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Y E A S T

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

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M.Th. Smith, Delft, Netherlands 39
S.J. Jong, Rockville, Maryland 39
C.P. Kurtzman, Peoria, Illinois 41
John D. Buck, Noank, Connecticut 42
M.A. Lachance, London, Canada 44
Anita D. Panek, Rio de Janeiro, Brazil 45
J.R. Villanueva, Salamanca, Spain 46
F. Radler, Mainz, West Germany 48
Byron F. Johnson, Ottawa, Canada 49
Dieter H. Wolf, Freiburg, West Germany 51
J.J. Miller, Ontario, Canada 52
Pier P. Puglisi, Parma, Italy 52
J. Schwencke, Gif Sur Yvette, France 56
W.A. Scheffers, Delft, Netherlands 57
Tamotsu Morita, Shizuoka, Japan 60
Seymour H. Hutner, New York, New York 61
P. Niederberger and R. Hutter, Zurich, Switzerland 62
E.D. Garber, Chicago, Illinois 63
Morton C. Kielland-Brandt, Copenhagen Valby, Denmark 64
Masamichi Takagi, Bunkyo-ku, Japan 65
Robert K. Mortimer and David Schild, Berkeley, California 65
J.F.T. Spencer, London, England 70
Olga Bendova and Vladimir Vondrejs, Prague, Czechoslovakia 72
Veijo Makinen, Espoo, Finland 75
P.A. Biacs, Budapest, Hungary 74
W.M. Ingledew, Saskatoon, Canada 75
P.A. Martin and D.A. Lovett, Burton-on-Trent, England 76
H. Suomalainen, Helsinki, Finland 77
E. Minarik, Matuskova, Czechoslovakia 78
C. Badour, Epernay, France 79
Books and Other Publications 81
Meetings 84
Brief News Items 94

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

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

Herman J. Phaff
Editor

I. Centraalbureau voor Schimmelcultures, Julianalaan 67a, 2628 BC Delft, the Netherlands. Communicated by M. Th. Smith.

Three publications have recently appeared from the CBS-Yeastdivision.

1. Buhagiar, R.W.M., Yarrow, D. & Barnett, J.A., 1983. Bullera crocea and Bullera armeniaca, Two new Yeasts from fruit and vegetables. Journal of General Microbiology 129:3149-3155.

2. Shivas, R.G. & Rodrigues de Miranda, L., 1983. Cryptococcus phylloplanus and Cryptococcus hinnuleus, two new yeast species. Antonie van Leeuwenhoek 49:153-158.

3. Shivas, R.G. & Rodrigues de Miranda, L., 1983. Two new species of the genus Sporobolomyces and a new Rhodotorula species from leaf surfaces. Antonie van Leeuwenhoek 49:159-166.

* * *

List of Cultures, 30th Edition

An up to date 30th edition of the CBS List of Cultures is now available at Hfl. 35,-- (microfiche edition: Hfl. 10,--). The new version has been extended with approximately 3,000 cultures and now lists a total of 28,000 strains, including 21,500 filamentous fungi, 5,000 yeasts and 1,500 actinomycetes. About 3500 of these are type strains. Each strain is listed with its source and date of isolation, depositor, possible mating type and any known biochemical properties.

The List of Cultures may be ordered by writing to the Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, the Netherlands.

Cultures are supplied on request at Hfl. 85,-- (dutch florins) per culture for commercial organizations and Hfl. 40,-- per culture for non-profit organizations.

Reductions are given for orders of 10 or more, or subscriptions of 10 cultures per year, viz. Hfl. 65,-- and Hfl. 30,-- per culture, respectively.

* * *

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

The strains listed have been added to the ATCC since May 23, 1983. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

Saccharomyces cerevisiae ATCC 52278	D. Botstein MIT Cambridge, MA

Saccharomyces cerevisiae ATCC 52284-52292	S.J. Silverman NINCDS, NIH Bethesda, MD
Lipomyces kononenkoae ATCC 52298	T. Placido Gulbenkian Institute of Science Portugal
Saccharomyces cerevisiae ATCC 52299-52300	P.S. Lazo Dept. Bioquimica Facultad Medicina Spain
Candida albicans ATCC 52301-52302	A. Sarachek Wichita State University Wichita, Kansas
Saccharomyces cerevisiae ATCC 52328	G. Loison Transgene S.A. France
Saccharomyces bisporus ATCC 52428	Linda Bengston American Type Culture Collection Rockville, MD
Candida tropicalis ATCC 52431	J. D'Souza University Bombay India
Saccharomyces cerevisiae ATCC 52432	"
Kluyveromyces fragilis ATCC 52466	J. Grant Wassink National Research Council Canada
Saccharomyces cerevisiae ATCC 52467-52469	H. Fukuhara Institut Curie France
Saccharomyces cerevisiae ATCC 52483	R. Davis Stanford University Stanford, CA
Kluyveromyces fragilis ATCC 52486	T. Placido Oeiras Portugal
Saccharomyces cerevisiae ATCC 52497-52500	H. Fukuhara Institut Curie France
Saccharomyces rouxii ATCC 52519	L. Restaino Hershey Foods Corp. Hershey, PA

Saccharomyces cerevisiae ATCC 52522-52530	C.E. Ballou University of California Berkeley, CA
Saccharomyces cerevisiae ATCC 52532-52537	M.L. Carbone University Degli Studi di Milano Italy
Candida chiropterorum ATCC 52547	R.M. Furman Center for Disease Control Atlanta, GA
Kluyveromyces fragilis ATCC 52633	D.A. Stevens Santa Clara Valley Medical Center San Jose, CA
Rhodosporidium toruloides ATCC 52636	M. Hofer Botanisches Institut, University Germany
Cryptococcus neoformans ATCC 52657	J.R. Graybill University Texas Health Science San Antonio, TX

* * *

III. United States Department of Agriculture, Agricultural Research Service, 1815 North University Street, Peoria, Illinois, 61604. Communicated by C.P. Kurtzman.

The following paper has been submitted to Mycotaxon:

Kurtzman, C.P. Resolution of varietal relationships within the species Hansenula anomala, Hansenula bimundalis and Pichia nakazawae through comparisons of DNA relatedness.

Abstract

Within the genera Hansenula and Pichia, varieties have been described for H. anomala (Hansen) H. et P. Sydow, H. bimundalis Wickerham et Santa Maria and P. nakazawae Kodama. These varieties were designated primarily on physiological characteristics. The relationship of these species with their varieties has been compared through estimates of DNA base sequence complementarity. Mol% G+C values for these taxa are: H. anomala var. anomala, 36.5%; H. anomala var. Schneggii, 36.6%; H. bimundalis var. bimunalis, 41.9%; H. bimundalis var. americana, 43.9%; P. nakazawae var. nakazawae, 39.4%; and P. nakazawae var. akitensis, 39.9%. DNA complementarity studies gave the following results. H. anomala var. anomala and H. anomala var. schneggii at 94% DNA relatedness were considered to be the same taxon, whereas the 19% relatedness betweenH. bimundalis var. bimundalis and H. bimundalis var. americana indicates the latter variety to be a separate species, described as H. americana. The

varieties of P. nakazawae exhibited 41% DNA relatedness and it is proposed that their varietal designations be maintained.

* * *

The following paper has been submitted to Antonie van Leeuwenhoek:

Kurtzman, C.P. Synonomy of the yeast genera Hansenula and Pichia demonstrated through comparisons of DNA relatedness.

Abstract

The relationship between the genera Hansenula and Pichia was examined through comparisons of DNA relatedness among phenotypically similar species. Hansenula minuta and Pichia lindneri showed 75% DNA base sequence complementarity. In other comparisons, H. nonfermentans was found to share nearly 50% of its DNA sequenes with both H. minuta and P. lindneri. Because of the high degree of relatedness observed, it is proposed that ability to assimilate nitrate, the sole distinction between Hansenula and Pichia, is of insufficient taxonomic value for the reliable separation of either species or genera. Hat-spored species of Hansenula H. et P. Sydow 1919 are being transferred to Pichia Hansen 1904. Species of Hansenula and Pichia with Saturn-shaped ascospores will be transferred to the genus Williopsis.

* * *

IV. The University of Connecticut, Institute of Marine Sciences, Marine Research Laboratory, Noank, Connecticut 06340. Communicated by John D. Buck.

For the past several years, I have been interested in the distribution of potentially pathogenic yeasts in environmental material and the occurrence of these organisms in captive marine mammals. Abstracts of some of this work follow below.

1. John D. Buck 1980. Occurrence of Human-Associated Yeasts in the Feces and Pool Waters of Captive Bottlenosed Dolphins (Tursiops truncatus). J. Wildlife Diseases 16:141-149.

Abstract

Total yeast counts at 20 and 37°C incubation from chlorinated salt water pools containing marine mammals averaged 40 per L and 12 per L, respectively. Candida albicans, the etiological agent of candidiasis in mammals, was found in 32% of 123 water samples although numbers were low (average of 1.2 cells per L). The yeast was isolated only once from feces from one Atlantic bottlenosed dolphin (Tursiops truncatus) but was recovered from three fecal samples from an asymptomatic beluga whale (Delphinapteras leucas) which suggested that this animal may be a carrier. Three yeasts (Candida tropicalis, C. parapsilosis, and Torulopsis glabrata) associated with human disease accounted for 73% and 88%, respectively, of the isolates from water and animals, on plates incubated at 37°C. The data indicate the routine presence of potentially pathogenic

yeasts in water and various marine mammals. Captive environments characterized by antimicrobial treatment (e.g., chlorine) may provide appropriate conditions for resistant microorganisms, including yeasts, to become opportunistic pathogens in susceptible marine mammals or to become established in others which act as healthy carriers.

* * *

2. J. Lawrence Dunn, John D. Buck and Stephen Spotte 1982. Candidiasis in captive cetaceans. J. American Vet. Med. Assn. 181:1316-1321.

Summary

Disseminated Candida albicans infections were found or suspected in 4 captive cetaceans. Ketoconazole at a dosage of 2.5 mg/kg, BID, administered orally for 18 days, followed by 8 biweekly oral doses of levamisole hydrochloride at the rate of 9 mg/kg, resulted in regression of clinical signs of candidiasis in an adult male Atlantic bottlenose dolphin (Tursiops truncatus). A higher dosage of ketoconazole (6 mg/kg, BID) was effective in eliminating the shedding of C. albicans from an adult male belukha whale (Delphinapterus leucas). A juvenile female harbor porpoise (Phocoena phocoena) treated with nystatin died with disseminated candidiasis, as did a juvenile male longfinned pilot whale (Globicephala melaena) treated with nystatin and levamisole. Three other adult bottlenose dolphins, a juvenile female belukha whale, and a female Commerson's dolphin (Cephalorhynchus commersonii) kept in the same water system never had evidence of candidiasis. A 5th bottlenose dolphin (an adult female) was culture-positive on one occasion, but never had signs of the disease.

* * *

3. John D. Buck 1983. Occurrence of Candida albicans in Fresh Gull Feces in Temperate and Subtropical Areas. Microbial Ecology 9:171-176.

Abstract

The occurrence of Candida albicans in fresh gull (Larus spp.) feces was compared in temperate and subtropical locations. Of 239 fresh samples, 133 were obtained in southeastern Connecticut and 106 from different sites on the southeastern and central western coasts of Florida. Overall, 60% of all feces contained C. albicans. Of the Connecticut samples, 78% were positive, whereas 38% of the Florida samples revealed the presence of the yeast. Only 1 of 24 samples of fresh brown pelican feces contained C. albicans. Differences in C. albicans occurrence in birds in various locations was ascribed to variations in habitat and feeding behavior. Samples of water from a municipal reservoir in Connecticut were routinely positive, with an average cell density of 20/liter. Two fresh gull samples obtained on the reservoir bank contained C. albicans at an average cell concentration of 5,200/g. The frequency of C. albicans in gull droppings was higher than reported by others, and the yeast is common in temperate waters. These findings have important public health implications.

* * *

V. Dept. of Plant Science, University of Western Ontario, London, Ontario N6A 5B7, Canada. Communicated by M.A. Lachance.

The following two abstracts represent studies from my laboratory.

1. D. Sidenberg and M.A. Lachance, 1983. Speciation, Species Delineation, and Electrophoretic Isoenzyme Patterns of the Type Strains of Kluyveromyces van der Walt emend. van der Walt. Internat. J. Syst. Bacteriol. 33:822-828.

Abstract

The type strains of the 20 species of the yeast genus Kluyveromyces sensu van der Walt 1970 were studied by gel electrophoresis of 11 isofunctional enzymes. These enzymes included five oxidoreductases (alcohol dehydrogenase [EC 1.1.1.1], lactate dehydrogenase [EC 1.1.1.27], malate dehydrogenase [EC 1.1.1.37], catalase [EC 1.11.1.6], and superoxide dismutase [EC 1.15.1.1]), five hydrolases (esterase [EC 3.1.1.1], alkaline phosphatase [EC 3.1.3.1], α-glucosidase [EC 3.2.1.20], β-glucosidase [EC 3.2.1.21], and exo-β-glucanase [EC 3.2.1.58]), and one lyase (aldolase [EC 4.1.2.12]). Polymorphism was evident in most of the enzymes studied. Each type strain had a unique pattern when all enzymes were considered. The results of a multivariate analysis of the electrophoretic patterns supported the division of the genus into 13 species, 2 of which comprised four and five taxa, respectively, which were recognized in 1970. Enzyme electrophoresis provided evidence that widespread gene flow does not necessarily occur between yeasts which are able to hybridize in the laboratory.

* * *

2. A. Margaritis, P. Bajpai, and M.A. Lachance, 1983. The Use of Free and Immobilized Cells of Debaryomyces polymorphus to Produce Ethanol from Jerusalem Artichoke Tubers. J. Fermentation Technol. 61(No. 5), in press.

Abstract

A newly isolated strain of Debaryomyces polymorphus UWO(PS)79-131 was used to produce ethanol from inulin sugars present in Jerusalem artichoke (Helianthus tuberosus) tubers. Both free and immobilized cells were tested. Using free cells, batch fermentation was completed in 32h, obtaining 42.5 g ethanol/l from 100 g sugars/l initial concentration. The maximum specific growth rate was $\mu_{max}=0.2h^{-1}$, and ethanol yield and % initial sugars utilization were found to be $Y_{p/s}=0.45$g ETOH/g sugars (88% of theoretical yield) and 91%, respectively. Debaryomyces polymorphus cells were also immobilized by entrapment within small Na-alginate beads and used batchwise for the total fermentation of 100g sugars/l in only 8h to give 47.2 g ETOH/l and an ethanol yield $Y_{p/s}=0.49$ g ETOH/g sugars (96% of theoretical yield). The same immobilized cells were recycled and used repeatedly for 11 batch cycles with fresh medium charged into the fermentor at the beginning of each cycle. It was found that the immobilized cells

were extremely stable and the ethanol yield was almost constant at 95% of the theoretical during the 11 batch cycles.

* * *

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

Below follow abstracts of a Master's Thesis and a recent paper from our Institute.

1. Partial Purification and Characterization of the Two Forms of Trehalase from Saccharomyces cerevisiae: Activation by a cAMP-Dependent Protein Kinase

Gisela M. Dellamora-Ortiz and Anita D. Panek

Summary

The inactive form of trehalase from Saccharomyces cerevisiae as well as the active form of the enzyme, resulting from activation by a cAMP-dependent protein kinase, were partially purified.

The following steps were used to isolate cryptic trehalase (trehalase-c): treatment with protamine sulphate; ammonium sulphate precipitation (35-50% saturation) and desalting by centrifugation over Sephadex G-50; DEAE-cellulose column chromatography (equilibrated with 0.1M phosphate buffer, pH 6.2), followed by dialysis, and Sephadex G-200 chromatography. Activation of cryptic trehalase by cAMP-dependent protein kinase, in the presence of cAMP and ATP, Mg, was used to follow purification. An inhibitor of the activation process or of the resulting active form of trehalase seems to have been removed during the purification.

Column chromatography on DEAE-cellulose (pH 6.2) resulted in the separation of trehalase-c and protein kinase. The activation assay conditions regarding the dependence on protein concentration of each of the isolated enzymes were re-evaluated. Variation of the concentration of trehalase-c at fixed protein kinase concentration gave rise to a hyperbolic plot of activation and the apparent K_m of protein kinase for trehalase-c was $3{,}94 \times 10^{-8} \pm 0.71 \times 10^{-8}$ M. On the other hand, when protein kinase concentration was varied, with fixed trehalase-c, a sigmoidal activation plot was obtained and the concentration ratio necessary to reach half-maximal activation of trehalase was 110: 1 of protein kinase : trehalase-c.

The active trehalase, resulting from activation, was isolated from trehalase-c and protein kinase by DEAE - cellulose column chromatography (equilibrated with 0.02 M Tris-HCl buffer, pH 7.5). The isolated enzyme (trehalase-a) showed absolute specificity towards the substrate and the apparent K_m for trehalose was $3.79 \times 10^{-3} \pm 0.11 \times 10^{-3}$ M.

