novobiocin, nalidixic acid, chloroquine and rifampicin at the concentrations of no growth inhibitory effects on the original strain, S. cerevisiae C658. Strain C658-K42 was considered to have a defect in cell membrane.

These osmotic sensitive mutants were tested for the screening of well known procarcinogens (promutagens), dimethylnitrosamine, 3,4-benzpyrene and 2-

acetylaminofluorene. The response of these mutants in a mutagenicity assay (Trp^+ reversion) apparently increased compared with that of the original strain. The yeast cells which were harvested from a late logarithmic phase culture could activate procarcinogens to genetically active forms without any exogeneously added metabolic activation system.

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2. Hamada, H., Toshimitsu, N., Kojima, M. and Morita, T.: Effects of methanol and magnesium chloride on the induction of respiration-deficient mutants in yeast by metal ions. Chem. Pharm. Bull. 33(4), 1985.

Abstract

The effects of methanol and magnesium chloride on the induction of cytoplasmic respiration-deficient (RD or petite) mutants of yeast, Saccharomyces cerevisiae, by metal compounds were investigated. At the concentration of almost no growth repression, $ZnCl_2$, $HgCl_2$, $PbCl_2$, $NiCl_2$, $MnCl_2$, $CuCl_2$ and $CdCl_2$ were weakly mutagenic. The RD mutation by these metal compounds was repressed to a spontaneous level (1.4%) by the addition of 4% methanol. At higher concentration, $CoCl_2$, $NiCl_2$, $MnCl_2$, and $CdCl_2$ were strongly mutagenic, but the mutagenicity of these metal compounds was also repressed by the addition of 4% methanol, except for $MnCl_2$. The induction frequency of RD mutants by 1×10^{-2}M $MnCl_2$ increased from 13.5% to 60.7% by the addition of methanol.

The enhancement of $MnCl_2$-induced RD mutation by methanol was repressed almost completely by the addition of $MgCl_2$ into the culture medium. The RD mutation by $MnCl_2$ without methanol was also repressed to a spontaneous level by $MgCl_2$. The effects of methanol and $MgCl_2$ on the RD mutation in yeast are discussed.

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XV. Department of Biophysics, Faculty of Science, Kyoto University, Kyoto, Japan. Communicated by Mitsuhiro Yanagida.

Papers published in 1984 from our group on Schizosaccharomyces pombe are as follows:

(1) Y. Nakaseko, O. Niwa and M. Yanagida (1984). A meiotic mutant of the fission yeast Schizosaccharomyces pombe that produces mature asci containing two diploid spores. J. Bacteriol. 157:334-336.

(2) T. Toda, Y. Nakaseko, O. Niwa and M. Yanagida (1984). Mapping of rRNA genes by integration of hybrid plasmids in Schizosaccharomyces pombe. Current Genetics 8:93-97.

(3) T. Toda, Y. Adachi, Y. Hiraoka and M. Yanagida (1984). Identification of the pleiotropic cell division cycle gene NDA2 as one of two different α-tubulin genes in Schizosaccharomyces pombe. Cell 37:233-242.

(4) T. Uemura and M. Yanagida (1984): Isolation of type I and II DNA topoisomeraase mutants from fission yeast: single and double mutants show different phenotypes in cell growth and chromatin organization. EMBO Journal 3:1737-1744.

(5) Y. Hiraoka, T. Toda and M. Yanagida (1984). The NDA3 gene of fission yeast encodes β-tubulin: a cold sensitive nda3 mutation reversibly blocks spindle formation and chromosome movement in mitosis. Cell (in press) (December part 1 issue).

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

1. Aprotic Polar Solvents Induce only Aneuploidy but not Mitotic Recombination or Point Mutation in Yeast and Interfere with the Assembly in vitro of Porcine Brain Tubulin. U. Groschel Stewart, Institut fur Zoologie, F.K. Zimmermann, V.W. Mayer, Genetic Toxicology Branch, FDA, Washington, DC.

We used strain D61.M to detect induction of mitotic aneuploidy by chemicals. Expression of three recessive markers is indicative of a monosomic condition: cyh2 is the selected marker, ade6 on the other side of the centromere changes the normal red colony (homozygous condition for ade2) to white, leu1 is the centromere marker. Monosomics grow on a medium with 1.5 ppm cycloheximide and form white colonies of leucine-requiring cells. Efficient induction of monosomics was achieved with a number of mostly aprotic polar solvents when growing cells were exposed for 4 h at 28°C, then stored in ice overnight, and again incubated for 4 h at 28°C before plating on the selective medium. Induction was only weak when incubation was continuous at 28°C. Induction of monosomics was usually the only genetic changes observed in strain D61.M which also signals induction of mitotic recombination and point mutation by an increase in red resistant colonies which do not express ade6. This specific induction of aneuploidy and the strong enhancement of induction by an intermittant incubation in ice suggested that tubulin and microtubules could be the major targets of the induction of aneuploidy. This hypothesis was tested in an assay in vitro following the assembly of twice recycled porcine brain tubulin. It could be shown that with the exception of fumaric acid dinitrile, all aprotic polar solvents interfered with tubulin aggregation in vitro and usually in the concentration range effective for induction of aneuploidy in yeast. The chemicals either inhibited aggregation or they enhanced the rate and extent of assembly. The results obtained with aneuploidy inducing agents are shown in the following table. The control values of monosomics in cultures of D61.M varied between none observed and about 2.5×10^{-6}. The highest induced aneuploidy frequencies and the concentrations needed for the reported effect are listed. The effects on tubulin assembly are given as inhibitory or accelerating. The concentrations are only given if they differ considerably from those needed for induction of aneuploidy.

Chemical	Aneuploidy per 10^6	Tubulin Assembly
Methyl acetate	28.63 at 3.38%	inhibitory
ethyl acetate	916.67 at 2.20%	inhibitory
2-methoxyethyl acetate	47.02 at 3.38%	accelerating at 0.01%
methyl n-propionate	15.51 at 1.48%	inhibitory
ethyl n-propionate	7.83 at 1.04%	inhibitory
acetone	42.56 at 6.98%	accelerating
methyl ethyl ketone	168.29 at 3.54%	inhibitory
diethyl ketone	126.96 at 1.48%	inhibitory
methyl n-propyl ketone	53.09 at 1.72%	inhibitory
-butyrolactone	47.17 at 3.38%	inhibitory
-valerolactone	38.80 at 2.91%	inhibitory
acetonitrile	178.26 at 4.31%	inhibitory at 0.1%
pivalinic acid nitrile	46.40 at 0.99%	accelerating
phenylacetonnitrile	26.85 at 0.20%	inhibitory
fumaric acid dinitrile	133.33 at 74.4 ppm	no effect up to 180 ppm
DMSO	18.26 at 9.91%	accelerating
pyridine	27.07 at 1.28%	inhibitory
miscellaneous chemicals		
LiCl	9.76 at 7.5 mg/mL	-
oncodazole (nocodazole)	169.23 at 1.49 ppm	-

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2. Regulation of the Synthesis of Glycolytic Enzymes. J. Heinisch.

Pyruvate kinase is one of the glycolytic enzymes which is not constitutive. Its activity increases between 2-5 fold when a cell culture is transferred from a glycerol-ethanol medium to a glucose medium. Mutants with a defect in phosphoglucose isomerase have the same high pyruvate kinase activities on media with and without sugars. The pyruvate kinase PYK1 gene was cloned from a genomic library of K. Nasmyth in multicopy vector YEP13 by complementation of a pyk1 mutant. This sequence was used to probe for PYK1 mRNA. It could be shown that the pyruvate kinase activities under different growth conditions and in mutant and wild type strains are correlated with different levels of mRNA.

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3. Molecular Taxonomy of Yeast-like Organisms. T. Seehaus.

Yeast taxonomy has been difficult because of the simple structure of these fungi. We have used specific gene probes to test for taxonomical relatedness at the DNA level. We used as probes the PYK1 gene of Heinisch (above), PDC1 of Schmitt et al. (MGG 192, 247, 1983), URA3 of YIp5 (Hinnen et al., PNAS 75, 1929, 1978) and TRP1 of YRp7 (Struhl et al., PNAS 76, 1035, 1979). Genes PDC1 and PYK1 were quite conserved among the yeasts tested whereas URA3 and TRP1 had no homologous counterparts in species not belonging to the genus Saccharomyces.

Hybridizations of Saccharomyces cerevisiae genes to chromosomal DNA from different yeast species

species	PDC1	PYK1	TRP1	URA3
Saccharomyces cerevisiae	++	++	++	++
Hansenula anomala	++	++	-	-
Hansenula polymorpha	-	+	-	-
Hansenula jadinii	++	++	-	-
Kluyveromyces fragilis	++	++	-	-
Kluyveromyces lactis	+	++	-	-
Pichia farinosa	+	++	-	-
Pichia guilliermondii	++	++	-	-
Candida albicans	+	+	-	-
Candida boidinii	++	+	-	-
Candida curvata	-	-	-	-
Hanseniaspora uvarum	++	++	-	-
Pachysolen tannophilus	++	++	-	-
Dekkera intermedia	-	+	-	-
Schwanniomyces occidentalis	+	+	-	-
Metschnikowia pulcherrima	-	+	-	-
Geotrichum candidum	-	-	-	-

Intensity of hybridization: ++ strong
+ intermediate
- negative

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XVII. Istituto di Genetica, Universita di Parma, Borgo Carissimi 10, 43100 Parma, Italy. Communicated by Pier P. Puglisi.

(This communication was received May 30, 1984 and was too late for inclusion in the Spring issue).

1. Nucleo-mitochondrial interaction in the regulation of L-lactate ferricytochrome c oxidoreductase (cytochrome b_2) in Saccharomyces cerevisiae
   I. Ferrero, R. Rambaldelli, M.R. Ingrosso, C. Donnini
   Acta of VII Meeting of National Group of Cybernetics and Biophysics of CNR
   VI Meeting of Italian Society of Pure and Applied Biophysics, Camogli, 1983

Abstract. In Saccharomyces cerevisiae, the dependence from the mitochondrial function of the induction of L- and D-lactate ferricytochrome c oxidoreductase is under nuclear genetic control. Strains have been identified in which the induction of L-LDH (cytochrome b_2) may also occur in condition of respiratory inhibition or of rho$^-$ mutation. These strains will be a useful tool in the further investigation of the role of the mitochondrion in the induction and in the maturation of this protein.

2. ALG/alg: a single gene controlling the utilization of lactate in the presence of antimycin in the yeast Saccharomyces cerevisiae
   I. Ferrero, R. Rambaldelli, A.M. Genga, C. Donnini, P.P. Puglisi
   Accepted by Current Genetics, 1984

Abstract. A strain dependent growth on lactate in the presence of antimycin A has been observed. In particular, the strain D261 can grow on lactate and AA, whereas in the strain K8/6C antimycin A prevents the utilization of lactate and the induction of LDH.

The genetic analysis demonstrates that the character 'growth on lactate in the presence of AA' segregates from D261 as controlled by a single nuclear factor which is indicated by ALG1 and alg1 in its dominant and recessive configuration. alg1 complements the gene(s) which give(s) rise to the same phenotype in K8/6C.

The analysis of the regulation by lactate of LDH in the absence and presence of AA and in rho$^-$ conditions shows that the growth on lactate and antimycin A is not correlated to the induction by lactate of LDH.

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3. Effect of erythromycin upon the protein pattern of heat shocked S. cerevisiae. Identification of new classes of heat-shock and heat-stroke products
N. Marmiroli, T. Lodi
Accepted by Current Genetics, 1984.

Abstract. Conventional and two dimensional (2D) electrophoresis on ultrathin horizontal slab gels showed that heat shock proteins are synthesized and heat stroke proteins are curtailed after the transfer of Saccharomyces cerevisiae strain 2270 from 23°C to 37°C. Upon addition of the mitochondrial inhibitor erythromycin to cell cultures which incorporated labeled methionine at 23°C and after the transfer at 37°C, we have shown that: a) in extracts of cells labeled at 23°C, translational products sensitive to erythromycin could be evidenced on 2D gels; the synthesis of some of these proteins was enhanced, whereas the synthesis of some others declined when the labeling was carried out 20 min after the transfer at 37°C; b) there existed heat shock proteins whose induction at 37°C was prevented by erythromycin; c) a number of proteins fainted at 37°C, but not at 23°C, when the labeling was done in the presence of erythromycin; d) two proteins were detectable only in samples labeled at 37°C in the presence of erythromycin.

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4. Antimycin- and Hydroxamate-insensitive respiration in yeast
T. Lodi, A.M. Viola, C. Rossi, I. Ferrero
Submitted to Antonie van Leeuwenhoek, 1984.

Abstract. In this paper evidence is presented for the mitochondrial localization of the antimycin A (AA) + hydroxamate (SHAM) - insensitive respiration of the yeasts Kluyveromyces lactis, Endomycopsis capsularis and Hansenula saturnus. Such a respiration, which can be sustained by NADH and NADPH but not by succinate, is inhibited by high concentrations of azide. AA+SHAM-insensitive respiration is not phosphorylating and its postulated physiological role is to oxidize NADH.

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5. Nuclear genetic control of the mitochondrial-dependent adaptation to maltose, raffinose and melibiose in the yeast Saccharomyces cerevisiae
A.A. Algeri, P. Goffrini, T. Lodi, P.P. Puglisi
Submitted to Current Genetics

Abstract. Pleiotropic effect of IMP1 gene was investigated. IMP1 appears specific for the rescue of galactose-positive phenotype in rho⁻ strains, but other nuclear genes operate like it for other carbon sources (maltose, raffinose).

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6. N. Marmiroli and T. Lodi. Modifications of nuclear gene expression in a Matα/Mata diploid of Saccharomyces cerevisiae induced by sporulation medium and by inhibition of mitochondrial function. Submitted to Molecular and General Genetics.

Abstract. The transfer of S. cerevisiae from growth to sporulation medium was accompanied by the disappearance of some "mitotic" polypeptides and by the appearance of a new set of "meiotic" polypeptides. Erythromycin, an inhibitor of mitochondrial protein synthesis, caused the disappearance of several "meiotic" polypeptides when added to sporulation cultures. These meiotic polypeptides, though sensitive to erythromycin, were however localized in the cytosol and they were also sensitive to cycloheximde, demonstrating that they were not mitochondrial translational products. Since erythromycin did not affect either protein synthesis or sporulation in mitochondrially inherited ery^r mutants, we conclude that mitochondrial protein synthesis is needed for the expression of some of those nuclear genes which are triggered in sporulation medium.

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7. Sporulation of Saccharomyces cerevisiae in the absence of mitochondrial translation is arrested before completion of meiosis I. Analysis of the ultrastructural variations accompanying the nuclear and cytosolic compartments.
N. Marmiroli, F. Tedeschi, C. Ferrari, P.P. Puglisi
Submitted to Protoplasma

Abstract. Upon transfer in sporulation medium, diploid cells of the yeast Saccharomyces cerevisiae undergo a series of developmental events which lead to meiosis and ascospore formation. When the inhibitor of mitochondrial protein synthesis, erythromycin, was added to the sporulation medium within the first 2-4 hrs after the transfer, the meiotic development was partway arrested between intragenic and intergenic recombination. In fact, intragenic recombination occurred at normal rates, whereas intergenic recombination was reduced and commitment to meiosis and haploidization did not occur (Marmiroli et al., 1983). The ultrastructural analysis reported here has shown that in the absence of mitochondrial protein synthesis, cells formed axial cores (leptotene), synaptonemal complex and poly-complexes (Zygotene-pachytene) and that sometimes two spindle pole bodies which remained side-by-side on the nuclear envelope (diplotene-diakinesis) are formed, but after this the cells did not complete the first meiotic division. The ultrastructural analysis has also evidenced that the mitochondrial internal architecture was maintained in the erythromycin-treated sporulation culture; in this condition, the oxygen

uptake and the cytochrome content were as in the untreated control, suggesting that upon this condition mitochondrial respiration could occur normally. The nucleolus of cells exposed to the sporulation medium in the presence of erythromycin became condensed, cap-shaped with associated dense bodies and laid on the nuclear envelope as the typical diplotene nucleolus. The final location of the various types of membrane bound vesicles was also affected in the absence of mitochondrial protein synthesis. The inhibition of the sporulation by erythromycin was only partial when the drug was added 4 hrs after the transfer into the sporulation medium and normal sporulation was re-established when the drug was added after 8 hrs and thereafter.

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XVIII. Department of Microbiology, Shizuoka College of Pharmacy, 2-2-1, Oshika, Shizuoka, 422 Japan. Communicated by Yoshihisa Iwamoto.

