

A Newsletter for Persons Interested in Yeast

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

Herman J. Phaff
Editor

NOTICE TO OUR READERS

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

We have explored with the University of California the possibility of direct transfer of the subscription fee on the bank account of the University of California. Unfortunately, this is not possible because of the large size of the University on nine campuses in the State of California with its numerous accounts. It is suggested that subscribers may wish to purchase dollars and pay cash in order to save the high service charge or use a postal money order.

H.J. Phaff
Editor

- I. Centraalbureau Voor Schimmelcultures, Yeast Division, Julianalaan 67a, 2628 BC DELFT (Netherlands). Communicated by M.TH. Smith.

Below follow several news items from the CBS.

1. Drs. L. Rodrigues de Miranda retired at the end of October after 20 years in the Yeast Division at Delft. He had specialized in basidiomycetous yeasts and contributed chapters on the genera Bullera, Cryptococcus and yeast phases of Sirobasidiaceae, as well as on the ascomycetous genus Sporopachydermia, to the 3rd Edition of The Yeasts. His farewell contribution to the yeasts is an article prepared in co-operation with Dr. Weijman and submitted for publication to Antonie van Leeuwenhoek.

* * *

2. Recent acquisitions:

Arthroascus schoenii (Nadson & Krasil'nikov) Babeva & al.
7223 = VKM 107, T, ex exudate of oak, USSR, G.S. de Hoog.

Asteropora lycoperdoides haploid yeast state.
7213, 7214, ex Russula cf. nigricans, Germany, H. Prillinger.

Bullera derxii Nakase & Suzuki
7225 = IFO 10177 = JCM 5280 = N0-92, T. ex dead leaf of Oriza sativa, Japan, T. Nakase.

Bullera intermedia Nakase & Suzuki
7226 = IFO 10178 = JCM 5291 = N0-157, T. ex dead leaf of Oryza sativa, Japan, T. Nakase.

Bullera pseudoalba Nakase & Suzuki
7227 = IFO 10179 = JCM 5290 = N0-165, T, ex dead leaf of Oryza sativa, Japan, T. Nakase.

Candida blankii Buckley & van Uden
7205, horse's uterus, New Zealand, resistant to gentamicin and cycloheximide, A. Woodgyer.

Candida catenulata Diddens & Lodder
7230, ex soil, UK, grows on 3-mono-chloropropionic acid, S. Hughes.

Candida valida (Leberle) van Uden & Buckley
7217, patent application strain for utilization of alcohol wastes (received as C. rugosa), D.P. Henry.

Candida vini (Lodder) van Uden & Buckley
7218, ex Sikeae harbour, atypical strain, K. Gustafsson.

Cryptococcus neoformans (Sanfelice) Vuillemin var. shanghaiensis Liao & al.
7229 = Liao's S 8012, T, ex case of meningitis, China, J.A. Barnett.

Malassezia furfur (Robin) Baillon
7231, ex high vaginal swab from 27-year-old woman, New Zealand, A. Woodgyer

Malassezia minutissima Simmons & al.
7222 = GSU-RBS-8541, ex human ear, E. Gueho.

Myxozyma geophila van der Walt
7219 = CSIR Y-907, T, ex surface soil, Transvaal, J.P. van der Walt.

Rhodotorula acheniorum (Buhagiar & Barnett) Rodrigues de Miranda
7221, maize leaves, Netherlands, W.J. Middelhoven.

Sporobolomyces oryzaicola Nakase & Suzuki
7228 = IFO 10180 = JCM 5299, T, ex dead leaf of *Oryza sativa*, Japan, T. Nakase.

Taphrina epiphylla haploid yeast state
7216, ex *Alnus glutinosa*, Germany, H. Prillinger.

Tremella foliacea haploid yeast state
7215, ex *Pinus mugo*, Germany, H. Prillinger.

* * *

Publications

- de Hoog, G.S. & Smith, M.T. 1986. Key to the species of Hyphozyma (yeast-like Hyphomycetes and description of H. rosenigra sp. nov. *Antonie van Leeuwenhoek* 52, 39-44.

Abstract

The new species, Hyphozyma roseonigra, characterized by pink colonies, budding cells with minute annellated zones and brown, septate hyphae, is described. It is non-fermentive, shows no colouration with Diazonium Blue B, and has a ascomycete-type cell wall ultrastructure. A key to the accepted species and varieties of Hyphozyma is given.

* * *

Smith, M.T. & Batenburg-van der Vegte, W. 1986. Pichia triangularis sp. nov., the teleomorph of Candida polymorpha Ohara et Nonomura, nom. nud. *Antonie van Leeuwenhoek* 52, 437-443.

The type strain of Candida polymorpha Ohara & Nonomura, nom. nud. was found to produce hat-shaped ascospores. On the basis of its morphology and physiology, it is considered a new species of the genus Pichia and is described as Pichia triangularis sp. nov.

* * *

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

The strains listed below have been added to the ATCC since April 30, 1986. Complete information on these strains may be obtained upon request from the Mycology & Botany Department of ATCC.

New Yeast Strains

Name	ATCC No.	Depositor and Strain	Significance and Reference
<u>Candida albicans</u>	62342	D.W. Warnock, B41628	Human pathogen (J. of Med. & Vet. Mycology 24:133-144, 1986)
<u>Candida albicans</u>	62376- 62379	D.H. Howard, FC18 FC18-1, FC18-3, FC18-6	Human pathogen (J. of Bacteriology 145: 896-903, 1981)
<u>Cryptococcus neoformans</u>	62066- 62072	T. Mitchell, 6, 15, 98, 110, 145, 184, 602	Clinical isolate (Infect. Immun. 5: 491-498, 1972)
<u>Saccharomyces cerevisiae</u>	62013- 62017	E.D. Sancho, 614.1, 504.3, 519.5, 510.2 44.2	Lithium sensitive (Appl. & Environ. Microbiol. 51: 395-397, 1986)
<u>Saccharomyces cerevisiae</u>	62418- 62422	M.R. de van Broock D-101, D-314, LP-a, LP 4	Mating type (Biotechnology Letters 6: No. 3 171-176, 1984).
<u>Torulaspora hansenii</u>	60977- 60981	F. Fatichenti, FOF9(4), F3512(1), F3514(3), SG1S1(5), T2F14(2)	Produces antimicrobial metabolites (Dairy Res. 50: 449-457, 1983)

* * *

III. Japan Collection of Microorganisms Riken, Wako-shi, Saitama, 351-01 Japan. Communicated by T. Nakase.

Below follow abstracts of two papers from JCM.

1. Takashi Nakase and Motofumi Suzuki. 1986. Bullera megalospora, A New Species of Yeast Forming Large Ballistospores Isolated from Dead Leaves of Oryza sativa, Miscanthus sinensis and Sasa sp. in Japan. J. Gen. Appl. Microbiol. 32:225-240.

Detailed taxonomic studies were made of thirty-five strains of psychrophilic yeasts with large ballistospores. These strains were isolated from dead leaves of Oryza sativa, Miscanthus sinensis and Sasa sp. in Japan and were found to comprise a single, hitherto undescribed species of the genus Bullera. The species is described here as Bullera megalospora Nakase et

Suzuki. Bullera megalospora resembles Bullera piricola and Sporobolomyces puniceus, but it can be distinguished from B. piricola by its inability to assimilate lactose, melibiose, and inositol, and from Sp. puniceus in its lack of assimilation of inositol. Electrophoretic comparison of ten enzymes clearly demonstrated the differences among these three yeasts at the specific level; the similarities in their enzyme patterns were below 22%. Sporobolomyces puniceus was considered to be more closely related to B. megalospora and B. piricola than any of the other species of the genus Sporobolomyces. We propose to transfer this species to the genus Bullera as Bullera punicea (Komagata et Nakase) Nakase et Suzuki comb. nov.

* * *

2. Takashi Nakase and Motofumi Suzuki. 1986. The Ubiquinone System in Strains of Species in The Ballistospore-Forming Yeast Genera Sporidiobolus, Sporobolomyces and Bullera. J. Gen. Appl. Microbiol. 32:251-258.

The ubiquinone system of twenty-four strains of ballistospore-forming yeasts in the genera Sporidiobolus, Sporobolomyces, and Bullera was investigated. Most strains are type strains. They are twenty-one known species and two unidentified species. Sporidiobolus johnsonii, Sporid. pararoseus, Sporid. ruinenii, Sporid. salmonicolor, Sporobolomyces alborubescens, Sp. foliicola, Sp. gracilis, Sp. holsaticus, and Sp. roseus had Q-10 as the major ubiquinone and Q-9 as the minor component. Bullera alba, B. armeniaca, B. aurantiaca, B. crocea, B. dendrophila, B. globospora, B. piricola, B. punicea, B. salicina, B. singularis, and B. tsugae also had Q-10 as the major ubiquinone and Q-9 as the minor component. The amounts of Q-9 are rather high (15.0-25.4%) in Sporid. pararoseus, Sporid. ruinenii, B. piricola, B. punicea, and B. salicina. The major ubiquinone of Sp. elongatus was Q-10 (H₂). This yeast has Q-9 (H₂) and Q-10 as the minor components. This is the 2nd finding of Q-10 (H₂) in yeasts as the major ubiquinone. Three unidentified strains of two species of Bullera had Q-9 as the major ubiquinone and Q-8 and Q-10 as the minor components. Taxonomically, these strains resemble B. singularis in spite of the difference in ubiquinone systems. These yeasts may be yeast phases of certain species of the genus Itersonilia or may represent a genus yet to be established.

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- IV. Shizuoka University, Faculty of Agriculture, 836 Ohya, Shizuoka 422, Japan. Communicated by Yuzo Yamada.

The following are recent publications from my laboratory:

1. Yuzo Yamada. 1986. Holleya gen. nov., an Ascosporegenous Yeast Genus from the Q₉-equipped Organism Whose Ascospores are Needle-shaped with Smooth Surfaces in their Anterior Half and Concentric Ridges in their Posterior Half and Without Appendage. J. Gen. Appl. Microbiol. 32(5) in press.

Cells are globose, ovoid, ellipsoid to cylindrical, reproducing by multilateral budding. Pseudomycelium and mycelium are formed. Asci arise by transformation of vegetative cells. Ascospores are needle-shaped, 8 per ascus, smooth in anterior half, concentrically ridged in posterior half and

without appendage. Fermentation is present. Coenzyme Q-9[Q-8] system is present.

Type species: Holleya sinecauda (Holley) Yamada comb. nov. (Basionym: Nematospora sinecauda Holley, Antonie van Leeuwenhoek 50:309 1984).

Typus: CBS 8199

Etymology: Latin prep. sine without; Latin nom. fem. n. cauda tail; M. Latin n. sinecauda without tail.

The genus Holleya is placed in the family Spermophthoraceae.

2. Yuzo Yamada, Kana Aizawa, and Isao Banno. 1986. The Coenzyme Q System and An Electrophoretic Comparison of Enzymes in the Strain of the Anamorphic Yeast Species, Sterigmatomyces fuzhouensis. J. Gen. Appl. Microbiol. 32, 367-370. Short Communication.
3. Yuzo Yamada. 1986. The Coenzyme Q System in Strains of Species in the Anamorphic Yeast Genus Myxozyma. J. Gen. Appl. Microbiol. 32, 259-261. Short Communication.

* * *

- V. Universita Degli Studi Di Perugia, Dipartimento di Biologia Vegetale, I - 06100 Perugia, Borgo XX Giugno, 74, Italy. Communicated by Ann Vaughan Martini.

Our publications for the year 1985/86 are listed below. Please note that as of November 1985, the official name of our institution has become: Dipartimento di Biologia Vegetale.

1. Vaughan Martini, A., Martini, A. 1986. Three newly delimited species of Saccharomyces sensu strictu. Antonie van Leeuwenhoek in press.

Abstract

Deoxyribonucleic acid reassociation studies of 24 different strains of wine and beer-associated Saccharomyces confirmed the presence of three separate species. S. cerevisiae and S. bayanus strains had only 22% of their genomes in common. S. pastorianus, with intermediate hybridization values between S. cerevisiae and S. bayanus, (52 and 72%, respectively) could possibly be a natural hybrid of the two species. This epithet replaces S. carlsbergensis, with which it is homologous for 93% of its genome, since S. pastorianus was described first by Hansen in 1904. These data do not agree with the results of traditional physiological tests.

* * *

2. A. Vaughan Martini, A. Martini. 1985. Perfect-imperfect Relationship within the Yeast Genus Kluyveromyces. Ann. Microbiol. (Milan), 35, 93-97.

Abstract

A deoxyribonucleic acid reassociation study of thirteen perfect and imperfect yeast strains associated with the yeast genus Kluyveromyces confirmed the premise of previous authors that a perfect-imperfect relationship exists between some of these strains. It was also demonstrated that the latest conventional classification of the genus does not entirely agree with the results of DNA/DNA reassociations.

* * *

3. Gianfranco Rosini. 1986. Wine-making by cell-recycle-batch fermentation process. *Appl. Microbiol. Biotechnol.* 24:140-143.

Summary

In order to check the overall validity of more efficient fermentation systems to reduce wine-making costs, we carried out an off-skins fermentation of clarified Trebbiano toscano grape-juice, making use of a non-conventional "cell-recycle-batch fermentation" process. The results showed that the process causes a reduction of the fermentation length as well as an improvement in ethanol productivity and yield and can be conveniently applied to the production of ordinary table wines.

* * *

4. F. Federici and M. Petruccioli. 1985. Effect of some Cultural Conditions on polygalacturonase Production by Cryptococcus albidus var. albidus. *Ann. Microbiol. (Milan)*, 35, 235.

Summary

The influence of carbon source, initial cell concentration, aeration, medium pH and incubation temperature on polygalacturonase production by the yeast strain Cryptococcus albidus var. albidus IMAT-4735 was studied. All the experiments were performed in a medium containing pectin as carbon source; the enzyme production was depressed when pectin was replaced by a mixed carbon source such as glucose or sucrose and pectin. Maximum polygalacturonase activity was obtained when the microorganism was cultivated at 24°C, the medium pH was 4.6, the pectin concentration 35.0 g/l, the aeration was gradually shifted from 1 to 0.15 VVm and the initial cell number 30.5×10^6 per ml. The time course of growth and enzyme production in batch culture at optimized growth conditions is reported.

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

Recent publications from this laboratory include the following.

1. Barnett, J.A., Payne, R.W. & Yarrow, D. (1985). Yeast Identification Program. Cambridge University Press.
Floppy disk version obtainable: IBM PC (DOS), DEC Rainbow (CP/M), DEC Rainbow (MS-DOS), Superbrain (CP/M).

2. McCann, A.K. & Barnett, J.A. (1986). The utilization of starch by yeasts. Yeast 2, 109-115.
3. Barnett, J.A. (1986). The stability of biological nomenclature: yeasts. Nature 322, 599.
4. McCann, A.K., Hilberg, F., Kenworthy, P. & Barnett, J.A. (1987). An unusual hexose-ATP-kinase with two catalytic sites and a role in catabolite repression in the yeast Schwanniomyces occidentalis. Journal of General Microbiology in the press.

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

Below follows the abstract of a paper that has been accepted for publication by Mycologia.

C.P. Kurtzman. 1987. Two New Species of Pichia From Arboreal Habitats. Abstract. Among hat-spored isolates of the genus Pichia collected in frass from trees in the U.S. and Japan were strains of two taxa that could not be identified with presently described species. These new homothallic species, described as P. hampshirensis (type strain, NRRL YB-4128 = CBS 7208) and P. japonica (type strain, NRRL YB-2750 = CBS 7209), show little nuclear DNA relatedness to P. wickerhamii, P. rhodanensis, P. mississippiensis, P. toletana, and other phenotypically similar taxa.

* * *

VIII. All-Union Collection of Microorganisms, Inst. Biochem. and Physiol. of Microorganisms, USSR Academy of Sciences, Pushchino, Moscow region 142292, USSR. Communicated by W.I. Golubev.

The following are recent publications from our Institute.

1. Golubev, W.I., 1986. Yeasts from arctic East-Siberian tundra - Izvestija Akad. Nauk SSSR, ser. biol., N4, 609-612.

The total yeast counts were (per g dry sample) several hundred or less for tundra soils and up to several hundred thousand for plant materials. The yeasts averaged a third of the micromycetous flora. The species diversity of yeast flora was limited, and most abundant yeasts were Cryptococcus aerius, Cr. magnus, Cr. uniguttulatus and Cr. albidus. The majority of the isolates were characterized by minimum growth temperatures of -1° to -2°C and maxima of 30°C or below.

2. Golubev, W.I., Naumov, G.I., Bibikova, I.I., Blagodatskaya, V.M., Vustin, M.M., Nikitina, T.N., Buzurg-zade, D.L., and Gradova, N.B., 1986. Reidentification of hydrocarbon strongly assimilating yeast strains of the genus Candida. - Biotechnologia, N 5, 17-21.

26 yeast strains used for the production of single-cell protein in the USSR from n-paraffins were examined. Of these, 23 strains were reidentified as Candida maltosa Komagata et al. and 3 strains as C. tropicalis (Castellani) Berkhout.

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A list of of newly accepted yeast cultures by the All-Union Collection of Micro-organisms is published in Prikladnaya biochimia i mikrobiologia, 22, N 4, 587-591, 1986.

* * *

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

The following 2 papers have been published recently.

1. Butler, M.J. and M.A. Lachance. 1986. Quantitative binding of azure A to melanins of the black yeast Phaeococcomyces sp. Exptl. Mycol. 10:166-170.

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2. Lachance, M.A., H. J. Phaff, W.T. Starmer, A. Moffitt, and L.G. Olson. 1986. Interspecific discontinuity in the genus Clavispora Rodriguez de Miranda by phenetic analysis, genomic deoxyribonucleic acid reassociation, and restriction mapping of ribosomal deoxyribonucleic acid. Int. J. Syst. Bacteriol. 36:524-530.

Summary

Heterothallic strains belonging to the biologically distinct yeast species Clavispora opuntiae and Clavispora lusitaniae were studied by three different methods. The type cultures of the two yeast species exhibited 8% relatedness as measured by reassociation of unique deoxyribonucleic acid. Ten strains of C. opuntiae and nine strains of C. lusitaniae were compared on the basis of their physiological phenotypes and the restriction maps of their ribosomal deoxyribonucleic acids (rDNAs). Although the two species possessed many similarities as well as certain amounts of intraspecific variation by both approaches, they appeared to constitute well-defined entities. Unlike C. opuntiae, C. lusitaniae always utilized L-rhamnose as the sole carbon source and was resistant to 10 mg of cycloheximide per liter. Strains of C. opuntiae did not utilize L-lysine as the sole nitrogen source or utilized it very weakly, whereas all strains of C. lusitaniae grew rapidly on this compound. By contrast, the hydrolysis of Tween 80 and the utilization of lactic acid, citric acid, and hexadecane tended to be more pronounced in C. opuntiae. The rDNA repeating unit was 9.0 kilobases long in C. lusitaniae, as compared with 7.6 kilobases in C. opuntiae. The conserved region identified previously in the rDNA of C. opuntiae was found almost intact in the rDNA of C. lusitaniae, but the variable regions differed substantially between the two species.

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3. Butler, M.J., and M.A. Lachance. 198-. Inhibition of melanin synthesis in the black yeast Phaeococcomyces sp. by growth on low pH ascorbate medium: production of spheroplasts from albinized cells. Can. J. Microbiol. (accepted October 1986).

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4. Ganter, P.F., W.T. Starmer, M. A. Lachance, and H.J. Phaff. 198-. Yeast communities from host plants and associated Drosophila in southern Arizona: new isolations and analysis of the relative importance of hosts and vectors on community composition. Oecologia (accepted June 1986).

Summary

The yeast communities from slime fluxes of three deciduous trees (Prosopis juliflora, Populus fremontii and Quercus emoryi) and the necroses of two cacti (Opuntia phaeacantha and Carnegiea gigantea) were surveyed in the region of Tucson, Arizona. In addition, the yeasts carried by dipterans associated with the fluxes or necroses (Drosophila carbonaria, D. brooksae, D. nigrospiracula, D. mettleri, and Aulacigaster leucopeza) were sampled. The results indicate that each host sampled had a distinct community of yeasts associated with it. The dipterans, which can act as vectors of the yeasts, deposited yeasts from other sources in addition to those found on their associated hosts. It is argued that host plant physiology is relatively more important than the activity of the vector in determining yeast community composition. Furthermore, the average number of yeast species per flux or necrosis is not different from the average number of yeast species per fly. It is hypothesized that the vector may affect the number of species per individual flux or rot, and that the number is lower than the rot or necrosis could potentially support.

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- X. University of Medicine & Dentistry of New Jersey, Robert Wood Johnson Medical School. Department of Molecular Genetics and Microbiology, Piscataway, New Jersey 08854-5635. Communicated by M.J. Leibowitz.

Below are abstracts of three recent papers from this laboratory.

1. E.M. Hannig, T.L. Williams and M.J. Leibowitz. 1986. The internal polyadenylate tract of yeast killer virus M₁ double-stranded RNA is variable in length. Virology 152:149-158.

A stable mRNA-dependent cell-free translation system from Saccharomyces cerevisiae, prepared by a modification of the method of Hofbauer et al. (Eur. J. Biochem. 122 (1982) 199-203) was active in translation of exogenous homologous and heterologous mRNAs. Optimal translational activity required the addition of polyamines and yeast tRNA. The m transcript of the M dsRNA segment, synthesized in vitro using the killer virus-associated RNA polymerase, directed the synthesis of preprotoxin polypeptide (M-p32), which was immunologically identified using antitoxin antibody. Sindbis virus capsid protein and rabbit globin were also translated from their mRNAs. Translation was inhibited by puromycin, sparsomycin and anisomycin. Analogues of the 5'-

terminal caps present on most eukaryotic mRNA molecules inhibited translation of added mRNAs, including capped mRNAs and the uncapped killer virus mRNA.

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2. I. Hussain and M.J. Leibowitz. 1986. Translation of homologous and heterologous messenger RNAs in a yeast cell-free system. *Gene* 46:13-23.

The 1.8 kilobase pair (kbp) M_1 double-stranded (ds) RNA from type 1 killer strains of *Saccharomyces cerevisiae* contains an internal 200 bp adenine- and uracil-rich region. We have previously demonstrated that this region consists primarily of adenine residues on the plus strand of M_1 dsRNA and on the full-length, in vitro synthesized (+) transcript (denoted m) of M_1 dsRNA, neither of which contains 3'-terminal polyadenylate. We now show that there is variability in the length of the polyadenylate tracts of m transcripts synthesized in vitro by virions purified from either of the K_1 diploid killer strains A364A x S7 or A364A x 1384. This variability reflects size differences seen in the corresponding M_1 dsRNA genomes. This, along with other data presented here, localizes the variability in the length of M_1 dsRNA to the adenine- and uracil-rich region.

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3. D.E. Georgopoulos, E.M. Hannig and M.J. Leibowitz. 1987. In R.B. Wickner, A. Hinnebusch, A. Lambowitz, I.C. Gunsalus, A. Hollaender, J. Preer, Jr., L. Mets and R. Gumpert (eds.). Extrachromosomal Elements in Lower Eukaryotes, Plenum Press, New York, pp. 203-213.

A full-length cDNA copy of the M_1 -2 region of the double-stranded genome of the killer virus of yeast was synthesized by reverse transcription utilizing the m in vitro transcript as template and synthetic primers for both strands. The sequence lacks any long open reading frames. The internal portion of the M_1 -2 region includes the sequence which is linked to the subterminal 229 bases of the M_1 -1 homologous region in the S3 defective-interfering mutant of killer virus dsRNA. Thus the probable site at which the deletion occurred in S3 has been identified.

* * *

- XI. Institut für Physiologische Chemie, Ruhr-Universität Bochum D-4630 Bochum 1, FRG. Communicated by W. Duntze.

The following abstract summarizes part of our recent work on the structure of the mating hormone α -factor.

Amino acid sequences of α -factor mating peptides from *Saccharomyces cerevisiae*

R. Betz, J.W. Crabb*, H.E. Meyer, R. Wittig and W. Duntze

*W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, N.Y. 12946 U.S.A.

Abstract. The molecular structure of a-factor, the mating hormone produced by mating type a cells of S. cerevisiae has been investigated. In culture filtrates of a cells four oligopeptides (a1 to a4) exhibiting a-factor activity have been found. These peptides have been isolated and their amino acid sequences determined. The a-factor peptides form two (apparently identical) pairs a1/a2 and a3/a4 which differ in an interchange at position 6 of a valine in a1/a2 for a Leucine in a3/a4. a1 and a4 which can be obtained by oxidation with H_2O_2 of purified a2 and a3, respectively, obviously represent oxidation artifacts formed under the conditions of culture.

The amino acid sequences determined for the a-factor peptides are Tyr-Ile-Ile-Lys-Gly-Leu/Val-Phe-Trp-Asp-Pro-Ala-Cys. Several lines of evidence suggest that the carboxy-terminal cysteine residue is S-alkylated by a hydrophobic aliphatic residue.

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XII. National Research Council Canada, Division of Biological Sciences, Ottawa, Canada K1A 0R6. Communicated by Byron F. Johnson and A. Nasim.

The following are abstracts of publications from the laboratory of B.F. Johnson and the Molecular Genetics Section (A. Nasim).

1. Byron F. Johnson, G.B. Calleja, and M. Zuker. 1984. Mating-Type Gene Switching in a Homothallic Fission Yeast. *J. theor. Biol.* 110, 299-312.

When single cells of a homothallic strain of the fission yeast (Schizosaccharomyces pombe 968h⁹⁰) are plated upon sporulation agar, a couple of cell divisions yield four preconjugal cells ordered in a line. Within a line, conjugation occurs either between sibs (the pair at either end of the line) or between cousins (the central pair of cells) or not at all. Miyata & Miyata (1981) have shown that sib matings are favored over cousin matings, the ratio of sib:cousin:sterile being 96:23:10. To have mating within these 4-cell clones means that the mating types of some of the cells have switched. In a further analysis of their data, we come to a series of deductions, one of which is that switching of mating-type genes in the fission yeast must be asymmetrical. We propose a random model and a deterministic model based upon asymmetrical switching. Either model could generate the ratios provided, but the models are sufficiently different that experimental tests should be able to discriminate between the two.

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2. Machiko Miyata^{1,3}, Hisao Miyata^{2,3}, and Byron F. Johnson³. 1986. Establishment of Septum Orientation in a Morphologically Altered Fission Yeast, Schizosaccharomyces pombe. *J. Gen. Microbiol.* 132, 2535-2540.

¹Gifu Pharmaceutical University, 6-1, Mitahora-higashi 5 chome, Gifu 502, Japan.

²Department of Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464, Japan.

Among the spheroidal fission-yeast (Schizosaccharomyces pombe) cells resulting from aculeacin A treatment cells were found whose putative growth axis and polarity differed from those of their progenitor, that is, they were changed in the orientation of their septum. The ratio of cell length (measured perpendicular to the septum plane) to septum diameter of these cells equalled or exceeded unity without exception, whether the septum orientation changed or not and whether the cellular shape was spherical, spheroidal or cylindrical. From these results we conclude that the septum is always oriented perpendicular to the plane including the longest axis of the cell even if the morphology is irregular or the new septum has become perpendicular to the previous septum. There is no cellular region forbidden to new septa.

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3. Machiko Miyata, Hisao Miyata, and Byron F. Johnson. 1986. Asymmetric Location of the Septum in Morphologically Altered Cells of the Fission Yeast Schizosaccharomyces pombe. J. Gen. Microbiol. 132: 883-891.