Both forms of the enzyme (cryptic and active trehalase) showed a molecular weight of 160,000 ± 10,000.

The activation of trehalase-c by protein kinase was completely dependent upon cAMP concentration and showed a sigmoidal plot. A maximum of trehalase activity was obtained with 1 μM of the cyclic nucleotide. Trehalase-c was found to be a totally inactive form of the enyzme which was not activated by any of the usual allosteric activators.

The present study contributes to the hypothesis that in Saccharomyces cerevisiae trehalase is activated by phosphorylation by a cAMP-dependent protein kinase.

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

* * *

2. Trehalose: Its Role in Germination of Saccharomyces cerevisiae.

Anita D. Panek and Edilson J. Bernardes

Mutants with specific lesions were used to differentiate between the functions of glycogen and trehalose in S. cerevisiae. Diploids which harbor the glc1/glc1 mutation depend upon the phosphorylated, less active form of glycogen synthase and show a more active, phosphorylated form, of the enzyme trehalase. These conditions are due to a lesion in the regulating subunit of cAMP-dependent protein kinase. Such cells are unable to sporulate. Diploids which contain the sst1/sst1 mutation have normal glycogen metabolism but their trehalose-6-phosphate synthase is not active. Such strains sporulate but germination is poor and only single-spore asci are formed. These results confirm that glycogen is needed to trigger sporulation while trehalose plays a role in the germination process. Different systems, I and II, of trehalose accumulation were proposed. System I would require the UDPG-linked trehalose synthase, whereas system II would constitute an alternative pathway, specifically induced or activated by the expression of a MAL gene. The presence of system II in its constitutive form in the constructed diploids would favour trehalose synthesis during growth on glucose; however, it did not overcome the glycogen deficiency during sporulation nor the lack of trehalose for germination. It seems that only system I, namely trehalose 6-P-synthase, plays a role in the germination process.

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

* * *

VII. Department of Microbiology, Faculty of Biological Sciences, University of Salamanca, Salamanca, Spain. Communicated by J.R. Villanueva.

Theses:

Rosa Esteban Canibano
"Studies on β-xylanases and β-glucanases in species belonging to the genus Bacillus: Synthesis and regulation."

Rosa Maria Varona Valle
"Studies on the mode of action of antifungal antibiotics papulacandin B and aculeacin A on Schizosaccharomyces pombe".

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

1. Teresa Ruiz., Julio R. Villanueva and Luis Rodriguez. Activation of yeast mannan synthetase by α-factor pheromone. FEBS Letters, in press.

The Saccharomyces cerevisiae mating pheromone α factor induces the activation of yeast mannan synthetase both "in vivo" and "in vitro". "In vivo" the activating effect of the pheromone on the synthetase is specific for cells of the a mating type, while "in vitro" α factor is able to exert its action on the synthetase of either cell type (MAT a, MAT α and MAT a/MATα).

* * *

2. Teresa Ruiz., Julio R. Villanueva and Luis Rodriguez. Influence of carbon catabolite repression on the G1 arrest of Saccharomyces cerevisiae MAT a cells by α factor. Journal of General Microbiology, in press.

Cells of the yeast Saccharomyces cerevisiae of the a mating type were arrested at the G1 phase of the cell division cycle after treatment with α factor in a culture medium containing a high concentration (2% w/v or higher) of a catabolite-repressing sugar. In media containing either a lower concentration of sugar or a non-fermentable carbon source, the extent of G1 arrest induced by the pheromone was reduced or became undetectable. Under catabolite-derepressing conditions α factor was inactivated by a cells at a higher rate than that found in repressing media. These results indicate the existence of a close correlation between the action of α factor on a cells and conditions of catabolite repression or derepression. A joint mechanism of action of α factor and catabolite-repressing carbon sources on a cells is postulated.

* * *

3. Angeles Sanchez., Angel R. Nebreda., Julio R. Villanueva and Tomas G. Villa. Post-secretional modification of exo-1, 3-β-D-glucanases from Saccharomyces cerevisiae. Biochemical Journal, in press.

Exo-1, 3-β-D-glucanase secreted by Saccharomyces cerevisiae undergoes extracellular modifications in its carbohydrate moiety which changes the affinity towards the lectin concanavalin A. The transition of negatively-reacting enzyme to a positively-reacting one depends on temperature. Results from experiments using glucono-δ-lactone and in vitro treatments with hydrolases suggest a glycosidase-mediated mechanism.

* * *

VIII. Johannes Gutenberg - Universität Mainz. FB 21/Biologie, Institut für Mikrobiologie und Weinforschung, Universität, Postfach 3980, D-6500 Mainz, West Germany. Communicated by F. Radler.

Below follow summaries of some recent publications of this institute.

The institute has been moved to a site on the Campus of the University (Becherweg 15). The postal address is:

Institut für Mikrobiologie und Weinforschung Universität Mainz, Postfach 3980 D-6500 Mainz, Germany

1. Schwartz, H., Steitz, H.-O, and Radler, F. 1983. Partial purification and characterization of succinyl-CoA synthetase from Saccharomyces cerevisiae. Antonie van Leeuwenhoek 49:69-78.

Succinyl-CoA synthetase from Saccharomyces cerevisiae was partially purified (20-fold) with a yield of 44%. The Michaelis-Menten constants were determined: K_m (succinate) = 17 mM; K_m (ATP) = 0.13 mM; K_m (CoA) = 0.03 mM. The succinyl-CoA synthetase has a molecular weight of about 80,000 (as determined by polyacrylamide gradient gel electrophoresis). The pH optimum is at 6.0. During fermentation the activity of succinyl-CoA synthetase is lower than in aerobically grown yeast cells. The presence of succinyl-CoA synthetase in fermenting yeasts may be regarded as an indication for the oxidative formation of succinate. In fermenting yeast cells succinyl-CoA synthetase is repressed by glucose if ammonium sulphate serves as nitrogen source. This catabolite repression is not observed with disaccharides or when amino acids are used as nitrogen source.

* * *

2. W. Emmerich and F. Radler, 1983. The Anaerobic Metabolism of Glucose and Fructose by Saccharomyces bailii. Journal of General Microbiology 129, 3311-3318.

In contrast to most yeasts, which ferment glucose more rapidly than fructose, Saccharomyces bailii ferments fructose first, then glucose. Thus, in a medium containing fructose and glucose, diauxic growth results. Cells of S. bailii that were grown on fructose were unable to ferment glucose when suspended in a glucose-containing buffer solution. Fructose-grown cells were cryptic for glucose fermentation but contained the enzymes for glucose metabolism. When suspended for 2 h in a growth medium containing glucose, fructose-grown cells acquired the ability to ferment glucose, due to the synthesis of a carrier protein. This induction was prevented by cycloheximide. In S. bailii, fructose was transported into the cells by a constitutive carrier system that was insensitive to uranyl ions. The inducible glucose carrier system was completely inhibited by 10^{-4} M-uranyl ions. If subsequent metabolism of hexoses was inhibited by iodoacetic acid, the uptake of hexoses could be measured by the increase in their intracellular concentrations. Fructose-grown cells took up only fructose whereas glucose-grown cells possessed an inducible glucose carrier and uptake of both glucose and fructose was observed. A model is proposed to explain the sequential fermentation of fructose and glucose in S. bailii.

* * *

3. F. Radler and E. Lang, 1982. Formation of malate by yeast. Wein-Wissenschaft 37, 391-399.

Summary

It was observed that yeasts produce malic acid during the fermentation of a liquid culture medium. Strains of the species Saccharomyces uvarum formed about 2 g malate per liter of medium. This malate formation was greatly influenced by the culture conditions. The formation of malate was high at sugar concentration of 20 to 30%, at pH-values of about pH 5, at limiting concentrations of nitrogen compounds (100-250 mg N/l) and in the presence of carbon dioxide. Malic acid that is present in the fermentation medium is partially decomposed during fermentation. These observations indicate, that depending on the conditions in media containing malic acid some part of the acid found after fermentation might have been synthesized by yeasts.

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IX. National Research Council Canada, Division of Biological Sciences, Ottawa, Canada K1A 0R6. Communicated by Byron F. Johnson.

The following are abstracts of recent papers from our laboratory.

1. Calleja, G.B., Johnson, B.F. & Walker, T. (1982) Sexual development in a homothallic fission yeast: synthesis of readiness proteins resolved by gel electrophoresis. Can. J. Biochem. 60:693-704.

Abstract

Sexual development of a homothallic strain of Schizosaccharomyces pombe was monitored by radiolabelling and sodium dodecyl sulfate (SDS) - polyacrylamide gel electrophoresis. Of more than 60 bands detected by Coomassie brilliant blue and by autoradiography, about 30 bands synthesized during development were discrete enough for experimental analysis.

About a dozen bands are preferentially vegetative, another dozen preferentially developmental. However, vegetative bands as a group are also synthesized during development. Their synthesis is relatively unaffected by low concentrations of cycloheximide or by chloramphenicol and is not temperature sensitive at 37°C nor catabolite repressible. Only band 40 (ca. 40,000 daltons) seems to be exclusively vegetative.

The synthesis of developmental bands 13, 18 24, 30 and α, all of which first appear during late-log phase, is catabolite repressible. Developmental band 51 is also synthesized throughout the vegetative phase. The synthesis of bands 24, 30, 51 and α is temperature sensitive at 37°C during the development, but that of band 18 is not. The synthesis of band 13 during development is not temperature sensitive, but its earlier synthesis during late-log phase is. The synthesis of all these six developmental bands is immediately inhibited by cycloheximide, but not by

chloramphenicol. Their appearance as a group of radioactive bands is greatly diminished in cultures grown in cycloheximide, in chloramphenicol, or in ethidium bromide.

Developmental bands 13, 18, 24, and 30 may be called readiness proteins. They first appear prior to the earliest morphological signs of sexual activity. Their developmental synthesis is inhibited by conditions that inhibit sexual development. Such inhibitory conditions include anaerobiosis, restrictive temperature, aging in stationary phase, the presence of inhibitors of cytoplasmic protein synthesis and of mitochondrial function, and catabolite repression. Readiness proteins may be regulating the switch from vegetative metabolism.

* * *

2. I.J. McDonald, G.B. Calleja and Byron F. Johnson, 1982. Conjugation in Chemostat Cultures of Schizosaccharomyces pombe. Journal of General Microbiology 128: 1981-1987.

Abstract

In chemostat cultures, Schizosaccharomyces pombe (NCYC 132 and derivative strains) formed conjugants in glucose-limited but not in ammonia-limited synthetic medium. Conjugation occurred at a low growth rate (doubling time, t_d approx. 11h), low aeration rate (0-1 fermenter vol. air min^{-1}) and 32°C, but not at an increased growth rate (t_d approx. 4-5 h) or at lower temperatures (20-25°C). Conjugation of S. pombe 968^{h90} in chemostat culture was greater under glucose-limitation than ammonia-limitation. Batch cultures of S. pombe 968^{h90} in synthetic medium produced conjugants when the glucose supply was limited, but when glucose was in excess the amount of nitrogen influenced the degree of conjugation. Conjugation of strain 968^{h90} was inhibited in batch cultures in synthetic medium under conditions such that a large quantity of glucose was metabolized, and in cultures grown in synthetic medium containing added ethanol (0-5%). Inhibition of conjugation also occurred in batch cultures of S. pombe NCYC 132 and 968^{h90} in malt extract broth containing added ethanol.

* * *

3. B.F. Johnson and I.J. McDonald, 1983. Cell division: a separable cellular sub-cycle in the fission yeast Schizosaccharomyces pombe. Journal of General Microbiology 129: in press.

Abstract

Carbon-limited growth of Schizosaccharomyces pombe in chemostats under certain conditions of dissolved oxygen concentration and temperature gave rise to multiseptate and branched hyphal cells. On the basis of these observations it is suggested that fission can be uncoupled from growth, nuclear processes and septation.

* * *

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

Below follows an abstract of our recent work concerning α-factor biosynthesis. The paper is in press in Biochem. Biophys. Res. Commun. (1983).

Yeast Pheromone α-Factor is Synthesized as a High Molecular Weight Precursor

O. Emter, B. Mechler, T. Achstetter, H. Müller and D.H. Wolf

Sexual conjugation of the two haploid cell types a and α of the yeast Saccharomyces cerevisiae is triggered by two oligopeptide pheromones, called a and α-factor (1). α-Factor is a tridecapeptide of the sequence Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr (1), exhibiting a molecular weight of approximately 1,700. Recent DNA sequencing studies revealed that the nucleotide sequence coding to α-factor is present in four repeats on a DNA stretch, which could potentially encode a 165 residue long α-factor precursor polypeptide (2). We investigated the question whether a high molecular weight precursor is actually part of the in vivo biosynthetic pathway of α-factor pheromone.

We prepared antibodies in rabbits against synthetic α-factor which had been coupled to bovine serum albumin. Yeast cells were grown and protein was labeled with L- $|^{35}S|$ methionine. Sodium dodecyl sulfate lysates of cells were prepared, α-factor antibody was added, the immunocomplexes formed were bound to protein A-Sepharose, washed, eluted, sized on sodium dodecyl sulfate polyacrylamide gels and visualized by fluorography. No α-factor precursor molecule could be found in Saccharomyces cerevisiae α-cells wild type for secretory functions, indicating a very short half life of the potentially existing α-factor precursor. When using α-cells of a mutant (sec18), conditionally blocked in the transfer of proteins from the endoplasmatic reticulum to the Golgi (3) an α-factor antibody cross reacting protein of approximately M_r = 28,000 could be found. The appearance of this protein is α-cell specific as it does not occur in a-cells. The DNA sequence implies three asparagine linked glycosyl residues present in the α-factor precursor molecule (2). When α-cells were labeled in the presence of tunicamycin, an inhibitor of asparagine linked glycosylation, three precursor species of lower molecular weight appeared, indicating three carbohydrate residues linked to the α-factor precursor molecule. A molecular weight of 18,000 was estimated for the unglycosylated precursor. This molecular weight is in excellent agreement with the molecular weight of the α-factor precursor molecule predictable from the DNA sequence. The identification of the processing proteinases of the pheromone precursor is a challenging problem.

(1) Betz, R., Manney, T.R., and Duntze, W. (1981), Gamete Res. 4, 571-584.
(2) Kurjan, J., and Herskowitz, I. (1982), Cell 30, 933-943.
(3) Schekman, R. (1982), Trends Biochem. Sci. 7, 243-246.

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XI. Department of Biology (Yeast Group), McMaster University, Hamilton, Ontario, Canada L8S 4K1. Communicated by J.J. Miller.

The following are the titles of three publications and a thesis from this laboratory:

Bilinski, C.A. and Miller, J.J. Translocation of zinc from vacuole to nucleus during yeast meiosis. Canadian Journal of Genetics and Cytology 25: 415-419, 1983.

Bilinski, C.A., Miller, J.J. and Girvitz, S.C. Events associated with restoration by zinc of meiosis in apomictic yeast. Journal of Bacteriology 155: 1178-1184, 1983.

Miller, J.J. In vitro experiments concerning the state of polyphosphate in the yeast vacuole. Canadian Journal of Microbiology. In Press.

Bilinski, C.A. Regulation of nuclear division and ascosporogenesis in apomictic strains of Saccharomyces cerevisiae. Ph.D. Thesis. McMaster University, Hamilton, Canada. 123 pp. 1983.

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

Below follow titles and abstracts of papers recently published in various journals, and of those still in press or recently submitted.

1. Effect of antibiotics on mitochondrial function and morphology during vegetative growth and gametes formation in the yeast Saccharomyces cerevisiae. N. Marmiroli, M. Ferri, P.P. Puglisi, C. Ferrari, F. Tedeschi, A. Sabatini, B. Fratello. Basic and Applied Histochemistry, 26 suppl. (1982).

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2. Sporulation and respiratory metabolism in the "petite negative" yeast Hansenula saturnus. A.M. Viola, N. Marmiroli. Current Genetics 7:37-45 (1983).

Abstract

Upon transition from growth medium to acetate sporulation medium buffered at pH 6.1 with 0.2 M PIPES, Hansenula saturnus showed a respiratory activity which was 88% antimycin A sensitive (1st) and 12% high azide sensitive (3rd) as in acetate complete growth medium. After ≈ 10h, 3rd respiration declined and oxygen consumption was inhibited by the simultaneous addition of antimycin A and hydroxamate, a situation which lasted until the appearance of the first asci. Later on, 1st and 3rd respiration reappeared and asci formation was completed under these respiratory conditions. The growth in the presence of antimycin A or erythromycin affected only quantitatively the ascospore production and this is because in sporulation medium there was a de novo synthesis of the

mitochondrial components of the respiratory chain. Cells which were devoid of 1st respiration but possessed 2nd or 3rd respiration could sporulate, indicating that these alternative respirations also have a role in the process. This was confirmed by the inhibition of sporulation as occurred in the presence of inhibitors of 1st, 2nd and 3rd respiration in sporulation medium.