The following two papers have been published recently:

1. Yoshihisa Iwamoto and Ichiji Mifuchi (1984). Induction of "Petite" Mutants of Yeast, Saccharomyces cerevisiae, by Photodynamic Action of Acriflavine. Chem. Pharm. Bull. 32:2759-2765.

Abstract. Treatment of yeast cells with acriflavine followed by illumination with fluorescent lamps resulted in extensive "petite" induction in addition to rapid cell inactivation and nuclear gene mutation. There was no petite induction or cell inactivation in the un-illuminated cells. Such photobiological damage induced by acriflavine was not observed under deoxygenated conditions, such as in the presence of NaN_3, which is a scavenger of singlet oxygen ($^1O_2$). Photodynamic treatment of yeast cells did not cause marked changes in the CsCl sedimentation profile of mitochondrial DNA. These results showed that the petite induction and the cell inactivation after acriflavine treatment are mainly due to type II photodynamic action.

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2. Y. Iwamoto[1], I. Mifuchi[1], L.W. Yielding[2], W.J. Firth III[2] and K.L. Yielding[3] (1984). Induction of cytoplasmically inherited respiration-deficient ('petite') mutants by photodynamic action of acridine compounds. Mutation Research, 125:213-219.

[1]Department of Microbiology, Shizuoka College of Pharmacy, Shizuoka-shi (Japan), and Departments of[2] Biochemistry and[3] Anatomy, University of South Alabama, College of Medicine, Mobile, AK 36688 (U.S.A.)

Summary

All acridines used (acriflavine, proflavine, acridine orange and 3-azido-10-methylacridinium chloride) produced killing in yeast cells when activated with visible light. Acriflavine, proflavine and 3-azido-10-methylacridinium chloride, but not acridine orange, produced petite and sectored colonies. Both cell killing and petite induction by light activation of acriflavine resulted apparently from photodynamic action mediated by singlet oxygen ($^1O_2$) since the effects were prevented by either sodium azide or anaerobiosis. The biological effects of 3-azido-10-methylacridinium chloride, which was developed as a potential photoaffinity probe for studying the binding and

biological effects of acridines, appeared to be due to a photodynamic action analogous to that of acriflavine. Sodium azide or anaerobiosis prevented the light-activated effects of 3-azido-10-methylacridinium chloride despite the fact that the initial chemical breakdown of the azido derivative induced by light was not affected. Cells suspended in D_2O demonstrated an enhanced response to 3-azido-10-methylacridinium chloride with irradiation. These results indicate that singlet oxygen mediates the light-activated biological effects of both acriflavine and 3-azido-10-methylacridinium chloride.

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XIX. Laboratoire de Génétique Physiologique, Institut de Biologie Moleculaire et Cellulaire du C.N.R.S., 15, rue R. Descartes, 67084 Strasbourg Cedex, France. Communicated by F. Lacroute.

Below follows the abstracts of our recent work concerning the yeast regulatory gene PPR1. The papers are in press in The Journal of Molecular Biology:

Yeast Regulatory Gene PPR1

1. Nucleotide sequence, restriction map and codon usage.
B. Kammerer, A. Guyonvarch and J.C. Hubert

The PPR1 gene of Saccharomyces cerevisiae controls the transcription of two unlinked structural genes URA1 and URA3. The primary structure of this eukaryotic regulatory gene and its flanking regions has been established by the dideoxynucleotide chain termination method. Our data show an open reading frame of 2712 nucleotides, corresponding to 904 amino acid residues. The 3' untranslated messenger RNA region presents consensus yeast termination and polyadenylation sequences. The pattern of codon usage in the gene is clearly random. This result is discussed in relation to protein abundance and is compared with the codon usage in 20 yeast structural and regulatory genes and with that found for Escherichia coli genes.

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2. Chromosomal localization, meiotic map, suppressibility, dominance/recessivity and dosage effect.
P. Liljelund, R. Losson, B. Kammerer and F. Lacroute

The Saccharomyces cerevisiae gene PPR1 encodes a positive regulator of the expression of the two unlinked structural genes URA1 and URA3. The gene has been mapped to a position 6.5 cM from the centromere of chromosome XII. Uninducible alleles have been selected and used to establish a meiotic map. Suppressible alleles have been identified. The sequencing of a suppressible allele confirms the nonsense nature of the mutation as well as the reading frame deduced from the nucleotide sequence. No evidence of intracistronic complementation was found, and enzymatic analysis of leaky mutants did not reveal any mutations dissociating regulation of URA1 from that of URA3. Three in vitro constructed deletions of PPR1 have been integrated at the chromosomal locus, giving strains with a completely negative phenotype. These deletion mutants display the wild type basal level of URA1 and URA3 expression and show a semi-dominant phenotype in heteroallelic ~~ppr1$^+$~~/ppr1-Δ diploids. Amplifying PPR1 by introduction into yeast on a multicopy vector increases the induction factor of URA1 and URA3 expression. These results show that the extent of

regulation of the two structural genes is dependent on the concentration of the active PPR1 protein.

A new current research interest is centered on the last steps of the pyrimidine pathway, i.e., phosphorylation of UMP and UDP and amination of UTP by selecting thermosensitive mutants affected in those steps.

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XX. Instituto de Ciências Biologicas, Universidade Federal do Rio Grande do Sul, Porto Alegre, 90.000, RS & Instituto de Quimica, Universidade de São Paulo, São Paulo, 01498, SP, Brazil. Communicated by Ana Clara Schenberg Frascino.

The following is an abstract of the work presented at the XVI Latin-American Congress on Chemistry, held at Rio de Janeiro, Brazil, from October 14 to 20, 1984.

A Novel PSO Gene Controlling Induced Mutagenesis in Yeast

K.V.L. Silva*, J.A.P. Henriques*, E.V. Galembeck** and A.C. Schenberg Frascino**, *Depto. de Fisiologia, Farmacologia e Biofisica, ICB, UFRGS, Porto Alegre, RS; **Depto. de Bioquimica, Instituto de Quimica da USP, Sao Paulo, SP, Brasil.

In a previous work, the Saccharomyces cerevisiae xs9 mutation was shown not to be allelic to any of the known pso mutations and to confer sensitivity specifically to photoaddition of the bi-functional furocoumarin 8-methoxypsoralen (8-MOP). This defect in repair capacity is more pronounced in cells treated during the G2 phase of the cell cycle. In order to further characterize the xs9 mutation, we now examined its effect on induced mutagenesis under 8-MOP and 3-carbethoxypsoralen (3-CPs) photo addition or 254 nm UV light irradiation. Reversions of mutations at the LYS2 locus are induced by these different treatments on the isogenic wild type strain while they are completely blocked in the xs9 mutant strain. In contrast, the induction of cytoplasmic respiratory deficient mutants is enhanced in the mutant strain in comparison with the wild type. With regard to 8-MOP photoaddition, the product of the PSO gene XS9 is thus involved in the repair of both lethal and pre-mutagenic lesions. Although in the case of 3-CPs photoaddition and 254nm UV light irradiation, survival is not affected by the xs9 mutation, it is clear that the XS9 gene product also plays an important role in induced mutagenesis. The results show that mutagenesis following a variety of different DNA structural lesions is under XS9 control.

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XXI. Department of Biology, York University, Faculty of Science, 4700 Keele Street, Downsview, Ontario M3J 1P3, Canada. Communicated by R.H. Haynes.

(The following two items were received May 30, 1984 and were too late for inclusion in the Spring issue).

1. Evan M. McIntosh, K. Laurie Maus and Robert H. Haynes. Deoxycytidylate Deaminase in Saccharomyces cerevisiae. Partial Characterization of the Enzyme and Isolation of a Mutant Strain Deficient in this Activity.

Deoxycytidylate deaminase activity in Saccharomyces cerevisiae has been partially characterized. The yeast enzyme was found to exhibit properties similar to those of dCMP deaminases isolated from higher eukaryotes. A mutant strain completely deficient in dCMP deaminase activity was isolated by selection for resistance to 5-fluoro-2'-deoxycytidylate (FdCMP) followed by screening for cross-sensitivity to 5-fluoro-2'-deoxyuridylate (FdUMP), a potent inhibitor of the yeast thymidylate synthetase. We have designated this new allele dcd1. A strain exhibiting an auxotrophic requirement for dUMP was isolated following mutagenesis of a dcd1 tup7 haploid. Genetic analysis reveals that this auxotrophic phenotype results from a combination of the dcd1 allele and a second, unlinked, nuclear mutation that we designate dmp1. This allele, which by itself conveys no readily discernable phenotype, presumably impairs efficient synthesis of dUMP from UDP. The auxotrophic requirement of dcd1 dmp1 tup7 strains also can be satisfied by exogenous dTMP but not deoxyuridine. The dcd1 allele had a locus specific effect on spontaneous mutation rate as measured by fluctuation tests. Forward mutation rate to canavanine resistance and reversion rate to tryptophan prototrophy (trp5-48) was 3.8 and 2.7 times that of control values, respectively. Reversion rate to arginine prototrophy (arg4-17) was significantly reduced in the strain deficient in dCMP deaminase activity.

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2. B.A. Kunz, G.R. Taylor and R.H. Haynes. Induction of Intra-Chromosomal Recombination by Inhibition of Thymidylate Bioxynthesis in Saccharomyces cerevisiae.

For several years, we have been interested in the genetic and biochemical consequences of thymidylate (dTMP) stress. To study these effects, dTMP biosynthesis has been blocked by defects in thymidylate synthetase or by employing drugs to inhibit this enzyme. Previously, we have demonstrated that dTMP starvation in Saccharomyces cerevisiae is lethal, mildly mutagenic for certain nuclear genes and provokes DNA strand breakage and mitotic inter-chromosomal recombination (crossing-over and gene conversion). Recently, we have examined the influence of dTMP stress on intra-chromosomal recombination in yeast. We have found that dTMP depletion induces unequal sister chromatid crossing-over (Current Genetics, in press) and intra-chromosomal gene conversion (manuscript in preparation).

The strains used to study exchange between sister chromatids were leu2 haploids obtained from Drs. T. Petes and L. Prakash and contained a LEU2 gene inserted in the rDNA cluster on chromosome XII. Deletion of the LEU2 insert from the reiterated rDNA leads to a Leu$^-$ phenotype because the insert bears the only functional copy of LEU2. Unequal crossing-over between sister chromatids, facilitated by misalignment of the rDNA sequences, can result in duplication of the LEU2 insert in one chromatid and its deletion from the other chromatid. In haploid strains, this gives rise to Leu$^+$/Leu$^-$ sectored colonies. Thymidylate depletion, caused by treatment with fluorodeoxyuridylate or folate antagonists, led to 7-30 fold enhancements in the production of both sectored (Leu$^+$/Leu$^-$) and non-sectored (Leu$^-$) colonies in wild-type strains. Concurrent provision of dTMP eliminated the induced

loss of the LEU2 insert. Hybridization patterns obtained by Southern blot analysis of DNA from the Leu$^+$ and Leu$^-$ halves of sectored colonies were consistent with the occurrence of unequal sister chromatid recombination. The induction of sectored colonies was prevented by the rad52-1 mutation but not by defects in RAD6. However, non-sectored Leu$^-$ colonies were induced in both rad52-1 and RAD6 strains.

To examine intra-chromosomal gene conversion, we constructed a haploid strain in which the ochre allele leu2-1 was inserted adjacent to the double frameshift allele leu2-3,112. As both leu2 alleles are defective, intra-chromosomal gene conversion is signaled by the emergence of leucine prototrophs. This strain segregated leucine prototrophs spontaneously at frequencies approximately 20-fold greater than the corresponding values for a strain carrying only leu2-1 (for all practical purposes, strains bearing only the leu2-3,112 allele fail to produce Leu$^+$ clones). Genetic characterization revealed that the vast majority of leucine protrophs was not due to suppression of the leu2-1 allele. Presumably, they arose as a consequence of intra-chromosomal gene conversion since at least two independent events would be required to generate Leu$^+$ clones by intra-chromosomal crossing-over. Antifolate-induced thymidylate starvation caused 10-20 fold increases in the frequency of leucine prototroph formation. This induction of Leu$^+$ clones was prevented by concomitant provision of dTMP. Control experiments demonstrated that neither direct reversion nor suppression of the leu2-1 accounted for the induced formation of leucine prototrophs. We conclude that dTMP depletion provokes intra-chromosomal gene conversion.

Thus, our results have shown that inhibition of thymidylate biosynthesis in Saccharomyces cerevisiae can induce not only inter-chromosomal exchange but also intra-chromosomal recombination.

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XXII. Laboratoire De Génétique Physiologique, Institut De Biologie Moleculaire Et Cellulaire Du C.N.R.S., 15, rue R. Descartes, 67084 Strasbourg Cedex, France. Communicated by M.R. Chevallier.

Approaches for the Isolation of the Uracil Transport Protein of Saccharomyces cerevisiae.

R. Jund, E. Weber and M.R. Chevallier

The uracil transport protein of the yeast Saccharomyces cerevisiae has been characterized both physiologically and genetically (1). The gene coding for the transport protein was cloned (2). One of the reasons for the cloning was to study the transcription and the regulation of the transport activity at the transcription level. The main purpose, however, was to make use of the cloned gene for the isolation of the transport protein.

Sequencing of the gene is under completion and the data obtained so far show that (i) an internal part of the protein is hydrophobic and rich in tryptophan residues (ii) and the N-terminal end of the protein is very hydrophilic. Beside our efforts to complete the determination of the DNA sequence they are as well directed towards the production of specific antibodies in order to track the protein during purification.

A peptide of 23 amino acids was synthesized by R. Jund and J.P. Briand which corresponds to a portion of the hydrophilic N-terminal end of the protein. This peptide is now used for the production of specific antibodies and detection of the protein in immuno-assays on Western blots of membrane extracts.

A second approach directed towards the production of specific antibodies was devised recently making use of the coding DNA sequence of the gene introduced into the Vaccinia virus under the control of a virus promoter. Depending on the expression of the foreign gene, after injection into rabbits, antibodies against the whole protein may be obtained and purified.

A third line of experiments was performed to obtain yeast strains in which the amount of the transport protein is greatly enhanced. The uracil transport protein gene was put under control of the GAL10 promoter on a multicopy plasmid. After induction by galactose, up to 60 times enrichment relative to the wild type was observed.

On the other hand, as a control for the purification, we would need strains not producing the transport protein. Such strains have been constructed, replacing the original chromosomal gene by one having a defined deletion produced in vitro.

(1) Jund et al., J. Membr. Biol. (1977) 36, 233-251.
(2) Chevallier, Mol. Cell. Biol. (1982) 2, 977-984.

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Identification of the purine-cytosine transport system in Saccharomyces cerevisiae as a glycoprotein.

R. Schmidt*, M.F. Manolson*, R. Ackermann* and M.R. Chevallier

[2-^{3}H] 8-azido adenine was used as a photoaffinity label for the purine-cytosine transport system. After irradiation in the presence of the photoaffinity label, the cells were converted into protoplasts, their plasma membranes purified, the membrane proteins extracted and separated by SDS-PAGE. The radioactivity was specially incorporated into a protein with a molecular weight of 120 K. Photoaffinity labelling of this protein could be blocked by irradiating in the presence of natural substrates for the transport system. The molecular weight determined by SDS-PAGE was found to be twice as high as calculated from the mRNA analysis of the cloned gene. Incubation of exponentially growing cells with tunicamycin, an antibiotic that inhibits the glycosilation of proteins, resulted in a 40% decrease in the overall initial uptake rate which correlates with a similar reduction of the labeled 120 K protein. The treatment of the extracted labeled plasma membrane proteins with glycosidic enzymes resulted in the disappearance of the 120 K peak and the appearance of two new peaks at 60 K and 70 K. These findings represent strong evidence that the purine-cytosine transport protein is a glycoprotein.

This work is under publication in Proc. Natl. Acad. Sci. (November 1984).

*Institut fur Biophysik, Freie Universität Berlin, D-1000 Berlin 33 (RFA).

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XXIII. National Research Council Canada, Division of Biological Sciences, Ottawa, Canada K1A OR6. Communicated by G.B. Calleja.

Since the last issue of the newsletter, the following publications from our lab have appeared:

1. G.B. Calleja (1984). Microbial Aggregation. 276 pp. CRC Press, Boca Raton, FL. ISBN 0-8493-5708-0. $92.00.

Aggregation, the gathering together of cells to form stable, multicellular associations, is comprehensively reviewed in this volume. Gametic agglutination in unicellular algae, bacterial star formation, mating-aggregate formation during bacterial conjugation, agglutination associated with competence to genetic transformation, aggregation in myxobacteria, dental plaque formation, and sex-directed flocculation in fission yeast are some of the topics presented.