Cells of the fission yeast Schizosaccharomyces pombe, normally sausage-shaped, changed to a round-bottomed flask (RBF)-like morphology during growth in the presence of aculeacin A (Acu), an antifungal antibiotic. The volume of RBF-like cells was comparable to that of the control cells. After being transferred to normal conditions (without Acu at 25°C), the RBF-like cells continued to grow at the cylindrical and or spherical end(s) and then the septum at the subsequent division of the cells was formed without exception at the boundary plane between the spheroidal and the cylindrical region: it is at this boundary that the nucleus was located before mitosis. Hence the RBF-like cell divided into a spheroidal and a cylindrical sib at the first cell division. At the end of the second cell cycle, the spheroidal and the cylindrical progeny divided into two spheroidal and two cylindrical sibs respectively. The values of the mean length (long/short) and volume (big/small) ratios of paired sibs were larger in order of (a) cylindrical normal, with both mean ratios 1.06; (b) cylindrical control; (c) cylindrical progeny of RBF-like cell; (d) spheroidal progeny of RBF-like cell; and (e) RBF-like cell, whose mean length ratio was 1.25 but whose mean volume ratio was 1.94. That is, the more the morphology deviated from the cylindrical form, the greater was the degree of asymmetry. There was no rule relating the biases to the growth pole in these asymmetries.

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4. Miyata, H., M. Miyata, and B.F. Johnson. 1986. Patterns of extension growth of the fission yeast, Schizosaccharomyces pombe. Can. J. Microbiol. 32:528-530.

The growth of sausage-shaped cells of the fission yeast, Schizosaccharomyces pombe (strain NCYC 132), was followed in the second or third cycle by time-lapse photomicrography. Experimental cells were harvested from glucose-limited (0.2% glucose EMM3) chemostat culture (dilution rate, 0.125/h) and were plated onto a slide with EMM3 agar (2% glucose). By observing their extension patterns, we found some rules of extension growth.

Thus, (1) all sibs with walls newly formed in the previous cycle, whose progenitor cells grew at the old end (followed Mitchison's rule), grow at the old end (also follow Mitchison's rule) (2) Sibs with old walls whose progenitor cell followed Mitchison's rule behave in one of three ways: (i) growth at the old end (follow Mitchison's rule); (ii) growth at the new end (violate Mitchison's rule); or (iii) growth at both ends (bipolar), (3) Both sibs whose progenitor grew at both ends (bipolar) always grow at the old end (follow Mitchison's rule).

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5. J.A. Erratt and A. Nasim. 1986. Allelism within the DEX and STA gene families in Saccharomyces diastaticus. Mol. Gen. Genet. 202:255-256.

Summary. Saccharomyces diastaticus produces an extracellular glucoamylase and is therefore capable of hydrolyzing and fermenting starch. Tamaki (1978) studied starch utilization in S. diastaticus and found three polymeric genes controlling this function: STA1, STA2 and STA3. Independently, Erratt and Stewart (1978) studied dextrin utilization by the yeast S. diastaticus and designated the gene, which they identified, DEX1. Erratt and Stewart (1981a, b) later described two other genes which controlled glucoamylase production in S. diastaticus: DEX2 and a third which was allelic to STA3. At that time STA1 and STA2 were not available to test for allelism in the DEX gene family. In this study strains containing the remaining 4 genes have been examined to determine if further allelism exists between the two gene families. It was ascertained that DEX1 is allelic to STA2 and DEX2 is allelic to STA1. Therefore, no new gene controlling starch utilization has been identified and these two nomenclatures can now be consolidated into one. Based on the fact that the glucoamylase from S. diastaticus can hydrolyze both dextrin and starch, dextrin being the term used to describe partially hydrolyzed starch, and the more wide use of the nomenclature STA, we propose to retain STA as the designation for genes coding for glucoamylase production in S. diastaticus.

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6. J.A. Erratt and A. Nasim. 1986. cloning and Expression of a Saccharomyces diastaticus Glucoamylase Gene in Saccharomyces cerevisiae and Schizosaccharomyces pombe. J. Bacteriol. 166:484-490.

A recombinant plasmid pool of the Saccharomyces diastaticus genome was constructed in plasmid YEp13 and used to transform a strain of Saccharomyces cerevisiae. Six transformants were obtained which expressed amylolytic activity. The plasmids each contained a 3.9-kilobase (kb) BamHI fragment, and all of these fragments were cloned in the same orientations and had identical restriction maps, which differed from the map of the STA1 gene (I. Yamashita and S. Fukui, Agric. Biol. Chem. 47:2689-2692, 1983). The glucoamylase activity exhibited by all S. cerevisiae transformants was approximately 100 times less than that of the donor strain. An even lower level of activity was obtained when the recombinant plasmid was introduced into Schizosaccharomyces pombe. No expression was observed in Escherichia coli. The 3.9-kb BamHI fragment hybridized to two sequences (4.4 and 3.9 kb) in BamHI-digested S. diastaticus DNA, regardless of which DEX (STA) gene S. diastaticus contained, and one sequence (3.9 kb) in BamHI-digested S. cerevisiae DNA. Tetrad analysis of crosses involving untransformed S. cerevisiae and S. diastaticus

indicated that the 4.4-kb homologous sequence cosegregated with the glucoamylase activity, whereas the 3.9-kb fragment was present in each of the meiotic products. Poly(A)⁺ RNA fractions from vegetative and sporulating diploid cultures of S. cerevisiae and S. diastaticus were probed with the 3.9-kb BAMHI fragment. Two RNA species, measuring 2.1 and 1.5 kb, were found in both the vegetative and sporulating cultures of S. diastaticus, whereas one 1.5-kb species was present only in the RNA from sporulating cultures of S. cerevisiae.

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7. A. Nasim, E.R. Stephen, and J.A. Erratt. 1985. Replicating instabilities in yeast: occurrence in different mutational systems. Mol. Gen. Genet. 199:152-153.

Summary. Following mutagenesis of yeast cells with nitrosoguanidine, primary mosaic colonies exhibiting prototrophic/auxotrophic phenotypes were obtained. Upon replating of these primary mosaics, numerous secondary mosaics were present in the progeny. This study shows that replicating instabilities occur at many different loci within the Schizosaccharomyces pombe genome. In addition, the ade-1 gene of Saccharomyces cerevisiae (causing red pigmentation) was used to show that the phenomenon also occurs in this yeast.

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8. Susan A. Nadin-Davis, Robert C.A. Yang, Saran A. Narang, and Anwar Nasim. 1986. The Cloning and Characterization of a RAS Gene from Schizosaccharomyces pombe. J. Mol. Evol. 23:41-51.

Summary. We have cloned and determined the complete nucleotide sequence of a RAS gene from the yeast Schizosaccharomyces pombe (SP-RAS). The putative RAS protein of 214 amino acids is encoded by two noncontiguous reading frames separated by an intron of 86 bp. The SP-RAS gene product shares extensive homology with the proteins of the Saccharomyces cerevisiae (SC), Dictyostelium, Drosophila, and human RAS genes in its N-terminal region but not in its C-terminal region. The extended C-terminal regions found in the SC-RAS genes have no counterpart in the SP-RAS gene. Thus the RAS genes of these two yeasts are structurally quite distinct. The SP-RAS sequence was expressed in vivo.

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9. S.A. Nadin-Davis, A. Nasim and D. Beach. Involvement of RAS in Sexual Differentiation but not in Growth Control in Fission Yeast. EMBO Journal (in press).

Abstract. The function of the ras⁺ gene of Schizosaccharomyces pombe has been studied by constructing null and activated alleles of this gene. An activated allele (ras_{va112}) inhibits conjugation but has no effect on cell growth, entry into stationary phase or sporulation. The phenotype of ras_{va112} is distinct from that caused by elevating the intracellular level of cAMP. This supports the hypothesis that ras of fission yeast does not modulate adenylate cyclase in a manner analogous to S. cerevisiae RAS. Introduction of a human ras sequence into fission yeast cells containing a non-functional null allele of ras restored the sexual differentiation process thus indicating that

the human sequence can complement Schiz. pombe ras. Our data suggest that although ras genes are highly conserved across a considerable evolutionary divide, the cellular function of the ras gene product varies in different organisms.

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XIII. Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo, 113, Japan. Communicated by Masamichi Takagi.

Below follow abstracts of recent publications from our laboratory.

1. M. Takagi, S. Kawai, Y. Takata, N. Tanaka, M. Sunairi, M. Miyazaki and K. Yano. 1985. Induction of cycloheximide resistance in Candida maltosa by modifying the ribosomes. J. Gen. Appl. Microbiol., 31, 267-275.

Abstract. When Candida maltosa IAM12247 was grown in the presence of cycloheximide, the growth was repressed for a distinct time depending on the concentration of cycloheximide, then the growth recovered. The cell-free translation experiments using polyuridylic acid as mRNA indicated that the protein-synthesizing activity was altered in the cells adapted to resist cycloheximide. A reconstituted cell-free system was constructed consisting of ribosomes and soluble fraction, and it was concluded that ribosomal modification was induced during the cultivation of the cells in the presence of cycloheximide.

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2. M. Takagi, S. Kawai, I. Shibuya, M. Miyazaki and K. Yano. 1986. cloning in saccharomyces cerevisiae of a cycloheximide resistance gene from the Candida maltosa genome which modified ribosomes. J. Bacteriol. in press.

Abstract. We have previously shown that cycloheximide resistance can be induced in a strain of Candida maltosa by modifying ribosomes. The present paper describes the cloning of the gene involved in this resistance by using a host-vector system of Saccharomyces cerevisiae.

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3. M. Takagi, S. Kawai, M.C. Chang, I. Shibuya and K. Yano. 1986. Construction of a host-vector system of Candida maltosa using an ARS site isolated from its genome. J. Bacteriol. in press.

Abstract. To construct a host-vector system in a n-alkane-assimilating yeast, Candida maltosa, isolation of an ARS site from its genome was attempted which replicated autonomously in C. maltosa. Leu⁻ mutants of C. maltosa were transformed with a gene library prepared using YEp13 (LEU⁺) as a vector, and Leu⁺ transformants were obtained at a high frequency. A plasmid named pCS1 was isolated from the recipient cells. pCS1 contained a 6.3 kb fragment of C. maltosa genome, and a 3.8 kb fragment with ARS activity was subcloned and designated as the TRA (transformation ability) region. Vectors (pTRA1 and pTRA11) for C. maltosa (Leu⁻) were constructed consisting of this 3.8 kb fragment, pBR322 and the LEU2 gene of S. cerevisiae. Transformation of C. maltosa (Leu⁻) with these plasmids was successful by both spheroplast- and Li-acetate-methods. Southern blot analysis suggested that the copy number of

pTRAI in C. maltosa was between 10 and 20, and it was stably maintained during growth without selective pressure in the medium. It was also found that these vectors could transform S. cerevisiae (leu2⁻) to LEU⁺, suggesting that the TRA region contained an ARS site or ARS sites that were specific not only for C. maltosa but also for S. cerevisiae.

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XIV. Academy of Sciences of the GDR, Central Institute of Microbiology and Experimental Therapy, Laboratory of Yeast Genetics, DDR-69 Jena, Beutenbergstr. 11. Communicated by H. Weber.

In the yeast genetics laboratory of our institute during the past several years a contribution was made to develop the genetics of Yarrowia (Saccharomycopsis) lipolytica. After establishing genetical methods own strains suitable for genetic studies were constructed. Main attention has been paid to improve conjugation and sporulation parameters as well to develop genetically well marked and defined strains. By means of these strains investigations on life cycle and regulation of glyoxylate cycle enzymes are now in progress.

Below follows a publications list from our laboratory:

1. H. Weber. 1979. Substructural studies on sporulation of Saccharomycopsis lipolytica. Z. Allg. Mikrobiologie 19, 283-297.
2. L. Spata and H. Weber. 1980. A study on protoplast fusion and parasexual hybridization of alcane utilizing yeast. Advances in Protoplast Research, S. 131-137, Akademia Kiado, Budapest and Pergamon Press, Oxford.
3. H. Weber, W. Förster, H.-E. Jacob und H. Berg. 1980. Enhancement of yeast protoplast fusion by electric field effects, Advances in Biotechnology: Proceedings V. Int. Symposium on yeast, London, Ontario 1980, Pergamon Press, 219-224.
4. H. Weber and L. Spata. 1980. Characterization of yeast protoplast-fusion products: Advances in Biotechnology: Proceedings V. Int. Symposium on yeasts, London, Ontario, 1980, Pergamon Press, 213-218.
5. H. Weber, W. Förster, H.-E. Jacob, H. Berg. 1981. Microbiological implications of electric field effects III. Stimulation of yeast protoplast fusion by electric field impulses. Z. Allg. Mikrobiologie, 555-562.
6. H. Weber, W. Förster, H.-E. Jacob und H. Berg. 1981. Parasexual Hybridization by Electric Field Stimulated Fusion of Protoplasts. Current Genetics, 165-166.
7. H. Weber. 1982. Zellbiologie und Genetik der Hefen. Methoden und Arbeitstechniken. 171 p., Akademie-Verlag Berlin/DDR.
8. G. Barth and H. Weber. 1983. Genetic studies on the yeast Saccharomycopsis lipolytica. Inactivation and mutagenesis. Z. Allg. Mikrobiol. 23: 147-157.

9. C. Kurischko, S.G. Inge-Vechtomov and H. Wever. 1983. Development of breeding stocks of the yeast Saccharomycopsis lipolytica by methods of moderate inbreeding. Z. Allg. Mikrobiol. 23, 513-515.
10. H. Berg, E. Bauer, W. Förster, M. Hartmann, H.-E. Jacob, A. Kurischko, P. Mühlig, H. Weber. 1983. Cell fusion by electric field pulses. Studia biophysica 94, 93-96.
11. I. Hones. 1983. Untersuchungen zur Regulation der Enzyme des Glyoxylatzyklus bei Saccharomycopsis lipolytica. 1. Einfluss der C-Quelle auf die Aktivität der Isocitratlyase und Malatsynthase. Z. Allgem. Mikrobiologie 23, 163-171.
12. I. Hones. 1984. Untersuchungen zur Regulation der Enzyme des Glyoxylatzyklus bei Saccharomycopsis lipolytica. 2. Wirkung von Glucose und Itaconsäure auf die Isocitratlyase-Regulation. Z. Allgem. Mikrobiologie 24, 9.
13. G. Barth and H. Weber. 1984. Use of nystatin for random spore analysis in the yeast Saccharomycopsis lipolytica. Z. Allgem. Mikrobiologie 24, 125-127.
14. G. Barth and H. Weber. 1984. Improved conditions for mating of the yeast Saccharomycopsis lipolytica. Z. Allgem. Mikrobiologie 24, 403-405.
15. H. Berg, K. Augsten, E. Bauer, W. Förster, H.-E. Jacob, P. Mühlig, A. Kurischko and H. Weber 1984. Possibilities of cell fusion and transformation by electrostimulation. Bioelectrochemistry and Bioenergetics 12, 119-133.
16. C. Kurischko. 1984. Analysis of genetic markers in new breeding stocks of the yeast Saccharomycopsis. Z. Allg. Mikrobiologie 24, 545-550.
17. G. Barth and H. Weber. 1985. Improvement of sporulation in the yeast Yarrowia lipolytica. Antonie van Leeuwenhoek, Journal of Microbiology 51, 167-177.
18. G. Barth. 1985. Genetic regulation of isocitrate lyase in the yeast Yarrowia lipolytica. Current Genetics 10, 119-124.
19. C. Kurischko and H. Weber. 1986. Temporal relationship of diploidization and haploidization in the yeast Yarrowia lipolytica. J. Basic Microbiol. 26, 2.
20. C. Kurischko. 1986. Spontaneous haploidization in early zygote progeny and its use for mapping in the yeast Yarrowia lipolytica. Current Genetics 10.

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

Below follow abstracts of two recent papers from my laboratory.

1. Y. Iwamoto¹, Y. Yanagihara¹, and L.W. Yielding². 1986. Petite Induction in Yeasts, Saccharomyces cerevisiae, by Photoactivation of 3-Azido-6-Amino-10-Methylacridinium Chloride. Photochemistry and Photobiology, Vol. 43, No. 2, pp. 139-144.

¹Department of Microbiology, Shizuoka College of Pharmacy, Shizuoka-shi, Japan and ²Department of Anatomy, University of South Alabama College of Medicine, Mobile, Alabama 36688, USA.

Abstract

The photoinduction of petite colonies and cell toxicity in non-growing yeast, Saccharomyces cerevisiae, by 3-azido-6-amino-10-methylacridinium chloride (AAMAC) has been examined. The results presented here indicate that mitochondrial DNA damage in resting yeast which occurs following irradiation of AAMAC-treated cells for short time periods is probably mediated through a covalent adduct between AAMAC and DNA. Furthermore, the photoreaction which contributes to biological activity is dependent on the presence of oxygen. Pre-irradiated AAMAC, which no longer exhibited the short-term photo-induction of biological effects showed a second biological activity. In this case longer irradiation time, e.g., 30 min, were required to induce petites for resting yeast. Again there was a strong dependence on the presence of oxygen. These results suggest that both processes may be effected through oxygen intermediates (photodynamic processes).

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2. Yoshihisa Iwamoto,^a Yasutake Yanagihara^a, Lerena W. Yielding^b, and K. Lemone Yielding^b. 1986. Petite Induction in Yeast, Saccharomyces cerevisiae, by Phenanthridinium Compounds: Promotive Effects of Propidium Iodide on Mutagenesis by Ethidium Bromide or 8-Deaminoethidium Chloride. Chem. Pharm. Bull. 34(4)1735-1739.

Shizuoka College of Pharmacy,^a 2-2-1 Oshika, Shizuoka-shi 422, Japan and School of Medicine, University of South Alabama,^b Mobile, Alabama 36688, USA.

Promotive effects of propidium iodide (PI) on petite induction by 8-deaminoethidium chloride (8-DAEC) were examined. 8-DAEC was a potent petite inducer in growing yeast cells but not in resting yeast cells. Addition of PI promoted the petite induction by 8-DAEC throughout the cultivation time at a concentration that was ineffective by itself. In resting cells, petites were scarcely induced by either 8-DAEC or PI or both, even after prolonged incubation. Notable sectorial colony induction was observed after prolonged incubation of the yeast cells with either 8-DAEC or PI in phosphate-buffer. The petite induction by ethidium bromide (EB) was not accelerated but was delayed in PI-pretreated cells, whereas the simultaneous presence of PI promoted the EB mutagenesis.

These results suggested that the simultaneous presence of PI and petite inducer in growth medium was required in order to exhibit the promotive

effects. Possible mechanisms of the differences in the process of petite induction between resting and growing cells are discussed.

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XVI. Institut für Genetik, TH Darmstadt, Schnittspahnstr. 10, 6100 Darmstadt, FR Germany. Communicated by Stefan Hohmann.

The following is a summary of a paper about invertase genes in Saccharomyces cerevisiae.

1. Stefan Hohmann and Friedrich K. Zimmermann. 1986. Cloning and Expression on a multicopy vector of five invertase genes of Saccharomyces cerevisiae. Current Genetics (in press).

Summary

Six unlinked loci for invertase structural genes are known in the yeast Saccharomyces cerevisiae: SUC1-SUC5 and SUC7. These genes are similar in structure and expression but not identical. Different yeast strains possess none, one or several of these genes.

We have isolated the genes SUC1-SUC5, subcloned them into the multicopy vector YEp24 and compared the expression of the five SUC genes in one recipient strain.

SUC2 was isolated by transformation of a suc0 strain with a gene pool and complementation to sucrose fermentation. SUC4 was cloned from a minipool of chromosomal fragments which were shown to contain SUC4 by Southern hybridization. SUC1, SUC3, and SUC5 were isolated using the method of plasmid eviction. A plasmid was integrated next to these SUC genes. The plasmid together with the SUC genes were then cut out off the chromosome using an appropriate restriction endonuclease.

The length of chromosomal DNA fragments containing the different SUC genes were 4.8 kb for SUC1, 5.2 kb for SUC2, 4.8 kb for SUC3, 12.8 kb for SUC4 and 17.2 kb for SUC5.

Fragments containing the complete SUC genes and the sequences controlling their expression were subcloned into YEp24 and transformed into a strain without any active invertase gene. Invertase activity of transformants was measured after growth on repressing (8% glucose) and derepressing (2% raffinose) conditions. As expected from results with strains carrying the individual SUC genes in a chromosomal location, the SUC genes were expressed to a different extent.

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XXVII. The University of Western Ontario, Department of Plant Sciences, Biological & Geological Building, London, Canada N6A 5B7. Communicated by R.B. Gardiner and A.W. Day.

The following are abstracts of recently published articles from our laboratory.

1. Day, A.W., R.B. Gardiner, R. Smith, A.M. Svircev, and W.E. McKeen. 1986. Detection of fungal fimbriae by protein A - gold immunocytochemical labelling in host plants infected with Ustilago heufleri or Peronospora hyoscyami f. sp. tabacina. Can. J. Microbiol. 32: 577-584.

Sections of leaves of Nicotiana tabacum L. infected with Peronospora hyoscyami De Bary f. sp. tabacina (Adam) Skalicky and of Erythronium americanum Ker. infected with Ustilago heufleri Fuckel were treated with an antiserum directed against the fimbriae of U. violacea Fuckel and other fungi. The sections were then treated with protein A - gold complexes to detect the presence and location of fimbriae antigens following transmission or scanning electron microscopy. Control preparations involved sections of uninfected leaves, as well as a range of serological control treatments. The infected leaf sections were heavily labelled with gold particles indicating the presence of fimbrial antigens, whereas the control preparations showed only a low background level of labelling. Gold particles were detected on the sections of hyphae, on haustoria, and on the nearby plant cells. The intensity of labelling was much higher for P. hyoscyami f. sp. tabacina than for U. heufleri and was particularly high in the walls of the former species. Relatively high levels of labelling occurred over the cells of infected hosts, but little or none occurred over the cells of uninfected host tissues, or of the infected host tissues treated with a range of serological controls. This high level of labelling was not associated with specific host structures in P. hyoscyami, but was frequently associated with the chloroplasts in U. heufleri. The antigens detected inside the host plant cells appear to indicate that fungal fimbrial protein, either as polymerized fibrils or as isolated subunits, can penetrate the host plasma membrane and therefore enter the host cytoplasm. An alternative possibility is that these antigens derive from host produced proteins synthesized as a result of infection. These results suggest the possibility that fungal fimbriae may play an important role in the molecular interaction between pathogen and host.

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2. Alan J. Castle and Alan W. Day. 1986. Diploid Derivatives of Ustilago violacea with Altered Mating-Type Activity III. Constitutive Mating Strains. Bot. Gaz. 147(1):110-115.

Freshly isolated diploids of Ustilago violacea, heterozygous for mating type (a_1/a_2), give rise to frequent (5×10^{-3}) derivative forms that appear to form after mitotic crossing-over near the mating-type locus. One such derivative, termed op-C, constitutively produces mating tubes at temperatures below 20 C on complete medium or 25 C on minimal medium but buds vegetatively at higher temperatures. Op-C strains remain diploid and have no detectable alterations, compared with the original diploid, in all the marked chromosomes except for the mating-type locus. Op-C strains yield segregants, which are either a_1 in mating type (both diploids and haploids) or haploids that produce mating tubes constitutively. The op-C phenotype arises after alterations that affect the a_2 allele in a_1/a_2 diploids and that therefore change the development of these cells. Op-C and other opaque-derivative forms provide valuable strains to study the action of the mating type locus as a developmental master switch.

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XVIII. Departamento de Genetica, Facultad de Biologia, Universidad de Sevilla, Apartado 1095, Sevilla, Spain. Communicated by Tahia Benitez.

The following papers have been published recently:

1. Andres Aguilera and Tahia Benitez. 1985. Role of mitochondria in ethanol tolerance of Saccharomyces cerevisiae. Arch. Microbiol. 142:389-392.

The presence of active mitochondria and oxidative metabolism is shown to be essential to maintain low inhibition levels by ethanol of the growth rate (μ), fermentation rate (v) or respiration rate (ρ) of Saccharomyces cerevisiae wild type strain S288C. Cells which have respiratory metabolism show K_i (ethanol inhibition constant) values for μ , v , and ρ , higher ($K_i > 1M$) than those of "petite" mutants or "grande" strains grown in anaerobiosis ($K_i = 0.7 M$). In addition, the relationship between μ or v and ethanol concentration is linear in cells with respiratory metabolism and exponential in cells lacking respiration. When functional mitochondria are transferred to "petite" mutants, the resulting strain shows K_i values similar to those of the "grande" strain and the inhibition of μ and v by increasing ethanol concentrations becomes linear.

2. Juan Jiménez and N. van Uden. 1985. Use of Extracellular Acidification for the Rapid Testing of Ethanol Tolerance in Yeast. Biotechnol. Bioeng. 27:1596-1598.

The exponential constant of ethanol-enhanced passive proton diffusion across the plasma membrane of yeast can be estimated rapidly and easily. It occurred to us that this might constitute a rapid method for the preliminary evaluation of ethanol tolerance in yeasts, such as would be convenient to use when large numbers of mutants and other strains have to be screened. To test its feasibility we verified, using a number of strains, whether a correlation existed between the values of the exponential constant (k) of ethanol-enhanced proton influx and the ethanol concentration (K_i) that reduced the specific growth rate by 50%.

3. Andres Aguilera and Tahia Benitez. 1986. Ethanol-sensitive mutants of Saccharomyces cerevisiae. Arch. Microbiol. 143:337-344.

Saccharomyces cerevisiae mutants unable to grow at ethanol concentrations at which the wild type strain S288C does grow, have been isolated. Some of them show additional phenotypic alterations in colony size, temperature sensitivity and viability in ethanol, which cosegregate with the growth sensitivity in ethanol. 21 selected monogenic ethanol-sensitive mutants define 20 complementation groups, denominated ETA1 to ETA20, which indicates that there is a high number of genes involved in the ethanol tolerance/sensitivity mechanism.

Out of 21 selected monogenic mutants, 20 are not altered in the glycolytic pathway since, when maintained in glucose-supplemented medium, they can produce as much ethanol as the wild type and at about the same velocity. Nor do any of the mutants seem to be altered in the lipid biosynthetic pathway since, whether grown in the absence or in the presence of ethanol, their

concentration of fatty acids and ergosterol is similar to that of the wild type under the same conditions. Therefore, growth sensitivity to ethanol does not seem necessarily to be related to carbohydrate or lipid metabolism.

4. Alfonso Pina, Isabel L. Calderon and Tahia Benitez. 1986. Intergeneric Hybrids of *Saccharomyces cerevisiae* and *Zygosaccharomyces fermentati* Obtained by Protoplast Fusion. Appl. Environ. Microbiol. in press.

To obtain strains that are able to efficiently produce ethanol from different carbohydrates, mainly cellulose hydrolysates, several species of the genus *Candida* and a *Zygosaccharomyces fermentati* strain were examined for their ability to utilize cellobiose and produce ethanol, as well as for their thermotolerance and the possibility of genetic manipulation. *Candida obtusa* and *Zygosaccharomyces fermentati* tolerated the maximal temperature for growth, possessed the highest cellobiase activity, and offered the possibility of genetic manipulation although neither of them proved to be a good producer of ethanol. Intergeneric hybrids of *Saccharomyces cerevisiae* and *Z. fermentati* were obtained after protoplast fusion. They were selected as prototrophic strains, after isolation of auxotrophic mutants from *Z. fermentati* and fusion with an *S. cerevisiae* strain which was also auxotrophic. The hybrids, which appeared at a frequency of 2×10^{-7} presented characteristics of both parents, such as resistance to certain drugs and the ability to grow with either cellobiose or lactic acid as the sole carbon source; they were very stable, even under nonselective conditions. These hybrids may have important industrial applications as good fermenting strains.