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3. Mitochondrial NAD, L-lactate dehydrogenase and NAD, D-lactate dehydrogenase in the yeast Saccharomyces cerevisiae. A.M. Genga, F. Tassi, T. Lodi, I. Ferrero. Microbiologica 1:1-8 (1983).

Summary

Mitochondrial NAD-linked L- and D-lactate dehydrogenase activities have been found in the yeast Saccharomyces cerevisiae grown at high (3%) but were absent at low (0.6%) glucose concentrations. The inhibition of mitochondrial protein synthesis by chloramphenicol and of primary respiration by antimycin A determines the appearance of the two activities even at low (0.6%) glucose concentration. Two respiratory deficient strains belonging respectively to the mit$^-$ class (which maintains mitochondrial protein synthesis) and the the ~~rho~~$^-$ class (which loses mitochondrial protein synthesis) display the activities even at low (0.6%) glucose concentration. L- and D-lactate have been detected in the growth medium when the cultures had been undertaken at high glucose concentrations, but were absent at low glucose concentrations.

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4. The role of glucose and amino acid starvation on the sensitivity of protein and RNA synthesis to cycloheximide and erythromycin in the yeast Saccharomyces cerevisiae. F. Tassi, I. Ferrero, C. Donnini, N. Marmiroli. Microbiologica 1:9-18 (1983).

Summary

In Saccharomyces cerevisiae dependence of ribonucleic acid synthesis on protein synthesis occurring during nutritional shift down conditions was evidenced. The results obtained indicated that yeast had "stringent control" of ribonucleic acid synthesis and that this control was reversed by cycloheximide only under glucose starvation or ammonia starvation in the presence of acetate as carbon source (phenotypic relaxation).

Therefore, it appeared that the "phenotypic relaxation" of RNA synthesis depended on the carbon source present in the medium suggesting that the process could be negatively controlled by glucose or by some glucose catabolite(s). Such a phenotypic relaxation was sensitive to erythromycin treatment. On the other hand, total protein synthesis carried out during amino-acid starvation in the presence of glucose or ammonia starvation in the presence of acetate was 30-40% inhibited by erythromycin, showing that in these conditions sensitivity to a nonlegitimate inhibitor could be triggered off in a haploid strain, and that such sensitivity did not depend on the presence of glucose in the medium.

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5. Involvement of mitochondrial protein synthesis in sporulation: Effects of erythromycin on macromolecular synthesis, meiosis and ascospore formation in Saccharomyces cerevisiae. N. Marmiroli, M. Ferri, P.P. Puglisi. J. Bacteriology 154, 118-129 (1983).

Cells of strain Z270 (MATα/MATa) of Saccharomyces cerevisiae did not undergo ascospore formation in buffered or unbuffered acetate sporulation medium in the presence of erythromycin. The drug inhibited sporulation when added within the first 6 to 8 h and affected to different extents some of the metabolic and sporulation-specific events that normally occur during this period. In sporulation medium, protein synthesis was highly sensitive to erythromycin whereas RNA synthesis was unaffected and premeiotic DNA synthesis was partially inhibited. Intragenic recombination occurred at normal rates for the various heteroallelic loci tested, but rates of intergenic recombination were markedly reduced and commitment to haploidization did not occur; hence development was evidently arrested between intragenic and intergenic recombination. Cells kept for 8 h in acetate sporulation medium that were ready for sporulation in water without erythromycin failed to sporulate in water containing the drug, indicating that erythromycin can inhibit sporulation independent of acetate utilization.

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6. The role of the nuclear gene "mitochondrial mutability control" (MMC1) in the process of mutability of the mitochondrial genome by different mutagens in Saccharomyces cerevisiae. C. Donnini, P.P. Puglisi, N. Marmiroli. Molec. Gen. Genet. (1983).

Summary

The accumulation of respiratory deficient (RD) mutants in Saccharomyces cerevisiae depended upon the mutagens used and upon the presence of the nuclear gene previously identified as MMC1 (one) which we showed to control the spontaneous and the erythromycin-induced RD mutability.

In this paper data are reported about the accumulation of RD mutants in the presence of manganous ions (Mn^{++}) and UV which was higher in the mmc1 (one) than in MMC1 strains. We found that the characters "low spontaneous" and "low induced" RD mutability by erythromycin, manganous ions and UV, were controlled by the same genetic determinant. In the presence of manganous ions, also the frequency of antibiotic resistant mutants cap^R and ery^R was higher in the mmc1 strains. Moreover, the accumuation of RD mutants in the presence of berenil, 5-fluorouracil and basic fuchsin was higher in the mmc1 than in MMC1 strains. In contrast, RD mutants accumulation by acriflavine and ethidium bromide treatments did not appear affected by the MMC1 genetic constitution.

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7. Respiratory pathways in Hansenula saturnus. A.M. Viola, F. Tassi, P. Goffrini, T. Lodi, I. Ferrero. Antonie van Leeuwenhoek, in press (1983).

Abstract

Hansenula saturnus is a petite negative yeast species which displays a primary, antimycin-sensitive respiration, a secondary, hydroxamate-sensitive respiration and an antimycin+hydroxamate-insensitive respiration. The three respiratory activities are all associated to the mitochondrial fraction.

H. saturnus displays a different pattern of respiration depending on the age of the cultures. In fact, the respiration is sensitive to antimycin A in the early exponential phase, is sensitive only to the simultaneous addition of antimycin A and hydroxamate in the middle exponential phase and is sensitive to hydroxamate in the late exponential and stationary phase.

The presence of antimycin A in the growth medium determines the induction of the antimycin+hydroxamate insensitive respiration which is 50% inhibited by 5 mM azide. On the contrary, the presence of erythromycin in the growth medium, which inhibits mitochondrial protein synthesis in this yeast species and the synthesis of cytochromes aa_3 and b, totally prevents the appearance of antimycin+hydroxamate-insenstive respiration. Moreover, the antibiotic affects cell viability, suggesting a role in mitochondrial protein synthesis in the cell cycle of H. saturnus.

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8. 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, C. Ferrari, F. Tedeschi, P.P. Puglisi. Submitted to J. Cell Biology (1983).

Abstract

Upon transfer in sporulation medium, diploid cells of the yeast Saccharomyces cerevisiae undergo a series of developmental events which lead to meiosis and ascospores 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 part-way 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. The ultrastructural analysis has shown that the cells formed in this condition axial cores (leptotene), synaptonemal complex and polycomplexes (zygotene-pachytene) and sometime formed two spindle pole bodies which remained side-by-side on the nuclear envelope (diplotene-diakinesis) but after this the cells did not complete the first meiotic division. The oxygen uptake and the cytochrome content of cells exposed to erythromycin in sporulation medium were not affected, suggesting that upon

this condition mitochondrial respiration could occur normally. The ultrastructural analysis has also evidenced that the mitochondrial internal architecture was maintained in the erythromycin-treated sporulation culture. The dynamics and the structure of the nucleolus of cells exposed to the sporulation medium in the presence of erythromycin showed that these organelles became condensed, cap-shaped with associated dense bodies, and laid on the nuclear envelope as the typical diplotene nucleolus. The behaviour of these and of other subcellular organelles such as the lysosomes and of the various types of material whose traffic accompanied normal sporulation was also affected in the absence of mitochondrial protein synthesis, indicating that the events: nuclear differentiation - cytoplasmic modification and traffics, are coordinately regulated under sporulation condition. 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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XIII. Centre National de la Recherche Scientifique, Laboratoire d'Enzymologie - Gif-sur-Yvette (France). Communicated by J. Schwencke.

The following paper has been recently published:

Jaime Schwencke, Hervé Canut* and Alberto Flores** (1983). Simultaneous isolation of the yeast cytosol and well preserved mitochondria with negligible contamination by vacuolar proteinases. FEBS Letters 156:274.

Abstract

Disruption of yeast spheroplasts by DEAE dextran in isoosmotic conditions allows isolation of relatively undamaged subcellular fractions from yeast. For the strains assayed disruption in 0.6 M sorbitol proved to permit the preservation of vacuoles and mitochondria and subsequently the isolation of the cytosol with negligible contamination (about 5%) by vacuolar proteinases. Low contamination was further indicated by the excellent stability shown by glutamic dehydrogenase (NAD-linked) in the cytoplasmic extract. Good preservation of mitochondrial structure was indicated by the good retention of their cytochrom C content.

The method, with small variations, can be applied to a variety of strains and allows the obtention of sub-cellular fractions almost uncontaminated by vacuolar proteinases and other vacuolar hydrolases such as alkaline phosphatase and ribonuclease.

*) H. Canut present address: Centre de Physiologie Végétale (L.A. CNRS 214) Université Paul Sabatier, 31062 Toulouse, France.

**) A. Flores: On leave from Universidad de Guanajuato, Mexico. Fellow from CONACYT, Mexico.

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

The following six papers, abstracts of which have already been presented in Yeast Newsletter Vol. XXXI Nr. II, p. 67-70, now have been published:

1. Peter M. Bruinenberg, Johannes P. van Dijken and W. Alexander Scheffers, A Theoretical Analysis of NADPH Production and Consumption in Yeasts, Journal of General Microbiology 129 (1983) 953-964.

2. Peter M. Bruinenberg, Johannes P. van Dijken and W. Alexander Scheffers, An enzymatic analysis of NADPH production and consumption in Candida utilis, Journal of General Microbiology 129 (1983) 965-971.

3. K. Nicolay, W.A. Scheffers, P.M. Bruinenberg, and R. Kaptein. Phosphorus-31 Nuclear Magnetic Resonance Studies of Intracellular pH, Phosphate Compartmentation and Phosphate Transport in Yeasts. Archives of Microbiology 133 (1982) 83-89.

4. C. Verduyn, J.P. van Dijken and W.A. Scheffers. A simple, sensitive and accurate alcohol electrode. Biotechnology and Bioengineering 25 (1983) 1049-1055.

5. W.A. Scheffers, J.P. van Dijken, Guniz Kaytan, M. Kloosterman, M.R. Wijsman and B.H.A. van Kleeff. Effect of Oxygen on Growth, Alcohol, Acetic Acid, and Glycerol Production by the Yeasts Brettanomyces intermedium and Zygosaccharomyces bailii. In: H. Dellweg (ed.) - Energie durch Biotechnologie. 5. Symposium Technische Mikrobiologie. Institut für Gärungsgewerbe und Biotechnologie, Berlin. 1982, p. 214-220.

6. P.M. Bruinenberg, J.P. van Dijken and W.A. Scheffers. Production and Consumption of Redox Equivalents during Growth of Candida utilis CBS 621 on Xylose. In: H. Dellweg (ed.) - Energie durch Biotechnologie. 5. Symposium Technische Mikrobiologie. Institut für Gärungsgewerbe und Biotechnologie, Berlin 1982, p. 208-213.

Besides, the following two papers have appeared:

7. K. Nicolay, W.A. Scheffers, P.M. Bruinenberg, and R. Kaptein. 1983. In vivo ^{31}P NMR studies on the role of the vacuole in phosphate metabolism in yeasts. Arch. Microbiol. 134:270-275.

Abstract

^{31}P NMR was used to study the dynamics of phosphate pools during substrate utilization by aerobic and anaerobic suspensions of the yeast Candida utilis and by aerobic suspensions of the yeast Brettanomyces intermedius. In both yeasts, the cytoplasmic pH was monitored: in C. utilis also the vacuolar pH could be measured. When glucose was used as a substrate for C. utilis, the vacuolar store of inorganic phosphorus (both orthophosphate and polyphosphate) was mobilized to replenish cytoplasmic phosphate which had become very low due to the build-up of high sugar

phosphate levels. The hydrolysis of polyphosphate was glucose-dependent: it did not occur with ethanol as the substrate. After glucose depletion resynthesis of polyphosphate occurred only under aerobic conditions: anaerobic C. utilis cells continued to hydrolyze vacuolar polyphosphate. This difference between the aerobic and anaerobic suspension could be related to differences in cellular ATP levels. When ethanol was employed as a substrate, both Candida utilis and Brettanomyces intermedius exhibited a substantial increase in polyphosphate levels. These observations suggested a dual role for polyphosphate in yeasts both as a phosphate and an energy store. The cytoplasmic pH in C. utilis displayed characteristic responses to metabolic changes during glucose degration. B. intermedius experienced a strong cytoplasmic acidification upon ethanol utilization due to acetic acid formation. The mechanism of transport of P_i across the vacuolar membrane in C. utilis appeared to be different from that reported for the plasma membrane.

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8. P.M. Bruinenberg, P.H.M. de Bot, J.P. van Dijken, and W.A. Scheffers. 1983. The role of redox balances in the anaerobic fermentation of xylose by yeasts. Eur. J. Appl. Microbiol. Biotechnol. 18:287-292.

Abstract

The kinetics of glucose and xylose utilization by batch cultures of Candida utilis were studied under aerobic and anaerobic conditions during growth in complex media. Rapid ethanol formation occurred during growth on glucose when aerobic cultures were shifted to anaerobic conditions. However, with xylose as a substrate, transfer to anaerobiosis resulted in an immediate cessation of metabolic activity, as evidenced by the absence of both ethanol formation and xylose utilization. The inability of the yeast to ferment xylose anaerobically was not due to the absence of key enzymes of the fermentation pathway, since the addition of glucose to such cultures resulted in the immediate conversion of glucose to ethanol. Furthermore, when the enzyme xylose isomerase was added to an anaerobic xylose culture, immediate conversion of xylose to ethanol was observed. This indicates that the inability of the yeast to form ethanol from xylose under anaerobic conditions is caused by metabolic events associated with the conversion of xylose to xylulose. A hypothesis is put forward which explains that ethanol production from xylose by yeast under anaerobic conditions is negligible. It is suggested that the failure to ferment xylose anaerobically is due to a discrepancy between production and consumption of NADH in the overall conversion of xylose to ethanol. When a hydrogen acceptor (i.e. acetoin) was added to anaerobic cultures of C. utilis, xylose utilization resumed, and ethanol and acetate were produced with the concomitant stoicheiometric reduction of acetoin to 2,3-butanediol.

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The following three papers are forthcoming:

9. C. Verduyn, J.P. van Dijken, and W.A. Scheffers. Colorimetric alcohol assays with alcohol oxidase. J. Microbiol. Methods, in press.

Abstract

A method is described for manufacturing crude alcohol oxidase (EC 1.1.3.13) preparations which are suitable for application in colorimetric alcohol assays. The procedure involves a one-step removal of catalase activity from a partially purified preparation of alcohol oxidase from the yeast Hansenula polymorpha via dialysis against 3-amino-1,2,4-triazole and hydrogen peroxide. Thus, the irreversible inactivation of more than 90% of the catalase present was achieved, which is a prerequisite for the use of alcohol oxidase preparations in colorimetric alcohol assays via peroxidase-mediated oxidation of dyes. This type of assay was shown to be rapid, accurate and sensitive. The influence of the relative concentrations of the various assay constituents such as alcohol oxidase, catalase and peroxidase is discussed. It is concluded that this colorimetric alcohol assay is particularly suitable for the determination of ethanol in fermentation broths, both in qualitative and in quantitative tests.

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10. P.M. Bruinenberg, P.H.M. de Bot, J.P. van Dijken, and W.A. Scheffers. NADH-linked aldose reductase: the key to anaerobic alcoholic fermentation of xylose by yeasts. Eur. J. Appl. Microbiol. Biotechnol., in press.

Abstract

The kinetics and enzymology of D-xylose utilization were studied in aerobic and anaerobic batch cultures of the facultatively fermentative yeasts Candida utilis, Pachysolen tannophilus and Pichia stipitis. These yeasts did not produce ethanol under aerobic conditions. When shifted to anaerobiosis cultures of C. utilis did not show fermentation of xylose; in Pa. tannophilus a very slow rate of ethanol formation was apparent, whereas with Pi. stipitis rapid fermentation of xylose occurred. The different behaviour of these yeasts is most probably explained by differences in the nature of the initial steps of xylose metabolism: in C. utilis xylose is metabolized via an NADPH-dependent xylose reductase and an NAD^+-linked xylitol dehydrogenase. As a consequence, conversion of xylose to ethanol by C. utilis leads to an overproduction of NADH which blocks metabolic activity in the absence of oxygen. In Pa. tannophilus and Pi. stipitis, however, apart from an NADPH-linked xylose reductase also an NADH-linked xylose reductase was present. Apparently xylose metabolism via the NADH-dependent reductase circumvents the imbalance of the NAD^+/NADH redox system, thus allowing fermentation of xylose to ethanol under anaerobic conditions. The finding that the rate of xylose fermentation in Pa. tannophilus and Pi. stipitis corresponds with the activity of the NADH-linked xylose reductase activity is in line with this hypothesis. Furthermore, a comparative study with various xylose-assimilating yeasts showed that significant alcoholic fermentation of xylose only occurred in those organisms which possessed NADH-linked aldose reductase.