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2. B.F. Johnson, G.B. Calleja, & M. Zuker (1984). Mating-type gene switching in a homothallic fission yeast. J. Theor. Biol. 110:299-312.

Abstract. When single cells of a homothallic strain of the fission yeast (Schizosaccharomyces pombe $968h^{90}$) are plated upon sporulation agar, a couple of cell divisions yield four preconjugal cells ordered in a line. Within a line, conjugation occurs either between sibs (the pair at either end of the line) or between cousins (the central pair of cells) or not at all. Miyata & Miyata (1981) have shown that sib matings are favored over cousin matings, the ratio of sib:cousin:steril being 96:23:10. To have mating within these 4-cell clones means that the mating types of some of the cells have switched. In a further analysis of their data, we come to a series of deductions, one of which is that switching of mating-type genes in the fission yeast must be asymmetical. We propose a random model and a deterministic model based upon asymmetrical switching. Either model could generate the ratios provided, but the models are sufficiently different that experimental tests should be able to discriminate between the two.

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3. G.B. Calleja, S. Levy-Rick, F. Moranelli, & A. Nasim (1984). Thermosensitive export of amylases in the yeast Schwanniomyces alluvius. Plant Cell Physiol. 25:757-761.

Cultures of Schwanniomyces alluvius were grown aerobically at 30°C in a phosphate-buffered defined medium containing soluble starch as sole carbon source. Extracellular amylolytic activity, minimal when cells were most actively dividing, increased dramatically at the end of exponential growth and became maximal during stationary phase. When cultures were grown at 37°C, no increase in extracellular amylolytic activity was detectable. This lack was only partly ascribable to thermal inactivation of the amylases. When cultures growing at 30°C were shifted to 37°C, excretion was arrested. Cultures growing at the restrictive temperature could be made to excrete full amylolytic activity at the permissive temperature, only if the shift was made

before the end of exponential growth.

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XXIV. Centre National de la Recherche Scientifique, Laboratoire d'Enzymologie. Gif-sur-Yvette (France). Communicated by J. Schwencke.

The following paper has been recently published:

Carmen Bordallo, Jaime Schwencke* and Maripaz Suarez Rendules. Localization of the thermosensitive X-prolyl dipeptidyl aminopeptidase in the vacuolar membrane of Saccharomyces cerevisiae (reference not supplied, editor).

Departamento Interfacultative de Bioquimica, Facultad de Medicine, Universidad de Oviedo, Spain and *Laboratoire d'Enzymologie du CNRS, Gif-sur-Yvette, France.

Abstract

Most of the X-prolyl dipeptidyl aminopeptidase activity of Saccharomyces cerevisiae was found to be associated with purified vacuolar membranes (specific activity approx. 75-times higher than in the protoplast lysate). The tonoplast-bound enzyme is thermosensitive. Another heat-resistant enzyme was found in the protoplast lysate. The tonoplast-bound thermosensitive enzyme shows an apparent Km of 0.06 mM against L-alanyl-L-prolyl-p-nitroanilide while the heat-resistant enzyme shows an apparent Km of 0.4 mM against the same substrate.

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

The following is an abstract of recent work from our Institute.

Tilman Achstetter and Dieter H. Wolf*. Hormone Processing and Membrane-Bound Proteinases in Yeast.

Search for maturating peptidases of the precursor protein of the mating hormone (pheromone) α-factor of Saccharomyces cerevisiae (1,2) was performed using short model peptides representing those sequences of the precursor protein where cleavage is thought to occur in vivo. This search was done in a mutant lacking several of the unspecific vacuolar peptidases (3). The chromogenic peptide Cbz-Tyr-Lys-Arg-4-nitroanilide led to the detection of a membrane-bound enzyme called proteinase yscF. Cleavage of the synthetic peptide derivative occurs after the basic amino acid pair, a proposed signal for hormone processing. Optimum pH for the reaction is 7.2. The enzyme does not cleave after single basic amino acid residues indicating that it is distinct from trypsin-like proteinases. Proteolytic activity is enhanced by Triton X-100. The enzyme is strongly inhibited by EGTA, EDTA and mercurials but insensitive to phenylmethyl-sulfonyl fluoride. The enzyme activity is strongly dependent on Ca^{2+} ions. In a mutant (kex2) (4) which accumulates an overglycosylated α-factor precursor, no proteinase yscF activity can be found. Membrane-bound peptidase activity possibly involved in removal of the

arginyl and lysyl residues remaining at the carboxy terminus of the α-factor pheromone peptide after the initial cut of the precursor molecule could be identified by using the model peptides Cbz-Tyr-Lys-Arg and Cbz-Tyr-Lys.

(1) Emter, O., Mechler, B., Achstetter, T., Müller, H., and Wolf, D.H. (1983) Biochem. Biophys. Res. Commun. 116, 822-829.

(2) Julius, D., Schekman, R., and Thorner, J. (1984) Cell 36, 309-318.

(3) Wolf, D.H. (1982) Trends Biochem. Sci. 7, 35-37.

(4) Bussey, H. (1981) Adv. Microb. Physiol. 22, 93-122.

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XXVI. Institute of Animal Physiology, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji, Czechoslovakia. Communicated by Ladislav Kováč.

Below follows a summary of data from this laboratory on the action of ionophoric antibiotics on S. cerevisiae.

The yeast cell offers unique opportunities for the elucidation of the mechanism of action of ionophores in biological membranes which is apparently more complex than in artificial lipid membranes. Valinomycin, nigericin, A 23187 and oleficin inhibit growth of a large number of strains of S. cerevisiae on non-fermentable substrates at concentrations at which they do not substantially affect fermentative growth on glucsoe (1,2). The antibiotics enter the cell but do not act as ionophores in the plasma membrane (1,2) although they function as ionophores in the mitochondrial membrane in vitro (3) and probably also in vivo (1,2). The latter can be demonstrated by measuring fluorescence of the cyanine dye diS-C_3 (5) in a yeast suspension which is a qualitative indicator of yeast mitochondrial and plasma membrane potentials (4). The interaction of the antibiotics with mitochondria in vivo is also reflected by the fact that oleficin (2) and valinomycin and nigericin (3) induce mass formation of cytoplasmic respiration-deficient mutants. The membrane selectivity of the action of the antibiotics suggests that proteins may be involved in their ionophoric potency in biological membranes. To test this possibility mutants resistant to the antibiotics have been isolated and their genetic and biochemical properties are under study.

1. L. Kováč, E. Böhmerová, P. Butko (1982) Biochim. Biophys. Acta 721:341-348.

2. L. Kováč, V. Poliachová, I. Horváth (1982) Biochim. Biophys. Acta 721:349-356.

3. J. Kolarov, J. Šubik, L. Kováč (1972) Biochim. Biophys. Acta 267:465-478.

4. L. Kováč, L. Varečka (1981) Biochim. Biophys. Acta 637:209-216.

5. L. Kováč, V. Klobučníková (1983) In Mitochondria 1983 (R.J. Schweyen, K. Wolf, F. Kaudewitz, Eds.) pp. 65-68. Walter de Gruyter, Berlin-New York.

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

The following publications have appeared since the last communications. The abstracts of reports have been given in Yeast Newsletter 33 (1984):1, 30-31.

John Londesborough and Kaija Varimo (1984). Characterization of two trehalases in baker's yeast. Biochemical Journal 219:511-518.

Kari Suoranta and John Londesborough (1984). Purification of intact and Nicked forms of a zinc-containing Mg^{2+}-dependent, low Km cyclic AMP phosphodiesterase from baker's yeast. The Journal of Biological Chemistry 259:6964-6971.

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Kari Suoranta. Cyclic AMP Phosphodiesterase Activities in Growing Cells of Baker's Yeast (Saccharomyces cerevisiae). Journal of Cyclic Nucleotide Research (in press).

The activities of two cyclic AMP phosphodiesterases of baker's yeast (Saccharomyces cerevisiae) were measured during diauxic batch growth on 2% glucose. The specific activity (units/mg of yeast protein) of the Mg-independent, high K_m phosphodiesterase increased 20-fold throughout the 108 h cultivation. The specific activity of the Mg-dependent, low K_m phosphodiesterase about doubled during glucose utilization and fell back to the initial level as the cells entered stationary phase.

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XXVIII. Johannes Gutenberg-Universität Mainz, Institut für Mikrobiologie und Weinforschung Universität, Postfach 3980, D-6500 Mainz, West Germany. Communicated by F. Radler.

The following paper is in press in the journal Antonie van Leeuwenhoek.

K. Baranowski and F. Radler (1984). The glucose-dependent transport of L-malic acid in Zygosaccharomyces bailii. Antonie van Leeuwenhoek 50 000-000.

Zygosaccharomyces bailii possesses a constitutive malic enzyme, but only small amounts of malate are decomposed when the cells ferment fructose. Cells growing anaerobically on glucose (glucose cells) decompose malate, whereas fructose cells do not. Only glucose cells show an increase in the intracellular concentration of malate when suspended in a malate-containing solution. The transport system for malate is induced by glucose, but it is repressed by fructose. The synthesis of this transport system is inhibited by cycloheximide. Of the two enantiomers L-malate is transported preferentially. The transport of malate by induced cells is not only inhibited by addition of fructose but also inactivated. This inactivation is independent of the presence of cycloheximde. The transport of malate is inhibited by uranyl ions; various other inhibitors of transport and phosphorylation were of little influence. It is assumed that the inducible protein carrier for malate operates by facilitated diffusion. Fructose cells of Z. bailii and cells of Saccharomyces cerevisiae do not contain a transport system for malate.

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XXIX. Technische Hogeschool Delft, Laboratorium voor Microbiologie, Julianalaan 67A, 2628 BC Delft, The Netherlands. Communicated by W.A. Scheffers.

The following three papers, abstracts of which have already been presented in Yeast Newsletter Vol. XXXII Nr. II, p. 58-60, now have been published:

1. Cornelis Verduyn, Johannes P. van Dijken and W. Alexander Scheffers (1984). Colorimetric alcohol assays with alcohol oxidase. Journal of Microbiological Methods 2:15-25.

2. Cornelis Verduyn, Timotheus P.L. Zomerdijk, Johannes P. van Dijken and W. Alexander Scheffers (1984). Continuous measurement of ethanol production by aerobic yeast suspensions with an enzyme electrode. Applied Microbiology and Biotechnology 19:181-185.

3. Peter M. Bruinenberg, Peter H. de Bot, Johannes P. van Dijken and W. Alexander Scheffers (1984). NADH-linked aldose reductase: the key to anaerobic alcoholic fermentation of xylose by yeasts. Applied Microbiology and Biotechnology 19:256-260.

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The following three papers have been published:

4. Martin R. Wijsman, Johannes P. van Dijken, Bastiaan H.A. van Kleeff and W. Alexander Scheffers (1984). Inhibition of fermentation and growth in batch cultures of the yeast Brettanomyces intermedius upon a shift from aerobic to anaerobic conditions (Custers effect). Antonie van Leeuwenhoek 50:183-192.

Abstract. Aerobic growth of the yeast Brettanomyces intermedius CBS 1943 in batch culture on a medium containing glucose and yeast extract proceeded via a characteristic pattern. In the first phase of growth glucose was fermented to nearly equal amounts of ethanol and acetic acid. After glucose depletion, growth continued while the ethanol produced in the first phase was almost quantitatively converted to acetic acid. Finally, after a long lag phase, growth resumed with concomitant consumption of acetic acid.

When the culture was made anaerobic during the first phase, growth, glucose consumption and metabolite production stopped immediately. This Custers effect (inhibition of alcoholic fermentation as a result of anaerobic conditions) was transient. After 7-8 h the culture was adapted to anaerobiosis, and growth and ethanol production resumed. The lag phase could be shortened at will by the introduction of hydrogen acceptors, such as oxygen or acetoin, into the culture. Glycerol production was not observed during any phase of growth. These results support the hypothesis that the Custers effect in this yeast is due to a disturbance of the redox balance, resulting from the tendency of the organism to produce acetic acid, and its inability to restore the balance by production of glycerol.

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5. Ansa Toivola, David Yarrow, Eduard van den Bosch, Johannes P. van Dijken and W. Alexander Scheffers, (1984). Alcoholic fermentation of D-xylose by yeasts. Applied and Environmental Microbiology 47:1221-1223.

Abstract. Type strains of 200 species of yeasts able to ferment glucose and grow on xylose were screened for fermentation of D-xylose. In most of the strains tested, ethanol production was negligible. Nineteen were found to produce between 0.1 and 1.0 $g.l^{-1}$ ethanol. Strains of the following species produce over 1 $g.l^{-1}$ ethanol in the fermentation test with 2% xylose: Brettanomyces naardenensis, Candida shehatae, Candida tenuis, Pachysolen tannophilus, Pichia segobiensis, and Pichia stipitis. Subsequent screening of these yeasts for their capacity to ferment D-cellobiose revealed that only Candida tenuis CBS 4435 was a good fermenter of both xylose and cellobiose under the test conditions employed.

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6. J.P. van Dijken and W.A. Scheffers (1984). Studies on Alcoholic Fermentation in Yeasts. Progress in Industrial Microbiology, 20:497-506.

Abstract. The capacity for alcoholic fermentation of sugars is found in numerous yeasts, including many organisms hitherto considered as non-fermentative.

The manifestation of fermentative capacity depends on a number of variables: the type of sugar, its concentration, and the availability of oxygen. Each of these variables may have a different effect in different yeasts.

Results of fundamental studies on alcoholic fermentation and its regulation in various yeasts, of potential importance for industrial applications, are discussed.

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7. H. van Doorne, W.A. Scheffers, Melanie Hadiutomo and E. van den Bosch. Microbial contamination of a vitamin A formulation, prepared in local pharmacies, and its preservation against yeasts and moulds. Antonie van Leeuwenhoek, in press.

Abstract. The microbial contamination of forty-four samples of a vitamin A preparation containing sucrose syrup was investigated. The contaminants were almost exclusively yeasts and moulds. Microbiological and physico-chemical studies demonstrated that sorbic acid was the preservative of choice for this formulation. The results are discussed with respect to the preservation of non-sterile pharmaceuticals.

8. Cornelis Verduyn, Ronald van Kleef, Johannes Frank Jzn, Henk Schreuder, Johannes P. van Dijken and W. Alexander Scheffers. Properties of the NAD(P)H-dependent aldose reductase from the xylose-fermenting yeast Pichia stipitis. Biochemical Journal (in press).

Abstract. Xylose reductase from the xylose-fermenting yeast Pichia

stipitis was purified to electrophoretic and spectral homogeneity via ion-exchange, affinity and high-performance gel chromatography. The enzyme was active with various aldose substrates, such as DL-glyceraldehyde, L-arabinose, D-xylose, D-ribose, D-galactose and D-glucose. Hence, the xylose reductase of Pichia stipitis is an aldose reductase (EC 1.1.1.21). Unlike all aldose dehydrogenases characterized so far, the enzyme from this yeast was active with both NADPH and NADH as coenzyme. The activity with NADH was approximately 70% of that with NADPH for the various aldose substrates. $NADP^+$ was a potent inhibitor of both the NADPH- and NADH-linked xylose reduction, whereas NAD^+ showed strong inhibition only with the NAD-linked reaction. These results are discussed in the context of the possible use of Pichia stipitis and similar yeasts for the anaerobic conversion of xylose into ethanol.

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

The following papers were published recently or accepted for publication:

1. Review papers

1.1. N. van Uden (1984). Cardinal temperatures of yeasts. In: Laskin and Lechevaliers (Eds), Handbook of Microbiology, 2nd ed., Vol. VI, pp. 1-8, CRC Press, Boca Raton, Florida.

1.2. N. van Uden (1984). Effects of ethanol on the temperature relations of viability and growth in yeasts, CRC Critical Reviews in Biotechnology, 1:263-272.

1.3. N. van Uden (1984). Temperature profiles of yeasts, Advances in Microbial Physiology, 25:195-248.

1.4. N. van Uden (1984). Ethanol toxicity and ethanol tolerance in yeasts, Annual Reports on Fermentation Processes, 8:000-000.

2. Research papers

2.1. C. Cabeça-Silva and A. Madeira-Lopes (1984). Temperature relations of yield, growth and thermal death in the yeast Hansenula polymorpha, Zeitschr. Allg. Mikrobiol., 24:129-132.

Summary

The temperature profile of thermal death and growth was studied in a strain of the methylotrophic yeast Hansenula polymorpha, together with the temperature dependence of the growth yield coefficients on glucose. The profile was typically dissociative with a maximum temperature for growth around 48°C, above which the yield was found to decline sharply.