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- XIX. Albert-Ludwigs-Universität Freiburg Im Breisgau, Medizinische Fakultät, Biochemisches Institut. Communicated by Helmut Holzer.

Below follows an abstract of a paper which is scheduled for the March 1987 issue of the Journal of Biological Chemistry.

Doris Horn¹ and Helmut Holzer^{1,2}. Fructose-1,6-bisphosphatase(4P) Dephosphorylating Protein-Phosphatase from *Saccharomyces cerevisiae*. Journal of Biological Chemistry, March 1987.

- 1) Biochemisches Institut der Universität Freiburg, D-7800 Freiburg i. Br. (West Germany).
- 2) Gesellschaft für Strahlen- und Umweltforschung, Geschäftsführung, D-8042 Neuherberg (West Germany).

Phosphorylation of fructose-1,6-bisphosphatase with cyclic AMP-dependent protein kinase from yeast is accompanied by a 50% decrease in the catalytic activity (G. Pohlig and H. Holzer (1985) J. Biol. Chem. 260, 13818-13823). Using reactivation of phosphorylated fructose-1,6-bisphosphatase as assay, a protein phosphatase was about 2,000-fold purified to electrophoretic homogeneity from *Saccharomyces cerevisiae*. Upon incubation with phosphorylated fructose-1,6-bisphosphatase the purified protein phosphatase not only reverses the 50% inactivation caused by phosphorylation, but also the previously observed change in the pH optimum and in the ratio of activity with

Mg²⁺ or Mn²⁺. The phosphatase is strongly inhibited by heparin and fluoride. L-Carnitine, orthophosphate, pyrophosphate and succinate inhibit to 50% at concentrations from 1-10 mM. The molecular mass of the native phosphatase was found to be 180,000 Da. SDS-gel electrophoresis suggested four subunits with a molecular mass of 45,000 Da each. Half maximal activity was observed with 5 mM Mg²⁺ or Mn²⁺, the pH-optimum of activity was found at pH 7. Using polyclonal antibodies disappearance of ³²P-labeled fructose-1,6-bisphosphatase and concomitant liberation of the expected amount of inorganic ³²P phosphate was demonstrated.

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XX. Chaire de Génétique, et de Microbiologie, Ecole Nationale Supérieure Agronomique, 34060 Montpellier Cedex, FRANCE. Communicated by Pier Galzy.

The following are articles recently published by workers in my laboratory.

GONDE P., RATOMAHENINA R., ARNAUD A., and P. GALZY. 1985.
Purification and properties of the exocellular β -glucosidase of Candida molischiana (ZIKES) MEYER and YARROW capable of hydrolyzing soluble cellodextrins.
Can J. Biochem. Cell Biol. (Can) 63:1160-1166.

LECLERC M., BLONDIN B., RATOMAHENINA R., ARNAUD A., and P. GALZY. 1985.
Selection and study of Dekkera intermedia and Candida wickerhamii mutants derepressed for β -glucosidase production.
FEMS Microbiol. Lett. (GBR) 30:389-392.

LECLERC M., ARNAUD A., RATOMAHENINA R. ET GALZY P. 1986.
Etude et amélioration génétique d'une souche de levure productrice de β -glucosidase.
Microbiologie - Aliments - Nutrition (Fr) 4:7-18.

LECLERC M., ARNAUD A., RATOMAHENINA R., and P. GALZY. 1986.
Physical and kinetic properties of the cellodextrinase and of the β -glucosidase of Candida wickerhamii. Regulation of their biosynthesis.
Xith International Specialized Symposium on Yeasts. Lisbon (Portugal), March 17-21.

GONDE P., LECLERC M., ARNAUD A., RATOMAHENINA R. and GALZY P. 1986.
Physical and kinetic properties of the exocellular β -glucosidase of Candida molischiana. Regulation of its biosynthesis.
Xith International Specialized Symposium on Yeast. Lisbon (Portugal), March 17-21.

LECLERC M., ARNAUD A., RATOMAHENINA R., and P. GALZY. 1986.
Genetic improvement of a strain of Candida wickerhamii by selection of mutants derepressed for β -glucosidase production.
Fifth International Symposium on the Genetics of Industrial Microorganism. Split (Yugoslavia), September 14-20.

LECLERC M., RATOMAHENINA R., ARNAUD A., and P. GALZY. 1986.
Sélection et amélioration de souches de levure pour la production de β -glucosidases.
Congrès 1986 de l'Association Tessier A. : Microbiologie - Biotechnologie - Nutrition, Paris 5-6 mars 1986.

J.M. MUDERHWA, R. RATOMAHENINA, M. PINA, J. GRAILLE and P. GALZY. 1986.
Purification and properties of the lipases from Rhodotorula pilimanae Hedrick and Burke. Appl. Microbiol. Biotechnol. 23:348-354.

D. MONTET, R. RATOMAHENINA, J.M. LABORBE, M. PINA, J. GRAILLE and P. GALZY.
Production de protéines d'organismes unicellulaires à partir de pâtes de neutralisation d'origine industrielle.
Oléagineux, 40, n° 10 (1985).

P. GALZY, R. RATOMAHENINA, J. GRAILLE et M. PINA. 1986.
Microbiologie appliquée à l'industrie des lipides.
Revue Française des Corps Gras - 33ème Année - n° 8-9. August-September.

RATOMAHENINA R., M. MUDERHWA, D. MONTET, M. PINA, J. GRAILLE et P. GALZY. 1986.
Study of lipases from various species of lipolytic yeasts.
XIth International Specialized Symposium on Yeasts. Lisbon-Portugal - March 17-21.

MALFAIT M.H., MOULIN G., GALZY P. 1986.
Ethanol inhibition of growth, fermentation and starch hydrolysis in Schwanniomyces castellii. J. Ferment. Technol. 69:279-284.

MOULIN G., POINSOT C. et P. GALZY. 1985.
Study of the respiratory and fermentative metabolism of Schwanniomyces castellii. Xth ISSY - Genetics and Molecular Biology - Varna - Bulgaria.

MOULIN G., POINSOT C., H. BOZE and P. GALZY. 1986.
Antimycin A and hydroxamate insensitive respiration in Schwanniomyces castellii. XI ISSY - Lisbon - Portugal.

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XXI. Instituto de Investigaciones Biomedicas del C.S.I.C., Facultad de Medicina de la Universidad Autonoma, Arzobispo Morcillo, 4, 28029 Madrid, Spain. Communicated by Carlos Gancedo and Rosario Lagunas.

The following are abstracts of recent publications from our Institute.

1. Francisco Portillo and Carlos Gancedo. 1986. Purification and properties of three intracellular proteinases from Candida albicans. Biochimica et Biophysica Acta 881:229-235.

Three intracellular proteinases termed A, B and C were purified to homogeneity from the unicellular form of the yeast Candida albicans. Enzyme A is an aspartic proteinase that acts on a variety of proteins. Its optimal pH is around 5 and it is displaced to 6.5 by KSCN. It is not significantly inhibited by PMSF, TLCK (Tos-Lys-CHCl₂) or soybean trypsin inhibitor but it is inhibited by pepstatin. Its molecular weight is 60000. Enzyme B is a

dipeptidase that acts on esters or on dipeptides without blocks in either the carboxyl or amino ends. Its pH optimum is around 7.5 and the molecular weight is 57000. It is inhibited by PMSF, TLCK and DANME (N₂Ac-Nle-OMe). Proteinase C is an aminopeptidase with an optimum pH around 8. Its molecular weight was 67000 when determined by SDS gel electrophoresis and 243000 when determined by gel filtration. It is active towards dipeptides in which at least one amino acid is apolar and is not active when the N-terminal amino acid is blocked. It is inhibited by EDTA or o-phenanthroline and activated by several divalent cations.

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2. Carlos Gancedo, Antonio Llobell, Juan-Carlos Ribas¹ and Francisca Luchi. 1986. Isolation and characterization of mutants from Schizosaccharomyces pombe defective in glycerol catabolism. Eur. J. Biochem. 159:171-174.

¹Instituto de Microbiologia Bioquímica del Consejo Superior de Investigaciones Científicas, Universidad de Salamanca.

Mutants unable to grow on glycerol were isolated from the fission yeast Schizosaccharomyces pombe. Two types of mutants were obtained: one type was able to grow on dihydroxyacetone while the other one did not grow on this compound. The first type of mutants was defective in glycerol dehydrogenase while the second one was affected both in the glycerol dehydrogenase and in dihydroxyacetone kinase. It was found that the second type was defective in the derepression of several enzymes. The mutations were nuclear and monogenic and defined two complementation groups. Spontaneous revertants, able to grow on glycerol, were obtained from the first type of mutants. They have regained the glycerol dehydrogenase activity. The results presented provide genetic evidence for a pathway of glycerol catabolism in Sch. pombe involving dehydrogenation of glycerol as the first step followed by phosphorylation of the dihydroxyacetone formed.

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3. Rosario Lagunas. 1986. Misconceptions About the Energy Metabolism of Saccharomyces cerevisiae. Yeast 2: (in press).

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Pasteur effect can be observed under special experimental conditions
Mechanism of appearance of Pasteur effect in resting cells: inactivation of the sugar transport system
Fermentation is more sensitive than respiration to changes in sugar uptake rate

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4. Ana Busturia and Rosario Lagunas. 1986. Catabolite Inactivation of the Glucose Transport System in Saccharomyces cerevisiae. J. of Gen. Microbiol. 132:379-385.

The sugar transport systems of Saccharomyces cerevisiae are irreversibly inactivated when protein synthesis is inhibited. This inactivation is responsible for the drastic decrease in fermentation observed in ammonium-starved yeast and is related to the occurrence of the Pasteur effect in these cells. Our study of the inactivation of the glucose transport system indicates that both the high-affinity and the low-affinity components of this system are inactivated. Inactivation of the high-affinity component evidently requires the utilization of a fermentable substrate by the cells, since (i) inactivation did not occur during carbon starvation, (ii) when a fermentable sugar was added to starved cells, inactivation began, (iii) when the fermentation inhibitors iodoacetate or arsenate were added in addition to sugars, the inactivation was prevented, (iv) when a non-fermentable substrate was added instead of sugars, inactivation was also prevented. The inactivation of the low-affinity component appeared to show similar requirements. It is concluded that the glucose transport system in S. cerevisiae is regulated by a catabolite-inactivation process.

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5. Carmen DeJuan and Rosario Lagunas. 1986. Inactivation of the galactose transport system in Saccharomyces cerevisiae. FEBS 207(2): October 258-261.

The galactose transport system of Saccharomyces cerevisiae consists of one component which shows a K_m value of approx. 4 mM in growing cells. A rapid and irreversible inactivation of this transport is detected on impairment of protein synthesis. This inactivation shows the following characteristics: (i) it is due to changes in the K_m and V_{max} of the transport system; (ii) it follows first-order kinetics, (iii) it is an energy-dependent process and is stimulated by the presence of an exogenous carbon source; (iv) fermentable substrates stimulate inactivation more efficiently than non-fermentable substrates.

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6. Rosario Lagunas, Carmen DeJuan, and Begona Benito. 1986. Inhibition of Biosynthesis of Saccharomyces cerevisiae Sugar Carriers by Tunicamycin. J. Bacteriol. 168(3) in press.

Tunicamycin apparently inhibited the biosynthesis of glucose, galactose, and maltose transport systems in Saccharomyces cerevisiae. Under the conditions used, the antibiotic also blocked the biosynthesis of invertase, a well-known yeast glycoprotein, as well as the glycosylation of a marker mannoprotein of the yeast cell wall. However, the antibiotic did not affect certain proteins which did not contain carbohydrate. It seems, therefore, that these sugar carriers are glycoproteins.

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XXII. Delft University of Technology, Department of Microbiology & Enzymology, Julianalaan 67, NL-2628 BC Delft, The Netherlands.
Communicated by W. Alexander Scheffers.

The following papers, abstracts of which have already appeared in Yeast Newsletter Vol. XXXV, Number I, now have been published.

1. J.P. van Dijken and W.A. Scheffers. Redox balances in the metabolism of sugars by yeasts. FEMS Microbiology Reviews 32 (1986) 199-224.
2. P.M. Bruinenberg, G.W. Waslander, J.P. van Dijken and W.A. Scheffers. A comparative radiorespirometric study of glucose metabolism in yeasts. YEAST 2 (1986) 117-121.
3. J.P. van Dijken, E. van den Bosch, J.J. Hermans, L. Rodrigues de Miranda and W.A. Scheffers. Alcoholic fermentation by 'non-fermentative' yeasts. YEAST 2 (1986) 123-127.
4. C. Purwin, K. Nicolay, W.A. Scheffers and H. Holzer. Mechanism of control of adenylate cyclase activity in yeast by fermentable sugars and carbonyl cyanide *m*-chlorophenyl-hydrazone. Journal of Biological Chemistry 261 (1986) 8744-8749.

Also, the following two papers have appeared.

5. P. de Jonge, F.C.M. de Jongh, R. Meijers, H.Y. Steensma and W.A. Scheffers. Orthogonal-field-alternation gel electrophoresis banding patterns of DNA from yeasts. YEAST 2 (1986) 193-204.

Abstract. Chromosomal DNAs from various yeast species were separated by orthogonal-field-alternation gel electrophoresis (OFAGE). To this end we developed a spheroplasting and lysis method to obtain intact DNA from both ascomycetous and basidiomycetous yeasts. The OFAGE banding patterns of 22 ascomycetous and four basidiomycetous yeast strains were compared. The strains represented species from the genera: Brettanomyces, Candida, Cryptococcus, Filobasidiella, Geotrichum, Hansenula, Kluyveromyces, Pachysolen, Pichia, Rhodosporidium, Rhodotorula, Saccharomyces, Saccharomycodes, Saccharomycopsis, Schizosaccharomyces and Zygosaccharomyces. Variations occurred in the number of bands and their positions in the gel, not only among strains of different genera but also among species from the same genus and even between varieties of the same species. The ascomycetous yeasts, with the exception of Saccharomyces cerevisiae, only showed one to five bands of DNA larger than 1000 kilobase pairs (kb) in general none smaller. The patterns of the four basidiomycetous yeasts revealed also a few large DNA bands but in addition one to six bands ranging in size from 500 to 1000 kb, with the exception of a single smaller chromosome in Rhodotorula mucilaginosa. From the OFAGE banding patterns of strains studied here it appears that in Sacch. cerevisiae the partitioning of DNA over chromosomes is unique. But rather than the large number of chromosomes, the presence of four chromosomes with less than 500 kb of DNA is characteristic of Sacch. cerevisiae.

6. P.M. Bruinenberg. The NADP(H) redox couple in yeast metabolism. *Antonie van Leeuwenhoek* 52 (1986) 411-429.

Abstract. Theoretical calculations of NADPH requirement for biomass formation indicate that in yeasts this parameter is strongly dependent on the carbon and nitrogen sources used for growth. Enzyme surveys of NADPH-generating metabolic pathways and radiorespirometric studies demonstrate that in yeasts the HMP pathway is the major source of NADPH. Furthermore, radiorespirometric data suggest that in yeasts the HMP pathway activities are close to the theoretical minimum. It may be concluded that the mitochondrial NADPH oxidation, which in yeasts may yield ATP, is quantitatively not an important process.

The inability of *C. utilis* to utilize the NADH produced in formate oxidation as an extra source of NADPH strongly suggests that transhydrogenase activity is absent. Furthermore, the absence of xylose utilization under anaerobic conditions in most facultatively fermentative yeasts indicates that also in these organisms transhydrogenase activity is absent. This conclusion is supported by the observation that anaerobic xylose utilization is observed only in those yeasts which possess a high activity of an NADH-linked xylose reductase. Hence in these organisms the redox-neutral conversion of xylose to ethanol is possible, since the second step in xylose metabolism is mediated by an NAD⁺-linked xylitol dehydrogenase.

7. On November 21, 1986, Dr. P.M. Bruinenberg (now at AVEBE, Foxhol, The Netherlands) has received the Kluiver prize 1986 of the Netherlands Society for Microbiology, during a meeting of the Society at Amsterdam.

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XXIII. Laboratory of Biochemistry and Physiology of Yeasts, Department of Enzyme Engineering, Institute of Microbiology, Czechoslovak Academy of Sciences, Videňská 1083, 142 20 Prague 4, Czechoslovakia.
Communicated by Ā. Novotný.

The following are the summaries of two papers from this laboratory which have been submitted for publication.

1. Ā. Novotný, B. Běhalová, L. Doležalová and J. Zajiček. 1987. Regulation of sterol synthesis by glucose in baker's yeast. *Acta Biotechnologica* 7:4, in press.

The ability of ten baker's yeast strains to synthesize sterols was checked. Ergosterol/24 (28)-dehydroergosterol (E/D) ratio had the value of 0.8 to 1 in most strains grown in a medium containing molasses as the carbon source. The ratio values were significantly increased in the cultures grown in a glucose medium. Both a decrease in the content of 24 (28)-dehydroergosterol and a slight increase of the ergosterol content were found to be responsible for the high values of E/D in the glucose-grown cells. The strains examined could be divided into three groups on the basis of their behaviour towards glucose. The effect of the type of cultivation on sterol accumulation is demonstrated by comparing the sterol content of the representatives of the three groups of baker's yeasts in the cells grown in a fermenter or in shaken flasks.

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2. Č. Novotný, B. Běhalová, B. Stružinský, M. Novák and J. Zajiček. Sterol composition of a $\Delta^{5,7}$ -sterol-rich strain of Saccharomyces cerevisiae during batch growth. Submitted for publication.

Sterol composition was examined during batch growth on a complex medium containing ethanol, molasses or glucose as the carbon source. The molasses-grown cells exhibited a balanced sterol composition throughout growth, maintaining the proportion of ergosterol/24:28-dehydroergosterol equal to 1.4. The negative effect of glucose (90 g per L) on sterol synthesis manifested itself by decreasing the accumulation of 24:28-dehydroergosterol and total sterols but not of ergosterol. Using ethanol as the sole carbon source, a large amount of 24:28-dehydroergosterol accumulated, partly on the account of other sterols. The intermittent addition of nitrogen source during growth significantly decreased the accumulation of ergosterol, 24:28-dehydroergosterol and of total sterols. The general scheme of regulation of sterol synthesis in the baker's yeast is discussed.

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

The following papers are in press or have been published since the last Yeast Newsletter.

1. Gregory P. Casey and W.M. Ingledew. 1986. Ethanol Tolerance in Yeasts. CRC Crit. Rev. in Microbiology 13(3):219-280.

The past 2 decades have seen an enormous increase in interest in the ethanol-tolerance properties of yeasts. Many recent findings, however, especially with brewing and wine yeasts, contradict the long-held belief that industrial strains of Saccharomyces yeasts vary considerably in their ability to tolerate ethanol. Of particular importance is the discovery that the inherent ethanol tolerance of any specific yeast strain is not the only factor regulating the maximum level of ethanol that can be produced in industrial alcoholic fermentations. This review, therefore, begins with an analysis of the methods used to define and measure ethanol tolerance. Subsequently, research exploring the mechanisms of ethanol toxicity, the influences of nutritional and environmental parameters, the role of yeast lipid composition, and the genetics of ethanol tolerance will all be explored. When these factors are all considered, the recent literature challenges many of the traditional views of ethanol tolerance in yeasts and should serve as an instrument to significantly improve the economics of ethanol-related industrial fermentations.

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2. W.M. Ingledew and N. Hazen. 1986. Active Dry Yeasts for Small Brewers. The New Brewer 3(4):1,14,15.

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3. W. M. Ingledew, F.W. Sosulski, and C.A. Magnus. An Assessment of Yeast Foods and Their Utility in Brewing and Enology. J. Amer. Soc. Brew. Chem. (in press).

Yeast foods are occasionally utilized in high-gravity brewing and enology to eliminate sluggish or stuck fermentations that may occur in these industries as a result of a deficiency of a key yeast nutrient such as low-molecular-weight nitrogen. Such foods are especially important when the carbohydrate content in the wort or juice is over 16%. Many yeast foods are now available commercially. In many cases, however, the brewer or enologist is unaware of the composition of the food, the purpose for which it was designed, how well it will serve as a nutrient for the yeast, or even the legality of its usage. This report identifies the major yeast-utilizable nitrogen sources in 15 such yeast foods and the levels of sulfate, phosphate, and major cations in each.

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4. C.A. Magnus, W.M. Ingledew, and G.P. Casey. High Gravity Brewing: Influence of High-Ethanol Beer on the Viability of Contaminating Brewing Bacteria. J. Amer. Soc. Brew. Chem. (in press).

It has been reported that when high-gravity brewers' worts were supplemented with a source of nitrogen and unsaturated lipids and sterol, ethanol concentrations up to 16.4% v/v could be achieved within normal fermentation times. As the resultant harvested yeast can be repitched over a number of generations, there appears to be no reason in industry to limit the gravities of commercial worts to 16% Plato, especially when the ester and fusel oil patterns of resultant beers may not be as elevated as previously thought. In this report the influence of fermenting high alcohol beer made from 28^o Plato wort on the viability of traditional bacterial brewing contaminants was examined. Lactobacillus and Pediococcus, Acetomonas, Acetobacter, and Zymomonas contaminants were able to survive levels of ethanol of 12-13% v/v. Hafnia (Flavobacterium or Obesumbacterium), Enterobacter, (including E.agglomerans), Citrobacter, and Klebsiella species, known to exist through most stages of the fermentation of traditional gravity wort, were completely eliminated by the elevated ethanol levels. Very high gravity fermentations therefore narrow the range of bacteria capable of spoiling the beer, thereby reducing the risk of bacterial spoilage problems. This work illustrates an additional production advantage in favor of increasing original gravities of worts.

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5. W.M. Ingledew. Schwanniomyces: A Potential Superyeast. CRC Crit. Rev. in Biotechnol. (in press).

The emphasis of this research has been on Schwanniomyces, a starch degrading-alcohol producing yeast. Its amylolytic capabilities have been examined in detail including isolation and purification of an alpha-amylase and a glucoamylase. Schwanniomyces is one of a very few yeasts which produces both these enzymes as well as having some fermentative ability to make ethanol from distressed grains or potato starches. Derepressed wild type strains were isolated that produce 3 to 4 fold higher levels of both amylases. The

reported levels of enzymes, or more, will be required if this microbe is to become industrially important for alcohol production, for amylase production or for single cell protein. Conservative genetic techniques (protoplast fusion) have been used to examine intergeneric fusion of Saccharomyces and Schwanniomyces, and interspecific fusion of 2 Schwanniomyces species with different enzyme complements. The objective has been to create superamylolytic yeasts capable of rapid fermentation of starch to ethanol. Although we have not yet succeeded in production of stable strains useable in industry, with what is now known, suitable genetic work-up may lead to such a strain. It may also be useful for expression of foreign proteins. This work summarizes the first reports on the use of protoplast fusion in this genus and the derepression of amylase enzymes in Schwanniomyces. A number of laboratories in Canada and the world are now also examining this microbe in detail.

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6. G.P. Casey. 1986. Molecular and Genetic Analysis of Chromosome X in Saccharomyces carlsbergensis. Carlsberg Res. Commun. 51:343-362

Molecular hybridization of electrophoretically separated and blotted chromosomes with ILV3 gene probes detected the presence of three chromosomes X in S. carlsbergensis 244 lager yeast. Meiotic segregants, which were disomic or trisomic for chromosome X were crossed with a karl -1 strain in S. cerevisiae (MAT α or MAT α arg3 met3 ilv3 cycl-1 cdcl1 hom6 ade2-40 cyh2 karl-1) for single chromosome transfer and isolation of two of the chromosomes X from the lager yeast. Type I chromosome X migrates slower than a reference chromosome X of S. cerevisiae K5-5A during chromosome separation by electrophoresis and contains the allele of ILV3 unique to the lager yeast. It recombines with chromosome X from S. cerevisiae, but only in the left arm. Type II chromosome X migrates faster than the reference chromosomes X and contains the allele of ILV3 also found in S. cerevisiae S288C. It recombines only in the right arm with chromosome X from S. cerevisiae. The third chromosome X from S. carlsbergensis, which co-migrates with the reference chromosome X of S. cerevisiae K5-5A, has yet to be isolated into the genetic background of a genetic standard strain.

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7. G.P. Casey. 1986. Cloning and Analysis of Two Alleles of the ILV3 Gene from Saccharomyces carlsbergensis. Carlsberg Res. Commun. 51:327-341.

A genomic library of a Saccharomyces carlsbergensis lager's yeast DNA was constructed in the yeast E. coli shuttle vector YRp17. Two alleles of the ILV3 were cloned from the library by complementation of the ilv3-12 mutation in strains of Saccharomyces cerevisiae yeast. Restriction site mapping and Southern hybridisation using an ILV3 probe from Saccharomyces cerevisiae S288C revealed one allele from the lager yeast to be closely related, or identical, to the ILV3 gene in S. cerevisiae S288C. The second allele has a different restriction site map and limited sequence homology with the ILV3 gene in S. cerevisiae S288C. The implications of these results in determining the genetic constitution of lager yeast and on research programs designed to genetically engineer lager yeast are discussed.

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XXV. National Research Council Canada, Molecular Genetics Section, Ottawa, Canada KIA OR6. Communicated by G.B. Calleja.

The following publications appeared recently:

1. G.B. Calleja, A. Nasim, C.V. Lusena, and S. Levy-Rick. 1986. Excretion of yeast amylases: requirement for oxygen and mitochondrial function. *Biochemistry International* 12:81-87.

Extracellular amylases are not detectable in hypoxic cultures of the starch-fermenting yeast Schwanniomyces alluvius. When aerobic cultures in the process of excretion after the end of exponential growth are made anoxic, excretion is immediately arrested. Respiratory inhibitors, such as cyanide and azide, have the same effect as anoxia. Grown on ethidium bromide for three generations, a culture exhibits no detectable extracellular amylolytic activity. This culture recovers full extracellular amylolytic activity only after the second sequential transfer in fresh medium without ethidium bromide. A dissolved-oxygen concentration of 10% saturation is necessary for the excretion of α -amylase and glucoamylase.

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2. G.B. Calleja, M. Yaguchi, S. Levy-Rick, J.R.H. Seguin, C. Roy, and C.V. Lusena. 1986. Single-cell protein production from potato starch by the yeast Schwanniomyces alluvius. *Journal of Fermentation Technology* 64:71-75.

Fully aerated cultures of Schwanniomyces alluvius grew on 4% soluble potato starch in a defined minimal medium at a doubling time of 1.5 h at 30°C. A recovery of 51% (0.51 g of dried biomass from 1 g of starch) was obtained. Yields and growth rates of cultures on starch were similar to those on glucose.

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3. G.B. Calleja, Susan Levy-Rick, F. Moranelli and A. Nasim. 1984. Thermosensitive Export of Amylases in the Yeast Schwanniomyces aluvius. *Plant & Cell Physiol.* 25:757-761.

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

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4. In Press:

- (a) G.B. Calleja. Cell aggregation. The Yeasts, 2nd Ed. (A.H. Rose & J.S. Harrison, eds.), Vol. 2, Chapter 7. Academic Press, London.
- (b) G.B. Calleja, S. Levy-Rick, A. Nasim & C.V. Lusena. Extracellular amylases of starch-fermenting yeast: pH effect on export and residence time in the periplasm. CRC Critical Reviews in Biotechnology 5 (2).