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11. C. Verduyn, T.P.L. Zomerdijk, J.P. van Dijken and W.A. Scheffers. Continuous measurement of ethanol production by aerobic yeast suspensions with an enzyme electrode. Eur. J. Appl. Microbiol. Biotechnol., in press.

Abstract

An alcohol electrode was constructed which consisted of an oxygen probe onto which alcohol oxidase was immobilized. This enzyme electrode was used, in combination with a reference oxygen electrode, to study short-term kinetics of alcoholic fermentation by aerobic yeast suspensions after pulsing with glucose. The results demonstrate that this device is an excellent tool in obtaining quantitative data on the short-term expression of the Crabtree effect in yeasts.

Samples from aerobic glucose-limited chemostat cultures of Saccharomyces cerevisiae not producing ethanol, immediately (within 2 min) exhibited aerobic fermentation after being pulsed with excess glucose. With chemostat-grown Candida utilis, however, ethanol production was not detectable even at high sugar concentrations. The Crabtree effect in S. cerevisiae was studied in more detail with commercial baker's yeast. Ethanol formation occurred only at initial glucose concentrations exceeding 150 $mg.l^{-1}$, and the rate of alcoholic fermentation increased with increasing glucose concentrations up to 1000 $mg.l^{-1}$ glucose.

Similar experiments with batch cultures of certain 'non-fermentative' yeasts revealed that these organisms are capable of alcoholic fermentation. Thus, even under fully aerobic conditions, Hansenula nonfermentans and Candida buffonii produced ethanol after being pulsed with glucose. In C. buffonii ethanol formation was already apparent at very low glucose concentrations (10 $mg.l^{-1}$) and alcoholic fermentation proceeded at a higher rate than in S. cerevisiae. With Rhodotorula rubra, however, the rate of ehtanol formation was below the detection limit, i.e., less than 0.1 mmol. g $cells^{-1}.h^{-1}$.

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

Below follow abstracts of two recent papers accepted for publication.

1. Hamada, H., Yamashita, M., Kojima, M. and Morita, T.: Effect of methanol on the induction of respiration deficient mutants by acriflavine in yeast. Chem. Pharm. Bull. 32(1), 1984.

Abstract

The effect of methanol was investigated on the induction of cytoplasmic respiration-deficient (RD) mutants of Saccharomyces cerevisiae by acriflavine (AF). After 24hr-incubation, AF at above 0.5 µg/ml induced approximately 100% RD mutants in surviving cells. Methanol prevented the RD mutation by AF at less than 1.5 µg/ml. The RD mutation by 0.15 µg/ml AF was completely repressed by the addition of 4% methanol into the culture.

The induction frequency of RD mutants by 0.5 μg/ml AF was reduced from 99% to 10% by the addition of 8% methanol.

The AF uptake by the yeast cells decreased to some extent in the presence of methanol. But this methanol induced repression in the RD mutation was not explained simply by the decrease of AF content in the yeast cells.

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2. Morita, T. and Mifuchi, I.: Ethanol enhancement of cytochrome P-450 contents in Saccharomyces cerevisiae D7. Chem. Pharm. Bull. 32(5), 1984.

Abstract

When Saccharomyces cerevisiae D7 was cultured in yeast extract-proteose peptone-2% glucose (YPG) liquid medium supplemented with 1.5 and 3% ethanol, the maximum cytochrome P-450 contents in the whole cell suspension increased 1.5 and 1.3 times, respectively, compared with that in YPG medium. The addition of 6% ethanol into YPG medium decreased the growth rate and the final cell population of the yeast markedly, while the cellular content of cytochrome P-450 was kept on a level with the cells from YPG medium. Though the logarithmic phase was extended under the glucose-repressed condition in YPG medium containing 10% glucose, there was no increase in the cytochrome P-450 content compared with the cells on 2% glucose. In the medium in which 2% ethanol was the sole carbon and energy source, the growth rate and the final cell population of the yeast showed a marked decrease compared with that grown on glucose. Cytochrome P-450 was not detectable in the cells grown on ethanol.

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XVI. Haskins Laboratories of Pace University, 41 Park Row [at Pace Plaza], New York, N.Y. 10038. Communicated by Seymour H. Hutner.

The following paper has been recently published from Haskins Laboratories and Dept. of Biology, Pace University, Pace Plaza, New York, New York*, and the Departments of Preventive Medicine and Medicine, New Jersey Medical School, Newark, New Jersey.**

Hutner, S.H.*, Karlawish, J.H.*, Werfel, S.B.*, Bacchi, C.J.*, and Baker, H.** (1983). Growth of a Saccharomyces cerevisiae spe10, a polyamine auxotroph; stimulation by choline and carnitine. J. Elisha Mitchell Sci. Soc. 97:145-151.

Abstract

An spe10 mutant of Saccharomyces cerevisiae requiring putrescine, spermidine, or spermine to initiate growth is shown here to respond also to choline or carnitine. Because this spe10 mutant was derived by mutagenizing a mutant (J25) which overproduced ornithine decarboxylase (ODC) 100-fold (ODC catalyzes putrescine synthesis), gene amplification of a regulatory gene for polyamine synthesis was thought to be involved. The

spe10 mutant was reported to have no detectable ODC; also, suppressor mutants in polyamine-limited agar cultures often appeared, outgrowing spe10. The sporadic and unexpectedly heavy growth observed by us in polyamine-limited liquid culture in defined media presumably is attributable to the same phenomenon. The connection if any between these suppressor mutants and the response to choline or carnitine (metabolites hitherto not associated with polyamines) remains to be elucidated.

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XVII. Mikrobiologisches Institut, Eidgenössische Technische Hochschule, ETH-Zentrum, CH-8092 Zürich, Switzerland. Communicated by P. Niederberger and R. Hütter.

We have recently published a paper with the title: Influence of the General Control of Amino Acid Biosynthesis on Cell Growth and Cell Viability in Saccharomyces cerevisiae. P. Niederberger, M. Aebi, and R. Hütter, J. Gen. Microbiol. (1983), 129, 2571-2583.

Summary

The general control of amino acid biosynthesis was shown to play an important role in the coordination between cell growth and cell division under amino acid limitations. Mutant strains defective in this regulatory system, as studied here mainly with mutant strain RH375 (ndr1-1), showed excessive and aberrant cell growth under mild limitation, and rapid loss of cell viability under severe amino acid limitation. Furthermore, wild-type (NDR1) cells were able to derepress, or at least maintain levels of enzymes subject to the general control under amino acid limitations. The ndr1-1 mutant cells showed significantly decreased enzyme levels under these conditions. The loss of viability of ndr1-1 mutant cells was not due to inability to accumulate at "Start" under amino acid limitation. In conclusion, we postulate that the aberrant behaviour of ndr-mutant cells is due to an inability to maintain adequate levels of amino acid biosynthetic enzymes throughout the mitotic cell cycle.

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Two publications have been accepted for publication in Current Genetics: Structure and Function of the TRP3 Gene of Saccharomyces cerevisiae: Analysis of Transcription, Promoter Sequence, and Sequence Coding for a Glutamine Amidotransferase. M. Aebi, R. Furter, F. Prantl, P. Niederberger, and R. Hütter.

Summary

The structure and function of the TRP3 gene of Saccharomyces cerevisiae were analyzed. Subcloning of an original 4.8 kb BamHI DNA fragment, carrying the yeast TRP3 gene, allowed for a localization of the gene on a 2.5 kb ClaI/BamHI fragment. Transcription was found to proceed from the ClaI site towards the BamHI site. Three major transcription start sites were determined at positions -92, -87, and -81 by S1-mapping. The synthesis of the TRP3 gene is regulated by the general control, and was found to take place at the transcriptional level. The sequence of the 5'-

noncoding region up to position -400 and part of the coding region to position 840 were determined. The 5'-noncoding region contains sequences common to most amino acid biosynthetic genes known so far, namely a presumptive ribosome binding site, "Goldberg-Hogness boxes", and a consensus sequence, possibly involved in the general control. For the coding region a single open reading frame was found. The deduced amino acid sequence was aligned with homologous amino acid sequences of Neurospora crassa, Pseudomonas putida and Escherichia coli. The exceptionally high homology (40-60%) between these sequences led us to postulate that the TRP3 gene product is of the structure NH_2-glutamine amidotransferase-indole-3-glycerol-phosphate synthase-COOH.

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Structure and Function of the TRP3 Gene of Saccharomyces cerevisiae: Analysis of 3'- and 5'-Deletions In Vivo and In Vitro. M. Aebi, R. Furter, F. Prantl, P. Niederberger, and R. Hütter.

Summary

Two sets of deletions, entering the TRP3 gene of Saccharomyces cerevisiae from the 3'- and the 5'-end were constructed. Complementation analysis with chromosomal trp3A, trp3B and trp3C mutation was done by introducing the 3'- and 5'-truncated gene on a multicopy 2µm-vector. The N-terminal glutamine amidotransferase function is encoded by a DNA fragment of 600-700 bp, and the C-terminal indole-3-glycerol-phosphate synthase function by a DNA fragment of about 900 bp, whereas both functions together are encoded by a contiguous DNA fragment of about 1500 bp. The bifunctional TRP3-peptide thus could be dissected into two catalytically independent peptides in vivo.

For the indole-3-glycerol-phosphate synthase activity, independent catalytic activity was also demonstrated in vitro: deletions entering the TRP3 gene from the 5'-end, and lacking large parts of the sequence coding for the glutamine amidotransferase function, still are able to express a peptide exhibiting functional indole-3-glycerol-phosphate synthase activity in vitro. Deletion plasmids pME505 DelC102 2µm and DelC10 2µm exhibited shorter TRP3 transcripts according to the deleted DNA-fragments (150 and 426 bp respectively) but yielded peptides of invariable M_r of 35,000. Transcription and translation of these peptides, which probably represent the independently folding indole-3-glycerol-phosphate synthase core are discussed.

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XVIII. Department of Biology, Barnes Laboratory, University of Chicago, Chicago, Illinos, 60637. Communicated by E.D. Garber.

Efficient methods have been developed to assign mutations to known or new linkage groups and to construct linkage maps for the phytopathogenic Heterobasidiomycete Ustilago violacea. The species is dimorphic, producing dikaryotic hyphae in host-tissue and budding sporidia in culture. There is reason to believe that these methods would be applicable to other species in the genus or the Ustilaginales: half-tetrad analysis, single selection

and double selection. These methods do not require microdissection of the basidiospores in the tetrad. Ustilago violacea has at least N = 14 and may have 15-20 linkage groups. The arms of the linkage groups are not longer than 32 cM, based on a sample of 51 mutations. Pericentric gene clustering is indicated by the observation that 66% of the 51 mutations are within 10 cM of their centromere.

The following publications pertinent to the mapping strategies have been or will be published:

Garber, E.D., O.H. Will III and J.M. Kokontis. 1981. Genetics of Ustilago violacea. X. The pseudohyphal mutation and tetrad analysis. Bot. Gaz. 142:589-591.

Garber, E.D., C. Eng, E.E. Puscheck, M.K. Weil and S. Ward. 1982. Genetics of Ustilago violacea. XII. Half-tetrad analysis and double selection. Bot. Gaz. 143:524-529.

Garber, E.D., C.D. Kerrigan and M.K. Weil. 1983. Genetics of Ustilago violacea. XVII. Half-tetrad analysis, single selection and double selection as mapping strategies. Bot. Gaz., in press.

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XIX. Carlsberg Laboratory, Department of Physiology, GL. Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark. Communicated by Morton C. Kielland-Brandt

The following is an abstract of a recently published paper.

Jens G. Litske Petersen, Steen Holmberg, Torsten Nilsson-Tillgren* and Morten C. Kielland-Brandt. 1983. Molecular Cloning and Characterization of the Threonine Deaminase (ILV1) Gene of Saccharomyces cerevisiae. Carlsberg Res. Commun. 48:149-159.

Abstract

The ILV1 gene from Saccharomyces cerevisiae coding for the anabolic threonine deaminase was isolated from a cosmid-yeast DNA gene pool on a 35 kb insert by transformation of a haploid ilv1 yeast mutant to isoleucine prototrophy. By subcloning, the functional gene was isolated on a 4.8 kb DNA fragment. Hybridization of radioactively labelled DNA fragments from the cloned region to electrophoretically separated yeast RNA showed that the ILV1 mRNA is about 2,000 ribonucleotides long. This analysis revealed also the approximate position of the transcribed region. The location of the ILV1 gene in the cloned region was confirmed by gene splitting: The transformation of a wild type strain with a plasmid containing an internal fragment of the cloned gene caused inactivation of the chromosomal ILV1 gene. By RNA hybridization (Northern) analysis, a 5-10 fold decrease in the threonine deaminase mRNA level was observed in wild type cells when minimal medium with leucine was supplemented with isoleucine and valine. This indicates that ILV1 is under transcriptional control.

*Institute of Genetics, University of Copenhagen, Øster Farimagsgade 2A, DK-1353 Copenhagen K.

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

Below follows the abstract of a recent paper from our Laboratory.

Masahide Kawamura, Masamichi Takagi and Keiji Yano. 1983. Cloning of a LEU gene and an ARS site of Candida maltose. Gene 24:157-162.

Summary

Gene libraries of DNA from an n-alkane-assimilating yeast strain, Candida maltosa IAM12247, were constructed, using Escherichia coli plasmid vector pBR322. A LEU gene from C. maltosa was cloned, and found to complement leu⁻ mutations in E. coli and Saccharomyces cerevisiae. In E. coli, the LEU gene in the cloned yeast DNA fragment was efficiently expressed when inserted into the vector in one orientation, while in the other orientation, it was expressed only weakly. In S. cerevisiae, the Candida LEU gene was efficiently expressed when inserted into a shuttle vector pRC3 in both orientations, suggesting that the isolated Candida DNA fragment contains a promoter sequence of Candida in front of the LEU gene, which is operative in S. cerevisiae but not in E. coli. In addition, our data suggest that the cloned LEU fragment also contains an ARS (autonomously replicating sequence) site of C. maltosa.

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XXI. Donner Laboratory and Department of Biophysics and Medical Physics, University of California, Berkeley, CA 94720. Communicated by Robert K. Mortimer and David Schild.

Characterization of Isolated Yeast DNA Repair Genes

Robert K. Mortimer, Isabel L. Calderon*, Rebecca Contopoulou, Herschell Emery, Susan T. Lovett, David Schild, Karen C. Sitney and John Takakuwa.

*Current Address: Departamento de Genetica, Facultad de Biologia, Universidad de Sevilla, Ap. 1095, Sevilla, Spain.

In the yeast Saccharomyces cerevisiae several different DNA repair pathways have been identified (reviewed in Game, 1983). These include recombinational repair, error-prone associated repair, excision repair and photoreactivation. Our research includes an examination of the RAD50 to RAD57 genes, a subset of the genes involved in DNA repair. The genes in the RAD50 to RAD57 epistasis group are thought to be involved in recombinational repair, since mutations in some of these genes decrease both repair and recombination. Although many phenotypes have been associated with mutations in the genes of this pathway, no enzyme or structural protein (such as a DNA binding protein) has been determined to

be the product of any of these genes. To study these genes at the molecular level and to isolate and characterize their primary gene products, we have cloned several of them. The cloning of these genes has recently been published (Schild et al., 1983a,b; Calderon et al., 1983). Here we report on work being done using these genes. In addition, while trying to obtain clones that would complement the RAD50 gene, an amber suppressor was isolated which suppressed the amber rad50-1 mutation. Work leading to the further characterization of the suppressor gene is also reported here.

RAD50

Nine mutant alleles of RAD50 have been studied genetically. Crosses of strains each carrying one of these mutations were made against strains containing either amber or ochre suppressors. This revealed that two of these mutations are nonsense, one ochre (rad50-3) and one amber (rad50-1).

In addition to the cloning of the suppressor gene (discussed below), a second plasmid was isolated which also complemented the rad50-1 mutation. This plasmid [YEp13-RAD50-210B] has since been shown to complement one of the other mutant alleles of RAD50, rad50-3. However, genetic analyses on the plasmid showed that it integrated near the centromere of chromosome XV, thightly linked to pet17, and not at the chromosomal site of RAD50, which has been shown to be on chromosome XIV proximal to met2. We have concluded that the [YEp13-RAD50-210B] clone has no homology to RAD50 sequences and thus renamed the plasmid [YEp13-210B]. The possibility that this plasmid may be a nonsense suppressor was ruled out by genetic studies. A second possibility that we are currently investigating is that the cloned gene codes for a protein that interacts in some way with RAD50, either structurally or in a regulatory capacity.

A 4.7 kb HindIII fragment of the [YEp13-210B] plasmid containing the complementing activity has been subcloned into the integrating plasmid YIp5 to determine whether this gene will still complement rad50 mutations when present in only one copy per cell.