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2.2. C. Leão and N. van Uden (1984). Effects of ethanol and other alkanols on the general amino acid permease of Saccharomyces cerevisiae, Biotechnol. Bioeng. 24:403-405.

2.3. C. Leão and N. van Uden (1984). Effects of ethanol and other alkanols on passive proton influx in the yeasts Saccharomyces cerevisiae. Biochim. Biophys. Acta, 774:43-48.

Summary

Ethanol, isopropanol, propanol and butanol enhanced the passive influx of protons into deenergized cells of Saccharomyces cerevisiae. The influx followed first-order kinetics with a rate constant that increased exponentially with the alkanol concentration. The exponential enhancement constants increased with the lipid solubility of the alkanols, which indicated hydrophobic membrane regions as the target sites. While the enhancement constants were independent of pH over the range tested (3.3-5.0), the rate constants decreased linearly with increasing extracellular proton concentration, indicating the presence of an additional surface barrier against proton penetration, the effectiveness of which increased with protonation. The alkanols affected the acidification curves of energized yeast suspensions in such a way that the final pH values were linear functions of the alkanol concentrations. These results were consistent with a balance between active and passive proton movements at the final pH, the exponential enhancement constants calculated from the slopes being nearly identical with those obtained with deenergized cells. It was concluded that passive proton influx contributes to the kinetics of acidification in S. cerevisiae and that uncoupling contributes to the overall kinetics of alkanol-inhibited secondary active transport across the yeast plasma membrane.

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2.4. M. Lemos-Carolino and A. Madeira-Lopes (1984). The effect of 5-fluorocytosine on the temperature profile of Candida albicans. Sabouraudia 22:351-354.

Summary

The temperature association of exponential thermal death with exponential growth, observed in a strain of Candida albicans, was disrupted by 5-fluorocytosine which, at a concentration of 2 $\mu g\ ml^{-1}$, shifted the maximum temperature for growth from 38°C to 33°C but did not affect thermal death.

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2.5. M.C. Loureiro-Dias and J.M. Peinado (1984). Transport of maltose in Saccharomyces cerevisiae - Effect of pH and potassium ions. Biochem. J. 222:293-298.

Summary

The transport of maltose in Saccharomyces cerevisiae has been generally accepted as a H^+-sugar symport, with a stoichiometrical ratio of 1:1. A simultaneous exit of K^+ from the cells with the initial uptake of maltose has been reported previously. By using a K^+-selective electrode and radioactive

maltose, we were able to measure the exit of 1 mol of K^+/mol of maltose taken up by the cells in the first 10-15s. This stoichiometrical ratio is pH-independent. So, uptake of proton in a non-buffered cell suspension or exit of K^+ in a buffered one can be used to measure initial rates of maltose uptake. We have used a K^+ electrode and a pH electrode to study the effect of external pH and K^+, respectively, on the kinetic parameters of maltose transport. The following results were obtained: the apparent half-saturation constant for maltose (K_m) increased from 5.2 mM at pH 5.8 to 38.0 mM at pH 7.8; the same increase in pH halved the apparent maximum uptake rate (V_{max}); K^+ had an inhibitory effect, decreasing V_{max} and increasing K_m at pH values above 5; K^+ had a stimulating effect at pH values below or equal to 4. Under physiological conditions, i.e., lower pH outside, neutral pH inside and much higher [K^+] inside the cell, and assuming symmetry of the system, a higher affinity for maltose is to be expected in the outer face of the plasma membrane. This behaviour of the system could explain, by itself, the maintenance of the high concentration of free maltose inside the cell (necessary because of the low affinity of the maltose), without significant back transport to the outside.

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2.6. A. Madeira-Lopes and C. Cabeça-Silva (1984). The dependence on temperature of thermal death, growth and yield of Candida tropicalis. Zeitschr. Allg. Mikrobiol. 24:133-135.

Summary

The temperature profile of thermal death and growth of a strain of the yeast Candida tropicalis was found to be dissociative. The yield coefficients on glucose did not significantly vary from 28°C up to 39°C, the maximum temperature for growth.

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2.7. I. Spencer-Martins (1984). Production and properties of an extracellular cyclodextrin hydrolase from the yeast Lipomyces kononenkoae. Int. J. Microbiol. 2:31-38.

Summary

Cyclodextrin hydrolase [cyclohepta (hexa)-D-glucan 4-glucanhydrolase, EC 3.2.1.12(13)] has been purified to homogeneity from the supernatant of a culture of the yeast Lipomyces kononenkoae IGC 4052 in a mineral medium with β-cyclodextrin as the carbon and energy source. The enzyme is a glycoprotein with a pH optimum of approximately 5.0. Range of pH stability: 3.5 to 5.5. After 1h at 60°C and pH 5.0 it loses 30% of its activity. Isoelectric point (pI): 5.2 to 5.4. K_m (40°C, pH 5) for β-cyclodextrin 0.16 mM, for maltose 1.3 mM and for soluble starch 19.7 gl^{-1}. Glucose is a competitive inhibitor. The enzyme produces glucose from maltose, p-nitrophenyl α-D-glucoside, soluble starch, α-, β- and γ-cyclodextrins. Attempts to separate cyclodextrinase and glucosidase activities by ion-exchange chromatography, gel filtration, electrophoresis, isoelectric focusing and heat inactivation have been unsuccessful.

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2.8. C. Cabeça-Silva, A. Madeira-Lopes and N. van Uden, The temperature profiles of growth, thermal death and ethanol tolerance of the cellobiose fermenting yeast Candida wickerhamii. Zeitschr. Allg. Mikrobiol. (accepted).

Summary

The temperature profile of growth and thermal death of the cellobiose fermenting yeast Candida wickerhamii was associative with the initial maximum, final maximum, optimum and minimum temperatures for growth around 38°C, 35°C, 31°C and 3°C, respectively. Ethanol enhanced thermal death by increasing the entropy of activation (entropy coefficient 7.2 entropy units mol^{-1} l^{-1}), shifted the supraoptimal part of the profile to lower temperatures without disrupting it and increased the minimum temperature for growth. The temperature profile of ethanol tolerance with respect to growth displayed a narrow temperature plateau (18-22°C) of maximum tolerance (limit 7.4% v/v, ethanol) while the toxic effects of ethanol increased steeply on either side of the plateau.

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2.9. I. Spencer-Martins and N. van Uden, Catabolite interconversion of glucose transport systems in the yeast Candida wickerhamii, Biochim. Biophys. Acta (accepted).

Summary

Candida wickerhamii IGC 3244 growing in glucose medium transported glucose by facilitated diffusion (at 25°C and pH 5 the K_s was 1.7 mM and the V_{max} 1.6 mmoles h^{-1} g^{-1} dry weight), while cells grown under derepressed conditions produced a glucose proton symport (at 25°C and pH 5 the K_s was 0.18 mM and the V_{max} 1.8-1.9 mmoles h^{-1} g^{-1} dry weight). In each case the Lineweaver-Burk plot of initial uptake rates was linear indicating the presence of a single system. In buffer with 2% glucose the symport suffered catabolite inactivation while the facilitated diffusion system emerged concomitantly in such a way that the combined V_{max} remained nearly constant. During the conversion process the Lineweaver-Burk plots were biphasic indicating the transitory co-existence of the two systems. A model is proposed that envisions the proton symport as composed of the facilitated diffusion system in association with (an)other transport protein(s), the latter being sensitive to carbon catabolite repression and inactivation.

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XXXI. Zymology Laboratory, School of Biological Sciences, University of Bath, Claverton Down, Bath, Avon BA2 7AY, England. Communicated by A.H. Rose.

The following are titles and summaries of papers which have recently been prepared and describe results of recent research in the Zymology Laboratory in the University of Bath.

1. Jill Calderbank, Michael H.J. Keenan, Anthony H. Rose and Geoffrey D. Holman (1983) Accumulation of Amino Acids by Saccharomyces cerevisiae

Y185 with Phospholipids Enriched in Different Fatty-acyl Residues: a Statistical Analysis of Data. J. Gen. Microbiol. 130:2817-2824.

Saccharomyces cerevisiae Y185, grown anaerobically in media containing ergosterol and palmitoleic, oleic or linoleic acids, synthesized phospholipids extensively enriched in the exogenously supplied fatty acid. A study was made of the effect of solute concentration on rates of accumulation of nine amino acids by organisms enriched in different fatty-acyl residues. Data were fitted using computer-aided statistical analysis to three equations to derive kinetic constants for accumulation. Analysis of data for two of the amino acids, namely L-threonine and L-histidine, showed different kinetics in organisms enriched in different fatty-acyl residues. Woolf-Hofstee plots for accumulation of L-threonine, as well as L-serine, showed abrupt changes in curvature at low concentrations with differently enriched organisms. Data for accumulation of both amino acids gave a significant fit to the model describing accumulation by one transport system without diffusion. Data for accumulation of L-histidine as well as L-aspartic acid best fitted a model describing accumulation by one transport system and diffusion. Values for K_T and the diffusion constant, but not V_{max}, differed only for accumulation of L-histidine in organisms with different fatty-acyl enrichments. A third model, describing accumulation by two separable transport systems, best fitted data for accumulation of L-glutamic acid and L-methionine. Data for accumulation of L-leucine, L-isoleucine and L-valine could not be fitted to any of the models. Woolf-Hofstee plots for accumulation of L-leucine and L-isoleucine by organisms enriched in oleyl or linoleyl residues were superimposable, although similar plots for accumulation of L-valine differed in shape.

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2. Jill Calderbank, Michael H.J. Keenan and Anthony H. Rose. Plasma-membrane Phospholipid Unsaturation Affects Expression of the General Amino-Acid Permease in Saccharomyces cerevisiae Y185. J. Gen. Microbiol. (1985) in press.

Saccharomyces cerevisiae Y185, enriched in linoleyl residues and incubated for up to 4 h in derepression buffer, more rapidly acquired GAP activity, as measured by the rate of accumulation of L-alanine, compared with organisms enriched in oleyl residues. A GAP-less mutant incubated under the same conditions did not acquire further L-alanine-accumulating ability, irrespective of the nature of the fatty-acyl enrichment. During derepression, K_T values for the GAP were virtually identical irrespective of the fatty-acyl enrichment, but V_{max} values were greater for linoleyl residue-enriched organisms, particularly after 1 h in derepression buffer. During incubation in derepression buffer, organisms with either fatty-acyl enrichment did not differ in the size of the amino-N pool, the concentration of L-alanine in that pool, rates of protein synthesis and glucose fermentation, or rate and extent of incorporation of label from $H_2{}^{32}PO_4^-$. Under conditions used to measure rates of L-alanine accumulation, organisms with either enrichment showed no evidence of metabolism of accumulated L-alanine.

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3. Malcolm Stratford and Anthony H. Rose. Hydrogen Sulfide Production by Saccharomyces cerevisiae Grown in the Presence of Sulphite and Sulphate. To be submitted shortly.

Saccharomyces cerevisiae TC8 produced hydrogen sulfide when grown under a variety of conditions in a medium containing sulfate and sulfite, but only after cessation of growth. Including methionine in the medium halved the rate of H_2S production, while lowering the concentration of sulfite lowered the rate of production. Sulfite, but not sulfate, was taken up by organisms, and sulfite was shown to be metabolized to give H_2S. Sulfite from a pyruvate-sulfite complex was also metabolized to give H_2S. Addition of ammonium sulfate to stationary-phase cultures producing H_2S led to further growth and cessation of H_2S production. Addition of cycloheximide to exponential-phase cultures led to cessation of growth and onset of H_2S production. Rates of H_2S production, induced by including different concentrations of ammonium ions in the medium or by supplementing cultures with cycloheximide, closely correlated with sulfite reductase activity in extracts of organisms.

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XXXII. Department of Biochemistry, Instituto de Quimica, Universidade Federal do Rio de Janeiro, Brasil. Communicated by Anita Panek.

The following are the summaries of two papers recently submitted for publication:

1. V.M.F. Paschoalin, V.L.A. Costa-Carvalho and A.D. Panek. Further Evidence for the Alternative Pathway of Trehalose Synthesis Linked to Maltose Utilization in Saccharomyces. Submitted to J. Biotechnol.

Summary

Yeast strains bearing a deficiency in trehalose-6-phosphate synthase activity are unable to accumulate trehalose on any carbon source unless they contain one of the MAL genes. If the gene is inducible then trehalose occurs specifically during growth on maltose; when the MAL gene is constitutive then trehalose accumulation can also be seen when cells are grown on glucose. Different systems for trehalose synthesis were suggested: one of them would require the UDPG-linked trehalose synthase, whereas the second would utilize an alternative pathway. We propose a mechanism by which the gene product of a MAL gene would serve as a common positive regulator for the expression of the genes coding for maltose permease, α-glucosidase and some component of the trehalose accumulation system. In order to elucidate this novel pathway a strain lacking UDPG-linked trehalose synthase activity and harboring a defect in maltose uptake was constructed. Excessive maltose uptake resulted in accumulation of intracellular maltose, and twice as much trehalose as in a control strain. Partial inhibition of hexokinase by xylose affected the ratio between internal maltose and trehalose and significantly reduced glycogen synthesis. Sodium fluoride also blocked glycogen synthesis but allowed trehalose accumulation. These results suggest that trehalose synthesis would require G-6-P formation derived from maltose. Such a deviation would allow for slowing down the glycolytic flux which, in turn, would favour efficient maltose utilization. Therefore, trehalose synthesis during growth in media containing glucose serves as an additional parameter for assessing constitutivity of MAL genes.

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2. M.H.M. Rocha-Leão, V.L.A. Costa-Carvalho and A.D. Panek. Some Aspects of Glycogen Metabolism in Yeast. Submitted to Arch. Biochem. Biophys.

Summary

Yeast mutants were used to evaluate the effects of catabolite repression and of the D → I conversion of glycogen synthase on glycogen accumulation during growth of cells of Saccharomyces cerevisiae. A thorough screening of growth conditions was carried out and glycogen was determined under nitrogen and carbon limitation. Glycogen increased along growth whenever cellular metabolism was oxidative. Otherwise, glycogen accumulation could only be demonstrated before the onset of diauxie (biphasic growth) or during stationary phase. Glycogen accumulation seems to be a function of the glycolytic flux irrespective of the ability of yeast cells to perform D → I conversion of glycogen synthase.

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The following is the abstract of a Master's thesis from our Institute.

Trehalose Synthesis in Saccharomyces

Rosane Charlab, Dulce E. Oliveira and Anita D. Panek

A specific deficiency in UDPG-linked trehalose-6-phosphate synthase in Saccharomyces has been associated with a single nuclear gene sst1 (Operti et al., 1982).

The sst1 mutation was first observed (Panek et al., 1979) in a revertant (Q_6R_2) of the pleiotropic fdp mutation described by van de Poll et al. (1974) which prevents growth on mannose, sucrose, glucose and fructose. The sst1 mutants utilized in this work are genetically derived from this revertant, a fact which may suggest a link between these two lesions.

Strain Q_6R_2 is a partial revertant for the fdp mutation, since it grew on glucose but maintained the mutant phenotype on fructose.

As in strain Q_6R_2, among the 38 strains tested, only strains with normal SST phenotypes grow on fructose in 24 hours. In addition, linkage studies revealed that the sst1 gene seems to map very close to the fdp gene and might even be the same gene. This result could indicate that the deficiency in trehalose synthase activity (sst1) represents one of the effects of the pleiotropic fdp mutation.

The cif mutation (Navon et al., 1979) prevents growth on fructose and has characteristics that are similar to the fdp mutation. Diploids combining the recessive mutant genes cif and sst1 are unable to grow on fructose and have almost undetectable trehalose synthase activity, indicating that the two mutant genes are allelic. This is other evidence that sst1 and fdp are genes controlling the same function.

Strains harbouring the sst1 mutation are capable of accumulating trehalose during growth on glucose medium providing a MAL^c gene is present (Operti et al., 1982). Two alternative pathways of trehalose synthesis were proposed. System I (Cabib & Leloir, 1958), dependent upon the activity of

trehalose-6-phosphate synthase and System II (Operti et al., 1982) controlled by the expression of the maltose fermentation genes and operative even when the synthase activity is undetectable.

A strain of MAL^c sst1 genotype (defective in System I) and a strain of a mal SST genotype (absence of System II) were studied under different conditions.

Different patterns of trehalose accumulation were observed. System I leads to trehalose accumulation when cells are grown on ethanol or incubated in non proliferating conditions. Net synthesis of trehalose is not observed during growth on glucose in strains which harbour System I, even though trehalose synthase activity is detected, probably due to low concentrations of substrates.