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XXVI. VTT Biotechnical Laboratory, Tietotie 2, SF-02150 Espoo 15, Finland. Communicated by Veijo Mäkinen.

J. Kronlöf, Hartwall Ltd. 1986. Optimizing of yeast handling in a brewery. Mallas ja Olut 1986, 22-27.

Yeast handling in a brewery is, like brewing in general, a synthesis of knowledge and traditions. In a well equipped control laboratory it is, however, to a certain extent possible to examine yeast and yeast handling and to apply the results in practice.

An example is presented, in which a clear connection between pitching rate and fermenting velocity was observed. Through a stepwise optimization of the yeast handling practice hanging fermentations could be eliminated and the average primary fermentation time was cut down by about 10 percent. This enabled a better process control and had a positive effect on the beer quality.

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XXVII. ALKO Research Laboratories, P.O. Box 350, SF-00101, Helsinki 10, Finland. Communicated by M. Korhola.

The following is a list of our work published since June 1986.

1. Matti Korhola and Kari Edelmänn. 1986. Metabolism of Trace Elements Related to Human Diseases. Nordic Symposium, Loen, Norway, 1985, Acta Pharmacol. Toxicol. 59, Suppl. VII, 148-151.

Baker's yeast grown under special conditions has been enriched with trace elements of dietary importance. Compared with yeast cultivated normally with beet molasses cells can be enriched with 0.5 mg selenium (about 12,000 times), 1.0 mg chromium (14,000 times) and 3.0 mg zinc (18 times) calculated per gram dry weight of yeast.

The dietary importance of these enriched yeasts is based on the argument that organically bound trace elements are physiologically more effectively utilized than inorganic ones.

Elements inextractable from yeast into water or dilute acid have in literature been regarded as organic forms. About 95% of Se, 93% of Cr and 56% of Zn could not be extracted from the enriched yeast cells by water. In acid treatment about 82% of Se, 73% of Cr and 37% of Zn were found to be organically bound.

To discover the distribution of trace elements in cell compartments, enriched yeasts were fractionated by disintegrating the cells, differential centrifugation and TCA precipitation of soluble proteins.

Of total selenium 15% was found in the cell walls, 9% in mitochondria and microsomes, 58% in soluble proteins and nucleic acids precipitated with TCA and, finally, 19% in the soluble fraction of small peptides, amino acids and inorganic acids.

Of total chromium about 70% was found particle-bound in the cell walls, mitochondria and microsomes, and the rest evenly distributed to soluble proteins and nucleic acids and to compounds of small molecular weight.

One half of the total zinc was bound to the cell walls and the other half to small soluble compounds.

One half of the total zinc was bound to the cell walls and the other half to small soluble compounds.

In the fractionation of enriched yeast selenium was mainly found in soluble proteins probably replacing some of the sulphur in the amino acids. Chromium and zinc possibly form complexes with yeast organic matter.

* * *

2. Roy S. Tubb. 1986. Amylolytic Yeasts For Commercial Applications. Trends in Biotechnology 4, 98-104.

Abstract

Significant recent progress has been made in constructing amylolytic strains of yeast and demonstrating their potential for a range of applications, particularly in ethanol or alcoholic beverage production. Nature offers a considerable assortment of amylolytic enzymes, and through recombinant DNA technology the merits of enzyme-producing yeasts for converting starch can now be compared directly with those of "added" enzymes. Over the next few years a number of amylolytic strains seem likely to find their way into commercial use.

* * *

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

The following are summaries of a book and papers recently published, accepted, or submitted for publication in 1986:

1. E. Minarik and A. Navara: Chemistry and Microbiology of Wine (in Slovak). [Russian, French and German summaries]. Priroda, Bratislava 1986, 547 p., Kcs 50.-

Important yeasts, bacteria and mold species having positive or negative influence on wine making, are thoroughly described. The ecology, physiology and biochemistry of yeasts most frequently occurring on grape berries, in spontaneously fermenting grape musts or causing wine spoilage are dealt

with. The biochemistry of alcoholic and malolactic fermentation are explained as well as the prevention of frequently occurring wine diseases caused by yeasts and bacteria are proposed. Chemical analysis of the wine and microbiological procedures in the identification of yeasts, bacteria and molds in wine are given in detail.

* * *

2. E. Minárik: Contribution to the identification of Zygosaccharomyces bailii (Lindner) Guilliermond (in Slovak). Vinohrad (Bratislava). Accepted for publication.

Zygosaccharomyces bailii belongs to the most widespread yeasts causing wine spoilage (haze, refermentation of sweet wines). A standard description of this osmo- and chemotolerant fructophilic yeast is given. Characteristic features of this species are described and an express method for their identification and classification is proposed.

* * *

3. E. Minárik: Determination of yeast number in wine by membrane filtration (in Slovak). Vinohrad (Bratislava) 24, 1986 (in press).

Principles of standard yeast counts by the membrane filtration method in wine control laboratories are described. The advantage of this method compared with the routine plating is explained.

* * *

4. E. Minárik, O. Jungová: Activation of alcoholic fermentation by biologic and non-biologic means. Kvasny prumysl (Prague).

Microcrystalline cellulose shows, similarly as yeast wall preparations and the activator Botrytis cinerea, a stimulating effect on the fermentation activity of the yeast Saccharomyces oviformis in grape must even under unfavorable fermentation conditions. The stimulating effect is evident by a more profound fermentation, higher alcohol content and lower volatile acid formation in the wine. This effect is markedly higher in musts with high sugar concentration.

* * *

5. The following is the summary of a paper submitted for publication to "Die Wein-Wissenschaft" (Wiesbaden), GFR, in 1986:

F. Malík, E. Michalčáková and E. Minárik: Acidification properties of active dry wine yeasts (in German). Wein-Wissenschaft 1987.

The reliable determination of the metabolism of active dry wine yeast preparations enables the estimation of acidification properties. The decrease of extracellular pH has been tested in 9 preparations of active dry wine yeasts. The highest decrease of extracellular pH was found in the preparation BLASTOSEL MV ($\Delta\text{pH}_{20} = 1.13$). The method of estimating acidification by the start and rate of metabolism after rehydration may be well used as a supplement to the characterization of such preparations.

* * *

XXIX. Department of Medicine E-5, Div. of Infectious Diseases, Harbor-UCLA Medical Center, Torrance, California 90509. Communicated by Marjorie Crandall.

Management of Allergic Candidiasis

DIAGNOSIS

History of Predisposing Factors: an allergic diathesis plus antibiotics - "Flagyl: and broad spectrum antibacterials, or steroids - cortisone and hormones. Also pregnancy, diabetes, and other immuno-deficiency states.

Mucosal Signs and Symptoms: mouth - bitter taste, burning of tongue, gums and lips, thrush patches; intestine - constipation, less frequently diarrhea, adverse food reactions, post-prandial tiredness or other mental symptoms, acid stomach, gas, peri-anal itching; vagina - recurrent yeast vaginitis, vulvar itching, burning, dysuria, dyspareunia, white cheesy discharge.

Medi-Trend Blood Test: Call 1-800-545-8900 to order the kit. A score of >140 is diagnostic for chronic/allergic candidiasis.

TREATMENT

"Nizoral" - oral ketoconazole

Liver Panel: must be tested before administration of Nizoral. There have been some deaths due to idiosyncratic hepatitis (read the Physicians Desk Reference in the library or doctor's office).

(Note: Nystatin is less toxic but also less effective)

Desensitization Injections with Candida Antigen = Immunotherapy

Diet Free of Yeasts and Molds: found in fermented foods and beverages such as beer, wine, cheese, bread, vinegar, pickles, soy sauce, and on the surface of fruits such as grapes and berries, etc. Restrict ingestion of sugar.

Anti-Inflammatory Prescriptions

PREVENTION

Avoid Airborne, Food, and Contact Allergens: avoid yeasts and molds, and avoid vaginal contact with all chemicals including douches, creams, soaps, spermicides, perfume, chlorinated pools, etc.

Clothing: wear only unrestrictive, white, cotton underwear

Avoid Antibiotics, Steroids, and other Predisposing Factors. If they are unavoidable, then follow a regimen of:

Antifungal Prophylaxis plus Anti-Candida Desensitization. This disease is both an infection and allergy to the infective agent.

XXXI. Meetings

1. International course on "Isolation, Identification and Maintenance of Yeasts"

An international theoretical and practical course on the "Isolation, Identification and Maintenance of Yeasts" will be held in the Gulbenkian Institute of Science, Oeiras, Portugal from 10-28 August, 1987. The principal lecturers are Prof. J. P. van der Walt (Pretoria), Dr. D. Yarrow (Delft), Drs. M. Smith (Delft), Drs. T. Boekhout (Baarn) and Prof. N. van Uden (Oeiras). The number of participants is limited to twenty. Participants may be accepted from any part of the world, should have previous experience in yeast biology and be under 35 years of age.

Information and application-for-admission forms:

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

Financial Aspects

The course is entirely financed by the Calouste Gulbenkian Foundation and no fees are charged to the participants. Travel and living expenses are the responsibility of the participants however. Lunch may be taken at the Gulbenkian Institute of Science (Monday through Friday) at a subsidized price.

Housing

Successful applicants should make their own hotel reservations through a travel agency or otherwise. Many hotels are located at a convenient distance from the institute (Estoril, Cascais, Carcavelos or Lisbon). A number of single rooms with shower in a student hostel near the institute are available free of charge. Applicants interested in such a room should indicate this on their application-for-admission form.

Synopsis

Lectures

The following topics will be covered: Historical background, Nomenclature, Life cycles (Ascomycetous yeasts), Life cycles (Basidiomycetous yeasts), Morphology (LM), Morphology (EM), Biochemical background of fermentation and assimilation tests, Temperature relations, Other methods used in identification (nitrogen compounds, vitamins, osmotolerance, extracellular polysaccharides, urease, fat-splitting, ester production, DBB test, Coenzyme Q etc.), Methods for estimating relatedness (hybridization, nucleic acids, proteins), Classification of the ascomycetous yeasts, Classification of the basidiomycetous yeasts, Classification of the imperfect yeasts, Selective

isolation techniques, Maintenance of culture collections, Use of computers and software.

Laboratory Work

Each participant will have the opportunity to use selective techniques for the isolation of yeasts from nature.

Furthermore he/she will identify five yeasts belonging to different species applying the identification techniques currently in use. The results will be discussed and shared with the other participants so that each participant will become acquainted in the laboratory with one hundred different yeast species.

Deadline For Application

May 1, 1987

2. XIIth International Specialized Symposium on Yeast, "Genetics of Non-Conventional Yeasts", September 13-19, 1987, Weimar, German Democratic Republic, Chairmen: H. Weber, Jena, F. Böttcher, Greifswald.

First Announcement

The International Commission for yeast decided at its meeting in Bombay (1983) that the XIIth International Specialized Symposium on Yeasts will be held on September 13-19, 1987 in Weimar, GDR.

The Scientific Programme will focus on the genetics of non-conventional yeasts. Results of genetic research on Saccharomyces cerevisiae and Schizosaccharomyces pombe have been discussed during several recent yeast meetings. It is, therefore, the aim of this symposium, to bring together scientists working on the genetics of yeasts other than these two species and to discuss latest results and trends of genetic studies on these yeasts with emphasis on industrially important features. The scientific programme will comprise plenary lectures and original communications presented orally or as posters.

Proposed topics:

1. Life cycles, sexuality, sporulation, parasexuality
2. Genome structure, recombination, extrachromosomal elements
3. Gene transfer and cloning
4. Regulation of gene expression
5. Applications in biotechnology

The language of the symposium will be English. Accompanying guests are also welcome. Social programme will be organized both for active and accompanying guests.

Information: Prof. Dr. H. Weber
Central Institute of Microbiology
and Experimental Therapy of the
Academy of Sciences of the GDR.
Beutenbergstr. 11
GDR-6900 Jena, GDR

3. FEBS Advanced Course on "Biochemistry and genetics of yeasts". Jerez de la Frontera, Cadiz, Spain. 7-24 September 1987. The course will consist in lectures (three per day) and practical work (five to six hours per day). Lecturers: T. Benitez (Seville), G. Bernardi (Paris); J. Conde (Seville); G.R. Fink (Cambridge); C. Gancedo (Madrid); J.M. Gancedo (Madrid); T. Ishikawa (Tokyo); P. Niederberger (Zurich); C. Nombela (Madrid); P. Russell (London); R. Serrano (Heidelberg); D. Van Loon (Bale); N. Van Uden (Oeiras); D.H. Wolf (Freiburg). Info: Carlos Gancedo. Instituto de Investigaciones Biomedicas CSIC. Facultad de Medicina UAM; Arzobispo Morcillo 4, 28029 Madrid, Spain. Deadline of applications: April 15, 1987.

4. The 19th Annual Meeting of the Yeast Genetics and Molecular Biology Society of Japan was held from July 21st through 23rd, 1986 at the Kyodai Hall in Kyoto. The following sixty-six topics were presented and discussed in thirteen Sessions: Session I, Structure of Genes (topics 1-7); II, Membrane and Secretion (8-13); III, Cytoplasmic Factors and Plasmids (14-18); IV, Meiosis and Sporulation (19-25); V, Chromosomes (26-29); VI, Regulation of Genes (30-35); VII, Nuclei and Nucleoids (36-40); VIII, Enzyme and Proteins (41-43); IX, Biochemistry and Comparative Study (44-47); X, cAMP and ras (48-54); XI, Genetic Analysis (55-56); XII, Cell Structure (57-61); XIII, Cell cycle (62-66). The abstracts of these presentations will be published in Japanese as "Yeast Genetics and Molecular Biology News, Japan" at the end of 1986. Communicated by Masayuki Yamamoto, Laboratory of Molecular Genetics, Institute of Medical Science, University of Tokyo, P.O. Takanawa, Tokyo 108, Japan.
 1. K. Morino, T. Uemura, S. Uzawa and M. Yanagida (Dept. Biophys., Kyoto Univ.). Cloning and disruption of the gene coding for S. pombe DNA topoisomerase I.
 2. T. Uemura and M. Yanagida (Dept. Biophysics, Kyoto Univ.). Gene structure and functional domains of the fission yeast DNA topoisomerase II.
 3. T. Takeda, M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Structure and function of calmodulin gene in S. pombe.
 4. A. Yasui (Research Institute for Tuberculosis and Cancer, Tohoku University). Homology of DNA repair gene between yeast and human.
 5. M. Mizukami and F. Hishinuma (Mitsubishi-Kasei Institute of Life Sciences). Sequence analysis of the URA3 gene of Kluyveromyces lactis.
 6. K. Hamasawa, S. Harada, K. Yoda and M. Yamasaki (Dept. Agric. Chem., Tokyo Univ.). The β -isopropylmalate dehydrogenase gene of Candida utilis.
 7. S. Miyamoto, Y. Ohya, Y. Ohsumi and Y. Anraku (Dept. Biol., Univ. of Tokyo). Primary structure of the CLS4 gene.
 8. M. Fukushima, K. Kiyono, Y. Kushima, I. Shibuya and A. Ohta (Dept. Biochemistry, Saitama Univ.). Amplification and Subcellar Distribution in Saccharomyces cerevisiae.

9. T. Yoshihisa, Y. Ohsumi and Y. Anraku (Department of Biology, Faculty of Science, University of Tokyo). Two Isoforms of Alpha-Mannosidase on Vacuolar Membranes of Yeast.
10. T. Yamazaki and H. Nonomura (Dept. Ferment. Technol., Yamanashi Univ.). Reversion of protoplasts of wine yeast Sacch. cerevisiae OC-2 and soy sauce yeast Zygosacch. rouxii Y-8 in PEG liquid medium.
11. T. Mizunaga, M. Izawa and Y. Maruyama (Dept. Agric. Chem., Univ. of Tokyo). Properties of Nonglycosylated Repressible Acid Phosphatase Secreted from Saccharomyces cerevisiae in the Presence of Tunicamycin under Low Temperature.
12. M. Tokunaga, N. Wada and F. Hishinuma (Molecular Genetics, Mitsubishi-Kasei Institute of Life Sciences). Expression and secretion of the killer toxin encoded on yeast linear plasmids. 2. Analysis expression and secretion of killer toxin using gene fusion technique.
13. T. Suzuki, Y. Ohya, S. Miyamoto, Y. Ohsumi, Y. Anraku, T. Shiomori* and M. Kasahara* (Fac. of Sci., University of Tokyo, *Fac. of Med., Teikyo University). Cloning of yeast genes complementing defects in secretion and endocytosis.
14. Tadashi Mabuchi and Kazuhiko Wakabayashi (Dept. Biochem., Medical School, The University of Yamanashi). Essential Structure of Yeast Mitochondrial ARS.
15. A. Jearnpipatkul, H. Araki and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Mechanism of holding stability of yeast plasmid pSR1.
16. H. Matsuzaki, N. Nakanishi, H. Araki and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Molecular mechanism of intramolecular recombination in a yeast plasmid, pSR1.
17. H. Araki and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). ARS (Autonomously Replicating Sequence) of yeast plasmid pSR1.
18. S. Sakamoto, T. Imura, I. Utatsu and A. Toh-e (Dept. Ferment. Technol., Hiroshima Univ.). Comparative study on 2 μ and 2 μ -like yeast plasmids.
19. M. Tsuboi (Dept. Biotechnol., Fukuyama Univ.). Order and timing of function of sporulation genes in Saccharomyces cerevisiae.
20. K. Kitamura and C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.). Analysis of mating-deficient mutants in the fission yeast, Schizosaccharomyces pombe.
21. Y. Iino, J. Sakaguchi-Inoue and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Interaction of cAMP and genes controlling meiosis in the fission yeast Schizosaccharomyces pombe.
22. C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.). Suppressor of meiosis-deficient mutation in the fission yeast.

23. Y. Watanabe, M. Yamamoto (Inst. Med. Sci., Tokyo Univ.). Attempt to raise antibodies against the mei2 gene product.
24. H. Fujioka and C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.). Cloning of mei4 gene essential for meiotic first division in the fission yeast Schizosaccharomyces pombe.
25. M. Kishida, Y. Nakaseko* and C. shimoda (Fac. Sci., Osaka City Univ., *Fac. Sci., Kyoto Univ.). Structural analysis of the mes1 gene essential for meiotic second division in the fission yeast.
26. T. Matsumoto, U. Chikashige, O. Niwa and M. Yanagida (Dept. Biophysics, Kyoto Univ.). Structure of S. pombe mini-chromosomes; Identification of chromosomal ends.
27. O. Niwa, T. Matsumoto, Y. Chikashige, O. Niwa and M. Yanagida (Dept. Biophysics, Faculty of Science, Kyoto Univ.). Structures of S. pombe minichromosomes and their meiotic segregation.
28. Y. Nakaseko, S. Funahashi, O. Niwa and M. Yanagida (Dept. Biophys., Kyoto Univ.). Structure of centromeric sequences and their implication in chromosome stability in fission yeast.
29. M Itaya*, P. Hieter**, and R. Crouch (Lab. Mol. Gen., NIH, **John's Hopkins Med. Sch., *present address; Mitsubishi-Kasei Inst. Life Sci.). Physical mapping of a yeast Saccharomyces cerevisiae gene by dividing chromosome at its gene site.
30. K. Yoshida, J. Kuromitsu, N. Ogawa and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Expression of the PH02, PH04, and PH081 regulatory genes in Saccharomyces cerevisiae.
31. N. Hayashi, Y. Kaneko*, and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., *IFO). Expression and regulation of the PH08 gene encoding repressible alkaline phosphatase in Saccharomyces cerevisiae.
32. T. Shimauchi, A. Toh-e (Dept. Ferment. Technol., Hiroshima Univ.). Function of negative factors of pho system.
33. Y. Suzuki, A. Abe*, Y. Nogi and T. Fukasawa (Keio Univ. Sch. Med. * Kitasato Inst.). Characterization of a Saccharomyces cerevisiae with an insertion mutation in the regulatory gene GAL11.
34. M. Igarashi*, T. Segawa, Y. Suzuki and T. Fukasawa (Keio Univ. Sch. Med.* Yamasa Shouyu Co. Ltd.). Mechanism for Regulated Expression of the Yeast Regulatory Gene GAL80.
35. K. Tanaka, K. Matsumoto*, T. Oshima and S. Tanaka (Suntory. Inst. Biomed. Res., DNAX. Res. Inst.). Regulation of expression of HSP70-100 gene in Saccharomyces cerevisiae.
36. I. Miyakawa, M. Miyamoto, N. Sando and T. Kuroiwa* (Biol. Inst., Fac. Sci., Yamaguchi Univ., *Dept. Cell Biol., Natl. Inst. Basic Biol.). Morphology of yeast mt-nucleoids and their protein components.

37. E. Tsuchiya and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Preparation and characterization of monoclonal antibodies against the yeast nuclear matrices.
38. T. Yata, E. Tsuchiya, T. Miyakawa and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Characterization of DNA fragments tightly attached to the yeast nuclear-matrix.
39. Y. Adachi and M. Yanagida (Dept. Biophys., Kyoto Univ.). Genetic analysis and characterization of cold sensitive mutants with aberrant nuclear chromatin distribution in fission yeast.
40. M. Nakagawa, E. Tsuchiya and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Immunochemical analysis of the yeast nuclear protein contracting DNA replication.
41. M. Iwabuchi, K. Kawasaki, O. Makino, and T. Shibata (Laboratory of Microbiology, RIKEN Institute). An immunochemical study on site-specific endonucleases of yeasts.
42. M. Uritani, H. Kagiya and M. Miyazaki (Inst. Molec. Biol. Nagoya Univ.). Property and Function of Polypeptide Elongation Factor-3 (EF-3) from yeast.
43. Y. Hayashi and C. W. Nakagawa (Inst. for Development Res., Aichi Colony). Cd-binding peptides of fission yeast. Isolation of cadystin A and B by TOYOPEARL DE-650 column.
44. H. Oh-ue and B. Ono (Fac. Pham. Sci., Okayama Univ.). Biochemical analyses of inorganic mercury resistant mutants of S. cerevisiae.
45. J. Ishiguro and Y. Azuma (Dept. of Biology, Faculty of Science, Konan University). Temporary changes in ribosomal and cytosol protein patterns in adenine-requiring mutant strains of K. lactis.
46. M. Yamamura and T. Kamihara (Dept. Indust. Chem., Kyoto Univ.). Petite induction at elevated temperatures: Stimulatory effect of casaminoacids and inorganic salts.
47. Y. Kaneko and I. Banno (Institute for Fermentation, Osaka). Are Saccharomyces bayanus and Saccharomyces cerevisiae conspecific?
48. I. Uno, T. Ishikawa, A. Toh-e*, K. Tanaka** and T. Oshima** (Inst. Appl. Micro., Univ. Tokyo; *Dept. Ferm. Tech., Hiroshima Univ.; **Suntory Inst. Biomedical Res.). Subcellular localization of cAMP-dependent protein kinase in yeast.
49. D. Shin, I. Uno, T. Ishikawa (Institute of Applied Microbiology Tokyo Univ.). Control of G1-G0 transition and G0 protein synthesis by cAMP in Saccharomyces cerevisiae.
50. H. Nakajima, I. Oshima, K. Yoda and M. Yamasaki (Dept. Agric. Chem., Tokyo Univ.). Glycogen accumulation and intracellular fine structure of temperature sensitive cyr1 mutant.

51. S. Yamano, A. Toh-e*, T. Oshima**, K. Tanaka** and K. Matsumoto*** (Kirin Brewery, *Dept. Ferment. Technol., Hiroshima Univ., **Suntory, ***DNAX). Structure and function of BCY1 gene.
52. T. Oshima, K. Tanaka, I. Uno*, H. Mitsuzawa* and T. Ishikawa* (Suntory Inst. Biomed. Res., *Inst. Appl. Microbiol. Univ. Tokyo). Expression of CYR1 and RAS2 genes of Saccharomyces Yeast in Escherichia coli.
53. H. Mitsuzawa, I. Uno, T. Ishikawa, K. Tanaka and T. Oshima* (Inst. Appl. Microbiol. Univ. Tokyo, *Suntory Biomed. Res.). Region of the yeast CYR1 gene that is involved in the interaction with the RAS products.
54. Y. Fukui and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Involvement of the ras1 gene function in recognition of the mating factor in Schizosaccharomyces pombe.
55. B. Ono, Y. Ishino, R. Fujimoto, Y. Ohno, N. Maeda and Y. Tsuchiya (Fac. Pharm. Sci., Okayama Univ.). UGA suppressor loci in Saccharomyces cerevisiae.
56. A. Toh-e (Dept. Fermentation Technology, Hiroshima University). Genetic analysis of revertants from a pho81-2 mutant.
57. M. Yamaguchi, T. Hirano, M. Araki* and K. Matsubara* (Cent. Res. Lab., Jikei Univ. Sch. Med., *Inst. Mol. Cell. Biol., Osaka Univ.). Electron microscopy of hepatitis B virus antigen-producing yeast cell.
58. K. Takeo (Res. Inst. Chemobiodynamics, Chiba Univ.). Correlation between the types of the plasma membranous particles and growth and non-growth zones of S. pombe.
59. A. Hirata (Inst. of Appl. Microbiol., Univ. of Tokyo). The fine structure of ascospore development in the fission yeast by freeze substitution - II.
60. K. Tanaka and T. Kanbe (Lab. Medical Mycol., Res. Inst. Disease Mech. Control, Nagoya Univ. Sch. Medicine). Ultrastructure of mitosis in Saccharomyces cerevisiae. Reinvestigation by freeze substitution method.
61. M. Osumi, N. Yamada, A. Taki, and N. Naito (Dept. of Biology and Laboratory of Electron Microscopy, Japan Women's University). Method for Study on the yeast cells by freeze-substitution fixation.
62. T. Tachikawa, T. Miyakawa, E. Tsuchiya and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). The role of Ca^{2+} /CaM for Mating Pheromone-Induced Sexual Differentiation in Heterobasidiomycetous Yeast.
63. K. Inokuchi, A. Nakayama, R. Nakano and F. Hishinuma (Mitsubishi-Kasei Institute of Life Sciences). Characterization of the promoter region of the MF α 1 gene in Saccharomyces cerevisiae.
64. H. Ohkura, Y. Adachi and M. Yanagida (Dept. Biophys., Kyoto Univ.). Isolation and analyses of the mutants which show abnormal spindle formation and unequal chromosome separation.

65. T. Hirano and M. Yanagida (Dept. Biophysics, Kyoto Univ.).
Identification of the NUC2 gene product in Schizosaccharomyces pombe.
66. Y. Kikuchi, M. Ando, H. Shimatake and A. Kikuchi* (Sch. Med., Toho Univ.,
*Inst. Life Sci., Mitsubishi-Kasei). Isolation of yeast mutants
defective in chromosomal segregation and characterization by gene
cloning.
5. The "Yeast Conference", organized by the Hungarian Scientific Society
for Food Industry, was held in Budapest, Hungary, 29th May, 1985.
Communicated by Peter A. Biacs, General Director of the Central Food
Research Institute, H-1022 Budapest, Herman Otto ut 15, Hungary.