RAD51

We originally isolated 6 plasmids that complemented rad51 and by restriction analyses these were all shown to be different. One of these six, [YEp13-RAD51-23], was shown to integrate at the site of rad51 on chromosome V. The remaining plasmids have been analyzed further and have been shown not to overlap [YEp13-RAD51-23] and also not to complement rad51. We deduce that the original yeast transformants contained at least two plasmids, only one of which complemented rad51. We are developing a detailed restriction mmap of [YEp13-RAD51-23] and plan to make subclones from this plasmid in order to localize the RAD51 gene in the insert.

RAD52

The rad52-1 mutation has been studied in many laboratories because of its pleotropic effects on both recombination and repair. Since it is possible that rad52-1 is a leaky mutation, we have isolated disruptions of this gene which should totally inactivate it. These disruptions were

constructed using the cloned RAD52 gene and LEU2 and TRP1 genes were substituted into the genome in a way similar to that used for the disruption of 210B (discussed above). The disruptions of RAD52 are viable, demonstrating that this gene codes for a protein that is nonessential for vegetative growth (Schild et al., 1983b). We are currently testing various phenotypes of these disruptions to compare them with the phenotypes of the rad52-1 mutation.

RAD54

We have initiated gene structure and regulation studies of RAD54. To determine if this gene is regulated at the transcriptional level, we have prepared RNA from yeast cultures grown various lengths of time following X-irradiation. The level of RAD54 mRNA in each RNA sample is now being determined by hybridization to internal fragments of the gene previously cloned in our lab. Similar experiments are also in progress to study RAD52 gene regulation. To utilize expression vectors for the isolation of gene product of the RAD54 gene, the structure of the gene is being studied. Using S1 nuclease mapping, we have determined the direction of RAD54 transcription and the approximate point of transcription initiation.

RAD55

The isolation of plasmids carrying the RAD55 gene has been reported by this lab (Calderon et al., 1983). Analysis of these plasmids has been continued. Restriction mapping data has shown that the plasmids share a region of 2.6 kb. A 1.7 kb HindIII restriction fragment from this conserved region was subcloned into the yeast vector plasmid YCp50 and shown to retain RAD55 complementing activity. Deletions of restriction fragments from these plasmids have defined two restriction sites internal to RAD55.

To continue the genetic analysis of RAD55, revertants of a rad55-3 strain (having a cold-sensitive mutant phenotype) were selected on MMS-containing medium. In addition to true revertants, we hoped to obtain pseudo-revertants; strains which maintain the original rad55-3 mutation but which have suffered changes in other genes which compensate for the rad55-3 defect. Such pseudo-reversions can occur by four different mechanisms: informational suppression (by changing tRNA interpretation of the mutant codon), regulatory suppression (by overproducing weakly-functional RAD55 gene product), bypass suppression (by providing a substitute pathway for RAD55 function), or interactive suppression (by making compensating mutations in gene products which interact with RAD55 gene product. These modes of suppression can be distinguished genetically by dominance/recessiveness and allele specificity of suppression, and by the phenotypic effects of the suppressor mutation itself.

Out of 120 rad55-3 "revertants", approximately 40 were found to be candidates for interactive suppressor strains; these had lost the cold-sensitive Rad phenotype and assumed a heat-sensitive Rad phenotype. This ts-radiation sensitivity was recessive. Genetic crosses with 20 of these strains have shown that their new phenotype occurs by alterations within RAD55; these strains carry either improper reversions of rad55-3 or mutations at other sites within RAD55 which compensate for rad55-3.

Incidental Cloning of a Gene for an Amber Suppressor in Yeast

In our attempt to clone the RAD50 gene of yeast we have incidentally isolated a plasmid which suppresses the rad50-1 mutation but fails to show homology to RAD50 sequences. We have shown that rad50-1 and trp1-289, both present in the recipient strain, are amber mutations, and that the cloned gene codes for an amber suppressor which suppresses both of these mutations as well as other known amber mutations in yeast. The suppressor gene is located on the 2.5 kb yeast insert of the plasmid [YEp13-SUP].

When a 1.5 kb restriction fragment from the insert was subcloned into the YRp7 replicating plasmid and yeast transformation carried out, all the transformants showed suppression of rad50-1 and other amber mutations, indicating that the suppressor gene is present in that fragment; when, however, the same 1.5 kb fragment was subcloned into the integrating plasmid YIp5 and yeast integrants isolated, none of the twelve studied showed suppression of rad50-1 or of any other amber mutations. We have concluded that we have cloned a weak suppressor which is expressed only when present in high copy number.

Genetic studies using standard genetic methods and the rad52 chromosome loss method showed that the plasmid bearing the 1.5 kb fragment of the supressor insert had integrated very close to the MAL4 gene located on the right arm of chromosome XI. MAL4 is one of a set of polymeric genes conferring ability to ferment maltose, and there is evidence for considerable homology between different genes in this set.

References

Calderon, I.L., Contopoulou, C.R., Mortimer, R.K. (1983). Isolation and Characterization of Yeast DNA Repair Genes II. Isolation of Plasmids that Complement the Mutations rad50-1, rad51-1, rad54-3, and rad55-3. Current Genetics 7:93-100.

Game, J.C. (1983). Radiation Sensitive Mutants and Repair in Yeast. In: Spencer, J.F.T., Spencer, D.M., Smith, A.R.W. Yeast Genetics: Fundamental and Applied Aspects. Springer Verlag, N.Y., New York.

Schild, D., Konforti, B., Perez, C., Gish, W., Mortimer, R.K. (1983a). Isolation and Characterization of Yeast DNA Repair Genes I. Cloning of the RAD52 gene. Current Genetics 7:85-92.

Schild, D., Calderon, I.L., Contopoulou, C.R., Mortimer, R.K. (1983b). Cloning of Yeast Recombination-Repair Genes and Evidence that Several are Nonessential Genes. In: Friedberg, E.C., Bridges, B.A. Cellular Responses to DNA Damage. Alan R. Liss, Inc., N.Y., New York.

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Cloning and Mapping of the Yeast Photoreactivation Gene PHR1

David Schild, John Johnston*, Caren Chang** and Robert K. Mortimer.

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The yeast Saccharomyces cerevisiae has several different repair mechanisms to deal with pyrimidine dimers caused by exposure to ultraviolet light. Although most dimers are removed by excision repair, they can also be repaired by directly splitting the dimers by photoreactivation. Photoreactivation involves a photolyase (or photoreactivating enzyme), a cofactor and the absorption of visible light of low wavelength. A yeast mutation (phr1) which is unable to photoreactivate dimers was isolated by M. Resnick in 1969. Although the PHR1 gene is clearly involved in photoreactivation, it is not known for which product the gene codes since: A) there appear to be two different photoreactivating enzymes in commercial batches of bakers yeast, one a monomeric enzyme and the other a heterodimer (1), B) each enzyme seems to require a cofactor (1), and C) a second photoreactivation-deficient mutation (phr2) has recently been isolated and shown to be loosely linked to phr1. To study the PHR1 gene at a molecular level and eventually to determine its primary gene product, we have cloned this gene.

A plasmid containing a 6.4 kb yeast DNA insert has been isolated and shown to restore photoreactivation in a phr1 strain. The plasmid was targeted to integrate into chromosomal DNA at a site homologous to the insert by cutting within the insert. Two of these integrants have been studied and shown to map on the right arm of chromosome XV; the integrants have been further mapped at approximately 13 cM from prt1. It has also been independently determined that phr1 maps at this location. Thus, we have determined the map position of PHR1 and also have shown that the plasmid contains PHR1, rather than a suppressor of the phr1 mutation.

Reference

Madden, J.J. and H. Werbin. 1983. Purification of two DNA photolyases from Saccharomyces cerevisiae. Photochem. Photobiol., in press.

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C. Ma and R.K. Mortimer. 1983. Empirical equation that can be used to determine genetic map distances from tetrad data. Molecular and Cellular Biology 3:1886-1887.

An empirical equation has been developed that can be used to calculate genetic map distances from tetrad data with good accuracy for distances of at least 120 centimorgans. The standard Perkins equation of X_p is applicable only to distances of 35 to 40 centimorgans because crossover ranks higher than 2 are ignored. The empirical equation

$$X_e = \frac{80.7\ X_p - 0.883\ X_p}{83.3 - X_p}$$

gives a good fit to the correct map distances determined by the method of Snow but without the use of a computer.

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

Below follow summaries of three recent publications.

J.F.T. Spencer[1,2], D.M. Spencer[2], and R. Miller[1] (1983). Non-Utilization of Sucrose by the Petite Mutant of a Distiller's Yeast. Current Genetics 7:47-50.

Summary

A number of yeast strains are known to be unable to metabolize several sugars (galactose, maltose, α-methylglucoside) when converted to their petite mutants. The basis of this phenomenon is considered to be the loss of the ability to transport the sugars across the cell membrane. However, sucrose is believed to be hydrolyzed before the products are transported into the cell, and the enzyme responsible (invertase) is thought to be either present in the periplasmic space or to be bound to the outer surface of the cytoplasmic membrane. Hence the loss of the ability to metabolize sucrose may infer the impairment of the mechanism for transport of invertase to its normal location outside the cytoplasm. We have found a distiller's yeast strain which has lost the ability to metabolize sucrose when it is converted to the petite mutant, and we report here some of its properties. We have shown that the cell produces invertase, which is present in the cell-free extract, but not in the pellet of cell walls and unbroken cells, though we have not determined whether the enzyme is present in the cytoplasm in the glycosylated or the unglycosylated form. The ability of the strain to ferment sucrose is also impaired in respiratory-competent cells, when the determination is made under anaerobic conditions.

[1]Department of Biological Sciences & Environmental Health, Thames Polytechnic,
Wellington Street, London SE18, UK.

[2]Department of Biology, Goldsmiths' College, University of London, London SE14, UK.

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J.F.T. Spencer[1,2], D.M. Spencer[2], P. Whittington-Vaughan[1] and R. Miller[1] (1983). Use of Mitochondrial Mutants in the Isolation of Hybrids Involving Industrial Yeast Strains. IV. Characterization of an Intergeneric Hybrid, Saccharomyces diastaticus x Hansenula capsulata, Obtained by Protoplast Fusion. Current Genetics 7:159-164.

Summary

A hybrid of Saccharomyces diastaticus x Hansenula capsulata was obtained by conversion of the S. diastaticus parent to a petite, protoplasting and fusion of the strains and isolation of the hybrids on selective media, containing glycerol or galactose. All of the single-cell clones obtained resembled S. diastaticus in morphology, and fermented glucose, galactose, maltose, sucrose and starch. The hybrid sporulated,

producing spores resembling those of S. diastaticus, but in asci which differed somewhat morphologically. The stability of the hybrid depended on the composition of the maintenance media, as hybrid clones maintained on glycerol medium lost their ability to sporulated after a few transfers and the cellular morphology altered to a form more closely resembling H. capsulata, and those maintained on glucose-containing medium lost the ability to utilize glycerol. Cultures maintained on yeast extract-starch medium retained the ability to sporulate and utilize glycerol. However, dissection of asci from the hybrid yielded clones, some of which had lost the ability to metabolize glycerol but which still sporulated. These clones had an unaltered cytochrome spectrum, and in other ways appeared to be respiratory competent. Washed cells of hybrids grown on glactose or starch as sole carbon source metabolized starch, as shown by respirometric measurements, but cells maintained on glycerol as sole carbon source had lost this ability. There was some evidence that the addition of vitamins stimulated oxygen uptake on starch by the hybrids. The formation of sporulating hybrids in the cross may permit the genetic analysis of other Saccharomyces x Hansenula crosses.

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J.F.T. Spencer, D.M. Spencer, and R. Miller (1983). Inability of Petite Mutants of Industrial Yeasts to Utilize Various Sugars, and a Comparison with the Ability of the Parent Strains to Ferment the Same Sugars Microaerophilically. Z. Naturforsch 38c, 405-407.

Summary

A number of industrial strains of Saccharomyces cerevisiae were converted to the petite form and tested for the ability to utilize galactose, maltose, sucrose, α-methyl glucoside and raffinose. The parent strains all metabolized these sugars aerobically. Twelve of the petite forms did not utilize galactose, six failed to utilize maltose, 17 did not utilize α-methyl glucoside, and 18 did not utilize raffinose. The petites of two distiller's yeast strains did not utilize sucrose. The respiratory-competent parent strains nearly all fermented galactose, maltose, sucrose and raffinose, though 19 strains did not ferment α-methyl glucoside microaerophilically. Three strains did not ferment galactose, two fermented it only after several days adaptation, one did not ferment raffinose, and two did not ferment sucrose under microaerophilic conditions. Six respiatory-competent strains which did not utilize galactose when in the petite form fermented higher (10%) concentrations of glucose and maltose under microaerophilic conditions, but only three of these fermented galactose. The implications of these findings for the use of such strains in industry are discussed briefly.

* * *

We are continuing our investigations of the genetic manipulation of industrial yeasts, with emphasis on interspecific and intergeneric crosses obtained by protoplast fusion, using respiratory-deficient mutants of industrial yeasts as one member of the crosses. We reported earlier that we had obtained sporulating crosses between Saccharomyces diastaticus and Hansenula capsulata, which were amenable to genetic analysis, and we have

recently obtained sporulating hybrids between S. diastaticus and a strain of Candida pseudotropicalis. Details of the characteristics of this cross will be reported later. We are beginning a study of the surface characteristics of industrial yeasts, induced by mitochondrial mutations.

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XXIII. Department of Genetics, Microbiology, and Biophysics, Faculty of Natural Sciences, Charles University, 128-44 Prague, Czechoslovakia. Communicated by Olga Bendová and Vladimir Vondrejs.

The following manuscripts are in preparation by members of our group:

1. Vondrejs, V., Plch, J., Janderová, B. A multiple "cascade" fusion of Saccharomyces cerevisiae protoplasts.

The procedure for selecting yeast hybrid clones, arising from protoplast fusion of a killer producing (K^+) auxotroph with a killer sensitive strain in minimal medium containing killer factor (Vondrejs et al., Folia biologica 29:372, 1983) may be used repeatedly in multiple "cascade" fusion of protoplasts. The hybrid formed upon the first fusion can be "cured" of the killer character and thus become killer sensitive strain (R^-) against a particular killer factor. In the second step a further killer-auxotrophic cell can be added through fusion and so a triploid is formed. This way of polyploidization was demonstrated in an experiment with Saccharomyces cerevisiae T158C (α, K^+, R^+, his^-) and S. cerevisiae S6/1 (α, K^-, R^-, his^+). Tetraploidy was a much less stable configuration than triploidy.

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2. Vernerová, J., Bendová, O., Janderová, B., Vondrejs, V. Characterization of the hybrid killer strain of brewer's yeast Saccharomyces uvarum P9-LK-12/1.

The new brewer's strain (Monatsschrift für Brauwissenschaft 36:167, 1983) produces a killer factor which eliminates sensitive strains of Saccharomyces sp. Yeasts belonging to this genus are the most common yeast contaminants of beer. The hybrid maintained industrially important properties of the parental brewer's strain S. uvarum P9. Semipilot plant wort fermentation proved enhanced rate of wort attenuation, good yeast sedimentation, and unchanged organoleptic character of the beer produced. These results indicate the possibility of using the new strain for production of beer with improved biological stability.

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3. Janderová, B., Davaasurengijn, T., Bendová, O., Vondrejs, V. 1983. Brewer's yeast hybrid strains with ability to degrade dextrins.

Protoplasts of Saccharomyces uvarum P9-LK-12/2 (K^+, R^+, mel^+, rd, dex^-, ery^S, met^+) a derivative of S. uvarum P9-LK-12(1) Bendová et al., Monatsschrift für Brauwissenschaft 36(4):167, 1983 were fused with S.

diastaticus TD 1 (K^-, R^-, mel$^-$, rs, dex$^+$, ery^r, met$^-$). Hybrid clones of phenotype:Killer producing (K^+), killer resistant (R^+), melibiose fermenting (mel$^+$), respiration sufitient (rs), dextrin degradating (dex$^+$), erythromycin resistant (ery^r), methionine independent (met$^+$), were selected in minimal medium with glycerol as a sole carbon source and tested for expected phenotype. Dextrin degradation ability was well pronounced during wort fermentation in four of six hybrids tested in more details, however, the smell of the beer was unusual.

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Since our last report three papers on yeasts appeared in scientific journals (for abstracts of manuscripts see Yeast Newsletter 31(I):30-31 (1982) and 32(II): 80,1982.

4. Vondrejs, V., Pseničká, I., Kupcová, L., Dostálová, R., Janderová, B., Bendová, O. 1983. The use of killer factor in the selection of hybrid strains. Folia biologica (Praha) 29:372-384.

5. Vondrejs, V., Gašková, D., Plášek, J., Prosser, V. 1982. N-phenyl-1-naphthylamine as a fluorescent probe for early event in the action of Yeast killer factor. Gen. Physiol. Biophys. 1: 435-445.

6. Bendová, O., Kupcová, L., Janderová, B., Vondrejs, V., Vernerová, J. 1983. Ein Beitrag zur Brauereihefe-Hybridisierung. Monatschrift für Brauwissenschaft 36(4):167-171.