On the other hand, accumulation of trehalose by System II in non-proliferating conditions was not observed under all experimental conditions examined. Trehalose accumulated by System I seems to serve as a storage carbohydrate while trehalose synthesized by System II is related to maltose metabolism.

This work was supported by grants from CAPES, CNPq, CEPG, FINEP and FUJB.

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XXXIII. Laboratory of Radiation Microbiology, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo, 113, Japan. Communicated by Masamichi Takagi.

The following paper will appear in the December issue of the Journal of Bacteriology:

Sunairi, M., Watabe, K., Takagi, M., and Yano, K., 1984. Increase of translatable mRNA for major microsomal proteins in n-alkane-grown Candida maltosa. J. Bacteriol.

Abstract

In an n-alkane-assimilating Candida sp., transfer from glucose- to n-alkane-containing medium induced changes in the microsomal proteins, and several distinctive polypeptides were deomonstrated in the solubilized microsomal fraction derived from n-alkane-grown cells. Long-term-labeling and pulse-labeling experiments in vivo demonstrated the synthesis of the specific microsomal polypeptides. The polypeptides were synthesized as in vitro translation products directed by polyadenylated RNA extracted from n-alkane-grown cells. Two major polypeptides were partially purified from the microsomal fraction from n-alkane-grown cells, and antiserum was prepared in a rabbit. Immunoprecipitation of these two polypeptides was accompanied by an increase in the amount of translatable mRNA. The molecular weights of the polypeptides derived from long-term-labeling, pulse-labeling and in vitro translation experiments appeared to be identical.

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XXXIV. Department of Bioscience and Biotechnology, Applied Microbiology Division, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, Scotland. Communicated by D.R. Berry.

The following three articles represent recent work from our Department.

1. C.M.M. Franco, J.E. Smith, and D.R. Berry (1984). Effect of Nitrogen and Phosphate on the Levels of Intermediates in Bakers' Yeast Grown in Continuous Culture. J. Gen. Microbiol. 130:2465-2472.

Abstract. Bakers' yeast (Saccharomyces cerevisiae) has been grown in continuous culture using a control medium and media which contained low levels of ammonium and phosphate. The effects of medium composition and growth rate on the levels of intermediates of the glycolytic pathways, the tricarboxylic acid cycle and the glyoxylate cycle were investigated. The energy charge varied only between 0.7 and 0.8 over the range of dilution rates studied; however, the level of ATP decreased by 50% at higher aerobic growth rates. Intermediates of the Embden-Meyerhof-Parnas pathway were higher at the low aerobic growth rates and decreased as the dilution rate was increased. However, higher levels of these intermediates were also observed at even higher dilution rates at which ethanol formation and fermentative metabolism occurred. Significant differences in levels of intermediates were observed between control experiments and fermentations using the low nitrogen and phosphate media. The greatest differences were observed in the levels of glucose 6-phosphate, 6-phosphogluconate, pyruvate, citrate and glyoxylate. Twenty-one different steady states were investigated and each was found to have a unique composition.

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2. C.M. Ramsay and D.R. Berry (1984). The effect of inoculum level on the formation of higher alcohols, fatty acids and esters in the malt whisky fermentation. Food Microbiol. 1:111-115.

Abstract. The levels of higher alcohols, fatty acids and esters formed in a small-scale whisky fermentation which had been inoculated with different amounts of yeast was investigated. The total level of higher alcohols increased with increasing inoculum levels. However, the relative levels of propanol, isobutanol, amyl alcohols and 2-phenyl ethanol were unaffected. The levels of octanoic, decanoic and dodecanoic acids and their ethyl esters were depressed at inoculum levels above 2×10^7 cells ml^{-1}. Varying the inoculum levels did not have a consistent effect on the acetate esters of the higher alcohols. However, the highest values were obtained at inoculum levels 4×10^7 cells ml^{-1}.

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3. C.M. Ramsay and D.R. Berry (1984). Effect of temperature and pH on the formation of higher alcohols, fatty acids and esters in the malt whisky fermentation. Food Microbiol. 1:117-121.

Abstract. The levels of higher alcohols, fatty acids and esters in small-scale whisky fermentations which were carried out at different temperatures and initial pH values were investigated. Neither the total higher alcohol content nor the relative abundance of different alcohols was

affected by varying the temperature between 20 and 30°C. However, the level of octanoic acid, decanoic acid, ethyl hexanoate and ethyl octanoate were depressed at higher temperatures. The highest level of acetate esters were observed at 26 and 30°C. Altering the initial pH of the wort had little effect on the level of higher alcohols. However, increasing the pH from 4.0 to 7.0 resulted in an increase in the level of octanoic, decanoic and dodecanoic acids. Maximum levels of acetate esters of higher alcohols were obtained when initial pH values of between 5.0 and 6.0 were used.

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XXXV. Institut D'Oenologie de L'Université de Bordeaux II, 351 cours de la Libération, 33405 Talence cedex, France. Communicated by Suzanne Lafon-Lafourcade.

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

S. Lafon-Lafourcade, C. Geneix et P. Ribereau-Gayon, 1984. Les modalités de mise en oeuvre des écorces de levure en vinification. Conn. Vigne Vin, 18:111-125.

The stimulatory action produced by yeast ghosts on alcoholic fermentation is greatest when they are added to the grape must after the fermentation of the first 50 g per liter of sugar. If they are added either before or after this point, their effect is diminished. The stimulation acts along with existing traditional fermentation activators. In stuck wine, the treatment with yeast ghosts must be followed by an inoculation of a yeast population in suitable physiological state.

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A. Joyeux, S. Lafon-Lafourcade et P. Ribereau-Gayon, 1984. Metabolism of acetic acid bacteria in grape must. Consequences on alcoholic and malolactic fermentations. Sci. Aliments 4, 247-255.

The development of G. oxydans and A. aceti, the main species of acetic acid bacteria developing on grapes, modifies the constitution of grape must. Glucose preferentially, but also fructose, malic and citric acids are degraded with formation of gluconic, lactic, succinic acids, acetaldehyde equally and ketonic substances. Nevertheless the level of combining sulfur dioxide remains low. Acetic acid derives from oxydation by G. oxydans of the small amount of ethanol already present in the grape must. A. aceti forms acetic acid but it is subsequently wholly degraded in the decline phase.

Previous development in the must of acetic bacteria, such as G. oxydans, inhibits growth and metabolism of S. cerevisiae. In contrast, malolactic degradation by L. oenos is generally stimulated.

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A. Joyeux, S. Lafon-Lafourcade et P. Ribereau-Gayon, 1984. Evolution of acetic acid bacteria during fermentation and storage of wine. Appl. Env. Microbiol. 48:153-156.

Acetic acid bacteria were present at all stages of wine making, from the mature grape through vinification to conservation. A succession of Gluconobacter oxydans, Acetobacter pasteurianus, and Acetobacter aceti during the course of these stages was noted. Low levels of A. aceti remained in the wine; they exhibited rapid proliferation on short exposure of the wine to air and caused significant increases in the concentration of acetic acid. Higher temperature of wine storage and higher wine pH favored the development and metabolism of these species.

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S. Lafon-Lafourcade et P. Ribereau-Gayon, 1984. Les altérations des vins par les bactéries acétiques et les bactéries lactiques.

Acetic acid and lactic acid bacteria were present at all stages of winemaking. A lower pH and a higher ethanol concentration limit the risks of alteration. During the storage, a low temperature, good use of SO_2 and frequent checkings ensure control of bacterial growth and metabolism.

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F. Larue, S. Lafon-Lafourcade et P. Ribereau-Gayon, 1984. Relationship between the inhibition of alcoholic fermentation by Saccharomyces cerevisiae and the activities of hexokinase and alcohol dehydrogenase. Biotechn. Letters. (in the press).

The cessation of fermentation by Saccharomyces cerevisiae in a medium rich in sugar is not due to an inhibition of the activities of hexokinase and alcohol dehydrogenase. The activity of alcohol dehydrogenase was significantly increased in a medium of low sugar concentration supplemented with toxic substances, ethanol and fatty acids.

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F. Larue, C. Geneix, S. Lafon-Lafourcade, A. Bertrand et P. Ribereau-Gayon, 1984. Premières observations sur le mode d'action des écorces de levure. Conn. Vigne Vin. 18:155-163.

When yeast ghosts are added to grape must they have the effect of limiting endocellular and parietal accumulations of fatty acids toxic to the yeasts. However the concentration of hexanoic, octanoic, decanoic and dodecanoic acids in the must is only slightly affected. The concentration of ethyl esters is unchanged. The result is particularly important for white wines, where these substances contribute to the aromatic characteristics.

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XXXVI. Instituto de Biotecnologia - Universidade de Caxias do Sul - Caixa Postal 1352, 95100 - Caxias do Sul - RS - Brasil. Communicated by Juan L. Carrau.

The following are abstracts of recent work in our laboratory.

1. Serafini, L.A., Carrau, J.L., Barros, N.M., Lemos, M.Z., and Souza, O.L.G. (1984). Studies on methods to detect natural markers in yeast clones for industrial use.

Abstract. The spheroplasts fusion method has offered to the industrial microbiologist a new tool to improve the quality of microorganisms making possible the addition of new characters in order to fulfill technological interests.

The present work shows the possibility of the use of the various methods for rapid yeast taxonomy developed by R. R. Davenport to recover spheroplasts fusion products. Also an analysis of the magnitude of the modification in each clone can be done.

The methods include the use of gradients, resistences, mixtures of commercial culture media and homemade ones. Combinations between these are also presented.

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2. Barros, N.M., Carrau, J.L., Serafini, L.A., and Dillon, A.J.P. (1984). The use of differential culture media for the control of outnumbering with cultures of Saccharomyces cerevisiae.

Abstract. The work shows the possibility of using some differential culture media to follow the occurrence of geometric models of outnumbering by Saccharomyces cerevisiae cultures.

The present work uses some Saccharomyces cerevisiae cultures that are used in enology - strains KI, IA and ZYMASIL. The KI line is the first Saccharomyces cerevisiae with the expression of the "Killer factor" that has been offered to the wine industry. The IA line has been isolated from the wine microbial flora present in the region of Caxias do Sul - RS; this fact gives special regional significance to the results.

The results presented are basically a comparative analysis between developed theoretical models of outnumbering based exclusively on growth rate and the experimental results observed using the various differential media. Different "Killer performances" of the KI strain are also demonstrated.

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XXXVII. Massey University, Department of Microbiology and Genetics, Palmerston North, New Zealand. Communicated by Roy J. Thornton.

The following communications have recently been presented or submitted for publication:

1. R.J. Thornton, 1984. The occurrence of "killer" capability in pure culture wine yeasts of Australia and New Zealand. Communication presented at the VIth International Symposium on Yeast, Montpellier, France. July 1984.

Twenty-five pure culture wine yeasts from culture collections and industry were examined for "killer" capability or sensitivity. Eight strains were classified as type K_2 "killers" and of these five could be described as "superkillers". All eight killer strains were sensitive to K_1 and K_3 toxins but the three "non super" killers did not kill K_3 strains. One of the remaining seventeen strains had a "neutral" phenotype and four were sensitive to all three killer types. Two strains were sensitive to K_1 and K_3 toxins and ten strains were sensitive to K_1 and K_2 toxins. Further investigation of active toxin production in low pH musts is proceeding.

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2. R.J. Thornton, 1984. The introduction of flocculation into a homothallic wine yeast, a practical example of the modification of winemaking properties by the use of genetic techniques. Communication presented at the 35th Annual Meeting of the Society for Enology and Viticulture, San Diego, California, U.S.A., June 1984 and accepted for publication by the American Journal of Enology and Viticulture (in press).

   The property of flocculation was introduced into a powdery wine yeast strain MD26 by mating spores of MD26 with haploid vegetative cells of laboratory yeast strain TE90 which carried a dominant gene for flocculation. Spore-vegetative cell mating was performed by micromanipulation and flocculent diploid hybrid strains were isolated. The fermentation performance of three hybrid strains was evaluated in small scale winemaking trials. Flocculent haploid strains were isolated from two of the hybrid strains and mated with MD26 spores. Five flocculent diploid hybrid strains were isolated from these matings and their winemaking properties evaluated. Three hybrid strains had similar fermentation patterns and produced similar wines to that of the original strain MD26. A scheme demonstrates how the winemaking properties of a yeast may be modified by selective hybridising and back breeding.

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3. R.J. Thornton, 1984. Improvement of desirable winemaking characteristics in wine yeasts by hybridisation. Communication presented to the International Symposium on Cool Climate Viticulture and Enology, Eugene, Oregon, U.S.A. June 1984.

   Glycerol may contribute significantly to the body and fullness of wines and a selective hybridisation programme was undertaken to develop strains of wine yeast which consistently produce relatively high levels of glycerol. Eight wine yeast strains were examined for glycerol production during the fermentation at 15°C of grape juice which contained 245 g/L fermentable sugar. Two homothallic strains producing 4.7 and 5.6 g/L were mated with three heterothallic haploid laboratory yeasts which produced 3.5, 4.1 and 7.7 g/L glycerol. Six diploid strains from these matings produced between 3.8 and 7.6 g/L glycerol. Seventy-one haploid strains were isolated from these six hybrid strains and produced between 2.7 and 10.3 g/L glycerol. High glycerol producing haploid strains were mated forming fifty-three diploid hybrids whose glycerol production ranged between 5.8 and 9.3 g/L. Ninety-five haploid strains were isolated from the five highest glycerol producing diploids and their

glycerol production ranged from 5.8 to 12.2 g/L. Sixteen diploid strains were bred from the highest glycerol producers and their glycerol production ranged from 9.9 to 11.4 g/L. Other winemaking properties were monitored throughout the hybridisation programme and the final sixteen hybrid strains fermented to dryness with efficient conversion of sugar to ethanol in the absence of off-flavours and odours or other negative winemaking properties. The level of glycerol production doubled from 5.6 to 11.4 g/L, and was consistent with the selection of the more active form of the enzyme glycerol-3-phosphate dehydrogenase. Commercial scale fermentation trials of two strains are in progress.

4. Dr. Susan B. Rodriguez (formerly of UC Davis and Heublein Wines) joined my laboratory in August 1984 for one year as a New Zealand University Grants Committee Postdoctoral Research Fellow to study malate utilisation by yeasts. She has developed an indicator medium which differentiates the most efficient malate-utilising wine yeast Schizosaccharomyces malidevorans from other malate-utilising wine yeasts. Colonies of S. malidevorans are easily distinguished from colonies of Saccharomyces cerevisiae and Zygosaccharomyces bailii by a colour difference. The medium could be used to screen for high-level malate-utilising clones resulting from genetic manipulations such as fusion and transformation. A paper is in preparation.

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

These are the summaries of papers recently published or have been accepted for publication:

1. F. Malik, E. Minarik and K. Kutlik: Investigations on dry wine yeast fermentation activity. Part I: Results of batch fermentation tests (in German). Wein-Wissenschaft (GFR) 39 178-183, 1984 (nr. 3).

Results of batch fermentation tests in using 8 preparations of dry wine yeasts (D-Hefix 1000; 2000, max; D-Ferotin; CH-Uvaferm BD; BC; F-Fermivin; CS-Viakvas 82). Ther fermentation course characterized by a decrease of substrate, production of alcohol and growth of biomass, is illustrated by graphical dependences $S = f_{(t)}$, $P = f_{(t)}$ and $X = f_{(t)}$. According to our results the following preparations had the most active metabolism: Uvaferm BD and Fermivin. In addition the samples of dry wine yeast were characterized by the total number as well as by number per gram of viable cells.

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2. F. Malik and E. Minarik: Importance of selected wine yeasts in wine technology. Part I.: Role and Position of Wine Yeast Cultures in Czechoslovak Wine Technology (in Slovak). Kvasny prumysl (Prague) 30, 1984, Nr. 8, pp. 173-176.

The introductory part of this work presents information on the utilization of the Yeast Collection of the Research Institute for Viticulture and Enology in Bratislava and on the production of liquid yeast starters. Selected wine yeast strains used in the Czechoslovak wine industry are briefly

characterized. Problems of pure wine yeast starter propagation, preparation of active dry wine yeasts and their application in enological practice are discussed.

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3. F. Malik and E. Minarik: Importance of selected wine yeasts in wine technology. Part II: Preparation and characteristics of "active dry" wine yeasts. Kvasny prumysl 30, 1984, Nr. 9, pp. 197-200.