List of Papers*

- L. Sagi: Manufacture of bakers' yeast in Hungary
GY Follath: Novelty in bakers' yeast production
T. Deak: Contradictions in taxonomy and identification due to new results
M. Sipiczki: Possibilities of the industrial applications of modern genetics
E. Novak: Sugar metabolism in yeasts
V. Tabajdi-Pinter: Yeasts as quality characteristics of foods
B. Matrai and A. Halasz: Study of bakers' yeast and brewers' yeast by
polyacrylamide gel electrophoresis
A. Maraz: Possibilities and results in the improvement of brewers' yeast
K. Szentpetery: Detection of wild yeasts in brewery samples
E. Farkas-Szucs: Determination of viable yeast count in fermenting brewage by
means of reducing capacity.
B. Sevelia and L. Nyeste: Trends in reactor research and technology
L. Boross, B. Szajani, ZS. Buzas, B. Polyak, K. Dallmann, P. Papp, V. Halmos,
GY. Copf and J. Zsolt: Growth and ethanol fermenting capacity of yeast
cells enclosed in gel
B. Kovacs: Up-to-date methods of alcohol fermentation
A. Asvany: General problems related to the use of wine yeasts
E. Polos: Study of psychrophilic yeasts
I. Wunsche: Possibilities of utilizing killer yeasts in wine fermentation
P. Sarkany: The role of yeasts in champagne manufacture

*Abstracts of the papers are published in Acta Alimentaria, Vol. 16 No. 1.

* * *

XXXII, Brief News Items

1. The following item appeared in the Alumnus Magazine of Southern
Illinois University (SIU).

Carol C. Lindegren, 89, the internationally recognized "father" of yeast
genetics and the founder of SIU's first laboratory designed solely for
research, died on Jan. 19, 1986. He was a resident of San Diego, Calif. Mr.
Lindegren retired from SIU in 1964, but continued to conduct research in the
Biological Research laboratory, which he organized after coming to SIU in
1948. He also established SIU's microbiology department and served as its
chairman until his retirement. SIU's Life Sciences I building was renamed
"Carl C. and Gertrude Lindegren Hall" in 1977. Gertrude Lindegren, Mr.
Lindegren's late wife, teamed with him as a research partner for more than 40

years. Their early discovery that yeast cells are sexed and can be cross-bred to form new strains opened up a new field of research in genetics and heredity. His wife, Zella, survives.

J.K. Bhattacharjee
Dept. of Microbiology
Miami University, Oxford,
Ohio 45056

2. The following paper will appear in a forthcoming issue of Letters in Appl. Microbiol.

"A revised method for the application of API 50 CH carbohydrate kits to yeasts".

A revised method is described for the application of the API 50 CH carbohydrate kit to yeasts. The method permits an objective assessment of positive assimilation with a high degree of reproducibility.

Renny Ison
The Lord Zuckerman Research Centre
The University
Whiteknights
PO Box 234
Reading RG6 2LA, England

* * *

3. "The Fungi of our Mouldy Earth" by Wm. Bridge Cooke, Beihefte zur Nova Hedwigia No 85, 467 pp., J. Cramer, Gebr. Borntraeger Verlagsbuchhandlung, Berlin. Stuttgart.

A number of yeast species are listed. There are three major parts in the book: (1) material from the Laboratory Guide, (2) keys to all categories, (3) a systematic list of species together with the locations from which each species had been isolated or reported.

Wm. Bridge Cooke
1135 Wilshire Ct.
Cincinnati, OH 45230

* * *

Dear Mr. [Name],

I have received your letter of the 10th of October regarding the [Topic].

The information you provided is being reviewed by the relevant departments.

We will contact you again once a final decision has been reached.

Thank you for your patience and understanding.

Yours faithfully,

[Signature]

[Name and Title]

[Address]

[City]

[Country]

[Phone Number]

[Fax Number]

A Newsletter for Persons Interested in Yeast

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

December 1986

Volume XXXV, Number II

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

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

Herman J. Phaff
Editor

NOTICE TO OUR READERS

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

We have explored with the University of California the possibility of direct transfer of the subscription fee on the bank account of the University of California. Unfortunately, this is not possible because of the large size of the University on nine campuses in the State of California with its numerous accounts. It is suggested that subscribers may wish to purchase dollars and pay cash in order to save the high service charge or use a postal money order.

H.J. Phaff
Editor

- I. Centraalbureau Voor Schimmelcultures, Yeast Division, Julianalaan 67a, 2628 BC DELFT (Netherlands). Communicated by M.TH. Smith.

Below follow several news items from the CBS.

1. Drs. L. Rodrigues de Miranda retired at the end of October after 20 years in the Yeast Division at Delft. He had specialized in basidiomycetous yeasts and contributed chapters on the genera Bullera, Cryptococcus and yeast phases of Sirobasidiaceae, as well as on the ascomycetous genus Sporopachydermia, to the 3rd Edition of The Yeasts. His farewell contribution to the yeasts is an article prepared in co-operation with Dr. Weijman and submitted for publication to Antonie van Leeuwenhoek.

* * *

2. Recent acquisitions:

Arthroascus schoenii (Nadson & Krasil'nikov) Babeva & al.
7223 = VKM 107, T, ex exudate of oak, USSR, G.S. de Hoog.

Asteropora lycoperdoides haploid yeast state.
7213, 7214, ex Russula cf. nigricans, Germany, H. Prillinger.

Bullera derxii Nakase & Suzuki
7225 = IFO 10177 = JCM 5280 = N0-92, T. ex dead leaf of Oriza sativa, Japan, T. Nakase.

Bullera intermedia Nakase & Suzuki
7226 = IFO 10178 = JCM 5291 = N0-157, T. ex dead leaf of Oryza sativa, Japan, T. Nakase.

Bullera pseudoalba Nakase & Suzuki
7227 = IFO 10179 = JCM 5290 = N0-165, T, ex dead leaf of Oryza sativa, Japan, T. Nakase.

Candida blankii Buckley & van Uden
7205, horse's uterus, New Zealand, resistant to gentamicin and cycloheximide, A. Woodgyer.

Candida catenulata Diddens & Lodder
7230, ex soil, UK, grows on 3-mono-chloropropionic acid, S. Hughes.

Candida valida (Leberle) van Uden & Buckley
7217, patent application strain for utilization of alcohol wastes (received as C. rugosa), D.P. Henry.

Candida vini (Lodder) van Uden & Buckley
7218, ex Sikeae harbour, atypical strain, K. Gustafsson.

Cryptococcus neoformans (Sanfelice) Vuillemin var. shanghaiensis Liao & al.
7229 = Liao's S 8012, T, ex case of meningitis, China, J.A. Barnett.

Malassezia furfur (Robin) Baillon
7231, ex high vaginal swab from 27-year-old woman, New Zealand, A. Woodgyer

Malassezia minutissima Simmons & al.
7222 = GSU-RBS-8541, ex human ear, E. Gueho.

Myxozyma geophila van der Walt
7219 = CSIR Y-907, T, ex surface soil, Transvaal, J.P. van der Walt.

Rhodotorula acheniorum (Buhagiar & Barnett) Rodrigues de Miranda
7221, maize leaves, Netherlands, W.J. Middelhoven.

Sporobolomyces oryzaicola Nakase & Suzuki
7228 = IFO 10180 = JCM 5299, T, ex dead leaf of *Oryza sativa*, Japan, T. Nakase.

Taphrina epiphylla haploid yeast state
7216, ex *Alnus glutinosa*, Germany, H. Prillinger.

Tremella foliacea haploid yeast state
7215, ex *Pinus mugo*, Germany, H. Prillinger.

* * *

Publications

- de Hoog, G.S. & Smith, M.T. 1986. Key to the species of Hyphozyma (yeast-like Hyphomycetes and description of H. rosenigra sp. nov. *Antonie van Leeuwenhoek* 52, 39-44.

Abstract

The new species, Hyphozyma roseonigra, characterized by pink colonies, budding cells with minute annellated zones and brown, septate hyphae, is described. It is non-fermentive, shows no colouration with Diazonium Blue B, and has a ascomycete-type cell wall ultrastructure. A key to the accepted species and varieties of Hyphozyma is given.

* * *

Smith, M.T. & Batenburg-van der Vegte, W. 1986. Pichia triangularis sp. nov., the teleomorph of Candida polymorpha Ohara et Nonomura, nom. nud. *Antonie van Leeuwenhoek* 52, 437-443.

The type strain of Candida polymorpha Ohara & Nonomura, nom. nud. was found to produce hat-shaped ascospores. On the basis of its morphology and physiology, it is considered a new species of the genus Pichia and is described as Pichia triangularis sp. nov.

* * *

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

The strains listed below have been added to the ATCC since April 30, 1986. Complete information on these strains may be obtained upon request from the Mycology & Botany Department of ATCC.

New Yeast Strains

Name	ATCC No.	Depositor and Strain	Significance and Reference
<u>Candida albicans</u>	62342	D.W. Warnock, B41628	Human pathogen (J. of Med. & Vet. Mycology 24:133-144, 1986)
<u>Candida albicans</u>	62376- 62379	D.H. Howard, FC18 FC18-1, FC18-3, FC18-6	Human pathogen (J. of Bacteriology 145: 896-903, 1981)
<u>Cryptococcus neoformans</u>	62066- 62072	T. Mitchell, 6, 15, 98, 110, 145, 184, 602	Clinical isolate (Infect. Immun. 5: 491-498, 1972)
<u>Saccharomyces cerevisiae</u>	62013- 62017	E.D. Sancho, 614.1, 504.3, 519.5, 510.2 44.2	Lithium sensitive (Appl. & Environ. Microbiol. 51: 395-397, 1986)
<u>Saccharomyces cerevisiae</u>	62418- 62422	M.R. de van Broock D-101, D-314, LP-a, LP 4	Mating type (Biotechnology Letters 6: No. 3 171-176, 1984).
<u>Torulasporea hansenii</u>	60977- 60981	F. Fatichenti, FOF9(4), F3512(1), F3514(3), SG1S1(5), T2F14(2)	Produces antimicrobial metabolites (Dairy Res. 50: 449-457, 1983)

* * *

III. Japan Collection of Microorganisms Riken, Wako-shi, Saitama, 351-01 Japan. Communicated by T. Nakase.

Below follow abstracts of two papers from JCM.

1. Takashi Nakase and Motofumi Suzuki. 1986. Bullera megalospora, A New Species of Yeast Forming Large Ballistospores Isolated from Dead Leaves of Oryza sativa, Miscanthus sinensis and Sasa sp. in Japan. J. Gen. Appl. Microbiol. 32:225-240.

Detailed taxonomic studies were made of thirty-five strains of psychrophilic yeasts with large ballistospores. These strains were isolated from dead leaves of Oryza sativa, Miscanthus sinensis and Sasa sp. in Japan and were found to comprise a single, hitherto undescribed species of the genus Bullera. The species is described here as Bullera megalospora Nakase et

Suzuki. Bullera megalospora resembles Bullera piricola and Sporobolomyces puniceus, but it can be distinguished from B. piricola by its inability to assimilate lactose, melibiose, and inositol, and from Sp. puniceus in its lack of assimilation of inositol. Electrophoretic comparison of ten enzymes clearly demonstrated the differences among these three yeasts at the specific level; the similarities in their enzyme patterns were below 22%. Sporobolomyces puniceus was considered to be more closely related to B. megalospora and B. piricola than any of the other species of the genus Sporobolomyces. We propose to transfer this species to the genus Bullera as Bullera punicea (Komagata et Nakase) Nakase et Suzuki comb. nov.

* * *

2. Takashi Nakase and Motofumi Suzuki. 1986. The Ubiquinone System in Strains of Species in The Ballistospore-Forming Yeast Genera Sporidiobolus, Sporobolomyces and Bullera. J. Gen. Appl. Microbiol. 32:251-258.

The ubiquinone system of twenty-four strains of ballistospore-forming yeasts in the genera Sporidiobolus, Sporobolomyces, and Bullera was investigated. Most strains are type strains. They are twenty-one known species and two unidentified species. Sporidiobolus johnsonii, Sporid. pararoseus, Sporid. ruinenii, Sporid. salmonicolor, Sporobolomyces alborubescens, Sp. follicola, Sp. gracilis, Sp. holsaticus, and Sp. roseus had Q-10 as the major ubiquinone and Q-9 as the minor component. Bullera alba, B. armeniaca, B. aurantiaca, B. crocea, B. dendrophila, B. globospora, B. piricola, B. punicea, B. salicina, B. singularis, and B. tsugae also had Q-10 as the major ubiquinone and Q-9 as the minor component. The amounts of Q-9 are rather high (15.0-25.4%) in Sporid. pararoseus, Sporid. ruinenii, B. piricola, B. punicea, and B. salicina. The major ubiquinone of Sp. elongatus was Q-10 (H₂). This yeast has Q-9 (H₂) and Q-10 as the minor components. This is the 2nd finding of Q-10 (H₂) in yeasts as the major ubiquinone. Three unidentified strains of two species of Bullera had Q-9 as the major ubiquinone and Q-8 and Q-10 as the minor components. Taxonomically, these strains resemble B. singularis in spite of the difference in ubiquinone systems. These yeasts may be yeast phases of certain species of the genus Itersonilia or may represent a genus yet to be established.

* * *

- IV. Shizuoka University, Faculty of Agriculture, 836 Ohya, Shizuoka 422, Japan. Communicated by Yuzo Yamada.

The following are recent publications from my laboratory:

1. Yuzo Yamada. 1986. Holleya gen. nov., an Ascosporegenous Yeast Genus from the Q₉-equipped Organism Whose Ascospores are Needle-shaped with Smooth Surfaces in their Anterior Half and Concentric Ridges in their Posterior Half and Without Appendage. J. Gen. Appl. Microbiol. 32(5) in press.

Cells are globose, ovoid, ellipsoid to cylindrical, reproducing by multilateral budding. Pseudomycelium and mycelium are formed. Asci arise by transformation of vegetative cells. Ascospores are needle-shaped, 8 per ascus, smooth in anterior half, concentrically ridged in posterior half and

without appendage. Fermentation is present. Coenzyme Q-9[Q-8] system is present.

Type species: Holleya sinecauda (Holley) Yamada comb. nov. (Basionym: Nematospora sinecauda Holley, Antonie van Leeuwenhoek 50:309 1984).

Typus: CBS 8199

Etymology: Latin prep. sine without; Latin nom. fem. n. cauda tail; M. Latin n. sinecauda without tail.

The genus Holleya is placed in the family Spermophthoraceae.

2. Yuzo Yamada, Kana Aizawa, and Isao Banno. 1986. The Coenzyme Q System and An Electrophoretic Comparison of Enzymes in the Strain of the Anamorphic Yeast Species, Sterigmatomyces fuzhouensis. J. Gen. Appl. Microbiol. 32, 367-370. Short Communication.
3. Yuzo Yamada. 1986. The Coenzyme Q System in Strains of Species in the Anamorphic Yeast Genus Myxozyma. J. Gen. Appl. Microbiol. 32, 259-261. Short Communication.

* * *

- V. Universita Degli Studi Di Perugia, Dipartimento di Biologia Vegetale, I - 06100 Perugia, Borgo XX Giugno, 74, Italy. Communicated by Ann Vaughan Martini.

Our publications for the year 1985/86 are listed below. Please note that as of November 1985, the official name of our institution has become: Dipartimento di Biologia Vegetale.

1. Vaughan Martini, A., Martini, A. 1986. Three newly delimited species of Saccharomyces sensu strictu. Antonie van Leeuwenhoek in press.

Abstract

Deoxyribonucleic acid reassociation studies of 24 different strains of wine and beer-associated Saccharomyces confirmed the presence of three separate species. S. cerevisiae and S. bayanus strains had only 22% of their genomes in common. S. pastorianus, with intermediate hybridization values between S. cerevisiae and S. bayanus, (52 and 72%, respectively) could possibly be a natural hybrid of the two species. This epithet replaces S. carlsbergensis, with which it is homologous for 93% of its genome, since S. pastorianus was described first by Hansen in 1904. These data do not agree with the results of traditional physiological tests.

* * *

2. A. Vaughan Martini, A. Martini. 1985. Perfect-imperfect Relationship within the Yeast Genus Kluyveromyces. Ann. Microbiol. (Milan), 35, 93-97.

Abstract

A deoxyribonucleic acid reassociation study of thirteen perfect and imperfect yeast strains associated with the yeast genus Kluyveromyces confirmed the premise of previous authors that a perfect-imperfect relationship exists between some of these strains. It was also demonstrated that the latest conventional classification of the genus does not entirely agree with the results of DNA/DNA reassociations.

* * *

3. Gianfranco Rosini. 1986. Wine-making by cell-recycle-batch fermentation process. *Appl. Microbiol. Biotechnol.* 24:140-143.

Summary

In order to check the overall validity of more efficient fermentation systems to reduce wine-making costs, we carried out an off-skins fermentation of clarified Trebbiano toscano grape-juice, making use of a non-conventional "cell-recycle-batch fermentation" process. The results showed that the process causes a reduction of the fermentation length as well as an improvement in ethanol productivity and yield and can be conveniently applied to the production of ordinary table wines.

* * *

4. F. Federici and M. Petruccioli. 1985. Effect of some Cultural Conditions on polygalacturonase Production by Cryptococcus albidus var. albidus. *Ann. Microbiol. (Milan)*, 35, 235.

Summary

The influence of carbon source, initial cell concentration, aeration, medium pH and incubation temperature on polygalacturonase production by the yeast strain Cryptococcus albidus var. albidus IMAT-4735 was studied. All the experiments were performed in a medium containing pectin as carbon source; the enzyme production was depressed when pectin was replaced by a mixed carbon source such as glucose or sucrose and pectin. Maximum polygalacturonase activity was obtained when the microorganism was cultivated at 24°C, the medium pH was 4.6, the pectin concentration 35.0 g/l, the aeration was gradually shifted from 1 to 0.15 Vvm and the initial cell number 30.5×10^6 per ml. The time course of growth and enzyme production in batch culture at optimized growth conditions is reported.

* * *

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

Recent publications from this laboratory include the following.

1. Barnett, J.A., Payne, R.W. & Yarrow, D. (1985). Yeast Identification Program. Cambridge University Press.
Floppy disk version obtainable: IBM PC (DOS), DEC Rainbow (CP/M), DEC Rainbow (MS-DOS), Superbrain (CP/M).

2. McCann, A.K. & Barnett, J.A. (1986). The utilization of starch by yeasts. Yeast 2, 109-115.
3. Barnett, J.A. (1986). The stability of biological nomenclature: yeasts. Nature 322, 599.
4. McCann, A.K., Hilberg, F., Kenworthy, P. & Barnett, J.A. (1987). An unusual hexose-ATP-kinase with two catalytic sites and a role in catabolite repression in the yeast Schwanniomyces occidentalis. Journal of General Microbiology in the press.

* * *

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

Below follows the abstract of a paper that has been accepted for publication by Mycologia.

C.P. Kurtzman. 1987. Two New Species of Pichia From Arboreal Habitats. Abstract. Among hat-spored isolates of the genus Pichia collected in frass from trees in the U.S. and Japan were strains of two taxa that could not be identified with presently described species. These new homothallic species, described as P. hampshirensis (type strain, NRRL YB-4128 = CBS 7208) and P. japonica (type strain, NRRL YB-2750 = CBS 7209), show little nuclear DNA relatedness to P. wickerhamii, P. rhodanensis, P. mississippiensis, P. toletana, and other phenotypically similar taxa.

* * *

VIII. All-Union Collection of Microorganisms, Inst. Biochem. and Physiol. of Microorganisms, USSR Academy of Sciences, Pushchino, Moscow region I42292, USSR. Communicated by W.I. Golubev.

The following are recent publications from our Institute.

1. Golubev, W.I., 1986. Yeasts from arctic East-Siberian tundra - Izvestija Akad. Nauk SSSR, ser. biol., N4, 609-612.

The total yeast counts were (per g dry sample) several hundred or less for tundra soils and up to several hundred thousand for plant materials. The yeasts averaged a third of the micromycetous flora. The species diversity of yeast flora was limited, and most abundant yeasts were Cryptococcus aerius, Cr. magnus, Cr. uniguttulatus and Cr. albidus. The majority of the isolates were characterized by minimum growth temperatures of -1° to -2°C and maxima of 30°C or below.

2. Golubev, W.I., Naumov, G.I., Bibikova, I.I., Blagodatskaya, V.M., Vustin, M.M., Nikitina, T.N., Buzurg-zade, D.L., and Gradova, N.B., 1986. Reidentification of hydrocarbon strongly assimilating yeast strains of the genus Candida. - Biotechnologia, N 5, 17-21.

26 yeast strains used for the production of single-cell protein in the USSR from n-paraffins were examined. Of these, 23 strains were reidentified as Candida maltosa Komagata et al. and 3 strains as C. tropicalis (Castellani) Berkhout.

* * *

A list of newly accepted yeast cultures by the All-Union Collection of Micro-organisms is published in Prikladnaya biochimia i mikrobiologia, 22, N 4, 587-591, 1986.

* * *

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

The following 2 papers have been published recently.

1. Butler, M.J. and M.A. Lachance. 1986. Quantitative binding of azure A to melanins of the black yeast Phaeococcomyces sp. Exptl. Mycol. 10:166-170.

* * *

2. Lachance, M.A., H. J. Phaff, W.T. Starmer, A. Moffitt, and L.G. Olson. 1986. Interspecific discontinuity in the genus Clavispora Rodriguez de Miranda by phenetic analysis, genomic deoxyribonucleic acid reassociation, and restriction mapping of ribosomal deoxyribonucleic acid. Int. J. Syst. Bacteriol. 36:524-530.

Summary

Heterothallic strains belonging to the biologically distinct yeast species Clavispora opuntiae and Clavispora lusitaniae were studied by three different methods. The type cultures of the two yeast species exhibited 8% relatedness as measured by reassociation of unique deoxyribonucleic acid. Ten strains of C. opuntiae and nine strains of C. lusitaniae were compared on the basis of their physiological phenotypes and the restriction maps of their ribosomal deoxyribonucleic acids (rDNAs). Although the two species possessed many similarities as well as certain amounts of intraspecific variation by both approaches, they appeared to constitute well-defined entities. Unlike C. opuntiae, C. lusitaniae always utilized L-rhamnose as the sole carbon source and was resistant to 10 mg of cycloheximide per liter. Strains of C. opuntiae did not utilize L-lysine as the sole nitrogen source or utilized it very weakly, whereas all strains of C. lusitaniae grew rapidly on this compound. By contrast, the hydrolysis of Tween 80 and the utilization of lactic acid, citric acid, and hexadecane tended to be more pronounced in C. opuntiae. The rDNA repeating unit was 9.0 kilobases long in C. lusitaniae, as compared with 7.6 kilobases in C. opuntiae. The conserved region identified previously in the rDNA of C. opuntiae was found almost intact in the rDNA of C. lusitaniae, but the variable regions differed substantially between the two species.

* * *

3. Butler, M.J., and M.A. Lachance. 198-. Inhibition of melanin synthesis in the black yeast Phaeococcomyces sp. by growth on low pH ascorbate medium: production of spheroplasts from albinized cells. Can. J. Microbiol. (accepted October 1986).

* * *

4. Ganter, P.F., W.T. Starmer, M. A. Lachance, and H.J. Phaff. 198-. Yeast communities from host plants and associated Drosophila in southern Arizona: new isolations and analysis of the relative importance of hosts and vectors on community composition. Oecologia (accepted June 1986).

Summary

The yeast communities from slime fluxes of three deciduous trees (Prosopis juliflora, Populus fremontii and Quercus emoryi) and the necroses of two cacti (Opuntia phaeacantha and Carnegiea gigantea) were surveyed in the region of Tucson, Arizona. In addition, the yeasts carried by dipterans associated with the fluxes or necroses (Drosophila carbonaria, D. brooksae, D. nigrospiracula, D. mettleri, and Aulacigaster leucopeza) were sampled. The results indicate that each host sampled had a distinct community of yeasts associated with it. The dipterans, which can act as vectors of the yeasts, deposited yeasts from other sources in addition to those found on their associated hosts. It is argued that host plant physiology is relatively more important than the activity of the vector in determining yeast community composition. Furthermore, the average number of yeast species per flux or necrosis is not different from the average number of yeast species per fly. It is hypothesized that the vector may affect the number of species per individual flux or rot, and that the number is lower than the rot or necrosis could potentially support.

* * *

- X. University of Medicine & Dentistry of New Jersey, Robert Wood Johnson Medical School. Department of Molecular Genetics and Microbiology, Piscataway, New Jersey 08854-5635. Communicated by M.J. Leibowitz.

Below are abstracts of three recent papers from this laboratory.

1. E.M. Hannig, T.L. Williams and M.J. Leibowitz. 1986. The internal polyadenylate tract of yeast killer virus M₁ double-stranded RNA is variable in length. Virology 152:149-158.

A stable mRNA-dependent cell-free translation system from Saccharomyces cerevisiae, prepared by a modification of the method of Hofbauer et al. (Eur. J. Biochem. 122 (1982) 199-203) was active in translation of exogenous homologous and heterologous mRNAs. Optimal translational activity required the addition of polyamines and yeast tRNA. The m transcript of the M dsRNA segment, synthesized in vitro using the killer virus-associated RNA polymerase, directed the synthesis of preprotoxin polypeptide (M-p32), which was immunologically identified using antitoxin antibody. Sindbis virus capsid protein and rabbit globin were also translated from their mRNAs. Translation was inhibited by puromycin, sparsomycin and anisomycin. Analogues of the 5'-

terminal caps present on most eukaryotic mRNA molecules inhibited translation of added mRNAs, including capped mRNAs and the uncapped killer virus mRNA.

* * *

2. I. Hussain and M.J. Leibowitz. 1986. Translation of homologous and heterologous messenger RNAs in a yeast cell-free system. *Gene* 46:13-23.

The 1.8 kilobase pair (kbp) M_1 double-stranded (ds) RNA from type 1 killer strains of *Saccharomyces cerevisiae* contains an internal 200 bp adenine- and uracil-rich region. We have previously demonstrated that this region consists primarily of adenine residues on the plus strand of M_1 dsRNA and on the full-length, in vitro synthesized (+) transcript (denoted m) of M_1 dsRNA, neither of which contains 3'-terminal polyadenylate. We now show that there is variability in the length of the polyadenylate tracts of m transcripts synthesized in vitro by virions purified from either of the K_1 diploid killer strains A364A x S7 or A364A x 1384. This variability reflects size differences seen in the corresponding M_1 dsRNA genomes. This, along with other data presented here, localizes the variability in the length of M_1 dsRNA to the adenine- and uracil-rich region.

* * *

3. D.E. Georgopoulos, E.M. Hannig and M.J. Leibowitz. 1987. In R.B. Wickner, A. Hinnebusch, A. Lambowitz, I.C. Gunsalus, A. Hollaender, J. Preer, Jr., L. Mets and R. Gumpert (eds.). Extrachromosomal Elements in Lower Eukaryotes, Plenum Press, New York, pp. 203-213.

A full-length cDNA copy of the M_1 -2 region of the double-stranded genome of the killer virus of yeast was synthesized by reverse transcription utilizing the m in vitro transcript as template and synthetic primers for both strands. The sequence lacks any long open reading frames. The internal portion of the M_1 -2 region includes the sequence which is linked to the subterminal 229 bases of the M_1 -1 homologous region in the S3 defective-interfering mutant of killer virus dsRNA. Thus the probable site at which the deletion occurred in S3 has been identified.

* * *

- XI. Institut für Physiologische Chemie, Ruhr-Universität Bochum D-4630 Bochum 1, FRG. Communicated by W. Duntze.

The following abstract summarizes part of our recent work on the structure of the mating hormone α -factor.

Amino acid sequences of α -factor mating peptides from *Saccharomyces cerevisiae*

R. Betz, J.W. Crabb*, H.E. Meyer, R. Wittig and W. Duntze

*W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, N.Y. 12946 U.S.A.