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XXIV. Technical Research Centre of Finland (VTT), Biotechnical Laboratory, Tietotie 2, 02150 Espoo 15, Finland. Communicated by Veijo Makinen.

The following papers have been published:

1. M.-L. Suihko and M. Drazic. Pentose Fermentation by Yeasts. Biotechnology Letters 5:107-112 (1983).

Summary

66 different yeast strains were screened for glucose, xylose and xylulose fermentation in shake flask cultures. None of the tested yeasts was able to grow or produce significant amounts of ethanol on xylose anaeobically. The best ethanol yields from xylulose were obtained with a wine yeast, two distillery yeasts, and a strain of Saccharomyces uvarum. The best conversion of xylulose to ethanol obtained was 76% of the theoretical yield. The optimal temperature was 30°C.

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2. Juha Ahvenainen. The Role of Lipids in Brewing. Technical Research Centre of Finland. Publication 12 (Academic Dissertation in General Microbiology).

Summary

Lipids are an important group of compounds affecting yeast metabolism and the physico-chemical properties of beer. In the present study the role of lipids in brewing was studied in laboratory and pilot scale. The analysis of total fatty acid content of wort was considered as a good method for controlling the separation processes during wort production and for evaluating the quality of finished wort. Amounts of fatty acids recommended on the basis of this work are 50-100 mg/l in the copper wort and 10-20 mg/l in the finished wort.

Yeast was enriched with lipids by supplying fatty acids in the wort or by propagating the yeast aerobically. Unsaturated fatty acids incorporated into the cells and into the complex lipids of yeast. They resulted in a reduction of the amount of medium chain length fatty acids in yeast and in beer, increased the synthesis of saturated acids by yeast and decreased the level of esters produced in beer fermentation. Unsaturated fatty acids also decreased the amount of squalene and increased the amount of sterols in yeast. In aerobically grown yeast cells the amounts of sterols and palmitoleic and oleic acids were highly increased compared with anaerobic cells.

Traditional, anaerobic pitching yeast was replaced with aerobically propagated yeast, which was rich in sterols and unsaturated fatty acids. A significant reduction in fermentation time and in ester production was achieved. The advantages of aerobically propagated yeast are most noticeable when worts of high specific gravity are fermented. No aeration of wort is required and the risk of oxidation of flavour compounds is eliminated.

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XXV. Central Food Research Institute, 1525 Budapest, II., Herman Otto ut 15. Postafiok: 76, Hungary. Communicated by P.A. Biacs.

The 12th International Symposium on Microbial Associations and Interactions was held in Budapest, in July, 1983. At this occasion a lecture, entitled "Alteration in the composition of Saccharomyces yeast during repeated beer fermentation", was delivered. The abstract of this paper follows below.

Alteration in the composition of Saccharomyces yeast during repeated beer fermentation.

P.A. Biacs, Katalin Gruiz, S. Klupacs

Brewer's yeast (Saccharomyces cerevisiae) is repeatedly used for beer fermentation in factories. This prolonged holding in the same fermenter causes alterations in yeast biomass: although a 2-3 times increase is observed during one brewing process, some quality problems arise by using the same yeast 6-8 times for fermenting malt wort to beer. Most attenuation difficulties happen because of physiological problems affecting the performance of the yeast. Attenuation difficulties merely reflect the fact that cells are reaching a resting state or becoming less tolerant to ethanol.

Under anaerobic conditions brewer's yeast becomes auxotrophic for sterol and unsaturated fatty acids. Lack of lipids, i.e., some of membrane constituents causes auxotrophism (starvation) and degradation of yeast cells. Samples taken directly from the brewery were dried and analysed, lipids were extracted and fatty acid composition examined by gas-chromatographic methods. Analysis of 6 consequent brewing processes showed a significant decrease of lipid extract and the ratio of fatty acids.

Table 1. Lipid extract and ratio of fatty acids in % of dry weight of yeast biomass.

No. of fermentation	Lipids	Fatty acids
1. brewing process	2.4%	1.0%
2. brewing process	1.9%	0.9%
3. brewing process	1.9%	0.8%
4. brewing process	1.5%	0.5%
5. brewing process	1.3%	0.5%
6. brewing process	1.0%	0.4%

During 6 consequent fermentation processes the percentage of short chain (C_8-C_{14}) fatty acids in membrane composition increased from 10% to 30%, but the ratio of $C_{18:1}$ (oleic acid) decreased from 8% to 4%, $C_{16:0}$ (palmitic acid) from 35% to 18%.

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XXVI. Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Canada S7N 0W0. Communicated by W.M. Ingledew - presently on leave until July 1984 at the Department of Viticulture and Enology, University of California, Davis, California 95616.

The following papers have been published or are in press.

Motiram R. Dhawale and W. Michael Ingledew (1983). Interspecific Protoplast Fusion of Schwanniomyces Yeasts. Biotechnology Letters (in press).

Abstract

Interspecific protoplast fusion of multiple auxotrophic mutants of Schwanniomyces castellii and Schw. alluvius resulted in fusion products displaying good stability and increased ploidy as a consequence of increased DNA content and cell volume. Following mitotic segregation analysis, recombinants were observed, suggesting that protoplast fusion resulted from nuclear fusion. Attempts to isolate NTG - induced derepressed mutants of one fusion product were successful; however, such mutants were similar in cell size and amylolytic activities to the auxotrophic strains from which they were initially derived.

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G.P. Casey and W.M. Ingledew (1983). High Gravity Brewing: Influence of Pitching Rate and Wort Gravity on Early Yeast Viability. J. Amer. Soc. Brewing Chemists 41(4): (in press).

Abstract

Poor yeast crop viability has been reported in high gravity brewing. When traditional pitching rates are used in high gravity worts of 19-39° Plato, yeast viability was a problem within the first 24 hour of the fermentation. Both immediate and continued losses of viability and fermentative ability were observed; the duration and severity increased with increasing wort gravity. By viable counting or methylene blue staining, however, early losses in viability in high gravity wort were significantly reduced by pitching at higher than usual pitching rates. The significance of this to brewing is that such drastic cell death reduces the real pitching rate. Such lower pitching rates have been said to be more inefficient in the utilization of oxygen for lipid synthesis. As a result, sluggish starts, protracted fermentations, and oxygen-deficient yeast crops are more likely to occur.

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G.P. Casey, C.A. Magnus, and W.M. Ingledew (1983). High Gravity Brewing: Nutrient Enhanced Production of High Concentrations of Ethanol by Brewing Yeasts. Biotechnology Letters 5(6): 429-434.

Abstract

Supplementation of high gravity brewing worts with 0.8% yeast extract, 24 ppm ergosterol and 0.24% (v/v) Tween 80 can result in the production of >14% (v/v) ethanol at 14°C within 5 days. Unsupplemented worts required up to 2 weeks to end ferment. Overcoming nitrogenous and lipid nutritional deficiencies results in substantial increases in cell mass production, decreased fermentation times and increased ethanol. It can be concluded that brewer's yeasts are tolerant to levels of ethanol previously associated only with winery and distilling yeasts, and that they do not require genetic manipulation or strain improvement to become tolerant to 14-16% ethanol.

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XXVII. Allied Breweries Limited, 107 Station Street, Burton-on-Trent DE14 1BZ. Communicated by P.A. Martin and D.A. Lovett.

Dr. W.J. Kilgour presented the following paper at the European Brewery Convention, London, 1983.

The Application of New Techniques for the Rapid Determination of Microbial Contamination in Brewing.
W.J. Kilgour, A. Day
Proc. Eur. Brew. Conv., 1983, 177-184.

Three techniques, employing modern instrumentation, were assessed in

relation to the varying requirements for speed, specificity and sensitivity within the brewing process. ATP bioluminescence detected the presence of viable contaminants down to 1 yeast or 100 bacterial cells ml^{-1} within 15 min. The DEFT (Direct Epifluorescent Filter Technique) showed the presence of yeast and bacteria, active and non-active, within 30 min, detection limit of 1 cell ml^{-1}. A/C conductance enumerated specific contaminants in the presence of other living microbes with a detection limit of 100 cells ml^{-1} within 18 hr. These techniques could provide the basis for rapid methods with consequent cost savings in the brewing industry.

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Dr. I.C. Cantrell exhibited the following poster at this convention.

Yeast Performance in Production Fermentations.
I.C. Cantrell, R.G. Anderson
Proc. Eur. Brew. Conv., 1983, 481-488

Production scale studies have been carried out to obtain a further understanding of the factors limiting fermentation performance. Particular emphasis has been placed upon the assessment of changes and interactions in the levels of fatty acids, sterols and glycogen in yeasts and fatty acids and oxygen in worts. The results indicate that differences in processing techniques play a key role both with regard to determining wort lipid content and influencing yeast physiology. Variations in wort fatty acids are illustrated and these, together with the role of yeast glycogen - which, under production conditions involving in-line pitching, does not influence fermentation performance - are discussed.

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

Below follow abstracts of our work published since June 1983.We organized in June of 1983 a 3-day international symposium for 20 invited speakers. The emphasis of the symposium was to cover recent advances in molecular biology of yeast, concentrating on special aspects of strain construction.

The symposium topics were:

Gene structure and gene expression
Vectors
Protein transport and modification
Strain construction

The proceedings of The Alko Yeast Symposium "Gene Expression in Yeast" make the first volume in a new publication series from the recently established Foundation for Biotechnical and Industrial Fermentation Research. The book (263 pages) will be available through Akateeminen Kirjakauppa, Keskuskatu 1, SF-00100 Helsinki 10, Finland and through Foundation for Biotechnical and Industrial Fermentation Research, POB 350, SF-00101 Helsinki 10, Finland for a price of USD 25.00. (See section Books for authors and titles).

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Matti Korhola. Improvement of Yeast Strains for Added Ethanol Tolerance.

Gene Expression in Yeast. Proceedings of the Alko Symposium Helsinki 1983, ed. by M. Korhola & E. Valsanen. Foundation for Biotechnical and Industrial Fermentation Research 1 (1983, 231-242).

Starting from an industrially used strain of baker's yeast with good fermentative characteristics and using the continuous culture technique with exogenously added ethanol to apply selection pressure, we have obtained strains with improved ethanol tolerance.

The characteristics of the starting strain MK 270 and the more ethanol tolerant strain MK 388 were compared. The fermentative activity of MK 388 was about 25% greater in continuous culture experiments than that of MK 270. In batch sugar-rich fermentation experiments MK 388 produced a final ethanol concentration about 1% higher than did the starting strain MK 270. The ploidy level determinations from DNA contents and from cell volumes gave contradictory results. The catalytic activities of alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and hexokinase in cell extracts of the two strains were inhibited by ethanol to about the same degree.

* * *

Hannu Poso and Anthony E. Pegg. 1983. Measurement of the Amount of Ornithine Decarboxylase in Saccharomyces cerevisiae and Saccharomyces uvarum by using α-[5-^{14}C]Difluoromethylornithine. Biochimica et Biophysica Acta 747: 209-214.

* * *

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

Below follow abstracts of three papers which have been published or submitted for publication.

1. E. Minarik: Influence of different yeast starter quantity on the course of must fermentation (in German). Mitteilungen Klosterneuburg, 34, 1984.

The alcohol level of wines produced in musts with high sugar content may be increased by the activator prepared from Botrytis cinerea. This level may even be increased by inoculation of the must with larger yeast starter quantities. Accordingly, the residual sugar of wines is considerably lower. The volatile acid content of wines fermented in the presence of the activator B. cinerea is much lower than in the control wine fermented without the activator. The quantity of yeast starter does not, however, influence the level of volatile acids in wines. A 3-5% yeast starter in musts with higher sugar content may be recommended.

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2. E. Minarik: Influence of some activators in the fermentation of must containing residual fungicides (in Slovak). Kvasny prumysl (Prague) 30, 1984.

The inhibitory effect of Botrytis-active fungicides on the alcoholic fermentation may be tempered or almost fully eliminated by the activator originating in the hyphal fungus Botryis cinerea. With 100-300 $mg.l^{-1}$ of the activator the fermentation start as well as the whole course of fermentation may be considerably stimulated, especially the first phase of fermentation of musts containing residual fungicides. Thiamine and ammonium phosphate have little or no stimulating activity on the fermentation rate of grape must.

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3. E. Minarik: First session of the Group of Experts "Microbiology" of the International Office of Viticulture and Enology (O.I.V.) Vinohrad (Bratislava) 21, 1983, p. 236 (in Slovak).

The working Group of Experts "Microbiology" of the O.I.V. met in Paris on 21 April 1983 at its first session after reorganization of the Sub-Commission "Microbiology of Wine". Delegates of 11 countries, members of the O.I.V. (Czechoslovakia, France, GFR, Hungary, Italy, Portugal, Rep. of South Africa, Soviet Union, Spain, Switzerland, Yugoslavia) elected Prof. Pierre Bidan chairman of the Group.

Main tasks of the Group:

1. To study international microbiological methods suitable in international wine practice and research,

2. To study modern microbiological procedures in enological research,

3. Co-operate with other O.I.V. sub-commissions, especially with the sub-commission for the Unification of Analytical Methods and Wine Evaluation.

For the next several years the following main topics have been recommended for discussion: taxonomy of wine yeasts and bacteria, methods of cultivation and characterization of yeasts and lactic acid bacteria, quick methods of cultivation and enumerating of wine yeasts and bacteria, methods of microbiological control of bottled wines, definition of synthetic media for wine yeast and bacteria, influence of strain of yeast species in the content of deuterium in wine, biogene amine formation by microorganisms in wine etc.

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XXX. Comité Interprofessionnel Du Vin De Champagne (CIVC), 5 Rue Henri-Martin - 51200 Epernay, France. Communicated by C. Badour.

Below follow several abstracts of our technological studies of yeast.

1. Valade, M., Moulin, J.P. (1983). Use of active dry yeasts for bottle fermentation. Preparation of a leaven from active dry yeasts. Le Vigneron Champenois, France, 1983 n° 1 pp 4-16.

Summary

A methodology is proposed to elaborate a leaven, from active dry yeasts, destined for the bottle fermentation.

A comparison of different starters obtained from liquid leaven (traditional methodology) and from active dry yeasts do not show any difference between the bottle fermentation kinetics, chemical analysis and flavour testings.

This methodology is also suitable to start fermentation again in case of stoppage of the alcoholic fermentation.

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2. Moulin, J.P., Valade, M. (1983). Yeasting in order to produce wines destined for bottle fermentation. Le Vigneron Champenois, France, 1983 n° 5 pp 237-250, n° 6 pp 283-306, n° 7/8 pp 345-375.

Summary

A summary is given of three years of experimentation dealing with yeasting with a view to alcoholic fermentation in the Champagne region.

This investigation was intended to study the influence of the strain of yeast, the advantage of a yeast strain selection, the yeasting techniques and the potentialities brought by active dry yeasts.

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3. Coulon, P., Duteurtre, B., Charpentier, M., Parenthoen, A. (Moët et Chandon)
Badour, C., Moulin, J.P., Valade, M., Laurent, M. (CIVC)
Lemenager, Y. (INRA et ENSBANA Dijon)
Outlooks in "Méthode champenoise". Utilization of immobilized yeasts for bottle fermentation.

Summary

The kinetics of bottle fermentation is quite similar with yeasts immobilized in calcium alginate gels and with traditional leaven.

The chemical and sensory analysis do not show significant differences between the tirage bottles obtained either from immobilized cells or not.

This technique is a possible answer the improve the shaking. After three years of experimentation it is reasonable to consider an industrial processing scale.

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XXXI. Books and Other Publications

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

At the occasion of the 9th International Specialized Symposium on Yeasts "Yeasts in Human Environment" held in Smolenice-Castle in April 1983, and the Anniversary of the Commission for Yeasts of the Czechoslovak Microbiological Society, the following publication has been issued:

Kocková-Kratochvilová, A. (ed.) et al. "20 Years of Activity in Czechoslovak Yeast Research", VEDA Publishing House of the Slovak Academy of Sciences, Bratislava 1983, 160 pp., Kcs 27.- The book comprises a review of nearly all publications of Czechoslovak yeast specialists on yeast research since 1974, including taxonomy, ecology, cytology, genetics, biochemistry, yeast growth and division, technology, immunology, pathogenicity, and antibiotics. There is also a review of the activity of the Commission for Yeasts, a list of yeast culture collections in Czechoslovakia, as well as a list of members of the Czechoslovak Commission for Yeasts.

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Yeast Genetics: Fundamental and Applied Aspects, J.F.T Spencer, D.M. Spencer, and A.R.W. Smith, editors. Springer-Verlag, New York, 1983. Cloth $56.00.

Table of Contents:

1. Genetic Control of Cell Proliferation
   B.L.A. Carter, J.R. Piggott, E.F. Walton, 1-28.

2. Genetic Control and Gene Expression During Meiosis and Sporulation in Saccharomyces cerevisiae
   Ian W. Dawes, 29-64

3. Meiotic Gene Conversion in Yeast: Molecular and Experimental Perspectives
   Seymour Fogel, Robert K. Mortimer, Karin Lusnak, 65-108.