The paper deals with results of pilot-plant preparations of pure yeast starters obtained by two methods. Under conditions of wine production the yeast biomass acquired by anaerobic propagation of the strain S. oviformis (Tokay 76/D) elaborated with Na-Montmorillonite the preparation "VIAKVAS-fix" (total number of cells $1.3 \times 10^9.g^{-1}$, viability 96.8%) was prepared. Under conditions of baker's yeast production by aerobic propagation and fluid dessication of the biomass of S. oviformis (Tokay 76/D) the preparation of active dry wine yeast "VIAKVAAS 82" was obtained. The viability of the preparation ($1.57 \times 10^9.g^{-1}$) after 8 months investigation was in conformity with the norm of the International Wine Office (O.I.V.) in Paris.

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4. F. Malik, P. Porubsky and E. Minarik: Importance of selected wine yeasts in wine technology. Part III: Investigations on dry wine yeast fermentation activity (in Slovak). Kvasny prumysl (Prague) 30, 1984, No. 10, pp. 222-225.

This paper deals with the fermentation activity of 6 foreign and 2 Czechoslovak "active dry" wine yeast preparations. The most complete fermentation in an 104 h "batch" fermentation in must (S_o = 17.1 g .100 ml^{-1}, 80.11% of sugar present) could be achieved with the preparation OENOFERM (Erbsloh Co., D-Geisenheim), the highest ethanol concentration with the preparations OENOFERM and BLASTOSEL KAPPA (Chimiciperdomini S.p.A., I-Verona) 5.73 and 5.19 vol.%, respectively. The highest product yield coefficients in the mentioned time were registered by BLASTOSEL VS ($Y_{P/S}$ = 0,333) and OENOFERM ($Y_{P/S}$ = 0,330). All other preparations of "active dry" wine yeasts tested confirmed their average properties in "batch" fermentation tests.

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5. E. Minarik and Z. Silharova: Zygosaccharomyces bailii, a harmful constituent of the microflora of bottled wines (in Slovak). Kvasny prumysl (Prague) 31, 1985 (in press).

Based on long-term investigations on the yeast flora of wines with residual sugar the occurrence of Zygosaccharomyces bailii in bottled wines could be confirmed. Causes of occurrence and possible localities in the wineries and suitable measures for the elimination of these yeasts are briefly discussed.

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XXXIX. Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Canada, S7N 0W0. Communicated by W.M. (Mike) Ingledew and G.P. Casey[1].

[1]c/o Carlsberg Laboratory, Dept. of Physiology, GL Carlsberg VEJ 10, DK 2500, Copenhagen, Valby, Denmark.

The following papers have been published or are in press:

Gregory P. Casey, Carol A. Magnus and W.M. Ingledew (1984). High-Gravity Brewing: Effects of Nutrition on Yeast Composition, Fermentative Ability and Alcohol Production. Appl. Env. Microbiol. 48(3):639-646.

Abstract. A number of economic and product quality advantages exist in brewing when high-gravity worts of 16 to 18% dissolved solids are fermented. Above this level, production problems such as slow or stuck fermentations and poor yeast viability occur. Ethanol toxicity has been cited as the main cause, as brewers' yeasts are reported to tolerate only 7 to 9% (vol/vol) ethanol. The inhibitory effect of high osmotic pressure has also been implicated. In this report, it is demonstrated that the factor limiting the production of high levels of ethanol by brewing yeasts is actually a nutritional deficiency. When a nitrogen source, ergosterol, and oleic acid are added to worts up to 31% dissolved solids, it is possible to produce beers up to 16.2% (vol/vol) ethanol. Yeast viability remains high, and the yeasts can be repitched at least five times. Supplementation does not increase the fermentative tolerance of the yeasts to ethanol but increases the length and level of new yeast cell mass synthesis over that seen in unsupplemented wort (and therefore the period of more rapid wort attenuation). Glycogen, protein, and sterol levels in yeasts were examined, as was the importance of pitching rate, temperature, and degree of anaerobiosis. The ethanol tolerance of brewers' yeast is suggested to be no different than that of sake or distillers' yeast.

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W.M. Ingledew and R.F. Kunkee. Factors Influencing Sluggish Fermentation of Grape Juice. Amer. J. Enol. Viticult. (in press).

Abstract. Attempts to overcome "sluggish" (very delayed or incomplete) vinifications were made by application of previous brewing and enological finds regarding requirements for oxygen and other nutrients. Ruby Cabernet juice, and a Chardonnay juice which was known to give an incomplete fermentation commercially, were totally end-fermented in only seven days at 14°C when large amounts of assimilable nitrogen and small amounts of headspace air were provided. Restriction of oxygen by nitrogen gas flushing of the headspace resulted in protracted, sluggish-to-stuck fermentations. Air and free amino nitrogen additions stimulated the duration of active yeast growth and final cell mass, thereby enhancing fermentation rates. Indications as to the timing of aeration and the concentration of yeast food required to prevent sluggish fermentations are provided.

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W.M. Ingledew and G.P. Casey. Rapid Production of High Concentrations of Ethanol using Unmodified Industrial Yeasts. Proceedings First Bioenergy Specialists Meeting on Biotechnology, Waterloo, Ontario, 1984 (in press).

Abstract. Very high gravity brewing fermentations above 20° Plato (or Brix) stick or become exceedingly sluggish when syrups or sugars are used as the additional adjunct source. Yeast viability problems early in such very high gravity fermentations can be avoided by using 1 to 2 x 10^6 CFU/ml per °Plato of extract. Even then, supplements of oxygen (or ergosterol and unsaturated fatty acid) and yeast-utilizable nitrogen are required to promote full attenuation. When oxygen and high levels of useable nitrogen are provided, rapid catabolism at 14°C occurs; up to 16% (v/v) ethanol can be made in 5-6 days in batch fermentations.

This conversion is mediated by unmodified production lager yeast and major losses in viability do not occur under these conditions. These results have led to a reassessment of ethanol tolerance in yeasts used in the beverage industry, and to an investigation of the potential uses of very high gravity "worts" in a number of fermentation industries.

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G.P. Casey and W.M. Ingledew. Reevaluation of Alcohol Synthesis and Tolerance in Brewer's Yeast. J. Amer. Soc. Brewing Chemists (in press).

Abstract. Ethanol tolerance in Saccharomyces yeasts is still not well understood. Recent developments in the measurements of ethanol tolerance, as well as studies dealing with mechanisms of ethanol toxicity and the role of "wort" lipid components on the cell membrane have been substantial. Recent research on production of up to 16% v/v concentrations of alcohol from very high gravity worts has also led to a reevaluation of the factors influencing tolerance in Saccharomyces uvarum. The industrial ramifications of such findings may be significant, as many of the factors influencing tolerance can be environmentally manipulated or altered by changes to the wort itself.

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XL. Kirin Brewery Co., Ltd., Brewing Science Laboratory, 3, Miyahara-cho, Takasaki-shi, Gunma 370-12, Japan. Communicated by T. Ohno and R. Takahashi.

Below follow abstracts of three papers from our laboratory.

1. Toshihiko Ohno and Reisuke Takahashi, 1983. Role of Wort Aeration in the Brewing Process (1) Biosynthesis of Lipids in Brewer's Yeast. Rep. Res. Lab. Kirin Brew. Co., No. 26, pp. 15-23.

Firstly, it was confirmed that the growth and fermenting ability of yeast in the brewing process increased with wort aeration. Then the effect of cultivation conditions on lipids biosynthesis was examined. The following results were obtained:

(1) The cultivation temperature affects lipid composition of yeast remarkably.

(2) The supply of oxygen was essential for unsaturation of saturated fatty acids and cyclation of squalene to lanosterol.
(3) Phospholipids and triacylglycerides were not synthesized in the absence of oxygen, and this limitation was based exclusively on the lack of unsaturated fatty acids.

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2. Toshihiko Ohno and Reisuke Takahashi, 1983. Role of Wort Aeration in the Brewing Process (2) Behavior of Yeast Lipids in the Brewing Process. Rep. Res. Lab. Kirin Brew. Co., No. 26, pp. 25-30.

In order to confirm the effect of wort aeration on lipid composition of yeast demonstrated at the experimental level[1], the lipid composition of yeast was examined in a practical brewing process.

The following results were obtained:

(1) Contents of unsaturated acyls, triacyl glycerides, and sterol esters in yeast increased and that of squalene decreased corresponding to the time of wort aeration.
(2) In addition to wort aeration, the cell growth cycle seemed to also affect the biosynthesis of functional lipids in membrane (free sterols and phospholipids).

The results indicate that the effect of wort aeration (by sparging) lies not only in mixing yeast with wort, but more on raising the physiological activity of yeast through promoting the biosynthesis of lipids essential for cell growth.

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3. Toshihiko Ohno and Reisuke Takahashi, 1984. Production of sulfite in the brewing process. Hakko-kogaku Kaishi, 62, No. 6, 401.

The effect of wort aeration on sulfite production in the brewing process was examined, and the following results were obtained:

(1) The sulfite productivity of yeast was lowered extremely by wort aeration of a short span of time.
(2) The decrease in the sulfite productivity and promotion of yeast cell growth occurred simultaneously, and these phenomena are physiologically closely correlated.
(3) The decrease of the sulfite productivity does not depend on an increased requirement for sulfur-containing amino acids for the accelerated growth, but rather depends on the accumulation of unsaturated acyls from the wort aeration which are necessary for active growth of the yeast.

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XLI. Department of Microbiology, College of Basic Sciences & Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, Distt. Nainital, India, 263145. Communicated by Bhavdish N. Johri.

Current studies by D.R. Modi are focussed on the isolation of active yeast strains from damaged and discarded apples with a view to utilise them for fermentation of the juice. On the basis of initial screening, two indigenous yeast strains (ApS & ApS1) have been found to effectively ferment apple juice to ethanol. For fermentations, mixed juice from several varieties of apple was used. This contained 11.0 to 11.4% sugar and had a pH of 4.5. The rate of alcohol production and fermentation efficiency of the two yeast strains is as follows:

Strain	Alcohol % (W/V)	Fermentation Efficiency (%)
ApS	5.5	72.75
ApS1	7.5	80.29

Attempts are presently underway to produce cider using juice from culled apples at various nitrogen levels and sugar concentrations. The purpose of the present investigation is to develop a suitable process for cider production utilizing locally available cheap fruit resources.

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XLII. Meetings

1. Symposium on Yeast Cell Biology, April 9-15, 1985, Keystone, Colorado, James Hicks Organizer

April 9	Registration
April 10	Cell cycle: Hormones and Receptors
	Oncogene Homologues and Meiosis
April 11	Cytoskeleton and Organelles
	The Nucleus and DNA Replication
April 12	Chromosome Structure
	Secretion and Membrane Biology
April 13	Protein Localization
	Workshop: Mitochondrial Protein Transport
April 14	Gene Regulation in Lower Eukaryotes
	Gene Evolution and Action
April 15	Departure

For further information write to:

UCLA SYMPOSIA
Molecular Biology Institute
University of California
Los Angeles, CA 90024 USA
phone (213) 206-6292
telex 910-342-7597

2. Below follow the minutes of the Yeast Commission Meeting that was held in Montpellier, France on July 9, 1984.

The issuing of these minutes marks an end to our term as Secretary and Chairman of the International Commission for Yeasts. We would like to thank all Commission Members for their assistance and support over the past four years and trust that we leave the Commission in a healthy state. We also are looking to the future with confidence. Professor P. Galzy will now take over as the New Chairman and Professor J.M. Bastide will be the new Secretary (address - Chaire de Genetique et Microbiologie, Ecole Nationale Superieure Agronomique, Place Viala, 34060, Montpellier, Cedex, France) and we wish them every success over the next four years.

G.G. Stewart, Chairman I. Russell, Secretary

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Meeting International Commission for Yeasts, July 9, 1984 held in Montpellier, France (VI ISY)

Members present at July 9, 1984 meeting of the ICY in Montpellier, France:

(Belgium) G. Verachtert, (Canada) B. Johnson, (Canada) I. Russell, (Canada) G.G. Stewart, (Czechoslovakia) O. Bendova - substitute for A. Kotyk, (Czechoslovakia) K. Sigler - substitute for E. Minarik, (England) A.H. Rose, (England) J.F.T Spencer, (Finland) M. Korhola, (France) J.M. Bastide, (France) P. Galzy, (France) M. Rochet - substitute for H. Heslot, (Ireland) A.J. Forage, (Japan) K. Iwata, (Poland) H. Oberman, (Scotland) J. Johnson - substitute for I.W. Dawes, (South Africa) J.P. van der Walt, (Sweden) M. Mortberg - substitute for K. Jarl, (Switzerland) O. Käppeli, (U.S.A.) C.P. Kurtzman, (U.S.S.R.) N. Elinov.

The following members sent their apologies for absence:

(Australia) B.C. Rankine, (Austria) H. Klaushofer, (Brazil) A.D. Panek, (Bulgaria) P.V. Venkov, (Czechoslovakia) A. Kockova-Kratochvilova, (Czechoslovakia) A. Kotyk, (Czechoslovakia) E. Minarik, (DDR) H.A. Koch, (DDR) P. Lietz, (DDR) W. Nordheim, (Denmark) A. Stenderup, (Egypt) A.E. ElNawawy, (Finland) H. Suomalainen, (France) H. Heslot, (Hungary) E.K. Novak, (India) T.V. Subbaiah, (Israel) C. Shalitin, (Japan) Y. Fukazawa, (The Netherlands) L.R. de Miranda (now retired), (New Zealand) M.G. Shepherd, (Poland) J.M. Jakubowska, (Poland) T. Lachowicz, (Portugal) N. van Uden, (Scotland) I.W. Dawes, (Spain) J. Gancedo, (Spain) R. Sentandreu, (Sweden) K. Jarl, (U.S.A.) H.J. Phaff, (U.S.A.) F. Sherman, (U.S.S.R.) I.P. Bab'eva, (U.S.S.R.) G.M. Shavlowsky, (West Germany) C.C. Emeis, (Yugoslavia) V. Johanides

The following new members were absent:

(Cuba) C. Pascual, (Denmark) P. Krogh, (Hungary) T. Deak, (Sweden) H. Neujahr, (U.S.S.R.) I.P. Bab'eva

The following new members were present:

(Finland) T.-M. Enari, (Italy) A. Martini, (The Netherlands) G.S. de Hoog, (The Netherlands) W.A. Scheffers, (The Netherlands) M.Th. Smith - substitute for L.R. de Miranda who has retired, (South Africa) J.C. du Preez

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Previous ISSY Meeting Minutes

The minutes of the Commission meetings held during the VIII ISSY in Bombay, India on January 27, 1983 and in Smolence, Czechoslovakia during the IX ISSY on April 19, 1983 were accepted.

Reports of the Officers of the ICY

The report of the Officers for the previous four years (1980 to 1984) was presented and discussed.

The following points arose during the discussion:

Dr. K. Sigler (Czechoslovakia) asked why in Professor N. van Uden's letter of March 22 the theme of the XI ISSY in 1986 in Portugal had been changed from "The Influence of Ethanol and Other Narcotics on Yeast" to "Regulation of Transport and Metabolism in Yeasts - Basic and Biotechnological Aspects". The Chairman stated he was not sure of the rationale for this change and stated that he would write to Professor van Uden requesting the reason behind this change.

Dr. Matti Korhola (Finland) asked about the financial status of the Commission. The Chairman replied that the Commission as such has no funds and that any costs incurred by the Commission (mainly clerical costs, mailings, etc.) either had to be borne by the Officer of the Commission or their employer. As a consequence of this, Dr. Korhola proposed a vote of thanks to the Labatt Brewing Company for their invaluable support over the past four years. The Chairman thanked the Commission for their gratitude and mentioned that he would pass the praise onto his principles. He stated that although the Commission was unable to financially support symposia that it sponsored, he felt it important to note that under certain circumstances funds can be solicited from IUMS and UNESCO.

Memberships of the Commission

1. Professor Nikolay Elinov (USSR) proposed that the name of Dr. G.M. Shavlovsky (also of the USSR) be deleted as a member of the Commission and replaed by Dr. I.P. Babeva. This motion was moved and passed unanimously.

2. The question of separate representation for England and Scotland was raised. Following considerable discussion it was agreed by a majority of votes that the representation of countries in the Commission be according to the list agreed by the United Nations. Consequently England and Scotland would no longer be permitted separate representation and would be now known as the United Kingdom and Northern Ireland. The Chairman asked Professor A.H. Rose to undertake the task of resolving the question

of U.K. representation on the ICY. Professor Rose agreed to do this and stated that he would report back on this matter at the next Commission meeting.