Abstract. The molecular structure of a-factor, the mating hormone produced by mating type a cells of *S. cerevisiae* has been investigated. In culture filtrates of a cells four oligopeptides (a1 to a4) exhibiting a-factor activity have been found. These peptides have been isolated and their amino acid sequences determined. The a-factor peptides form two (apparently identical) pairs a1/a2 and a3/a4 which differ in an interchange at position 6 of a valine in a1/a2 for a leucine in a3/a4. a1 and a4 which can be obtained by oxidation with H₂O₂ of purified a2 and a3, respectively, obviously represent oxidation artifacts formed under the conditions of culture.

The amino acid sequences determined for the a-factor peptides are Tyr-Ile-Ile-Lys-Gly-Leu/Val-Phe-Trp-Asp-Pro-Ala-Cys. Several lines of evidence suggest that the carboxy-terminal cysteine residue is S-alkylated by a hydrophobic aliphatic residue.

* * *

XII. National Research Council Canada, Division of Biological Sciences, Ottawa, Canada K1A 0R6. Communicated by Byron F. Johnson and A. Nasim.

The following are abstracts of publications from the laboratory of B.F. Johnson and the Molecular Genetics Section (A. Nasim).

1. Byron F. Johnson, G.B. Calleja, and M. Zuker. 1984. Mating-Type Gene Switching in a Homothallic Fission Yeast. *J. theor. Biol.* 110, 299-312.

When single cells of a homothallic strain of the fission yeast (*Schizosaccharomyces pombe* 968h⁹⁰) are plated upon sporulation agar, a couple of cell divisions yield four preconjugal cells ordered in a line. Within a line, conjugation occurs either between sibs (the pair at either end of the line) or between cousins (the central pair of cells) or not at all. Miyata & Miyata (1981) have shown that sib matings are favored over cousin matings, the ratio of sib:cousin:sterile being 96:23:10. To have mating within these 4-cell clones means that the mating types of some of the cells have switched. In a further analysis of their data, we come to a series of deductions, one of which is that switching of mating-type genes in the fission yeast must be asymmetrical. We propose a random model and a deterministic model based upon asymmetrical switching. Either model could generate the ratios provided, but the models are sufficiently different that experimental tests should be able to discriminate between the two.

* * *

2. Machiko Miyata^{1,3}, Hisao Miyata^{2,3}, and Byron F. Johnson³. 1986. Establishment of Septum Orientation in a Morphologically Altered Fission Yeast, *Schizosaccharomyces pombe*. *J. Gen. Microbiol.* 132, 2535-2540.

¹Gifu Pharmaceutical University, 6-1, Mitahora-higashi 5 chome, Gifu 502, Japan.

²Department of Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464, Japan.

³Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada.

Among the spheroidal fission-yeast (Schizosaccharomyces pombe) cells resulting from aculeacin A treatment cells were found whose putative growth axis and polarity differed from those of their progenitor, that is, they were changed in the orientation of their septum. The ratio of cell length (measured perpendicular to the septum plane) to septum diameter of these cells equalled or exceeded unity without exception, whether the septum orientation changed or not and whether the cellular shape was spherical, spheroidal or cylindrical. From these results we conclude that the septum is always oriented perpendicular to the plane including the longest axis of the cell even if the morphology is irregular or the new septum has become perpendicular to the previous septum. There is no cellular region forbidden to new septa.

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3. Machiko Miyata, Hisao Miyata, and Byron F. Johnson. 1986. Asymmetric Location of the Septum in Morphologically Altered Cells of the Fission Yeast Schizosaccharomyces pombe. J. Gen. Microbiol. 132: 883-891.

Cells of the fission yeast Schizosaccharomyces pombe, normally sausage-shaped, changed to a round-bottomed flask (RBF)-like morphology during growth in the presence of aculeacin A (Acu), an antifungal antibiotic. The volume of RBF-like cells was comparable to that of the control cells. After being transferred to normal conditions (without Acu at 25°C), the RBF-like cells continued to grow at the cylindrical and or spherical end(s) and then the septum at the subsequent division of the cells was formed without exception at the boundary plane between the spheroidal and the cylindrical region: it is at this boundary that the nucleus was located before mitosis. Hence the RBF-like cell divided into a spheroidal and a cylindrical sib at the first cell division. At the end of the second cell cycle, the spheroidal and the cylindrical progeny divided into two spheroidal and two cylindrical sibs respectively. The values of the mean length (long/short) and volume (big/small) ratios of paired sibs were larger in order of (a) cylindrical normal, with both mean ratios 1.06; (b) cylindrical control; (c) cylindrical progeny of RBF-like cell; (d) spheroidal progeny of RBF-like cell; and (e) RBF-like cell, whose mean length ratio was 1.25 but whose mean volume ratio was 1.94. That is, the more the morphology deviated from the cylindrical form, the greater was the degree of asymmetry. There was no rule relating the biases to the growth pole in these asymmetries.

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4. Miyata, H., M. Miyata, and B.F. Johnson. 1986. Patterns of extension growth of the fission yeast, Schizosaccharomyces pombe. Can. J. Microbiol. 32:528-530.

The growth of sausage-shaped cells of the fission yeast, Schizosaccharomyces pombe (strain NCYC 132), was followed in the second or third cycle by time-lapse photomicrography. Experimental cells were harvested from glucose-limited (0.2% glucose EMM3) chemostat culture (dilution rate, 0.125/h) and were plated onto a slide with EMM3 agar (2% glucose). By observing their extension patterns, we found some rules of extension growth.

Thus, (1) all sibs with walls newly formed in the previous cycle, whose progenitor cells grew at the old end (followed Mitchison's rule), grow at the old end (also follow Mitchison's rule) (2) Sibs with old walls whose progenitor cell followed Mitchison's rule behave in one of three ways: (i) growth at the old end (follow Mitchison's rule); (ii) growth at the new end (violate Mitchison's rule); or (iii) growth at both ends (bipolar), (3) Both sibs whose progenitor grew at both ends (bipolar) always grow at the old end (follow Mitchison's rule).

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5. J.A. Erratt and A. Nasim. 1986. Allelism within the DEX and STA gene families in Saccharomyces diastaticus. Mol. Gen. Genet. 202:255-256.

Summary. Saccharomyces diastaticus produces an extracellular glucoamylase and is therefore capable of hydrolyzing and fermenting starch. Tamaki (1978) studied starch utilization in S. diastaticus and found three polymeric genes controlling this function: STA1, STA2 and STA3. Independently, Erratt and Stewart (1978) studied dextrin utilization by the yeast S. diastaticus and designated the gene, which they identified, DEX1. Erratt and Stewart (1981a, b) later described two other genes which controlled glucoamylase production in S. diastaticus: DEX2 and a third which was allelic to STA3. At that time STA1 and STA2 were not available to test for allelism in the DEX gene family. In this study strains containing the remaining 4 genes have been examined to determine if further allelism exists between the two gene families. It was ascertained that DEX1 is allelic to STA2 and DEX2 is allelic to STA1. Therefore, no new gene controlling starch utilization has been identified and these two nomenclatures can now be consolidated into one. Based on the fact that the glucoamylase from S. diastaticus can hydrolyze both dextrin and starch, dextrin being the term used to describe partially hydrolyzed starch, and the more wide use of the nomenclature STA, we propose to retain STA as the designation for genes coding for glucoamylase production in S. diastaticus.

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6. J.A. Erratt and A. Nasim. 1986. cloning and Expression of a Saccharomyces diastaticus Glucoamylase Gene in Saccharomyces cerevisiae and Schizosaccharomyces pombe. J. Bacteriol. 166:484-490.

A recombinant plasmid pool of the Saccharomyces diastaticus genome was constructed in plasmid YEp13 and used to transform a strain of Saccharomyces cerevisiae. Six transformants were obtained which expressed amylolytic activity. The plasmids each contained a 3.9-kilobase (kb) BamHI fragment, and all of these fragments were cloned in the same orientations and had identical restriction maps, which differed from the map of the STA1 gene (I. Yamashita and S. Fukui, Agric. Biol. Chem. 47:2689-2692, 1983). The glucoamylase activity exhibited by all S. cerevisiae transformants was approximately 100 times less than that of the donor strain. An even lower level of activity was obtained when the recombinant plasmid was introduced into Schizosaccharomyces pombe. No expression was observed in Escherichia coli. The 3.9-kb BamHI fragment hybridized to two sequences (4.4 and 3.9 kb) in BamHI-digested S. diastaticus DNA, regardless of which DEX (STA) gene S. diastaticus contained, and one sequence (3.9 kb) in BamHI-digested S. cerevisiae DNA. Tetrad analysis of crosses involving untransformed S. cerevisiae and S. diastaticus

indicated that the 4.4-kb homologous sequence cosegregated with the glucoamylase activity, whereas the 3.9-kb fragment was present in each of the meiotic products. Poly(A)⁺ RNA fractions from vegetative and sporulating diploid cultures of S. cerevisiae and S. diastaticus were probed with the 3.9-kb BAMHI fragment. Two RNA species, measuring 2.1 and 1.5 kb, were found in both the vegetative and sporulating cultures of S. diastaticus, whereas one 1.5-kb species was present only in the RNA from sporulating cultures of S. cerevisiae.

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7. A. Nasim, E.R. Stephen, and J.A. Erratt. 1985. Replicating instabilities in yeast: occurrence in different mutational systems. Mol. Gen. Genet. 199:152-153.

Summary. Following mutagenesis of yeast cells with nitrosoguanidine, primary mosaic colonies exhibiting prototrophic/auxotrophic phenotypes were obtained. Upon replating of these primary mosaics, numerous secondary mosaics were present in the progeny. This study shows that replicating instabilities occur at many different loci within the Schizosaccharomyces pombe genome. In addition, the ade-1 gene of Saccharomyces cerevisiae (causing red pigmentation) was used to show that the phenomenon also occurs in this yeast.

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8. Susan A. Nadin-Davis, Robert C.A. Yang, Saran A. Narang, and Anwar Nasim. 1986. The Cloning and Characterization of a RAS Gene from Schizosaccharomyces pombe. J. Mol. Evol. 23:41-51.

Summary. We have cloned and determined the complete nucleotide sequence of a RAS gene from the yeast Schizosaccharomyces pombe (SP-RAS). The putative RAS protein of 214 amino acids is encoded by two noncontiguous reading frames separated by an intron of 86 bp. The SP-RAS gene product shares extensive homology with the proteins of the Saccharomyces cerevisiae (SC), Dictyostelium, Drosophila, and human RAS genes in its N-terminal region but not in its C-terminal region. The extended C-terminal regions found in the SC-RAS genes have no counterpart in the SP-RAS gene. Thus the RAS genes of these two yeasts are structurally quite distinct. The SP-RAS sequence was expressed in vivo.

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9. S.A. Nadin-Davis, A. Nasim and D. Beach. Involvement of RAS in Sexual Differentiation but not in Growth Control in Fission Yeast. EMBO Journal (in press).

Abstract. The function of the ras⁺ gene of Schizosaccharomyces pombe has been studied by constructing null and activated alleles of this gene. An activated allele (ras_{val12}) inhibits conjugation but has no effect on cell growth, entry into stationary phase or sporulation. The phenotype of ras_{val12} is distinct from that caused by elevating the intracellular level of cAMP. This supports the hypothesis that ras of fission yeast does not modulate adenylate cyclase in a manner analogous to S. cerevisiae RAS. Introduction of a human ras sequence into fission yeast cells containing a non-functional null allele of ras restored the sexual differentiation process thus indicating that

the human sequence can complement Schiz. pombe ras. Our data suggest that although ras genes are highly conserved across a considerable evolutionary divide, the cellular function of the ras gene product varies in different organisms.

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XIII. Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo, 113, Japan. Communicated by Masamichi Takagi.

Below follow abstracts of recent publications from our laboratory.

1. M. Takagi, S. Kawai, Y. Takata, N. Tanaka, M. Sunairi, M. Miyazaki and K. Yano. 1985. Induction of cycloheximide resistance in Candida maltosa by modifying the ribosomes. J. Gen. Appl. Microbiol., 31, 267-275.

Abstract. When Candida maltosa IAM12247 was grown in the presence of cycloheximide, the growth was repressed for a distinct time depending on the concentration of cycloheximide, then the growth recovered. The cell-free translation experiments using polyuridylic acid as mRNA indicated that the protein-synthesizing activity was altered in the cells adapted to resist cycloheximide. A reconstituted cell-free system was constructed consisting of ribosomes and soluble fraction, and it was concluded that ribosomal modification was induced during the cultivation of the cells in the presence of cycloheximide.

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2. M. Takagi, S. Kawai, I. Shibuya, M. Miyazaki and K. Yano. 1986. cloning in saccharomyces cerevisiae of a cycloheximide resistance gene from the Candida maltosa genome which modified ribosomes. J. Bacteriol. in press.

Abstract. We have previously shown that cycloheximide resistance can be induced in a strain of Candida maltosa by modifying ribosomes. The present paper describes the cloning of the gene involved in this resistance by using a host-vector system of Saccharomyces cerevisiae.

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3. M. Takagi, S. Kawai, M.C. Chang, I. Shibuya and K. Yano. 1986. Construction of a host-vector system of Candida maltosa using an ARS site isolated from its genome. J. Bacteriol. in press.

Abstract. To construct a host-vector system in a n-alkane-assimilating yeast, Candida maltosa, isolation of an ARS site from its genome was attempted which replicated autonomously in C. maltosa. Leu⁻ mutants of C. maltosa were transformed with a gene library prepared using YEpl3 (LEU⁺) as a vector, and Leu⁺ transformants were obtained at a high frequency. A plasmid named pCS1 was isolated from the recipient cells. pCS1 contained a 6.3 kb fragment of C. maltosa genome, and a 3.8 kb fragment with ARS activity was subcloned and designated as the TRA (transformation ability) region. Vectors (pTRA1 and pTRA11) for C. maltosa (Leu⁻) were constructed consisting of this 3.8 kb fragment, pBR322 and the LEU2 gene of S. cerevisiae. Transformation of C. maltosa (Leu⁻) with these plasmids was successful by both spheroplast- and Li-acetate-methods. Southern blot analysis suggested that the copy number of

pTRAI in C. maltosa was between 10 and 20, and it was stably maintained during growth without selective pressure in the medium. It was also found that these vectors could transform S. cerevisiae (leu2⁻) to LEU⁺, suggesting that the TRA region contained an ARS site or ARS sites that were specific not only for C. maltosa but also for S. cerevisiae.

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XIV. Academy of Sciences of the GDR, Central Institute of Microbiology and Experimental Therapy, Laboratory of Yeast Genetics, DDR-69 Jena, Beutenbergstr. 11. Communicated by H. Weber.

In the yeast genetics laboratory of our institute during the past several years a contribution was made to develop the genetics of Yarrowia (Saccharomycopsis) lipolytica. After establishing genetical methods own strains suitable for genetic studies were constructed. Main attention has been paid to improve conjugation and sporulation parameters as well to develop genetically well marked and defined strains. By means of these strains investigations on life cycle and regulation of glyoxylate cycle enzymes are now in progress.

Below follows a publications list from our laboratory:

1. H. Weber. 1979. Substructural studies on sporulation of Saccharomycopsis lipolytica. Z. Allg. Mikrobiologie 19, 283-297.
2. L. Spata and H. Weber. 1980. A study on protoplast fusion and parasexual hybridization of alcane utilizing yeast. Advances in Protoplast Research, S. 131-137, Akademia Kiado, Budapest and Pergamon Press, Oxford.
3. H. Weber, W. Förster, H.-E. Jacob und H. Berg. 1980. Enhancement of yeast protoplast fusion by electric field effects, Advances in Biotechnology: Proceedings V. Int. Symposium on yeast, London, Ontario 1980, Pergamon Press, 219-224.
4. H. Weber and L. Spata. 1980. Characterization of yeast protoplast-fusion products: Advances in Biotechnology: Proceedings V. Int. Symposium on yeasts, London, Ontario, 1980, Pergamon Press, 213-218.
5. H. Weber, W. Förster, H.-E. Jacob, H. Berg. 1981. Microbiological implications of electric field effects III. Stimulation of yeast protoplast fusion by electric field impulses. Z. Allg. Mikrobiologie, 555-562.
6. H. Weber, W. Förster, H.-E. Jacob und H. Berg. 1981. Parasexual Hybridization by Electric Field Stimulated Fusion of Protoplasts. Current Genetics, 165-166.
7. H. Weber. 1982. Zellbiologie und Genetik der Hefen. Methoden und Arbeitstechniken. 171 p., Akademie Verlag Berlin/DDR.
8. G. Barth and H. Weber. 1983. Genetic studies on the yeast Saccharomycopsis lipolytica. Inactivation and mutagenesis. Z. Allg. Mikrobiol. 23: 147-157.

9. C. Kurischko, S.G. Inge-Vechtomov and H. Wever. 1983. Development of breeding stocks of the yeast Saccharomycopsis lipolytica by methods of moderate inbreeding. Z. Allg. Mikrobiol. 23, 513-515.
10. H. Berg, E. Bauer, W. Förster, M. Hartmann, H.-E. Jacob, A. Kurischko, P. Mühlig, H. Weber. 1983. Cell fusion by electric field pulses. Studia biophysica 94, 93-96.
11. I. Hones. 1983. Untersuchungen zur Regulation der Enzyme des Glyoxylatzyklus bei Saccharomycopsis lipolytica. 1. Einfluss der C-Quelle auf die Aktivität der Isocitratlyase und Malatsynthase. Z. Allgem. Mikrobiologie 23, 163-171.
12. I. Hones. 1984. Untersuchungen zur Regulation der Enzyme des Glyoxylatzyklus bei Saccharomycopsis lipolytica. 2. Wirkung von Glucose und Itaconsäure auf die Isocitratlyase-Regulation. Z. Allgem. Mikrobiologie 24, 9.
13. G. Barth and H. Weber. 1984. Use of nystatin for random spore analysis in the yeast Saccharomycopsis lipolytica. Z. Allgem. Mikrobiologie 24, 125-127.
14. G. Barth and H. Weber. 1984. Improved conditions for mating of the yeast Saccharomycopsis lipolytica. Z. Allgem. Mikrobiologie 24, 403-405.
15. H. Berg, K. Augsten, E. Bauer, W. Förster, H.-E. Jacob, P. Mühlig, A. Kurischko and H. Weber 1984. Possibilities of cell fusion and transformation by electrostimulation. Bioelectrochemistry and Bioenergetics 12, 119-133.
16. C. Kurischko. 1984. Analysis of genetic markers in new breeding stocks of the yeast Saccharomycopsis. Z. Allg. Mikrobiologie 24, 545-550.
17. G. Barth and H. Weber. 1985. Improvement of sporulation in the yeast Yarrowia lipolytica. Antonie van Leeuwenhoek, Journal of Microbiology 51, 167-177.
18. G. Barth. 1985. Genetic regulation of isocitrate lyase in the yeast Yarrowia lipolytica. Current Genetics 10, 119-124.
19. C. Kurischko and H. Weber. 1986. Temporal relationship of diploidization and haploidization in the yeast Yarrowia lipolytica. J. Basic Microbiol. 26, 2.
20. C. Kurischko. 1986. Spontaneous haploidization in early zygote progeny and its use for mapping in the yeast Yarrowia lipolytica. Current Genetics 10.

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

Below follow abstracts of two recent papers from my laboratory.

1. Y. Iwamoto¹, Y. Yanagihara¹, and L.W. Yielding². 1986. Petite Induction in Yeasts, Saccharomyces cerevisiae, by Photoactivation of 3-Azido-6-Amino-10-Methylacridinium Chloride. Photochemistry and Photobiology, Vol. 43, No. 2, pp. 139-144.

¹Department of Microbiology, Shizuoka College of Pharmacy, Shizuoka-shi, Japan and ²Department of Anatomy, University of South Alabama College of Medicine, Mobile, Alabama 36688, USA.

Abstract

The photoinduction of petite colonies and cell toxicity in non-growing yeast, Saccharomyces cerevisiae, by 3-azido-6-amino-10-methylacridinium chloride (AAMAC) has been examined. The results presented here indicate that mitochondrial DNA damage in resting yeast which occurs following irradiation of AAMAC-treated cells for short time periods is probably mediated through a covalent adduct between AAMAC and DNA. Furthermore, the photoreaction which contributes to biological activity is dependent on the presence of oxygen. Pre-irradiated AAMAC, which no longer exhibited the short-term photo-induction of biological effects showed a second biological activity. In this case longer irradiation time, e.g., 30 min, were required to induce petites for resting yeast. Again there was a strong dependence on the presence of oxygen. These results suggest that both processes may be effected through oxygen intermediates (photodynamic processes).

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2. Yoshihisa Iwamoto,^a Yasutake Yanagihara^a, Lereña W. Yielding^b, and K. Lemone Yielding^b. 1986. Petite Induction in Yeast, Saccharomyces cerevisiae, by Phenanthridinium Compounds: Promotive Effects of Propidium Iodide on Mutagenesis by Ethidium Bromide or 8-Deaminoethidium Chloride. Chem. Pharm. Bull. 34(4)1735-1739.

Shizuoka College of Pharmacy,^a 2-2-1 Oshika, Shizuoka-shi 422, Japan and School of Medicine, University of South Alabama,^b Mobile, Alabama 36688, USA.

Promotive effects of propidium iodide (PI) on petite induction by 8-deaminoethidium chloride (8-DAEC) were examined. 8-DAEC was a potent petite inducer in growing yeast cells but not in resting yeast cells. Addition of PI promoted the petite induction by 8-DAEC throughout the cultivation time at a concentration that was ineffective by itself. In resting cells, petites were scarcely induced by either 8-DAEC or PI or both, even after prolonged incubation. Notable sectorial colony induction was observed after prolonged incubation of the yeast cells with either 8-DAEC or PI in phosphate-buffer. The petite induction by ethidium bromide (EB) was not accelerated but was delayed in PI-pretreated cells, whereas the simultaneous presence of PI promoted the EB mutagenesis.

These results suggested that the simultaneous presence of PI and petite inducer in growth medium was required in order to exhibit the promotive

effects. Possible mechanisms of the differences in the process of petite induction between resting and growing cells are discussed.

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XVI. Institut für Genetik, TH Darmstadt, Schnittspahnstr. 10, 6100 Darmstadt, FR Germany. Communicated by Stefan Hohmann.

The following is a summary of a paper about invertase genes in Saccharomyces cerevisiae.

1. Stefan Hohmann and Friedrich K. Zimmermann. 1986. Cloning and Expression on a multicopy vector of five invertase genes of Saccharomyces cerevisiae. Current Genetics (in press).

Summary

Six unlinked loci for invertase structural genes are known in the yeast Saccharomyces cerevisiae: SUC1-SUC5 and SUC7. These genes are similar in structure and expression but not identical. Different yeast strains possess none, one or several of these genes.

We have isolated the genes SUC1-SUC5, subcloned them into the multicopy vector YEp24 and compared the expression of the five SUC genes in one recipient strain.

SUC2 was isolated by transformation of a suc0 strain with a gene pool and complementation to sucrose fermentation. SUC4 was cloned from a minipool of chromosomal fragments which were shown to contain SUC4 by Southern hybridization. SUC1, SUC3, and SUC5 were isolated using the method of plasmid eviction. A plasmid was integrated next to these SUC genes. The plasmid together with the SUC genes were then cut out off the chromosome using an appropriate restriction endonuclease.

The length of chromosomal DNA fragments containing the different SUC genes were 4.8 kb for SUC1, 5.2 kb for SUC2, 4.8 kb for SUC3, 12.8 kb for SUC4 and 17.2 kb for SUC5.

Fragments containing the complete SUC genes and the sequences controlling their expression were subcloned into YEp24 and transformed into a strain without any active invertase gene. Invertase activity of transformants was measured after growth on repressing (8% glucose) and derepressing (2% raffinose) conditions. As expected from results with strains carrying the individual SUC genes in a chromosomal location, the SUC genes were expressed to a different extent.

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XXVII. The University of Western Ontario, Department of Plant Sciences, Biological & Geological Building, London, Canada N6A 5B7. Communicated by R.B. Gardiner and A.W. Day.

The following are abstracts of recently published articles from our laboratory.

1. Day, A.W., R.B. Gardiner, R. Smith, A.M. Svircev, and W.E. McKeen. 1986. Detection of fungal fimbriae by protein A - gold immunocytochemical labelling in host plants infected with Ustilago heufleri or Peronospora hyoscyami f. sp. tabacina. Can. J. Microbiol. 32: 577-584.

Sections of leaves of Nicotiana tabacum L. infected with Peronospora hyoscyami De Bary f. sp. tabacina (Adam) Skalicky and of Erythronium americanum Ker. infected with Ustilago heufleri Fuckel were treated with an antiserum directed against the fimbriae of U. violacea Fuckel and other fungi. The sections were then treated with protein A - gold complexes to detect the presence and location of fimbriae antigens following transmission or scanning electron microscopy. Control preparations involved sections of uninfected leaves, as well as a range of serological control treatments. The infected leaf sections were heavily labelled with gold particles indicating the presence of fimbrial antigens, whereas the control preparations showed only a low background level of labelling. Gold particles were detected on the sections of hyphae, on haustoria, and on the nearby plant cells. The intensity of labelling was much higher for P. hyoscyami f. sp. tabacina than for U. heufleri and was particularly high in the walls of the former species. Relatively high levels of labelling occurred over the cells of infected hosts, but little or none occurred over the cells of uninfected host tissues, or of the infected host tissues treated with a range of serological controls. This high level of labelling was not associated with specific host structures in P. hyoscyami, but was frequently associated with the chloroplasts in U. heufleri. The antigens detected inside the host plant cells appear to indicate that fungal fimbrial protein, either as polymerized fibrils or as isolated subunits, can penetrate the host plasma membrane and therefore enter the host cytoplasm. An alternative possibility is that these antigens derive from host produced proteins synthesized as a result of infection. These results suggest the possibility that fungal fimbriae may play an important role in the molecular interaction between pathogen and host.

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2. Alan J. Castle and Alan W. Day. 1986. Diploid Derivatives of Ustilago violacea with Altered Mating-Type Activity III. Constitutive Mating Strains. Bot. Gaz. 147(1):110-115.

Freshly isolated diploids of Ustilago violacea, heterozygous for mating type (a_1/a_2), give rise to frequent (5×10^{-3}) derivative forms that appear to form after mitotic crossing-over near the mating-type locus. One such derivative, termed op-C, constitutively produces mating tubes at temperatures below 20 C on complete medium or 25 C on minimal medium but buds vegetatively at higher temperatures. Op-C strains remain diploid and have no detectable alterations, compared with the original diploid, in all the marked chromosomes except for the mating-type locus. Op-C strains yield segregants, which are either a_1 in mating type (both diploids and haploids) or haploids that produce mating tubes constitutively. The op-C phenotype arises after alterations that affect the a_2 allele in a_1/a_2 diploids and that therefore change the development of these cells. Op-C and other opaque-derivative forms provide valuable strains to study the action of the mating type locus as a developmental master switch.

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XVIII. Departamento de Genetica, Facultad de Biologia, Universidad de Sevilla, Apartado 1095, Sevilla, Spain. Communicated by Tahia Benitez.

The following papers have been published recently:

1. Andres Aguilera and Tahia Benitez. 1985. Role of mitochondria in ethanol tolerance of Saccharomyces cerevisiae. Arch. Microbiol. 142:389-392.