4. Radiation-Sensitive Mutants and Repair in Yeast
   J.C. Game, 109-138.

5. Nucleic Acid Relatedness Among Yeasts
   C.P. Kurtzman, H.J. Phaff, S.A. Meyer, 139-166.

6. Genetic Approaches to the Study of Protease Function and Proteolysis in Saccharomyces cerevisiae
   Elizabeth W. Jones, 167-204.

7. Genetic Control of Flocculation
   J.R. Johnston, H.P. Reader, 205-224.

8. Protein and Lipid Composition of the Yeast Plasma Membrane
G.H. Rank, A.J. Robertson, 225-242.

9. Protoplast Fusion in Yeasts
Richard F. Freeman, John F. Peberdy, 243-254.

10. Genetic and Functional Aspects of Yeast Mitochondria
D. Wilkie, 255-268.

11. Molecular Genetic Aspects of Yeast Mitochondria
Ivor H. Evans, 269-370.

12. ScV "Killer" Viruses in Yeast
Diane J. Mitchell, E. Alan Bevan, 371-420.

13. Approaches to the Genetic Analysis and Breeding of Brewer's Yeast
Morten C. Kielland-Brandt, Torsten Nilsson-Tillgren, Jens G. Litske Petersen, Steen Holmberg, Claes Gjermansen, 421-438.

14. Genetic Improvement of Wine Yeast
Richard Snow, 439-460.

15. Aspects of the Biochemistry and Genetics of Sugar and Carbohydrate Uptake by Yeasts
G.G. Stewart, I. Russell, 461-484.

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Gene Expression in Yeast. Proceedings of The Alko Yeast Symposium, June 1-3, 1983, Helsinki, Finland. Edited by Matti Korhola and Eino Väisänen. Foundation for Biotechnical and Industrial Fermentation Research Volume 1 (see communication by Heikki Suomalainen for ordering details and price).

1. Positive regulation of genes under the general amino acid control in yeast
G.R. Fink, G. Lucchini and A.G. Hinnebusch

2. A relationship between chromatin structure and genetic elements at the yeast HIS3 locus
K. Strubl

3. A comparison of yeast and metazoan mRNA splicing
M. Rosbash, C.W. Pikielny, J.R. Rodriguez and J.L. Teem

4. Splicing of the transcripts of heterologous genes cloned in Saccharomyces cerevisiae
F.Z. Watts, V. Simanis, C. Castle and J.D. Beggs

5. Structure and function of two regulatory genes of the yeast pyrimidine pathway
R. Losson, P. Liljelund, F. Exinger, J.C. Hubert, B. Kammerer, A. Guyonvarch, R. Fuchs and F. Lacroute

6. The expression of bacterial β-lactamase and its applications to gene technology in yeast
C.P. Hollenberg, R. Roggenkamp, E. Erhart, K. Breunig and G. Reipen

7. Vectors for cloning in yeast: applications to studies of gene expression and gene function
D. Botstein

8. High efficiency expression vectors
S.M. Kingsman, M.J. Dobson, M.F. Tuite, J. Mellor, N.A. Roberts and A.J. Kingsman

9. Telomeres in yeast
J.W. Szostak

10. Secretion and organelle assembly in yeast
R. Schekman, D. Julius, S. Emr and J. Thorner

11. Inferences regarding the glycosylation pathway in yeast derived from structural studies on Saccharomyces cerevisiae mannoproteins
C.E. Ballou, P.-K. Tsai and L. Ballou

12. Control of intracellular proteolysis
H. Holzer

13. High expression and secretion of foreign proteins in yeast
A. Hinnen, B. Meyback and R. Tsapis

14. Current status in the application of recombinant DNA technology in the construction of cellulolytic yeast strains
V.L. Seligy, J.R. Barbier, K.D. Dimock, M.J. Dove, F. Moranelli, R. Morosoli, G.E. Willick and M. Yaguchi

15. The cloning of fungal cellulase genes
J.K.C. Knowles, K.M.H. Nevalainen, M.E. Penttilä, I.A. Salovuori and T.T. Teeri

16. Ty elements and the stability of the Saccharomyces cerevisiae genome
P. Philippsen, H. Eibel, J. Gafner and A. Stotz

17. Homeologous ILV genes in Saccharomcyes
J.G.L. Petersen, M.C. Kielland-Brandt, S. Holmberg, T. Nilsson-Tillgren and C. Gjermansen

18. Genetic manipulation of amylolytic enzyme production by yeasts
A.M. Sills, C.J. Panchal, I. Russell and G.G. Stewart

19. Constructing amylolytic strains of yeast for commercial applications
R.S. Tubb, B.A. Searle, K. Ogden, P.G.T. Meaden and M.A. Luker

20. Improvement of yeast strains for added ethanol tolerance
M. Korhola

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

Yeasts: Characteristics and Identification. J.A. Barnett, R.W. Payne and D. Yarrow (Cambridge University Press), price ≥75, published October 1983, has 811 + ix pages and about 500 photomicrographs.

This new book describes and photomicrographs all currently recognized species (about 470), for which the results of 83 physiological tests are given. There is a register of all yeast names (about 3,500) known to the authors, giving current synonyms where known and references. There is also a register of all specific epithets, to help people find the appropriate current and former generic names. Other features include about 1500 references, glossary and general index.

Like the previous work, A Guide to Identifying and Classifying Yeasts by the same authors, there are 18 identification keys, both general and specialized, and tables for confirming the identity of each species, using a minimum number of tests.

As computers were used extensively in preparing this book, the final compilation and production were very fast, so information about new species could be included remarkably close to the time of publication.

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XXXII. Meetings

1. The Sixteenth Annual Meeting of the Yeast Genetics and Molecular Biology Society of Japan was held from August 1st to 3rd, 1983, at the Center of Culture Exchange of the Osaka City University, Osaka, Japan. The following topics were presented and discussed.

Communicated by Masayuki Yamamoto
Lab. of Molecular Genetics
Inst. of Medical Science
University of Tokyo
P.O. Takanawa, Tokyo 108
Japan

Session 1: Structure and Function of Cell Organelles (Chairpersons, K. Tanaka and M. Osumi).

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

M. Nagano, M. Osumi and H. Yamaguchi (Dept. Biol., Japan Women's Univ. and Sch. Med. Teikyo Univ.) An electron microscopical study on the formation of yeast cell wall.

H. Tamaki (Doshisha Women's College) Characterization of additional chromosomes in the abortive products resulting from intergeneric protoplast fusion in yeasts.

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

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

K. Miura, A. Ohta and I. Shibuya (Dept. Biochem., Saitama Univ.) Analysis of Saccharomyces cerevisiae mutants defective in membrane phospholipid synthesis.

K. Yokoyama and K. Takeo (Res. Inst. Chemobiodynamics, Chiba Univ.) Differences of asymmetrical division between the pseudomycelial and yeast forms of Candida albicans.

Y. Yamada, Y. Tani and T. Kamihara (Seibo Women's Jr. College and Dept. Indust. Chem., Kyoto Univ.) Formation of glutathione by filamentous Candida tropicalis Pk 233.

Session 2: Radiation (Chairperson, K. Hieda).

S. Nakai and I. Machida (Div. Genet., Natl. Inst. Radiol. Sci.) Induction of sister chromatid recombination (SCR) by radiations in RAD and rad$^-$ mutants of Saccharomyces cerevisiae.

Session 3: Gene Structure (Chairpersons, A. Toh-e, N. Gunge and Y. Oshima).

Y. Nogi, M. Tajima and T. Fukasawa (Sch. Med., Keio Univ. and Shiseido Co.) The analysis of 5'-flanking region of GAL7 gene in Saccharomyces cerevisiae.

K. Azuma, T. Ogawa and H. Ogawa (Dept. Biol., Osaka Univ.) Transcripts from RAD52 locus in Saccharomyces cerevisiae.

K. Murakami (Cancer Res. Inst., Kanazawa Univ.) Characteristics of chromatin structure of autonomous replicating fragments.

J. Sakaguchi, N. Nakanishi, M. Sugiura and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo) Sequence analysis of a plasmid which propagates autonomously in S. pombe.

Y. Adachi, T. Toda, O. Niwa and M. Yanagida (Dept. Biophys., Kyoto Univ.) ARS sequence in S. pombe; application for centromere cloning.

M. Takagi, Y. Takada, N. Tanaka and K. Yano (Dept. Agr. Chem., Univ. Tokyo) Cloning and analysis of Leu and ARS of Candida yeast which are expressed in S. cerevisiae.

S. Matsumoto, K. Yasuda and M. Yanagida (Dept. Biophys., Kyoto Univ.) Cloning of histone genes in the fission yeast S. pombe.

T. Toda, Y. Adachi and M. Tanagida (Dept. Biophys., Kyoto Univ.) NDA2 codes for α-tubulin in fission yeast.

Session 4: Sporulation, Life Cycle (I) (Chairpersons, B. Ono and K. Matsumoto)

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

I. Miyakawa, T. Tsukamoto, N. Sando and T. Kuroiwa (Biol. Inst., Yamaguchi Univ. and Dept. Cell Biol., Natl. Inst. Basic Biol.) Configurational changes of mitochondrial nucleoids in meiotic spheroplasts of Saccharomyces cerevisiae.

M. Tsuboi and H. Tanaka (Dept. Biol. Osaka City Univ.) Mapping of sporulation genes and isolation of sporulation-deficient mutants in Saccharomyces cerevisiae.

Y. Nakaseko, O. Niwa and M. Yanagida (Dept. Biophys., Kyoto Univ.) Isolation and genetic analysis of meiotic mutants in Schizosaccharomyces pombe.

C. Shimoda and M. Kishida (Dept. Biol., Osaka City Univ.) Characterization of sporulation-deficient mutants in the fission yeast S. pombe.

A. Hirata, K. Tanaka and C. Shimoda (Inst. Appl. Microbiol., Univ. Tokyo, Fac. Med., Nagoya Univ. and Dept. Biol., Osaka City Univ.) Ultrastructure of sporulation-deficient mutants in the fission yeast Schizosaccharomyces pombe.

C. Shimoda and M. Uehira (Dept. Biol., Osaka City Univ.) Cloning of meiosis genes in the fission yeast S. pombe.

Y. Iino and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo) Suppressor mutation of mei1 defect in Schizosaccharomyces pombe.

Session 5: Gene Regulation (Chairpersons, Y. Nogi and M. Takagi).

K. Ogawa, Y. Tamai, Y. Kaneko, A. Toh-e and Y. Oshima (Dept. Ferment. Technol., Osaka Univ. and Dept. Ferment. Technol., Hiroshima Univ.) Regulation of phosphatase production in Saccharomyces cerevisiae.

I. Uno, K. Matsumoto and T. Ishikawa (Inst. Appl. Microbiol., Univ. Tokyo and Dept. Industr. Chem., Tottori Univ.) Characterization of cyclic AMP-requiring mutants altered in cyclic AMP-dependent protein kinase.

K. Matsumoto, I. Uno and T. Ishikawa (Dept. Industr. Chem., Tottori Univ. and Inst. Appl. Microbiol., Univ. Tokyo) Relationship between gene expression and cAMP-dependent protein kinase.

Y. Ohya, Y. Osumi and Y. Anraku (Dept. Biol., Univ. Tokyo) Genetic study of regulatory roles of Ca^{2+} on cell division cycle of Saccharomyces cerevisiae.

M. Yamamoto, J. Sakaguchi and K. Sakai (Inst. Med. Sci., Univ. Tokyo and Centr. Res. Lab., Asahi Brew. Co.) Transformation by copolymerization of plasmids in S. pombe.

T. Kamiryo and K. Okazaki (Fac. Integr. Arts and Sci., Hiroshima Univ.) Cloning of genes encoding the peroxisomal proteins of Candida tropicalis.

K. Suzuki, I. Yamashita, T. Maemura and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.) Molecular cloning of a glucoamylase-producing gene in Saccharomyces.

Session 6: Biochemistry (Chairpersons, T. Miyakawa, M. Yanagida, T. Ikemura and K. Murakami).

T. Fujimura, K. Nakamura and N. Gunge (Mitsubishi-Kasei Inst. Life Sci.) Secretion of killer toxin by Saccharomyces cerevisiae containing linear dsDNA plasmid pGK1-1.

M. Yamaguchi, K. Yoshida and N. Yanagishima (Biol. Inst., Nagoya Univ.) Cytoplasmic a agglutination substance in Saccharomyces cerevisiae.

H. Fujimura, K. Yoshida and N. Yanagishima (Biol. Inst., Nagoya Univ.) Purification and partial characterization of α-pheromone-binding glycoprotein in Saccharomyces cerevisiae.

T. Kamihara, Y. Nagami, T. Kuroiwa and I. Nakamura (Fac. Eng., Kyoto Univ. and Chukyo Jr. College) Respiratory depression at elevated temperatures in yeasts.

T. Sato, K. Kitamoto, Y. Osumi and Y. Anraku (Dept. Biol., Univ. Tokyo) Compartmentation of amino acids in S. cerevisiae.

E. Uchida, Y. Osumi and Y. Anraku (Dept. Biol., Univ. Tokyo) H^+-ATPase of the yeast vacuolar membrane.

H. Iida and I. Yahara (Tokyo Metrop. Inst. Med. Sci.) Possible involvement of heat shock proteins in regulation of cell proliferation: A study using heat shock-resistant mutants of Saccharomyces cerevisiae.

K. Huzimura, M. Miyazaki, S. Kageyama and K. Takahashi (Inst. Mol. Biol., Nagoya Univ. and Primate Res. Inst., Kyoto Univ.) The partial structure and function of peptide elongation factor EF-1α from yeast.

M. Uritani and M. Miyazaki (Inst. Mol. Biol., Nagoya Univ.) GTPase activity of peptide elongation factors from yeast.

T. Uemura and M. Yanagida (Dept. Biophys., Kyoto Univ.) Isolation of fission yeast mutants deficient in DNA topoisomerase and endonuclease.

M. Sunairi, K. Watanabe, M. Takagi and K. Yano (Dept. Agr. Chem., Univ. Tokyo) The regulation of synthesis of major microsomal proteins in n-alkane assimilating yeast.

K. Kimura, E. Tsuchiya, T. Miyakawa and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.) Fluctuation of intranuclear DNA polymerase activity during the cell division cycle of Saccharomyces cerevisiae.

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

T. Ikemura (Dept. Biophys., Kyoto Univ.) High resolutional separation of yeast mRNAs using two-deminsional gel electrophoresis.

Session 7: Mutation (Chairperson, S. Nakai)

Y. Iwamoto and I. Mifuchi (Dept. Microbiol., Shizuoka College Pharm.) Petite induction by photodynamic action of acridine dyes.

F. Miyamoto (Wakayama Univ.) Studies on the genetic character of respiratory deficient mutants induced by p-nitrophenol.

J. Ishiguro (Dept. Biol., Konan Univ.) I. Aneuploid mapping of a structural gene coding for ribosomal protein L23 of Saccharomyces cerevisiae. II. Genetic characterization of blasticidin S-resistant mutants of S. cerevisiae.

E. Sakamoto and B. Ono (Fac. Pharm. Sci., Okayama Univ.) Studies on inorganic mercury tolerance of Saccharomyces cerevisiae.

Session 8: Chromosome Map (Chairpersons, O. Niwa and H. Tamaki)

Y. Ishino and B. Ono (Fac. Pharm. Sci., Okayama Univ.) Genetic mapping of Saccharomyces omnipotent suppressors. I. Construction of mapping strains.

B. Ono, M. Tanaka, I. Awano and Y. Ishino (Fac. Pharm. Sci., Okayama Univ.) Genetic mapping of Saccharomyces omnipotent suppressors. II. Mapping of recessive omnipotent suppressors.

B. Ono, I. Awano and Y. Ishino (Fac. Pharm. Sci., Univ. Okayama) Genetic mapping of Saccharomyces omnipotent suppressors. III. Mapping of dominant omnipotent suppressors.

R. Fujimoto, Y. Ohno and B. Ono (Fac. Pharm. Sci., Okayama Univ.) Genetic analysis of UGA suppressor in Saccharomyces cerevisiae.

O. Niwa and M. Yanagida (Dept. Biophs., Kyoto Univ.) Occurrence of unstable aneuploid in Schizosaccharomyces pombe.

C. Shimoda (Dept. Biol., Osaka City Univ.) Systematic mapping of S. pombe genes by the use of random spore analysis.

Session 9: Sporulation, Life Cycle (II) (Chairpersons, N. Yanagishima and N. Sando)

T. Hatano, K. Hamada, E. Tsuchiya, T. Miyakawa and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.) Characterization of acid phosphatases specifically located on cell surface of gamate cells of Tremella mesenterica.

K. Hamada, K. Suzuki, E. Tsuchiya, T. Miyakawa and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.) Characterization of gene products specifically induced in Tremella mesenterica ab cells by Tremerogen A-10, a fungal sex hormone.