3. The following new members were elected.

Dr. J. du Preez (South Africa)
Dr. A. Martini (Italy)
Dr. H. Neujahr (Sweden)
Dr. C. Pascual (Cuba)
Dr. P. Krogh (Denmark)
Professor T-M. Enari (Finland)
Dr. T. Deak (Hungary)
Dr. M.Th. Smith (The Netherlands)
Dr. G.S. de Hoog (The Netherlands)
Dr. W.A. Scheffers (The Netherlands)

IUMS

The Chairman emphasized the sentiments expressed in the Officers' report that he was very pleased between the relationship of the ICY Commission and the IUMS Mycology Division. Professor N. Elinov (USSR) questioned what exactly that relationship was. The Chairman replied stating the ICY Commission was a Commission affliated to the Mycology Division of the IUMS. The Chairman also stated that during the Mycology Congress held in Tokyo in September 1983, there was some discussion between the Mycology Division of the IUMS and the organizers of the Mycology Congress as to whether there should be a closer relationship between these two bodies. The Commission was represented by Dr. B. Johnson (Canada) at this meeting and he reported that although there was some interest in a closer liaison between the two bodies, he doubted if there was any real interest in a consolidation of the two bodies.

XIV Intern. Congress of Microbiology

The Chairman reported that the XIV ICM is being held in Manchester, England during September 1986. The ICY is sponsoring a symposium on Candidosis and the convenor is Dr. L.J. Douglas of the University of Glasgow in Scotland. Dr. Douglas (who was present at the Montpellier meeting) reported that there was considerable interest in this symposium and she would shortly have a list of speakers. The Chairman reported that he hoped that the symposium on Candidosis could be published in the same manner as was the symposium sponsored by the Commission at the XIII ICM (Fundamental and Applied Aspects of Ethanol Production and other Microorganisms). However, the Chairman requested the views of the Commission for permission to embark upon publication of symposia it sponsors. It was proposed by Dr. B. Johnston and seconded by Dr. J.P. van der Walt that the Commission was indeed in favour of the publishing of symposia. This motion was passed unanimously. Dr. Douglas also stated that a pharmaceutical company in the United Kingdom has offered to subsidize the symposium on Candidosis at the XIV ICM. This led to a discussion on whether the Commission was in favour of symposia being subsidized by commercial bodies. Professor P. Galzy (France) and Dr. J.P. van der Walt (South Africa) proposed a motion that, in principle, the Commission agreed to this symposium and other symposia being sponsored by commercial bodies. The Chairman stated, he felt sure that he spoke on behalf of all Commission members, that he was grateful to Dr. Douglas for being the

organizer for the XIV ICM and he hoped that this involvement begun at the XIII ICM and continued at the XIV would become a tradition.

X ISSY

The Chairman reported that Dr. P. Venkov of Bulgaria had written to him stating that the symposium on "Molecular Genetics" will be held in Plovdiv, Bulgaria from October 14-19, 1985 and that the first circular would soon be available.

XI ISSY

The Symposium on "Regulation and Transport and Metabolism in Yeasts, Basic and Biotechnological Aspects" will be held in 1986 in Lisbon, Portugal.

XII ISSY

The XII ISSY will be held in Jena, DDR. Dr. John Johnston (Scotland) stated that as this was a symposium on non-Saccharomyces yeasts, care should be taken as far as possible to exclude Schizosaccharomyces pombe. The Chairman agreed to write to Dr. Weber expressing these sentiments.

ISSY Symposia Beyond 1988

It was agreed in principle, but not formally approved that in 1989 and 1990 consideration be given to holding ISSY meetings on the subjects of Taxonomy (possibly in the Netherlands) and Yeast Technology in relationship to alcoholic fermentation, (possibly in Belgium). A decision on this matter will be taken at a Commission meeting to be held in 1985/86.

The question was also raised whether there should only be one Specialized Symposium per year or whether there could be more than one if topics were somewhat diversified. After some dicussion it was decided that except in unusual circumstances (for example to accommodate climatic conditions, etc.) there would only be one yeast symposium per annum.

Location of VII ISY in 1988

The Chairman stated he had three formal proposals to host the VII ISY in 1988. These proposals were from Perugia, Italy, Bratislava, Czechoslovakia and Rio de Janeiro, Brazil. It was decided to hold a secret ballot and the Chairman stated that all Commission members (including those newly elected) and substitute members present would be entitled to vote. The voting resulted in a plurality vote for holding the next ISY in Perugia, Italy. Dr. A. Martini of Italy thanked the Commission for the confidence they had shown in his proposal and stated that he would do his utmost to hold a memorable yeast symposium.

UNESCO Training Course

The Chairman announced the Commission would be part sponsor of the UNESCO Yeast Training Course in Bangkok, Thailand in December 1984. He stated that he hoped the involvement of the Commission would be the first of many.

Yeast Newsletter

The Chairman announced that for the time being Professor H.J. Phaff will continue to be the Editor of the Yeast Newsletter. It was decided that the Chairman should write to Dr. Phaff on behalf of all Commission members, thanking him for all his efforts as Editor in past years and in the future.

New Officers of the ICY

The Chairman announced that in accordance with tradition, Professor P. Galzy will be the new Chairman, Dr. G.G. Stewart the Vice-Chairman and Professor J.M. Bastide the Secretary. This change in officers will occur once the present Officers have issued the minutes and all other necessary paperwork to close the books for their 1980-84 tenure in office.

The Chairman also stated that on behalf of all Commission member, he would write to Professor H. Klaushofer (present Vice-Chairman) and thank him for all his efforts over the past 10 years.

Any Other Business

There being no other business, the Chairman adjourned the meeting and mentioned the date and place of the next ICY meeting would be at the call of the new Chairman.

The Chairman on behalf of all Commission members and scientists present at the VI ISY, sincerely thanked Professors Galzy and Bastide for all their efforts in the organization of this symposium. The Chairman stated that although it was early in the week, the symposium was obviously an outstanding event both scientifically and socially.

G.G. Stewart, Chairman ICY I. Russell, Secretary ICY

Statute of the International Commission for Yeasts

Article 1. History

In the year 1966 the Council for Yeast Research, composed of prominent specialists in the field of yeasts was established in Bratislava. In 1971 the Council was transferred into the International Association of Microbiological Societies (IAMS).

Article 2. Name

The name of the organization shall be the International Commission for Yeasts (ICY).

Article 3. Structure

The International Commission for Yeasts shall be an organization within the IAMS. For this reason the ICY is subject to the statute of IAMS and shall determine only specialized by-laws.

Article 4. Affiliation

Any regional yeast commission organized within their national microbiological societies irrespective of size or location may seek affiliation with ICY.

Article 5. Headquarters of ICY

Headquarters of ICY shall be the offices of the Chairman and Secretary of ICY.

Article 6. Selection of the Chairman and Secretary

The Chairman and Secretary of ICY for the term of four years shall be the Chairman and Secretary of the Organizing Committee of the last General Symposium. The Vice-Chairman is automatically the retiring Chairman of the previous General Symposium.

Article 7. Membership

ICY shall consist of up to three members from every country.

Article 8. Objectives

The general objectives of ICY shall be: To establish an effective liaison between persons and organizations concerned with yeast investigations, and between them and the practical users of results of investigations including yeast culture collections.

ICY will use the "Yeast Newsletter" as a means of communication.

ICY shall sponsor conferences and symposia on topics and problems of common interest. Every five years a General Symposium and if possible each year in the meantime a Specialized Symposium shall be held. Members of ICY shall be informed about regional conferences of yeasts.

Article 9. Activities

The activities of the ICY shall be conducted through the Executive Board (EB), composed of the Chairman, Vice-Chairman and Secretary.

Article 10. Duties of the ICY and its officers

The Chairman shall preside at meetings of the ICY. The Vice-Chairman shall, in the absence of the Chairman, perform the duties and exercise the powers of the Chairman. The Secretary shall record the minutes of meetings of the ICY. He shall be responsible generally for the maintenance of effective liaison between the ICY and affiliated organizations. He shall prepare reports of EB for presentation to the ICY and send invitations for its meetings.

Article 11. Meetings

Meetings of the Commission (ICY) shall be held at every general and specialized symposium. The members of the Commission will officially be invited by the Headquarters to every meeting.

Article 12. Rights privileges and obligations of membership

All members shall enjoy full participation in the affairs of the ICY except where otherwise stated in statutes.

No member shall use his connection with the ICY to further interests of his or any other organization except as is provided for in the statutes. The ICY shall not be responsible for the utterance or acts of its individual members.

Article 13. Amendments to the statutes

Any proposals for amendments to the statutes may be made in writing to the Secretary of the ICY at least six months prior to a meeting of the ICY.

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3. Xth I.S.S.Y. Xth International Specialized Symposium on Yeast

"Genetics and Molecular Biology"

Organizing Committee

P. Venkov, Chairman, A. Hadjiolov, L. Waltschewa, L. Stateva, N. Nikolaev, V. Kostov, T. Guinova-Stojanova, M. Garabedian, P. Dandanov, Treasurer

First Circular

In accordance with the resolution of the International Commission for Yeast the Xth International Specialized Symposium on Yeast "Genetics and Molecular Biology" will be held in Plovdiv, Bulgaria, 24th-29th September, 1985.
The Scientific Programme will include different aspects of yeast genetics, molecular biology and their applications in biotechnology.
Membership will be open to all persons interested in the scientific programme. Accompanying members are also welcome.
The Language of the Symposium will be English.

For further information write to: Xth I.S.S.Y. Secretariat, Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.

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4. The Eleventh International Specialized Symposium on Yeasts (XI ISSY), an activity of the International Commission for Yeasts (ICY), will be held in Lisbon, Portugal (17-21 March, 1986) with the following theme:

Regulation of Transport and Metabolism in Yeasts -
- Basic and Biotechnological Aspects

Colleagues who wish to receive the first announcement should contact Prof. N. van Uden, Gulbenkian Institute of Science, Apartado 14, 2781 OEIRAS Codex, Portugal.

* * *

During 1986 (six) the following intensive workshops courses on Yeasts will be offered by the Gulbenkian Institute of Science, Oeiras, Portugal:

Regulation of metabolism in yeasts
(Workshop leaders: C. Gancedo, Madrid; K.D. Entian, Tubingen; R. Hutter, Zurich; P. Lable, Paris).

Systematics and evolution of basidiomycetous yeasts
(Workshop leaders: F. Oberwinkler, P. Blanz, G. Deml, Tubingen).

Protoplast fusion in yeasts and filamentous fungi
(Workshop leaders: L. Ferenczy, Szeged, and others).

Information: Prof. N. van Uden, Gulbenkian Institute of Science, Apartado 14, 2781 OEIRAS Codex, Portugal.

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Future Yeast Meetings ICY

1987 XII I.S.S.Y. Genetics of non-Saccharomyces yeasts. Jena. DDR.
1988 VII I.S.Y. General yeast symposium. Perugia, Italy.
1989 XIII I.S.S.Y. Taxonomy. The Netherlands (tentative).
1990 XIV I.S.S.Y. Alcoholic beverages. Belgium (tentative)

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5. The Seventeenth Annual Meeting of the Yeast Genetics and Molecular Biology Society of Japan was held from July 26th to 28th, 1984, at the Kakuyo-kaikan Hall of the School of Medicine, Nagoya University, Nagoya, Japan. The following topics were presented and discussed. The abstracts of these talks will be published in Japanese in "Yeast Genetics and Molecular Biology News, Japan" at the end of 1984. Communicated by Masayuki Yamamoto. Laboratory of Molecular Genetics, Institute of Medical Science, University of Tokyo, P.O. Takanawa, Tokyo 108, Japan.

Session 1: Sporulation & Life Cycles

1) N. Ikeda, S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Directionality-control of mating type interconversion in Saccharomyces cerevisiae.

2) Y. Ishino-Arao and B. Ono (Fac. of Pharm. Sci., Okayama Univ.). Inhibition of homothallic mating type switching in rho$^-$ mutants of Saccharomyces cerevisiae.

3) I. Uno, K. Matsumoto*, A. Hirata and T. Ishikawa (Inst. Appl. Microbiol., Univ. Tokyo, *Dept. Ind. Chem., Tottori Univ.). The roles of cyclic AMP on the meiotic process in yeast.

4) K. Matsumoto, I. Uno*, D.Y. Shin*, H. Iida** and T. Ishikawa* (Dept. Ind. Chem., Tottori Univ., *Inst. Appl. Microbiol., Univ. Tokyo, ** Tokyo Metropolitan Inst. Med. Sci.). Heat-shock resistance and protein phosphorylation in yeast.

5) M. Tsuboi and H. Tanaka (Dept. Biol., Osaka City Univ.). Genetic mapping of sporulation genes in S. cerevisiae.

6) H. Tanaka and M. Tsuboi (Dept. Biol., Osaka City Univ.). Cloning of a sporulation gene in Saccharomyces cerevisiae.

7) C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.). Expression of meiosis genes in the fission yeast S. pombe.

8) M. Kishida and C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.). Genetic mapping of spo genes and molecular cloning of the spo6 gene in the fission yeast Schizosaccharomyces pombe.

9) Y. Iino and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Characterization of a mutation, pat1, of S. pombe which allows sporulation from the haploid state.

10) M. Yamamoto and Y. Iino (Inst. Med. Sci., Univ. Tokyo). A negative control model for the initiation of meiosis.

11) T. Uemura, M. Yanagida (Dept. Biophys., Kyoto Univ.). Type I and II DNA topoisomerase mutants of fission yeast: single and double mutants show different phenotypes in cell division cycle and chromatin organization.

12) T. Suzuki, T. Kuroiwa* and K. Tanaka (Lab. Medical Mycology, Nagoya Univ. Sch. Medicine, *Natl. Inst. Basic Biology). Heterozygosity at a ploidy-determining gene in the imperfect yeast Candida albicans.

13) H. Tohoyama and N. Yanagishima* (Biol. Inst., Ehime Univ., Biol. Inst., Nagoya Univ.). Secretion mechanism of sexual agglutination substance in Saccharomyces cerevisiae.

14) K. Suzuki and N. Yanagishima (Biol. Inst., Facult. Sci., Nagoya Univ.). α-Mating-type specific mutation causing specific defect in sexual agglutinability in Saccharomyces cerevisiae.

Session 2: Gene Structure

15) Y. Hiraoka, T. Toda, T. Hirano and M. Yanagida (Dept. Biophys., Fac. Sci., Kyoto Univ.). Cloning of the fission yeast nuclear division gene NDA3.

16) Y. Adachi, T. Toda, O. Niwa and Y. Yanagida (Dept. Biophys., Kyoto Univ.). Two α-tubulin genes expressed in S. pombe.

17) S. Matsumoto and M. Yanagida (Dept. Biophysics, Kyoto Univ.). Organization of the histone genes in fission yeast (II).

18) Y. Nakaseko, Y. Adachi, O. Niwa and M. Yanagida (Dept. Biophys., Kyoto Univ.). Walking near the centromere in fission yeast.

19) S. Nagata, K. Nagashima, Y. Yokota, A. Naito and Y. Kaziro (The Institute of Medical Science, University of Tokyo). The gene structure of nuclear-coded mitochondrial and cytoplasmic EF-1α from S. cerevisiae.

20) K. Kiyono, K. Miura, A. Ohta and I. Shibuya (Dept. of Biochemistry, Saitama Univ.). Structural analysis of Saccharomyces cerevisiae PSS gene encoding phosphatidylserine synthase.

21) K. Suzuki, T. Ito, I. Yamashita, and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Structure and expression of glucoamylase-producing genes in yeast.

22) S. Nishiwaki, D.H. Chung, S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Analysis of 5' upstream region of the HIS5 gene in Saccharomyces cerevisiae.

23) Y. Nogi and T. Fukasawa (Lab. Mol. Genetics, Keio Univ. Sch. Med.). The DNA sequence of the GAL80 region of Saccharomyces cerevisiae.

24) T. Kamiryo, K. Okazaki and T. Takechi (Fac. Integrated Arts and Sci., Hiroshima Univ.). Cluster of peroxisomal genes in Candida tropicalis.

25) A. Yasui (Medical Biological Laboratory, TNO, Rijswijk and Cell Biology and Genetics, Erasmus Univ. Rotterdam, The Netherlands). Photoreactivation gene of the yeast Saccharomyces cerevisiae.

26) Y. Shimada, Y. Tominaga, S. Nakade*, S. Harashima* and Y. Oshima* (Osaka Municipal Technical Research Institute, *Dept. Ferment. Technol., Osaka Univ.). Minimum essential ARS1 region of Saccharomyces cerevisiae.