The presence of active mitochondria and oxidative metabolism is shown to be essential to maintain low inhibition levels by ethanol of the growth rate (μ), fermentation rate (v) or respiration rate (ρ) of Saccharomyces cerevisiae wild type strain S288C. Cells which have respiratory metabolism show K_i (ethanol inhibition constant) values for μ , v , and ρ , higher ($K_i > 1M$) than those of "petite" mutants or "grande" strains grown in anaerobiosis ($K_i = 0.7 M$). In addition, the relationship between μ or v and ethanol concentration is linear in cells with respiratory metabolism and exponential in cells lacking respiration. When functional mitochondria are transferred to "petite" mutants, the resulting strain shows K_i values similar to those of the "grande" strain and the inhibition of μ and v by increasing ethanol concentrations becomes linear.

2. Juan Jiménez and N. van Uden. 1985. Use of Extracellular Acidification for the Rapid Testing of Ethanol Tolerance in Yeast. Biotechnol. Bioeng. 27:1596-1598.

The exponential constant of ethanol-enhanced passive proton diffusion across the plasma membrane of yeast can be estimated rapidly and easily. It occurred to us that this might constitute a rapid method for the preliminary evaluation of ethanol tolerance in yeasts, such as would be convenient to use when large numbers of mutants and other strains have to be screened. To test its feasibility we verified, using a number of strains, whether a correlation existed between the values of the exponential constant (k) of ethanol-enhanced proton influx and the ethanol concentration (K_i) that reduced the specific growth rate by 50%.

3. Andres Aguilera and Tahia Benitez. 1986. Ethanol-sensitive mutants of Saccharomyces cerevisiae. Arch. Microbiol. 143:337-344.

Saccharomyces cerevisiae mutants unable to grow at ethanol concentrations at which the wild type strain S288C does grow, have been isolated. Some of them show additional phenotypic alterations in colony size, temperature sensitivity and viability in ethanol, which cosegregate with the growth sensitivity in ethanol. 21 selected monogenic ethanol-sensitive mutants define 20 complementation groups, denominated ETA1 to ETA20, which indicates that there is a high number of genes involved in the ethanol tolerance/sensitivity mechanism.

Out of 21 selected monogenic mutants, 20 are not altered in the glycolytic pathway since, when maintained in glucose-supplemented medium, they can produce as much ethanol as the wild type and at about the same velocity. Nor do any of the mutants seem to be altered in the lipid biosynthetic pathway since, whether grown in the absence or in the presence of ethanol, their

concentration of fatty acids and ergosterol is similar to that of the wild type under the same conditions. Therefore, growth sensitivity to ethanol does not seem necessarily to be related to carbohydrate or lipid metabolism.

4. Alfonso Pina, Isabel L. Calderon and Tahia Benitez. 1986. Intergeneric Hybrids of Saccharomyces cerevisiae and Zygosaccharomyces fermentati Obtained by Protoplast Fusion. Appl. Environ. Microbiol. in press.

To obtain strains that are able to efficiently produce ethanol from different carbohydrates, mainly cellulose hydrolysates, several species of the genus Candida and a Zygosaccharomyces fermentati strain were examined for their ability to utilize cellobiose and produce ethanol, as well as for their thermotolerance and the possibility of genetic manipulation. Candida obtusa and Zygosaccharomyces fermentati tolerated the maximal temperature for growth, possessed the highest cellobiase activity, and offered the possibility of genetic manipulation although neither of them proved to be a good producer of ethanol. Intergeneric hybrids of Saccharomyces cerevisiae and Z. fermentati were obtained after protoplast fusion. They were selected as prototrophic strains, after isolation of auxotrophic mutants from Z. fermentati and fusion with an S. cerevisiae strain which was also auxotrophic. The hybrids, which appeared at a frequency of 2×10^{-7} presented characteristics of both parents, such as resistance to certain drugs and the ability to grow with either cellobiose or lactic acid as the sole carbon source; they were very stable, even under nonselective conditions. These hybrids may have important industrial applications as good fermenting strains.

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- XIX. Albert-Ludwigs-Universität Freiburg Im Breisgau, Medizinische Fakultät, Biochemisches Institut. Communicated by Helmut Holzer.

Below follows an abstract of a paper which is scheduled for the March 1987 issue of the Journal of Biological Chemistry.

Doris Horn¹ and Helmut Holzer^{1,2}. Fructose-1,6-bisphosphatase(4P) Dephosphorylating Protein-Phosphatase from Saccharomyces cerevisiae. Journal of Biological Chemistry, March 1987.

- 1) Biochemisches Institut der Universität Freiburg, D-7800 Freiburg i. Br. (West Germany).
- 2) Gesellschaft für Strahlen- und Umweltforschung, Geschäftsführung, D-8042 Neuherberg (West Germany).

Phosphorylation of fructose-1,6-bisphosphatase with cyclic AMP-dependent protein kinase from yeast is accompanied by a 50% decrease in the catalytic activity (G. Pohlig and H. Holzer (1985) J. Biol. Chem. 260, 13818-13823). Using reactivation of phosphorylated fructose-1,6-bisphosphatase as assay, a protein phosphatase was about 2,000-fold purified to electrophoretic homogeneity from Saccharomyces cerevisiae. Upon incubation with phosphorylated fructose-1,6-bisphosphatase the purified protein phosphatase not only reverses the 50% inactivation caused by phosphorylation, but also the previously observed change in the pH optimum and in the ratio of activity with

Mg²⁺ or Mn²⁺. The phosphatase is strongly inhibited by heparin and fluoride. L-Carnitine, orthophosphate, pyrophosphate and succinate inhibit to 50% at concentrations from 1-10 mM. The molecular mass of the native phosphatase was found to be 180,000 Da. SDS-gel electrophoresis suggested four subunits with a molecular mass of 45,000 Da each. Half maximal activity was observed with 5 mM Mg²⁺ or Mn²⁺, the pH-optimum of activity was found at pH 7. Using polyclonal antibodies disappearance of ³²P-labeled fructose-1,6-bisphosphatase and concomitant liberation of the expected amount of inorganic ³²P phosphate was demonstrated.

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XX. Chaire de Génétique, et de Microbiologie, Ecole Nationale Supérieure Agronomique, 34060 Montpellier Cedex, FRANCE. Communicated by Pier Galzy.

The following are articles recently published by workers in my laboratory.

GONDE P., RATOMAHENINA R., ARNAUD A., and P. GALZY. 1985.
Purification and properties of the exocellular β -glucosidase of Candida molischiana (ZIKES) MEYER and YARROW capable of hydrolyzing soluble cellodextrins.
Can J. Biochem. Cell Biol. (Can) 63:1160-1166.

LECLERC M., BLONDIN B., RATOMAHENINA R., ARNAUD A., and P. GALZY. 1985.
Selection and study of Dekkera intermedia and Candida wickerhamii mutants derepressed for β -glucosidase production.
FEMS Microbiol. Lett. (GBR) 30:389-392.

LECLERC M., ARNAUD A., RATOMAHENINA R. ET GALZY P. 1986.
Etude et amélioration génétique d'une souche de levure productrice de β -glucosidase.
Microbiologie - Aliments - Nutrition (Fr) 4:7-18.

LECLERC M., ARNAUD A., RATOMAHENINA R., and P. GALZY. 1986.
Physical and kinetic properties of the cellodextrinase and of the β -glucosidase of Candida wickerhamii. Regulation of their biosynthesis.
XIth International Specialized Symposium on Yeasts. Lisbon (Portugal), March 17-21.

GONDE P., LECLERC M., ARNAUD A., RATOMAHENINA R. and GALZY P. 1986.
Physical and kinetic properties of the exocellular β -glucosidase of Candida molischiana. Regulation of its biosynthesis.
XIth International Specialized Symposium on Yeast. Lisbon (Portugal), March 17-21.

LECLERC M., ARNAUD A., RATOMAHENINA R., and P. GALZY. 1986.
Genetic improvement of a strain of Candida wickerhamii by selection of mutants derepressed for β -glucosidase production.
Fifth International Symposium on the Genetics of Industrial Microorganism. Split (Yugoslavia), September 14-20.

LECLERC M., RATOMAHENINA R., ARNAUD A., and P. GALZY. 1986.
Sélection et amélioration de souches de levure pour la production de β -glucosidases.
Congrès 1986 de l'Association Tessier A. : Microbiologie - Biotechnologie - Nutrition, Paris 5-6 mars 1986.

J.M. MUDERHWA, R. RATOMAHENINA, M. PINA, J. GRAILLE and P. GALZY. 1986.
Purification and properties of the lipases from Rhodotorula pilimanae Hedrick and Burke. Appl. Microbiol. Biotechnol. 23:348-354.

D. MONTET, R. RATOMAHENINA, J.M. LABORBE, M. PINA, J. GRAILLE and P. GALZY.
Production de protéines d'organismes unicellulaires à partir de pâtes de neutralisation d'origine industrielle.
Oléagineux, 40, n° 10 (1985).

P. GALZY, R. RATOMAHENINA, J. GRAILLE et M. PINA. 1986.
Microbiologie appliquée à l'industrie des lipides.
Revue Française des Corps Gras - 33ème Année - n° 8-9. August-September.

RATOMAHENINA R., M. MUDERHWA, D. MONTET, M. PINA, J. GRAILLE et P. GALZY. 1986.
Study of lipases from various species of lipolytic yeasts.
Xith International Specialized Symposium on Yeasts. Lisbon-Portugal - March 17-21.

MALFAIT M.H., MOULIN G., GALZY P. 1986.
Ethanol inhibition of growth, fermentation and starch hydrolysis in Schwanniomyces castellii. J. Ferment. Technol. 69:279-284.

MOULIN G., POINSOT C. et P. GALZY. 1985.
Study of the respiratory and fermentative metabolism of Schwanniomyces castellii. Xth ISSY - Genetics and Molecular Biology - Varna - Bulgaria.

MOULIN G., POINSOT C., H. BOZE and P. GALZY. 1986.
Antimycin A and hydroxamate insensitive respiration in Schwanniomyces castellii. XI ISSY - Lisbon - Portugal.

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XXI. Instituto de Investigaciones Biomedicas del C.S.I.C., Facultad de Medicina de la Universidad Autonoma, Arzobispo Morcillo, 4, 28029 Madrid, Spain. Communicated by Carlos Gancedo and Rosario Lagunas.

The following are abstracts of recent publications from our Institute.

1. Francisco Portillo and Carlos Gancedo. 1986. Purification and properties of three intracellular proteinases from Candida albicans. Biochimica et Biophysica Acta 881:229-235.

Three intracellular proteinases termed A, B and C were purified to homogeneity from the unicellular form of the yeast Candida albicans. Enzyme A is an aspartic proteinase that acts on a variety of proteins. Its optimal pH is around 5 and it is displaced to 6.5 by KSCN. It is not significantly inhibited by PMSF, TLCK (Tos-Lys-CHCl₂) or soybean trypsin inhibitor but it is inhibited by pepstatin. Its molecular weight is 60000. Enzyme B is a

dipeptidase that acts on esters or on dipeptides without blocks in either the carboxyl or amino ends. Its pH optimum is around 7.5 and the molecular weight is 57000. It is inhibited by PMSF, TLCK and DANME (N₂Ac-Nle-OMe). Proteinase C is an aminopeptidase with an optimum pH around 8. Its molecular weight was 67000 when determined by SDS gel electrophoresis and 243000 when determined by gel filtration. It is active towards dipeptides in which at least one amino acid is apolar and is not active when the N-terminal amino acid is blocked. It is inhibited by EDTA or o-phenanthroline and activated by several divalent cations.

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2. Carlos Gancedo, Antonio Llobell, Juan-Carlos Ribas¹ and Francisca Luchi. 1986. Isolation and characterization of mutants from Schizosaccharomyces pombe defective in glycerol catabolism. Eur. J. Biochem. 159:171-174.

¹Instituto de Microbiologia Bioquímica del Consejo Superior de Investigaciones Científicas, Universidad de Salamanca.

Mutants unable to grow on glycerol were isolated from the fission yeast Schizosaccharomyces pombe. Two types of mutants were obtained: one type was able to grow on dihydroxyacetone while the other one did not grow on this compound. The first type of mutants was defective in glycerol dehydrogenase while the second one was affected both in the glycerol dehydrogenase and in dihydroxyacetone kinase. It was found that the second type was defective in the derepression of several enzymes. The mutations were nuclear and monogenic and defined two complementation groups. Spontaneous revertants, able to grow on glycerol, were obtained from the first type of mutants. They have regained the glycerol dehydrogenase activity. The results presented provide genetic evidence for a pathway of glycerol catabolism in Sch. pombe involving dehydrogenation of glycerol as the first step followed by phosphorylation of the dihydroxyacetone formed.

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3. Rosario Lagunas. 1986. Misconceptions About the Energy Metabolism of Saccharomyces cerevisiae. Yeast 2: (in press).

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Fate of the ATP produced in yeast catabolism
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Misinterpretations by modern biochemists of Pasteur's data on growth yield
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Pasteur effect can be observed under special experimental conditions
Mechanism of appearance of Pasteur effect in resting cells: inactivation of the sugar transport system
Fermentation is more sensitive than respiration to changes in sugar uptake rate

Final remarks
References

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4. Ana Busturia and Rosario Lagunas. 1986. Catabolite Inactivation of the Glucose Transport System in Saccharomyces cerevisiae. J. of Gen. Microbiol. 132:379-385.

The sugar transport systems of Saccharomyces cerevisiae are irreversibly inactivated when protein synthesis is inhibited. This inactivation is responsible for the drastic decrease in fermentation observed in ammonium-starved yeast and is related to the occurrence of the Pasteur effect in these cells. Our study of the inactivation of the glucose transport system indicates that both the high-affinity and the low-affinity components of this system are inactivated. Inactivation of the high-affinity component evidently requires the utilization of a fermentable substrate by the cells, since (i) inactivation did not occur during carbon starvation, (ii) when a fermentable sugar was added to starved cells, inactivation began, (iii) when the fermentation inhibitors iodoacetate or arsenate were added in addition to sugars, the inactivation was prevented, (iv) when a non-fermentable substrate was added instead of sugars, inactivation was also prevented. The inactivation of the low-affinity component appeared to show similar requirements. It is concluded that the glucose transport system in S. cerevisiae is regulated by a catabolite-inactivation process.

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5. Carmen DeJuan and Rosario Lagunas. 1986. Inactivation of the galactose transport system in Saccharomyces cerevisiae. FEBS 207(2): October 258-261.

The galactose transport system of Saccharomyces cerevisiae consists of one component which shows a K_m value of approx. 4 mM in growing cells. A rapid and irreversible inactivation of this transport is detected on impairment of protein synthesis. This inactivation shows the following characteristics: (i) it is due to changes in the K_m and V_{max} of the transport system; (ii) it follows first-order kinetics, (iii) it is an energy-dependent process and is stimulated by the presence of an exogenous carbon source; (iv) fermentable substrates stimulate inactivation more efficiently than non-fermentable substrates.

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6. Rosario Lagunas, Carmen DeJuan, and Begona Benito. 1986. Inhibition of Biosynthesis of Saccharomyces cerevisiae Sugar Carriers by Tunicamycin. J. Bacteriol. 168(3) in press.

Tunicamycin apparently inhibited the biosynthesis of glucose, galactose, and maltose transport systems in Saccharomyces cerevisiae. Under the conditions used, the antibiotic also blocked the biosynthesis of invertase, a well-known yeast glycoprotein, as well as the glycosylation of a marker mannoprotein of the yeast cell wall. However, the antibiotic did not affect certain proteins which did not contain carbohydrate. It seems, therefore, that these sugar carriers are glycoproteins.

XXII. Delft University of Technology, Department of Microbiology & Enzymology, Julianalaan 67, NL-2628 BC Delft, The Netherlands.
Communicated by W. Alexander Scheffers.

The following papers, abstracts of which have already appeared in Yeast Newsletter Vol. XXXV, Number 1, now have been published.

1. J.P. van Dijken and W.A. Scheffers. Redox balances in the metabolism of sugars by yeasts. FEMS Microbiology Reviews 32 (1986) 199-224.
2. P.M. Bruinenberg, G.W. Waslander, J.P. van Dijken and W.A. Scheffers. A comparative radiorespirometric study of glucose metabolism in yeasts. YEAST 2 (1986) 117-121.
3. J.P. van Dijken, E. van den Bosch, J.J. Hermans, L. Rodrigues de Miranda and W.A. Scheffers. Alcoholic fermentation by 'non-fermentative' yeasts. YEAST 2 (1986) 123-127.
4. C. Purwin, K. Nicolay, W.A. Scheffers and H. Holzer. Mechanism of control of adenylate cyclase activity in yeast by fermentable sugars and carbonyl cyanide *m*-chlorophenyl-hydrazone. Journal of Biological Chemistry 261 (1986) 8744-8749.

Also, the following two papers have appeared.

5. P. de Jonge, F.C.M. de Jongh, R. Meijers, H.Y. Steensma and W.A. Scheffers. Orthogonal-field-alternation gel electrophoresis banding patterns of DNA from yeasts. YEAST 2 (1986) 193-204.

Abstract. Chromosomal DNAs from various yeast species were separated by orthogonal-field-alternation gel electrophoresis (OFAGE). To this end we developed a spheroplasting and lysis method to obtain intact DNA from both ascomycetous and basidiomycetous yeasts. The OFAGE banding patterns of 22 ascomycetous and four basidiomycetous yeast strains were compared. The strains represented species from the genera: Brettanomyces, Candida, Cryptococcus, Filobasidiella, Geotrichum, Hansenula, Kluyveromyces, Pachysolen, Pichia, Rhodosporidium, Rhodotorula, Saccharomyces, Saccharomycodes, Saccharomycopsis, Schizosaccharomyces and Zygosaccharomyces. Variations occurred in the number of bands and their positions in the gel, not only among strains of different genera but also among species from the same genus and even between varieties of the same species. The ascomycetous yeasts, with the exception of Saccharomyces cerevisiae, only showed one to five bands of DNA larger than 1000 kilobase pairs (kb) in general none smaller. The patterns of the four basidiomycetous yeasts revealed also a few large DNA bands but in addition one to six bands ranging in size from 500 to 1000 kb, with the exception of a single smaller chromosome in Rhodotorula mucilaginosa. From the OFAGE banding patterns of strains studied here it appears that in Sacch. cerevisiae the partitioning of DNA over chromosomes is unique. But rather than the large number of chromosomes, the presence of four chromosomes with less than 500 kb of DNA is characteristic of Sacch. cerevisiae.

6. P.M. Bruinenberg. The NADP(H) redox couple in yeast metabolism. *Antonie van Leeuwenhoek* 52 (1986) 411-429.

Abstract. Theoretical calculations of NADPH requirement for biomass formation indicate that in yeasts this parameter is strongly dependent on the carbon and nitrogen sources used for growth. Enzyme surveys of NADPH-generating metabolic pathways and radiorespirometric studies demonstrate that in yeasts the HMP pathway is the major source of NADPH. Furthermore, radiorespirometric data suggest that in yeasts the HMP pathway activities are close to the theoretical minimum. It may be concluded that the mitochondrial NADPH oxidation, which in yeasts may yield ATP, is quantitatively not an important process.

The inability of *C. utilis* to utilize the NADH produced in formate oxidation as an extra source of NADPH strongly suggests that transhydrogenase activity is absent. Furthermore, the absence of xylose utilization under anaerobic conditions in most facultatively fermentative yeasts indicates that also in these organisms transhydrogenase activity is absent. This conclusion is supported by the observation that anaerobic xylose utilization is observed only in those yeasts which possess a high activity of an NADH-linked xylose reductase. Hence in these organisms the redox-neutral conversion of xylose to ethanol is possible, since the second step in xylose metabolism is mediated by an NAD⁺-linked xylitol dehydrogenase.

7. On November 21, 1986, Dr. P.M. Bruinenberg (now at AVEBE, Foxhol, The Netherlands) has received the Kluyster prize 1986 of the Netherlands Society for Microbiology, during a meeting of the Society at Amsterdam.

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XXIII. Laboratory of Biochemistry and Physiology of Yeasts, Department of Enzyme Engineering, Institute of Microbiology, Czechoslovak Academy of Sciences, Videlska 1083, 142 20 Prague 4, Czechoslovakia.
Communicated by Ā. Novotný.

The following are the summaries of two papers from this laboratory which have been submitted for publication.

1. Ā. Novotný, B. Běhalová, L. Doležalová and J. Zajiček. 1987. Regulation of sterol synthesis by glucose in baker's yeast. *Acta Biotechnologica* 7:4, in press.

The ability of ten baker's yeast strains to synthesize sterols was checked. Ergosterol/24 (28)-dehydroergosterol (E/D) ratio had the value of 0.8 to 1 in most strains grown in a medium containing molasses as the carbon source. The ratio values were significantly increased in the cultures grown in a glucose medium. Both a decrease in the content of 24 (28)-dehydroergosterol and a slight increase of the ergosterol content were found to be responsible for the high values of E/D in the glucose-grown cells. The strains examined could be divided into three groups on the basis of their behaviour towards glucose. The effect of the type of cultivation on sterol accumulation is demonstrated by comparing the sterol content of the representatives of the three groups of baker's yeasts in the cells grown in a fermenter or in shaken flasks.

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2. Č. Novotný, B. Běhalová, R. Stružinský, M. Novák and J. Zajiček. Sterol composition of a $\Delta^{5,7}$ -sterol-rich strain of Saccharomyces cerevisiae during batch growth. Submitted for publication.

Sterol composition was examined during batch growth on a complex medium containing ethanol, molasses or glucose as the carbon source. The molasses-grown cells exhibited a balanced sterol composition throughout growth, maintaining the proportion of ergosterol/24:28-dehydroergosterol equal to 1.4. The negative effect of glucose (90 g per L) on sterol synthesis manifested itself by decreasing the accumulation of 24:28-dehydroergosterol and total sterols but not of ergosterol. Using ethanol as the sole carbon source, a large amount of 24:28-dehydroergosterol accumulated, partly on the account of other sterols. The intermittent addition of nitrogen source during growth significantly decreased the accumulation of ergosterol, 24:28-dehydroergosterol and of total sterols. The general scheme of regulation of sterol synthesis in the baker's yeast is discussed.

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

The following papers are in press or have been published since the last Yeast Newsletter.

1. Gregory P. Casey and W.M. Ingledew. 1986. Ethanol Tolerance in Yeasts. CRC Crit. Rev. in Microbiology 13(3):219-280.

The past 2 decades have seen an enormous increase in interest in the ethanol-tolerance properties of yeasts. Many recent findings, however, especially with brewing and wine yeasts, contradict the long-held belief that industrial strains of Saccharomyces yeasts vary considerably in their ability to tolerate ethanol. Of particular importance is the discovery that the inherent ethanol tolerance of any specific yeast strain is not the only factor regulating the maximum level of ethanol that can be produced in industrial alcoholic fermentations. This review, therefore, begins with an analysis of the methods used to define and measure ethanol tolerance. Subsequently, research exploring the mechanisms of ethanol toxicity, the influences of nutritional and environmental parameters, the role of yeast lipid composition, and the genetics of ethanol tolerance will all be explored. When these factors are all considered, the recent literature challenges many of the traditional views of ethanol tolerance in yeasts and should serve as an instrument to significantly improve the economics of ethanol-related industrial fermentations.

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2. W.M. Ingledew and N. Hazen. 1986. Active Dry Yeasts for Small Brewers. The New Brewer 3(4):1,14,15.

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3. W. M. Ingledew, F.W. Sosulski, and C.A. Magnus. An Assessment of Yeast Foods and Their Utility in Brewing and Enology. J. Amer. Soc. Brew. Chem. (in press).

Yeast foods are occasionally utilized in high-gravity brewing and enology to eliminate sluggish or stuck fermentations that may occur in these industries as a result of a deficiency of a key yeast nutrient such as low-molecular-weight nitrogen. Such foods are especially important when the carbohydrate content in the wort or juice is over 16%. Many yeast foods are now available commercially. In many cases, however, the brewer or enologist is unaware of the composition of the food, the purpose for which it was designed, how well it will serve as a nutrient for the yeast, or even the legality of its usage. This report identifies the major yeast-utilizable nitrogen sources in 15 such yeast foods and the levels of sulfate, phosphate, and major cations in each.

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4. C.A. Magnus, W.M. Ingledew, and G.P. Casey. High Gravity Brewing: Influence of High-Ethanol Beer on the Viability of Contaminating Brewing Bacteria. J. Amer. Soc. Brew. Chem. (in press).

It has been reported that when high-gravity brewers' worts were supplemented with a source of nitrogen and unsaturated lipids and sterol, ethanol concentrations up to 16.4% v/v could be achieved within normal fermentation times. As the resultant harvested yeast can be repitched over a number of generations, there appears to be no reason in industry to limit the gravities of commercial worts to 16% Plato, especially when the ester and fusel oil patterns of resultant beers may not be as elevated as previously thought. In this report the influence of fermenting high alcohol beer made from 28° Plato wort on the viability of traditional bacterial brewing contaminants was examined. Lactobacillus and Pediococcus, Acetomonas, Acetobacter, and Zymomonas contaminants were able to survive levels of ethanol of 12-13% v/v. Hafnia (Flavobacterium or Obesumbacterium), Enterobacter, (including E.agglomerans), Citrobacter, and Klebsiella species, known to exist through most stages of the fermentation of traditional gravity wort, were completely eliminated by the elevated ethanol levels. Very high gravity fermentations therefore narrow the range of bacteria capable of spoiling the beer, thereby reducing the risk of bacterial spoilage problems. This work illustrates an additional production advantage in favor of increasing original gravities of worts.

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5. W.M. Ingledew. Schwanniomyces: A Potential Superyeast. CRC Crit. Rev. in Biotechnol. (in press).

The emphasis of this research has been on Schwanniomyces, a starch degrading-alcohol producing yeast. Its amylolytic capabilities have been examined in detail including isolation and purification of an alpha-amylase and a glucoamylase. Schwanniomyces is one of a very few yeasts which produces both these enzymes as well as having some fermentative ability to make ethanol from distressed grains or potato starches. Derepressed wild type strains were isolated that produce 3 to 4 fold higher levels of both amylases. The

reported levels of enzymes, or more, will be required if this microbe is to become industrially important for alcohol production, for amylase production or for single cell protein. Conservative genetic techniques (protoplast fusion) have been used to examine intergeneric fusion of Saccharomyces and Schwanniomyces, and interspecific fusion of 2 Schwanniomyces species with different enzyme complements. The objective has been to create superamylolytic yeasts capable of rapid fermentation of starch to ethanol. Although we have not yet succeeded in production of stable strains useable in industry, with what is now known, suitable genetic work-up may lead to such a strain. It may also be useful for expression of foreign proteins. This work summarizes the first reports on the use of protoplast fusion in this genus and the derepression of amylase enzymes in Schwanniomyces. A number of laboratories in Canada and the world are now also examining this microbe in detail.

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6. G.P. Casey. 1986. Molecular and Genetic Analysis of Chromosome X in Saccharomyces carlsbergensis. Carlsberg Res. Commun. 51:343-362

Molecular hybridization of electrophoretically separated and blotted chromosomes with ILV3 gene probes detected the presence of three chromosomes X in S. carlsbergensis 244 lager yeast. Meiotic segregants, which were disomic or trisomic for chromosome X were crossed with a karl -1 strain in S. cerevisiae (MAT α or MAT α arg3 met3 ilv3 cycl-1 cdc11 hom6 ade2-40 cyh2 karl-1) for single chromosome transfer and isolation of two of the chromosomes X from the lager yeast. Type I chromosome X migrates slower than a reference chromosome X of S. cerevisiae K5-5A during chromosome separation by electrophoresis and contains the allele of ILV3 unique to the lager yeast. It recombines with chromosome X from S. cerevisiae, but only in the left arm. Type II chromosome X migrates faster than the reference chromosomes X and contains the allele of ILV3 also found in S. cerevisiae S288C. It recombines only in the right arm with chromosome X from S. cerevisiae. The third chromosome X from S. carlsbergensis, which co-migrates with the reference chromosome X of S. cerevisiae K5-5A, has yet to be isolated into the genetic background of a genetic standard strain.