T. Yasutake, T. Miyakawa, E. Tsuchiya and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.) Modulation of protein phosphorylation during sexual differentiation of a heterobasidiomycete, Rhodosporidium toruloides.

T. Kadota, T. Miyakawa, E. Tsuchiya and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.) Alteration in cell surface proteins during sexual differentiation of a heterobasidiomycete, Tremella mesenterica.

T. Suzuki, T. Kuroiwa, T. Kanbe and K. Tanaka (Nagoya Univ. Sch. Med. and Natl. Inst. Basic Biol.) Parasexual life cycle in the imperfect yeast Candida albicans.

K. Tanaka, T. Kanbe and T. Suzuki (Nagoya Univ. Sch. Med.) Reductional nuclear division in Candida albicans.

Y. Hiraoka and M. Yanagida (Dept. Biophys., Kyoto Univ.) Chromosome movement during mitosis in fission yeast.

Session 10: Plasmids (Chairpersons, Y. Kikuchi, I. Takano and M. Yamamoto)

Y. Shimada, Y. Tomonaga, S. Harashima and Y. Oshima (Osaka Munic. Tech. Res. Inst. and Dept. Ferment. Technol., Osaka Univ.) Characterization of yeast mini-plasmid originated from YRp7.

H. Araki, H. Tatsumi, K. Ushio, T. Sakurai, A. Jearnpipatkul, T. Muta and Y. Oshima (Dept. Ferment. Technol., Osaka Univ. and Higashmaru Shoyu Co.) Structural and functional analysis of yeast plasmid pSR1.

N. Gunge and C. Yamane (Mitsubishi-Kasei Inst. Life Sci.) Isolation of ars-element from pGKL-killer plasmid and its stability.

A. Takagi, C. Boonchird, S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.) Gene expression of a cloned gene in isogenic series of Saccharomyces cerevisiae polyploid strains.

Y. Kikuchi (Keio Univ. Sch. Med.) Requirements for a high copy YCp-plasmid.

I. Utatsu and A. Toh-e (Dept. Ferment. Technol., Hiroschima Univ.) Structure and function of 2-μm DNA-like plasmids.

K. Sugihara and A. Toh-e (Dept. Ferment. Technol., Hiroshima Univ.) A new circular plasmid derived from Saccharomyces bailii.

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

T. Toda, Y. Nakaseko, O. Niwa and M. Yanagida (Dept. Biophys., Kyoto Univ.) Mapping of rRNA genes by integration of hybrid plasmids in Schizosaccharomyces pombe.

* * *

2. The XIIth International Conference on Yeast Genetics and Molecular Biology will be held in Edinburgh from 17th to 21st September 1984. We aim to keep the cost of attending low: it is impossible to give the exact figure at this stage, but we hope to charge less than £200 per person for registration and accommodation combined. For further details write to:

Dr. Ian Dawes
Department of Microbiology
University of Edinburgh
Edinburgh EH9 3JG
U.K.

or

Dr. Peter Fantes
Department of Zoology
University of Edinburgh
Edinburgh EH9 3JT
U.K.

* * *

3. Fifth International Congress of Culture Collections, Bangkok, Thailand, November 25-December 1, 1984

Registration:

Basic registration fees for the Congress will be 50 $US for WFCC members, 65 $US non-WFCC members. The social program fee is 50 $US and the published proceedings are 25 $US.

Bangkok Microbiological Resource Center (MIRCEN), Ms. Poonsook Atthasampuna, Director will host the Congress and training courses. To receive further information, registration and abstract forms; write to Dr. Robert Stevenson, Chairman ICCC-V, American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 USA.

* * *

4. VIth International Symposium on Yeasts, July 9-13, 1984, Montpellier, France. For registration forms and informational circular write to:

Administrative secretariat
Midi-Contacts
Le Capoulié
6, rue Maguelone BP 1041
34006 Montpellier Cedex
France
Tel. (67) 92.42.72

* * *

5. Minutes of the Meeting of the International Commission for Yeasts, January 27, 1983, held in Bombay, India, (VIII ISSY).

Members present at the January 27, 1983 meeting of the ICY in Bombay, India.

(Austria) H. Klaushofer, (Belgium) H. Verachtert, (Bulgaria) P. Venkov, (Canada) G.G. Stewart, (East Germany - visitor) H. Weber, (Finland) M. Korhola, (France) H. Heslot, (India) T. Subbaiah, (Scotland) D. Watson for I. Dawes, (Switzerland) O. Kappeli, (U.S.S.R.) N. Elinov

The following members sent their apologies for absence.

(Australia) B.C. Rankine, (Brazil) A. Panek, (Canada) B. Johnson, (Canada) I. Russell, (Czechoslovakia) A. Kockova-Kratochvilova, (Czechoslovakia) A. Kotyk, (Czechoslovakia) E. Minarik, (DDR) H. Koch, (DDR) P. Lietz, (DDR) W. Nordheim, (Denmakr) A. Stenderup, (England) A.H. Rose, (England) J.F.T. Spencer, (Egypt) A. ElNawawy, (France) J.-M. Bastide, (France) P. Galzy, (Hungary) E Novak, (Ireland) A.J. Forage, (Israel) C. Shalitin, (Japan) Y. Fukazawa, (Japan) K. Iwata, (The Netherlands) L. Rodrigues de Miranda, (New Zealand) M.G. Shepherd, (Poland) J. Jakubowska, (Poland) T. Lachowicz, (Poland) H. Oberman, (Portugal) N. van Uden, (South Africa) J. van der Walt, (Spain) J. Gancedo, (Spain) R. Sentandreu, (Sweden) K. Jarl, (U.S.A.) C.P. Kurtzman, (U.S.A.) H. Phaff, (U.S.A.) F. Sherman, (USSR) I. Bab'eva, (USSR) G. Shavlovsky, (West Germany) C. Emeis, (Yugoslavia) V. Johanides.

The minutes of a Commission Meeting held during the XIII International Congress of Microbiology in Boston, Massachusetts on August 11, 1982 were accepted.

Membership of ICY

The Chairman reported he hadread with regret that Dr. Heiki Suomalainen of Finland would be retiring from the Yeast Commission. The Chairman expressed the opinion of all Commission Members when he stated that Dr. Suomalainen had performed outstanding service for the ICY and would send a letter thanking him for all his efforts over the years and best wishes during his retirement. It was also agreed that Dr. M. Korhola would replace Dr. Suomalainen as Finland's representative on the ICY.

The Chairman also stated he had received Professor Susumu Nagai's resignation due to retirement from the National Women's University in Japan. Professor Nagai has been an outstanding member of the Yeast Commission for a number of years and a letter would be forthcoming to express, on behalf of all Commission Members, our most profound gratitude for all the faithful years of service.

IUMS

The Chairman reported on the activities of the ICY at the XIII Microbiology Congress held in Boston, Massachusetts in August, 1982. It is to be hoped that the ICY will also be involved in future Microbiology Congresses.

Discussions have already been held regarding ICY involvement in the XIV Microbiology Congress to be held in Manchester, U.K. in September, 1986.

IX ISSY

The next International Specialized Symposium on Yeasts will be held in Smolenice Castle, Czechoslovakia in April, 1983, the theme being "Yeast in the Human Environment". The Chairman stated that due to other commitments, neither he nor the ICY secretary would be able to attend. Fortunately, however, the Vice-Chairman, Professor H. Klaushofer, would be able to attend the IX ISSY and he would be the official representative of the ICY officer.

VI ISY

The first circular for the VI ISY to be held in Montpellier, France has been mailed (copies were available at the VIII ISSY). All the arrangements were proceeding to plan, the Chairman stated he would discuss the situation with Professor Galzy when he met him at the European Brewery Convention in London, England in June, 1983.

Future ISSY and ISY Meetings

It was agreed that the following ISSY and ISY Meetings will be held.

X ISSY - 1985	Sofia, Bulgaria Theme - "Molecular Genetics" (Dr. P. Venkov)
XI ISSY - 1986	Portugal Theme - "The Influence of Ethanol and Other Narcotics in Yeast" (Prof. N. van Uden)
XII ISSY - 1987	Jena, DDR Theme - "The Genetics of Non-Saccharomyces Yeasts" (Dr. H. Weber)

The possibility of holding future meetings at one site - campus or hotel, was discussed and it was agreed that if at all possible, this was preferable.

VII ISY - 1988

It was agreed that the Chairman of the ICY should write to all ICY Members asking for proposals concerning a location for this symposium. The location is to be decided upon at the next ICY meeting to be held in Montpellier, France, (July 9-13, 1984) at the time of the VI ISY.

UNESCO Training Courses

The Chairman reported he had been contacted by Dr. E.J. DaSilva of UNESCO concerning the possibility of the ICY collaborating in the organization and teaching of UNESCO sponsored yeast training courses that will be held in

developing countries. It was agreed in principle this was an acceptable concept and the Chairman stated he would keep all ICY Members informed as matters developed.

There being no other business the Chairman closed the meeting and thanked all the Members present including Dr. T. Subbaiah for his excellent organization of the VIII ISSY in Bombay.

The next meeting of the ICY will be held in Montpellier, France, July 13-19, 1984.

G.G. Stewart
Chairman, ICY

6. Minutes of the Informal Meeting, International Commission for Yeasts, April 19th, 1983 held at Smolenice Castle, Smolenice, Czechoslovakia.

Members present at the April 19th, 1983 informal meeting of the ICY in Smolenica: H. Klaushofer (Austria), A. Kocková-Kratochvilová (CSSR), E. Minarik (CSSR), H. Koch (GDR), J.F.T. Spencer (G. Britain), E. Novak (Hungary), H. Oberman (Poland).

Visitors present: P. Biely (CSSR), O. Bendová (CSSR), C. Pascual (Cuba), P. Krogh (Denmark), F.H. Jacob (France), A. Martini (Italy) G.D. de Hoog (The Netherlands), C. Ramirez (Spain), H. Naujahr (Sweden), W.I. Golubev (USSR).

The meeting of ICY in Smolenice 1983 was opened by Dr. E. Minarik (CSSR). He announced the informal character of the meeting as well as the date of the next official ICY meeting at the occasion of the 6th ICY Symposium to be held in July, 1984 in Montpellier, France. Then he passed the word to the ICY vice-chairman Prof. H. Klaushofer who informed the present members and visitors about the January 1983 ICY meeting held in Bombay at the occasion of the VIIIth ISSY.

Future meetings: Prof. H. Klaushofer also presented information on future ISY symposia:
6th ISY - July 9-13, 1984, Montpellier, France (Dr. J.M. Bastide and Dr. P. Galzy).
Xth Specialized Symposium on Yeasts (ISSY), 1985, "Molecular and Genetic Aspects of Yeasts", Black Sea Resort, Bulgaria (Dr. P. Venkov), still to be confirmed.
XIth Specialized Symposium on Yeasts (ISSY), 1986, "Basic Research on Applied Aspects of Yeasts, Portugal (Dr. N. van Uden).

Dr. A. Martini (Instituto di Biologia Vegetale, Perugia, Italy) and Dr. H. Oberman (Institute of Fermentation, Technology and Microbiology, Technical University of Lodz, Poland) expressed their interest in organizing the yeast symposia in future years. They were advised to contact Dr. G. Steward, the chairman of ICY or to raise the question on the ICY meeting in Montpellier, 1984.

Coordination of activities: The members and visitors expressed their opinion that there should be a better distribution of information about other related meetings which may cover a part of yeast research

activities. As an example Dr. S.G. de Hoog (The Netherlands) quoted the 1983 Mycological Congress to be held in Tokyo, Japan. Dr. Kocková-Kratochvilová and Dr. Minarik suggested that the information should be effected through the members of the Mycology Council and by using the Yeast Newsletter.

Membership of ICY: In the term of the statutes of the ICY article 7, allowing up to three members from every country, the following scientists were proposed to become delegates to ICY:

Dr. A. Martini (Italy)
Dr. T. Deak (Hungary)
Dr. H. Neujahr (Sweden)
Dr. C. Pascual (Cuba)
Dr. P. Krogh (Denmark)

Election should be effected in Montpellier, 1984.

General comments and remarks: Dr. E. Novak (Hungary) on behalf of the ICY members, expressed sincere thanks and cordial congratulations to Dr. A. Kocková-Kratochvilová, Dr. P. Biely, Dr. J. Sandula and other members of the Organizing Committee for successful and well organized IXth ISSY Symposium in Smolenice.

There being no other business the informal ICY meeting was adjourned by its chairman Dr. E. Minarik.

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XXXIII. BRIEF NEWS ITEMS

1. Postdoctoral fellowship. The research project concerns biochemical aspects of the cell envelope of Sporothrix schenckii, a dimorphic fungus that is the causative agent of sporotrichosis. Particular attention will be addressed to the isozyme patterns of acid phosphatase displayed by cell extracts of yeast- and mycelial-phases. Preliminary evidence indicates important differences between the two cell types, as well as a multiplicity of cellular loci. We seek an associate with some training in enzymology as well as general microbiology. The grant period is for three years; the beginning salary is up to $16,000 per year (depending upon experience) plus fringe benefits. Other areas of research by the same group will include metabolic studies on whole cell suspensions by NMR techniques. Please send expressions of interest to Dr. Wilf Arnold, Professor of Biochemistry, University of Kansas Medical Center, Kansas City, Kansas, USA 66103. (Telephone: 913-588-7056).

* * *

2. Postdoctoral position available immediately for a person to study protein secretion in the yeast Saccharomycopsis lipolytica. Primary focus of the project is on the effects of mutations in the structural gene of a secreted protein on the processing and secretion of the protein. Candidates should have a Ph.D. in microbiology, genetics,

biochemistry or molecular biology. Knowledge of microbial physiology and genetics is essential and experience with recombinant DNA techniques is a plus. Starting salary: $16,000. Send curriculum vitae and the names of three references to Dr. David M. Ogrydziak, Institute of Marine Resources, University of California, Davis, California 95616; (916) 752-2506.

* * *

3. We have succeeded in the transformation of intact yeast cells without making protoplasts. The only thing we have to do is to treat yeast cells with alkali cations such as Li-acetate or CsCl etc. The paper concerned with this subject was published in the J. Bacteriol. 153, (1983). The summary of this paper was given in the Yeast Newsletter XXXII, No. 1.

   We have continued to study this technique in two ways.

   (1) To simplify this method further although the efficiency becomes lower.
   (2) To develop a more efficient simple method. Recently we have succeeded in both ways. The detailed results will be published elsewhere.

Akira Kimura
Kyoto University
The Research Institute for
Food Science
Uji, Kyoto, Japan 611

4. The following are two news items from our laboratory.

   R. Montrocher - Participated in the 3rd Internat. Mycol. Congress in Tokyo (Sept. 1983) with a lecture entitled: "Biochemical correlations among Candida and related yeasts"
   Abstract - A new taxonomic approach of yeasts by the determination of cytochrome absorption spectra after aerobic growth of cells is proposed. The information obtained among several Candida species and their assumed perfect form correlates with serological and DNA results and suggests that cytochrome spectrum is a marker assessing accurately the relatedness among the species.

   J.B. Fiol is pursuing the study of cytochromes in the sporogenous yeasts Pichia (including the Pichia spp. with spherical spores) and Hansenula (in collaboration with the Laboratory of Molecular Genetics of M. Claisse); a quantitative study of the coenzyme Q system of a number of yeasts; research on the possible usefulness of minor compounds in systematics.

M.C. Pignal, Section Levures
Bat. 405, Univ. Lyon I
43 Bd du 11 Nov. 1918
69622 Villeurbanne Cedex
France

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5. The following are three recent publications from our laboratory:

Multiperforate septa in Geotrichum and Dipodascus by J.P. van der Walt, J.A. von Arx and N.V.D.W. Liebenberg, in South African Journal of Botany, vol. 2, (1983).

Pichia euphorbiae sp. nov., a new haploid heterothallic yeast species by J.P. van der Walt and Annette Opperman, in Antonie van Leeuwenhoek, vol. 49 (1983) pp. 51-59.

Fermentation of D-xylose to ethanol by a strain of Candida shehatae by J.C. du Preez and J.P. van der Walt, in Biotechnology Letters, vol. 5 (1983) pp. 357-362.

J.P. van der Walt
Microbiology Research Group
South African CSIR
P.O. Box 395
Pretoria 0001 South Africa

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6. Dr. Gerald Reed, formerly with Amber Laboratories, writes that Amber Laboratories have been acquired by Universal Foods Corporation this summer. I am now a full time consultant for Universal Foods with an office at the following address:

Universal Foods Corp.
Technical Center
6143 N. 60th St.
Milwaukee, WI 53218 phone 414-271-6755

7. The Editorial Board of Acta Alimentaria deeply regrets to announce that its founder and Editor-in-chief, Director of the Central Food Research Institute, President of the Committee on Food Science of the Hungarian Academy of Sciences passed away on 22 November 1981 at the age of 62. Professor Vas was an internationally recognized leading personality of Hungarian food science. His scientific heritage is of such a variety that it is impossible, within the frame of this notice to give a full account of it.

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