27) T. Mabuchi, S. Nishikawa and K. Wakabayashi (Dept. of Biochemistry, Medical School, The University of Yamanashi). Structural features of mitochondrial arss in Saccharomyces cerevisiae.

Session 3: Chromosomes

28) Y. Kaneko, A. Toh-e* and Y. Oshima ** (Institute for Fermentation, Osaka, *Dept. Ferment. Technol., Hiroshima Univ., ** Dept. Ferment. Technol., Osaka Univ.). Genetic mapping and DNA sequencing of the Saccharomyes cerevisiae gene for specific p-nitrophenylphosphatase.

29) O. Niwa, T. Matsumoto and M. Yanagida (Kyoto Univ., Dept. Biophysics). Stable aneuploid in S. pombe containing a mini-chromosome.

Session 4: Cellular Structure and Function

30) T. Hirano, M. Yamaguchi and A. Tanaka* (The Jikei University, *Tokyo Metro. Inst. of Med. Sci.). Observation of Concanavalin A-Gold on the surface of yeast protoplasts.

31) M. Baba and M. Osumi (Dept. Biol., Japan Women's Univ.). Observation of the ultrastructure of yeast cells by the freeze-substitution method.

32) K. Takeo (Res. Inst. Chemobiodynamics, Chiba Univ.). Ultrastructural changes of the plasma membrane of Schizasaccharomyces pombe during the cell cycle.

33) K. Tanaka and T. Kanbe (Lab. Medical Mycology, Res. Inst. Disease Mech. Cont., Nagoya Univ. Sch. Medicine). Ultrastructure of mitosis and cytokinesis in the fission yeast Schizosaccharomyces pombe as revealed by freeze substitution-electron microscopy.

34) M. Ikeda, E. Tsuchiya, T. Miyakawa and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Visualization of the Intranuclear Structure of Saccharomyces cerevisiae.

35) I. Miyakawa, S. Kawano*, S. Nakamura*, N. Sando and T. Kuroiwa* (Biol. Inst., Yamaguchi Univ., *Natl. Inst. for Basic Biology). Isolation of yeast mitochondrial nucleoids.

36) M. Suda, A. Takagi, S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Application of transformation-associated cell fusion to breeding of industrial strains.

37) T. Yamazaki and H. Nonomura (Dept. Ferment. Technol., Yamanashi Univ.). Protoplast fusion between the wine yeast Saccharomyces cerevisiae OC-2 and the salt-tolerant yeast Zygosaccharomyces rouxii (Part 2).

Session 5: Radiation & Recombination

38) T. Ito and A. Ito (Inst. Phys., College Arts Sciences, Univ. Tokyo). A γ-ray induced beads-like cell clump of yeast and its colony forming ability.

Session 6: Mutation

39) H. Oh-ue, E. Sakamoto and B. Ono (Fac. of Pharm. Sci., Okayama Univ.). Characteristics of Saccharomyces cerevisiae mutant strains resistant to inorganic mercury.

40) E. Sakamoto and B. Ono (Fac. of Pharm. Sci., Okayama Univ.). Growth retardation of the mercury-sensitive strains of Saccharomyces cerevisiae on the medium containing excess fermentable sugars.

41) B. Ono and Y. Ishino-Arao (Fac. of Pharm. Sci., Okayama Univ.). Saccharomyces cerevisiae strains showing retarded growth on high pH media.

42) J. Ishiguro and M. Miyazaki* (Dept. of Biology, Konan Univ., *Inst. of Molec. Biology, Nagoya Univ.). Genetic and Biochemical Characterization of Blasticidin S-Resistant Mutants of Saccharomyces cerevisiae.

43) K. Kitamoto, K. Yoshizawa, Y. Ohsumi*, and Y. Anraku* (Nat. Res. Inst. Brewing; *Dept. Biol., Fac. Sci., Univ. Tokyo). Isolation of mutants defective in vacuolar functions of S. cerevisiae.

Session 7: Gene Regulation

44) K. Yoshida, Y. Tamai, J. Kuromitsu and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Isolation of DNA fragment complementing a pho2 mutation in Saccharomyces cerevisiae.

45) A. Toh-e (Dept. Ferment. Technol., Hiroshima University) Cloning and sequencing of the PHO80 gene of Saccharomyces cerevisiae.

46) K. Ogawa, K. Matsumoto*, A. Toh-e**, I. Uno***, Y. Oshima and T. Ishikawa *** (Dept. Ferment. Technol., Osaka Univ. Dept. Ind. Chem., Tottori Univ., **Dept. Ferment. Technol., Hiroshima Univ., ***Inst. Applied Microbiol., Univ. Tokyo). Transcriptional regulation of repressible acid phosphatase production by cAMP.

47) T. Masahiro*, Y. Nogi and T. Fukasawa (Lab. Mol. Genetics, Keio Univ. Sch. Med., *Shiseido Lab.). Two upstream regulatory sequences (URS) involved in controlled transcription of yeast GAL7 gene.

48) T. Fukasawa, T. Segawa, H. Shimada and M. Igarashi* (Keio Univ. Sch. Med., *Yamasa Shoyu Co.). Autogenous Regulation of Expression of Yeast GAL80 Gene.

49) Y. Ohya, S. Miyamoto, Y. Ohsumi and Y. Anraku (Dept. Biol., Fac. Sci., Univ. Tokyo) Genetic study of the roles of Ca^{2+} in the cell proliferation of Saccharomyces cerevisiae II. (Functions of CLS gene products).

50) K. Matsumoto, I. Uno* and T. Ishikawa * (Dept. Ind. Chem., Tottori Univ., *Inst. Applied Microbiol., Univ. Tokyo). Isolation and characterization of resistant mutants to calmodulin inhibitor in Saccharomyces cerevisiae.

51) K. Adzuma, T. Ogawa, and H. Ogawa (Dept. Biol., Osaka Univ.). Expression of the RAD52 gene of Saccharomyces cerevisiae.

52) Y. Takano, I. Yamashita and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Mating-type Control on the Expression of STA1 Gene which codes for an Extracellular Glucoamylase Activity in the Yeast Saccharomyces diastaticus.

53) T. Fujii, J. Watari, M. Takagi and K. Yano (Dept. Agr. Chem., Tokyo Univ.). Expression in S. cerevisiae of LEU gene from Candida maltosa in relation to nucleosome structure.

54) S. Miyake and M. Yanagida (Dept. Biophys. Kyoto Univ.). Molecular cloning of the fission yeast cell division cycle genes NDA1 and NDA4.

Session 8: Technical Aspects & Cytoplasmic Inheritance

55) K. Yoshida (Biol. Inst., Fac., Sci., Nagoya Univ.). Microcomputer softwares for computer aided genetic engineering.

56) M. Niwa, K. Ohashi and M. Umeda (Dept. Bacteriol., Osaka City Univ. Med. Sch.). Metachromatic stain of bacteria, fungi and yeast with carbocyanine dye.

57) F. Miyamoto and K. Okamoto (Dept. Education, Wakayama Univ.). Studies on supressiveness of petite mutants induced from one strain of S. cerevisiae: all mutants induced from it were highly suppressive.

Section 9: Biochemistry

58) S. Hasegawa, I Banno*, N. Yanagishima (Dept. Biol., Fact. Science, Nagoya Univ., *Institute for Fermentation, Osaka). Secreted sexual agglutination substances in the yeast Issatchenkia scutulata var. scutulata.

59) T. Mizunaga, K. Ikeda and Y. Maruyama (Dept. Agric. Chem., Univ. of Tokyo). Do ionophores and Ca^{2+} affect protein secretion in Saccharomyces cerevisiae?

60) H. Arakawa, T. Hikiji, A. Ohta and I. Shibuya (Dept. of Biochemistry, Saitama Univ.). Properties and intracellular distribution of Phosphatidylserine decarboxylase activity in Saccharomyces cerevisiae.

61) E. Tsuchiya, M. Ikeda, T. Miyakawa and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Characterization of biochemical activities in the nuclear matrix isolated from Saccharomyces cerevisiae.

62) T. Sato*, Y. Ohsumi and Y. Anraku (Dept. Biol., Fac. Sci., Univ. Tokyo; *Inst. Molecular Biology, Nippon Zeon). Amino acid transport systems of the vacuolar membrane in S. cerevisiae.

63) E. Uchida, Y. Ohsumi and Y. Anraku (Dept. Biol., Fac. Sci., Univ. Tokyo). Purification and properties of H^+ translocating, Mg^{2+} -ATPase from vacuolar membranes of S. cerevisiae.

64) Y. Ohsumi and Y. Anraku (Dept. Biol., Fac. Sci., Univ. Tokyo). Specific induction of Ca^{2+} uptake by α-factor.

65) H. Iida and I. Yahara (Tokyo Metrop. Inst. Med. Sci.). Purification and physical properties of yeast hsp48 and hsp46.

66) I. Uno, K. Matsumoto*, K. Adachi and T. Ishikawa (Inst. Appl. Microbiol., Univ. Tokyo, *Dept. Ind. Chem., Tottori Univ.). Isolation and characterization of a phosphoprotein phosphatase-deficient mutant in yeast.

67) M. Miyazaki and M. Uritani (Inst. Molec. Biol., Sch. Sci., Nagoya Univ.). Characterization of a Cycloheximide Sensitivity Factor (CH.SF) occurring in a Soluble Fraction from Yeast (Part 2).

68) Y. Hayashi, C.W. Nakagawa and A. Murasugi (Inst. for Developmental Res., Aichi Colony). Biosynthesis of Cd-binding peptide allomorphs in fission yeast.

69) S. Yamamoto, Y. Matsuse, Y. Hattori, B. Ono and H. Mori* (Fac. Pharm. Sci., Okayama Univ., *Noda Inst. Sci. Res.). Changes in polyamine concentrations of salt-tolerant, halophilic and sugar-tolerant yeasts induced by sodium chloride and sucrose.

70) M. Yamamura, T. Kuroiwa, I. Nakamura and T. Kamihara (Dept. Indust. Chem., Kyoto Univ.). Petite induction at elevated temperatures in Saccharomyces yeasts.

71) T. Kamihara, M. Nakai and I. Nakamura (Dept. Indust. Chem., Kyoto Univ.). Thiamine-induced respiratory deficiency in yeasts: Lipid composition of mitochondria.

72) M. Kaji, T. Miyakama, E. Tsuchiya and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). The role of sulfhydryls of surface protein of the recipient cell for the trans-membrane signaling of the mating pheromone rhodotorucine A.

73) T. Kadota, T. Miyakawa, E. Tsuchiya and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Analysis of cell surface proteins specific to gamete cells of Tremella mesenterica using monoclonal antibody.

Session 10: Plasmids

74) M. Umetsu, M. Kozaki and Y. Ikeda* (Dept. Agr. Chem., Tokyo Univ. Agr., *Nodai Res. Inst., Tokyo Univ. Agr.). 1.5-μm DNA plasmid in Kluyveromyces drosophilarum.

75) N. Gunge, C. Yamane (Mitsubishi-Kasei Institute of Life Sciences). Stabilization of pGKL-ars vector.

76) H. Araki, A. Jearnpipatkul, H. Tatsumi, Y. Kaisho and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Functional region of yeast plasmid pSR1.

77) K. Sugihara, A. Toh-e (Dept. Ferment. Technol. Hiroshima Univ.). Structure and function of 3μm-like plasmid derived from Zygosaccharomyces bailii.

78) J. Inoue, N. Nakanishi, M. Yamamoto and Y. Kikuchi* (Inst. Med. Sci., Univ. Tokyo and *Sch. Med., Keio Univ.). Characterization of ars's on the plasmids which form polymers in Schizosaccharomyces pombe.

Session 11: Biotechnology

79) H. Kojima, M. Wada, H. Takahashi and H. Saito (Institute of Applied Microbiology, University of Tokyo). Expression of APH Gene in S. cerevisiae.

80) A. Takagi, E. Chua, C. Sugiki, S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Effect of host ploidy on the productivity of a cloned gene.

81) K. Tanaka and T. Oshima (Suntory Institute for Biomedical Research). Construction of a high stability Yeast vector.

82) T. Oshima, M. Tujimoto, S. Tanaka and H. Nakazato (Suntory Institute for Biomedical Research). Secreted expression vector in Yeast.

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XLIII. Brief News Items

1. I organized the First National Intensive Training Course on Taxonomy and Biotechnology of Yeast which was held in the Technological Institute of Merida, Yucatan, Mexico from 1 to 12 October, 1984.

   The principal lecturers were: Dr. Sally A. Meyer, (Atlanta, Georgia); Dr. Clete P. Kurtzman, (Peoria, Illinois); M. Sc. Miguel Cedeño Cruz, (Yucatan, Mexico); M. Sc. Jovita Martinez Cruz, (Mexico); Fifteen students came from different states of the Mexican Republic.

   In the unanimous view of the students, the teachers and the host Institute the course had exceptional quality and was a full success both scientifically and socially.

   A Second National Course on the same subject is being planned for 1986.

Jovita Martinez Cruz
Centro de Investigacion y de Estudios
Avanzados del IPN
Apartado Postal 14-740
Mexico 14, D.F.
Departamento de Biotecnologia y Bioingenieria

2. The following are news items from our laboratory.

R. Montrocher has defended his thesis: "Etude des affinités interspécifiques dans le genre Candida (levures asporogènes). Approches sérologique, biochimique et mathématique.

We have received visits from Dr. C.P. Kurtzman and Dr. T. Nakase.

We have participated in the 6th ISY in Montpellier, in July, 1984, with the following communications:

M.C. Pignal and D. Lachaise - Levures isolées de Drosophiles au Cameroun.

F. Jacob, S. Poncet, J. Perrier and A. Michel - Utilisation de lactosérum "brut" pour la production de protéines levures.

R. Montrocher and S. Poncet - Application d'une méthode d'analyse numérique dans le genre Candida.

J.B. Fiol - Etude qualitative et quantitative des coenzymes Q des Kluyveromyces.

J.B. Fiol, R. Montrocher, Z. Hmama et M. Claisse - Les Pichia à spores sphériques: étude immunologique et biochimique; applications systématiques.

M.C. Pignal
University Claude Bernard (Lyon 1)
43 Blvd du 11 Novembre 1918
Villeurbanne, France

3. Brewing Science Laboratory of Kirin Brewery Co., Ltd. announces that Dr. Eiichi Kokubo, formerly General Manager, has been appointed General Manager of Hiroshima Plant: Dr. Takashi Inoue, formerly Manager of the Research Group has been appointed General Manager of the Brewing Science Laboratory.

Kirin Brewery Co., Ltd.
Brewing Science Laboratory
3, Miyahara-cho, Takasaki-shi
GUNMA 370-12, Japan

4. Change of address:

former address	new address
Dr. Rodney Rothstein Microbiology Department - MSB F607 CMDNJ - New Jersey Medical School 100 Bergen Street Newark, New Jersey 07103	Department of Human Genetics and Development Columbia University College of Physicians and Surgeons 701 W. 168th Street New York, NY 10032

5. Bio Expo 85 is an international conference and exhibition on biotechnology, which will be held in Boston, 14-16 May 1985. The Bio Expo 85 conference is sponsored by our monthly biotechnology journal ENZYME AND MICROBIAL TECHNOLOGY and also by the journal VACCINE, which is also published by Butterworth.

Further information about Bio Expo 85 can be obtained from: Bill Burris, Cahners Exposition Group, 7315 Wisconsin Avenue, P.O. Box 70007, Washington, DC 20088, USA.

6. Postdoctoral Fellowship

We seek a research associate with experience in enzymology and microbiology to collaborate with us in a program concerning biochemical and ultrastructural aspects of pathogenic yeasts. The beginning stipend is $16,480 per annum plus finge benefits.

The specific project, which is funded by N.I.H., centers on the purification and characterization of acid phosphatases from Sporothrix schenckii. Important differences between the yeast phase and the (saprophytic) mycelial phase have been documented. Particular attention will be paid to the possible role of one or more of the isoenzymes of acid phosphatase in the dephosphorylation of phospho-proteins, and the implications for control mechanisms affecting metabolism and development.

Other areas of concurrent interest within the same group include the modes of action of new antifungal drugs and metabolic studies on intact-cell suspensions by NMR techniques. Please contact:

Dr. Wilfred N. Arnold
Department of Biochemistry
University of Kansas Medical Center
Kansas City, Kansas, USA 66103
913-588-7056

7. 2 year postdoctoral fellowship available, salary $18,000 with:

Dr. Vincent Cirillo
Dept. of Biochemistry
State University of New York
Stony Brook, N.Y. 11101

Topic area: Reconstitution of transport proteins in yeast plasma membranes.

For more information, call 516-246-5039