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7. G.P. Casey. 1986. Cloning and Analysis of Two Alleles of the ILV3 Gene from Saccharomyces carlsbergensis. Carlsberg Res. Commun. 51:327-341.

A genomic library of a Saccharomyces carlsbergensis lager's yeast DNA was constructed in the yeast E. coli shuttle vector YRp17. Two alleles of the ILV3 were cloned from the library by complementation of the ilv3-12 mutation in strains of Saccharomyces cerevisiae yeast. Restriction site mapping and Southern hybridisation using an ILV3 probe from Saccharomyces cerevisiae S288C revealed one allele from the lager yeast to be closely related, or identical, to the ILV3 gene in S. cerevisiae S288C. The second allele has a different restriction site map and limited sequence homology with the ILV3 gene in S. cerevisiae S288C. The implications of these results in determining the genetic constitution of lager yeast and on research programs designed to genetically engineer lager yeast are discussed.

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XXV. National Research Council Canada, Molecular Genetics Section, Ottawa, Canada K1A 0R6. Communicated by G.B. Calleja.

The following publications appeared recently:

1. G.B. Calleja, A. Nasim, C.V. Lusena, and S. Levy-Rick. 1986. Excretion of yeast amylases: requirement for oxygen and mitochondrial function. *Biochemistry International* 12:81-87.

Extracellular amylases are not detectable in hypoxic cultures of the starch-fermenting yeast Schwanniomyces alluvius. When aerobic cultures in the process of excretion after the end of exponential growth are made anoxic, excretion is immediately arrested. Respiratory inhibitors, such as cyanide and azide, have the same effect as anoxia. Grown on ethidium bromide for three generations, a culture exhibits no detectable extracellular amylolytic activity. This culture recovers full extracellular amylolytic activity only after the second sequential transfer in fresh medium without ethidium bromide. A dissolved-oxygen concentration of 10% saturation is necessary for the excretion of α -amylase and glucoamylase.

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2. G.B. Calleja, M. Yaguchi, S. Levy-Rick, J.R.H. Seguin, C. Roy, and C.V. Lusena. 1986. Single-cell protein production from potato starch by the yeast Schwanniomyces alluvius. *Journal of Fermentation Technology* 64:71-75.

Fully aerated cultures of Schwanniomyces alluvius grew on 4% soluble potato starch in a defined minimal medium at a doubling time of 1.5 h at 30°C. A recovery of 51% (0.51 g of dried biomass from 1 g of starch) was obtained. Yields and growth rates of cultures on starch were similar to those on glucose.

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3. G.B. Calleja, Susan Levy-Rick, F. Moranelli and A. Nasim. 1984. Thermosensitive Export of Amylases in the Yeast Schwanniomyces aluvius. *Plant & Cell Physiol.* 25:757-761.

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

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4. In Press:

- (a) G.B. Calleja. Cell aggregation. The Yeasts, 2nd Ed. (A.H. Rose & J.S. Harrison, eds.), Vol. 2, Chapter 7. Academic Press, London.
(b) G.B. Calleja, S. Levy-Rick, A. Nasim & C.V. Lusena. Extracellular amylases of starch-fermenting yeast: pH effect on export and residence time in the periplasm. CRC Critical Reviews in Biotechnology 5 (2).

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XXVI. VTT Biotechnical Laboratory, Tietotie 2, SF-02150 Espoo 15, Finland. Communicated by Veijo Makinen.

J. Kronlöf, Hartwall Ltd. 1986. Optimizing of yeast handling in a brewery. Mallas ja Olut 1986, 22-27.

Yeast handling in a brewery is, like brewing in general, a synthesis of knowledge and traditions. In a well equipped control laboratory it is, however, to a certain extent possible to examine yeast and yeast handling and to apply the results in practice.

An example is presented, in which a clear connection between pitching rate and fermenting velocity was observed. Through a stepwise optimization of the yeast handling practice hanging fermentations could be eliminated and the average primary fermentation time was cut down by about 10 percent. This enabled a better process control and had a positive effect on the beer quality.

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XXVII. ALKO Research Laboratories, P.O. Box 350, SF-00101, Helsinki 10, Finland. Communicated by M. Korhola.

The following is a list of our work published since June 1986.

1. Matti Korhola and Kari Edelmann. 1986. Metabolism of Trace Elements Related to Human Diseases. Nordic Symposium, Loen, Norway, 1985, Acta Pharmacol. Toxicol. 59, Suppl. VII, 148-151.

Baker's yeast grown under special conditions has been enriched with trace elements of dietary importance. Compared with yeast cultivated normally with beet molasses cells can be enriched with 0.5 mg selenium (about 12,000 times), 1.0 mg chromium (14,000 times) and 3.0 mg zinc (18 times) calculated per gram dry weight of yeast.

The dietary importance of these enriched yeasts is based on the argument that organically bound trace elements are physiologically more effectively utilized than inorganic ones.

Elements inextractable from yeast into water or dilute acid have in literature been regarded as organic forms. About 95% of Se, 93% of Cr and 56% of Zn could not be extracted from the enriched yeast cells by water. In acid treatment about 82% of Se, 73% of Cr and 37% of Zn were found to be organically bound.

To discover the distribution of trace elements in cell compartments, enriched yeasts were fractionated by disintegrating the cells, differential centrifugation and TCA precipitation of soluble proteins.

Of total selenium 15% was found in the cell walls, 9% in mitochondria and microsomes, 58% in soluble proteins and nucleic acids precipitated with TCA and, finally, 19% in the soluble fraction of small peptides, amino acids and inorganic acids.

Of total chromium about 70% was found particle-bound in the cell walls, mitochondria and microsomes, and the rest evenly distributed to soluble proteins and nucleic acids and to compounds of small molecular weight.

One half of the total zinc was bound to the cell walls and the other half to small soluble compounds.

One half of the total zinc was bound to the cell walls and the other half to small soluble compounds.

In the fractionation of enriched yeast selenium was mainly found in soluble proteins probably replacing some of the sulphur in the amino acids. Chromium and zinc possibly form complexes with yeast organic matter.

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2. Roy S. Tubb. 1986. Amylolytic Yeasts For Commercial Applications. Trends in Biotechnology 4, 98-104.

Abstract

Significant recent progress has been made in constructing amylolytic strains of yeast and demonstrating their potential for a range of applications, particularly in ethanol or alcoholic beverage production. Nature offers a considerable assortment of amylolytic enzymes, and through recombinant DNA technology the merits of enzyme-producing yeasts for converting starch can now be compared directly with those of "added" enzymes. Over the next few years a number of amylolytic strains seem likely to find their way into commercial use.

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

The following are summaries of a book and papers recently published, accepted, or submitted for publication in 1986:

1. E. Minarik and A. Navara: Chemistry and Microbiology of Wine (in Slovak). [Russian, French and German summaries]. Priroda, Bratislava 1986, 547 p., Kcs 50.-

Important yeasts, bacteria and mold species having positive or negative influence on wine making, are thoroughly described. The ecology, physiology and biochemistry of yeasts most frequently occurring on grape berries, in spontaneously fermenting grape musts or causing wine spoilage are dealt

with. The biochemistry of alcoholic and malolactic fermentation are explained as well as the prevention of frequently occurring wine diseases caused by yeasts and bacteria are proposed. Chemical analysis of the wine and microbiological procedures in the identification of yeasts, bacteria and molds in wine are given in detail.

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2. E. Minárik: Contribution to the identification of Zygosaccharomyces bailii (Lindner) Guilliermond (in Slovak). Vinohrad (Bratislava). Accepted for publication.

Zygosaccharomyces bailii belongs to the most widespread yeasts causing wine spoilage (haze, refermentation of sweet wines). A standard description of this osmo- and chemotolerant fructophilic yeast is given. Characteristic features of this species are described and an express method for their identification and classification is proposed.

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3. E. Minárik: Determination of yeast number in wine by membrane filtration (in Slovak). Vinohrad (Bratislava) 24, 1986 (in press).

Principles of standard yeast counts by the membrane filtration method in wine control laboratories are described. The advantage of this method compared with the routine plating is explained.

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4. E. Minárik, O. Jungová: Activation of alcoholic fermentation by biologic and non-biologic means. Kvasny prumysl (Prague).

Microcrystalline cellulose shows, similarly as yeast wall preparations and the activator Botrytis cinerea, a stimulating effect on the fermentation activity of the yeast Saccharomyces oviformis in grape must even under unfavorable fermentation conditions. The stimulating effect is evident by a more profound fermentation, higher alcohol content and lower volatile acid formation in the wine. This effect is markedly higher in musts with high sugar concentration.

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5. The following is the summary of a paper submitted for publication to "Die Wein-Wissenschaft" (Wiesbaden), GFR, in 1986:

F. Malík, E. Michalčáková and E. Minárik: Acidification properties of active dry wine yeasts (in German). Wein-Wissenschaft 1987.

The reliable determination of the metabolism of active dry wine yeast preparations enables the estimation of acidification properties. The decrease of extracellular pH has been tested in 9 preparations of active dry wine yeasts. The highest decrease of extracellular pH was found in the preparation BLASTOSEL MV ($\Delta\text{pH}_{20} = 1.13$). The method of estimating acidification by the start and rate of metabolism after rehydration may be well used as a supplement to the characterization of such preparations.

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XXIX. Department of Medicine E-5, Div. of Infectious Diseases, Harbor-UCLA Medical Center, Torrance, California 90509. Communicated by Marjorie Crandall.

Management of Allergic Candidiasis

DIAGNOSIS

History of Predisposing Factors: an allergic diathesis plus antibiotics - "Flagyl" and broad spectrum antibacterials, or steroids - cortisone and hormones. Also pregnancy, diabetes, and other immuno-deficiency states.

Mucosal Signs and Symptoms: mouth - bitter taste, burning of tongue, gums and lips, thrush patches; intestine - constipation, less frequently diarrhea, adverse food reactions, post-prandial tiredness or other mental symptoms, acid stomach, gas, peri-anal itching; vagina - recurrent yeast vaginitis, vulvar itching, burning, dysuria, dyspareunia, white cheesy discharge.

Medi-Trend Blood Test: Call 1-800-545-8900 to order the kit. A score of >140 is diagnostic for chronic/allergic candidiasis.

TREATMENT

"Nizoral" - oral ketoconazole

Liver Panel: must be tested before administration of Nizoral. There have been some deaths due to idiosyncratic hepatitis (read the Physicians Desk Reference in the library or doctor's office).

(Note: Nystatin is less toxic but also less effective)

Desensitization Injections with Candida Antigen = Immunotherapy

Diet Free of Yeasts and Molds: found in fermented foods and beverages such as beer, wine, cheese, bread, vinegar, pickles, soy sauce, and on the surface of fruits such as grapes and berries, etc. Restrict ingestion of sugar.

Anti-Inflammatory Prescriptions

PREVENTION

Avoid Airborne, Food, and Contact Allergens: avoid yeasts and molds, and avoid vaginal contact with all chemicals including douches, creams, soaps, spermicides, perfume, chlorinated pools, etc.

Clothing: wear only unrestrictive, white, cotton underwear

Avoid Antibiotics, Steroids, and other Predisposing Factors. If they are unavoidable, then follow a regimen of:

Antifungal Prophylaxis plus Anti-Candida Desensitization. This disease is both an infection and allergy to the infective agent.

XXXI. Meetings

1. International course on "Isolation, Identification and Maintenance of Yeasts"

An international theoretical and practical course on the "Isolation, Identification and Maintenance of Yeasts" will be held in the Gulbenkian Institute of Science, Oeiras, Portugal from 10-28 August, 1987. The principal lecturers are Prof. J. P. van der Walt (Pretoria), Dr. D. Yarrow (Delft), Drs. M. Smith (Delft), Drs. T. Boekhout (Baarn) and Prof. N. van Uden (Oeiras). The number of participants is limited to twenty. Participants may be accepted from any part of the world, should have previous experience in yeast biology and be under 35 years of age.

Information and application-for-admission forms:

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

Financial Aspects

The course is entirely financed by the Calouste Gulbenkian Foundation and no fees are charged to the participants. Travel and living expenses are the responsibility of the participants however. Lunch may be taken at the Gulbenkian Institute of Science (Monday through Friday) at a subsidized price.

Housing

Successful applicants should make their own hotel reservations through a travel agency or otherwise. Many hotels are located at a convenient distance from the institute (Estoril, Cascais, Carcavelos or Lisbon). A number of single rooms with shower in a student hostel near the institute are available free of charge. Applicants interested in such a room should indicate this on their application-for-admission form.

Synopsis

Lectures

The following topics will be covered: Historical background, Nomenclature, Life cycles (Ascomycetous yeasts), Life cycles (Basidiomycetous yeasts), Morphology (LM), Morphology (EM), Biochemical background of fermentation and assimilation tests, Temperature relations, Other methods used in identification (nitrogen compounds, vitamins, osmotolerance, extracellular polysaccharides, urease, fat-splitting, ester production, DBB test, Coenzyme Q etc.), Methods for estimating relatedness (hybridization, nucleic acids, proteins), Classification of the ascomycetous yeasts, Classification of the basidiomycetous yeasts, Classification of the imperfect yeasts, Selective

isolation techniques, Maintenance of culture collections, Use of computers and software.

Laboratory Work

Each participant will have the opportunity to use selective techniques for the isolation of yeasts from nature.

Furthermore he/she will identify five yeasts belonging to different species applying the identification techniques currently in use. The results will be discussed and shared with the other participants so that each participant will become acquainted in the laboratory with one hundred different yeast species.

Deadline For Application

May 1, 1987

2. XIIth International Specialized Symposium on Yeast, "Genetics of Non-Conventional Yeasts", September 13-19, 1987, Weimar, German Democratic Republic, Chairmen: H. Weber, Jena, F. Böttcher, Greifswald.

First Announcement

The International Commission for yeast decided at its meeting in Bombay (1983) that the XIIth International Specialized Symposium on Yeasts will be held on September 13-19, 1987 in Weimar, GDR.

The Scientific Programme will focus on the genetics of non-conventional yeasts. Results of genetic research on Saccharomyces cerevisiae and Schizosaccharomyces pombe have been discussed during several recent yeast meetings. It is, therefore, the aim of this symposium, to bring together scientists working on the genetics of yeasts other than these two species and to discuss latest results and trends of genetic studies on these yeasts with emphasis on industrially important features. The scientific programme will comprise plenary lectures and original communications presented orally or as posters.

Proposed topics:

1. Life cycles, sexuality, sporulation, parasexuality
2. Genome structure, recombination, extrachromosomal elements
3. Gene transfer and cloning
4. Regulation of gene expression
5. Applications in biotechnology

The language of the symposium will be English. Accompanying guests are also welcome. Social programme will be organized both for active and accompanying guests.

Information: Prof. Dr. H. Weber
Central Institute of Microbiology
and Experimental Therapy of the
Academy of Sciences of the GDR.
Beutenbergstr. 11
GDR-6900 Jena, GDR

3. FEBS Advanced Course on "Biochemistry and genetics of yeasts". Jerez de la Frontera, Cadiz, Spain. 7-24 September 1987. The course will consist in lectures (three per day) and practical work (five to six hours per day). Lecturers: T. Benitez (Seville), G. Bernardi (Paris); J. Conde (Seville); G.R. Fink (Cambridge); C. Gancedo (Madrid); J.M. Gancedo (Madrid); T. Ishikawa (Tokyo); P. Niederberger (Zurich); C. Nombela (Madrid); P. Rusell (London); R. Serrano (Heidelberg); D. Van Loon (Bale); N. Van Uden (Oeiras); D.H. Wolf (Freiburg). Info: Carlos Gancedo. Instituto de Investigaciones Biomedicas CSIC. Facultad de Medicina UAM; Arzobispo Morcillo 4, 28029 Madrid, Spain. Deadline of applications: April 15, 1987.
4. The 19th Annual Meeting of the Yeast Genetics and Molecular Biology Society of Japan was held from July 21st through 23rd, 1986 at the Kyodai Hall in Kyoto. The following sixty-six topics were presented and discussed in thirteen Sessions: Session I, Structure of Genes (topics 1-7); II, Membrane and Secretion (8-13); III, Cytoplasmic Factors and Plasmids (14-18); IV, Meiosis and Sporulation (19-25); V, Chromosomes (26-29); VI, Regulation of Genes (30-35); VII, Nuclei and Nucleoids (36-40); VIII, Enzyme and Proteins (41-43); IX, Biochemistry and Comparative Study (44-47); X, cAMP and ras (48-54); XI, Genetic Analysis (55-56); XII, Cell Structure (57-61); XIII, Cell cycle (62-66). The abstracts of these presentations will be published in Japanese as "Yeast Genetics and Molecular Biology News, Japan" at the end of 1986. Communicated by Masayuki Yamamoto, Laboratory of Molecular Genetics, Institute of Medical Science, University of Tokyo, P.O. Takanawa, Tokyo 108, Japan.
 1. K. Morino, T. Uemura, S. Uzawa and M. Yanagida (Dept. Biophys., Kyoto Univ.). Cloning and disruption of the gene coding for S. pombe DNA topoisomerase I.
 2. T. Uemura and M. Yanagida (Dept. Biophysics, Kyoto Univ.). Gene structure and functional domains of the fission yeast DNA topoisomerase II.
 3. T. Takeda, M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Structure and function of calmodulin gene in S. pombe.
 4. A. Yasui (Research Institute for Tuberculosis and Cancer, Tohoku University). Homology of DNA repair gene between yeast and human.
 5. M. Mizukami and F. Hishinuma (Mitsubishi-Kasei Institute of Life Sciences). Sequence analysis of the URA3 gene of Kluyveromyces lactis.
 6. K. Hamasawa, S. Harada, K. Yoda and M. Yamasaki (Dept. Agric. Chem., Tokyo Univ.). The β -isopropylmalate dehydrogenase gene of Candida utilis.
 7. S. Miyamoto, Y. Ohya, Y. Ohsumi and Y. Anraku (Dept. Biol., Univ. of Tokyo). Primary structure of the CLS4 gene.
 8. M. Fukushima, K. Kiyono, Y. Kushima, I. Shibuya and A. Ohta (Dept. Biochemistry, Saitama Univ.). Amplification and Subcellar Distribution in Saccharomyces cerevisiae.

9. T. Yoshihisa, Y. Ohsumi and Y. Anraku (Department of Biology, Faculty of Science, University of Tokyo). Two Isoforms of Alpha-Mannosidase on Vacuolar Membranes of Yeast.
10. T. Yamazaki and H. Nonomura (Dept. Ferment. Technol., Yamanashi Univ.). Reversion of protoplasts of wine yeast Sacch. cerevisiae OC-2 and soy sauce yeast Zygosacch. rouxii Y-8 in PEG Liquid medium.
11. T. Mizunaga, M. Izawa and Y. Maruyama (Dept. Agric. Chem., Univ. of Tokyo). Properties of Nonglycosylated Repressible Acid Phosphatase Secreted from Saccharomyces cerevisiae in the Presence of Tunicamycin under Low Temperature.
12. M. Tokunaga, N. Wada and F. Hishinuma (Molecular Genetics, Mitsubishi-Kasei Institute of Life Sciences). Expression and secretion of the killer toxin encoded on yeast linear plasmids. 2. Analysis expression and secretion of killer toxin using gene fusion technique.
13. T. Suzuki, Y. Ohya, S. Miyamoto, Y. Ohsumi, Y. Anraku, T. Shiomori* and M. Kasahara* (Fac. of Sci., University of Tokyo, *Fac. of Med., Teikyo University). Cloning of yeast genes complementing defects in secretion and endocytosis.
14. Tadashi Mabuchi and Kazuhiko Wakabayashi (Dept. Biochem., Medical School, The University of Yamanashi). Essential Structure of Yeast Mitochondrial ARS.
15. A. Jearnpipatkul, H. Araki and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Mechanism of holding stability of yeast plasmid pSR1.
16. H. Matsuzaki, N. Nakanishi, H. Araki and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Molecular mechanism of intramolecular recombination in a yeast plasmid, pSR1.
17. H. Araki and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). ARS (Autonomously Replicating Sequence) of yeast plasmid pSR1.
18. S. Sakamoto, T. Imura, I. Utatsu and A. Toh-e (Dept. Ferment. Technol., Hiroshima Univ.). Comparative study on 2 μ and 2 μ -like yeast plasmids.
19. M. Tsuboi (Dept. Biotechnol., Fukuyama Univ.). Order and timing of function of sporulation genes in Saccharomyces cerevisiae.
20. K. Kitamura and C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.). Analysis of mating-deficient mutants in the fission yeast, Schizosaccharomyces pombe.
21. Y. Iino, J. Sakaguchi-Inoue and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Interaction of cAMP and genes controlling meiosis in the fission yeast Schizosaccharomyces pombe.
22. C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.). Suppressor of meiosis-deficient mutation in the fission yeast.

23. Y. Watanabe, M. Yamamoto (Inst. Med. Sci., Tokyo Univ.). Attempt to raise antibodies against the mei2 gene product.
24. H. Fujioka and C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.). Cloning of mei4 gene essential for meiotic first division in the fission yeast Schizosaccharomyces pombe.
25. M. Kishida, Y. Nakaseko* and C. shimoda (Fac. Sci., Osaka City Univ., *Fac. Sci., Kyoto Univ.). Structural analysis of the mes1 gene essential for meiotic second division in the fission yeast.
26. T. Matsumoto, U. Chikashige, O. Niwa and M. Yanagida (Dept. Biophysics, Kyoto Univ.). Structure of S. pombe mini-chromosomes; Identification of chromosomal ends.
27. O. Niwa, T. Matsumoto, Y. Chikashige, O. Niwa and M. Yanagida (Dept. Biophysics, Faculty of Science, Kyoto Univ.). Structures of S. pombe minichromosomes and their meiotic segregation.
28. Y. Nakaseko, S. Funahashi, O. Niwa and M. Yanagida (Dept. Biophys., Kyoto Univ.). Structure of centromeric sequences and their implication in chromosome stability in fission yeast.
29. M. Itaya*, P. Hieter**, and R. Crouch (Lab. Mol. Gen., NIH, **John's Hopkins Med. Sch., *present address; Mitsubishi-Kasei Inst. Life Sci.). Physical mapping of a yeast Saccharomyces cerevisiae gene by dividing chromosome at its gene site.
30. K. Yoshida, J. Kuromitsu, N. Ogawa and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Expression of the PH02, PH04, and PH081 regulatory genes in Saccharomyces cerevisiae.
31. N. Hayashi, Y. Kaneko*, and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., *IFO). Expression and regulation of the PH08 gene encoding repressible alkaline phosphatase in Saccharomyces cerevisiae.
32. T. Shimauchi, A. Toh-e (Dept. Ferment. Technol., Hiroshima Univ.). Function of negative factors of pho system.
33. Y. Suzuki, A. Abe*, Y. Nogi and T. Fukasawa (Keio Univ. Sch. Med. * Kitasato Inst.). Characterization of a Saccharomyces cerevisiae with an insertion mutation in the regulatory gene GAL11.
34. M. Igarashi*, T. Segawa, Y. Suzuki and T. Fukasawa (Keio Univ. Sch. Med.* Yamasa Shouyu Co. Ltd.). Mechanism for Regulated Expression of the Yeast Regulatory Gene GAL80.
35. K. Tanaka, K. Matsumoto*, T. Oshima and S. Tanaka (Suntory. Inst. Biomed. Res., DNAX. Res. Inst.). Regulation of expression of HSP70-100 gene in Saccharomyces cerevisiae.
36. I. Miyakawa, M. Miyamoto, N. Sando and T. Kuroiwa* (Biol. Inst., Fac. Sci., Yamaguchi Univ., *Dept. Cell Biol., Natl. Inst. Basic Biol.). Morphology of yeast mt-nucleoids and their protein components.

37. E. Tsuchiya and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Preparation and characterization of monoclonal antibodies against the yeast nuclear matrices.
38. T. Yata, E. Tsuchiya, T. Miyakawa and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Characterization of DNA fragments tightly attached to the yeast nuclear-matrix.
39. Y. Adachi and M. Yanagida (Dept. Biophys., Kyoto Univ.). Genetic analysis and characterization of cold sensitive mutants with aberrant nuclear chromatin distribution in fission yeast.
40. M. Nakagawa, E. Tsuchiya and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Immunochemical analysis of the yeast nuclear protein contracting DNA replication.
41. M. Iwabuchi, K. Kawasaki, O. Makino, and T. Shibata (Laboratory of Microbiology, RIKEN Institute). An immunochemical study on site-specific endonucleases of yeasts.
42. M. Uritani, H. Kagiya and M. Miyazaki (Inst. Molec. Biol. Nagoya Univ.). Property and Function of Polypeptide Elongation Factor-3 (EF-3) from yeast.
43. Y. Hayashi and C. W. Nakagawa (Inst. for Development Res., Aichi Colony). Cd-binding peptides of fission yeast. Isolation of cadystin A and B by TOYOPEARL DE-650 column.
44. H. Oh-ue and B. Ono (Fac. Pham. Sci., Okayama Univ.). Biochemical analyses of inorganic mercury resistant mutants of S. cerevisiae.
45. J. Ishiguro and Y. Azuma (Dept. of Biology, Faculty of Science, Konan University). Temporary changes in ribosomal and cytosol protein patterns in adenine-requiring mutant strains of K. lactis.
46. M. Yamamura and T. Kamihara (Dept. Indust. Chem., Kyoto Univ.). Petite induction at elevated temperatures: Stimulatory effect of casaminoacids and inorganic salts.
47. Y. Kaneko and I. Banno (Institute for Fermentation, Osaka). Are Saccharomyces bayanus and Saccharomyces cerevisiae conspecific?
48. I. Uno, T. Ishikawa, A. Toh-e*, K. Tanaka** and T. Oshima** (Inst. Appl. Micro., Univ. Tokyo; *Dept. Ferm. Tech., Hiroshima Univ.; **Suntory Inst. Biomedical Res.). Subcellular localization of cAMP-dependent protein kinase in yeast.
49. D. Shin, I. Uno, T. Ishikawa (Institute of Applied Microbiology Tokyo Univ.). Control of G1-G0 transition and G0 protein synthesis by cAMP in Saccharomyces cerevisiae.
50. H. Nakajima, I. Oshima, K. Yoda and M. Yamasaki (Dept. Agric. Chem., Tokyo Univ.). Glycogen accumulation and intracellular fine structure of temperature sensitive cyr1 mutant.

51. S. Yamano, A. Toh-e*, T. Oshima**, K. Tanaka** and K. Matsumoto*** (Kirin Brewery, *Dept. Ferment. Technol., Hiroshima Univ., **Suntory, ***DNAX). Structure and function of BCY1 gene.
52. T. Oshima, K. Tanaka, I. Uno*, H. Mitsuzawa* and T. Ishikawa* (Suntory Inst. Biomed. Res., *Inst. Appl. Microbiol. Univ. Tokyo). Expression of CYR1 and RAS2 genes of Saccharomyces Yeast in Escherichia coli.
53. H. Mitsuzawa, I. Uno, T. Ishikawa, K. Tanaka and T. Oshima* (Inst. Appl. Microbiol. Univ. Tokyo, *Suntory Biomed. Res.). Region of the yeast CYR1 gene that is involved in the interaction with the RAS products.
54. Y. Fukui and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Involvement of the ras1 gene function in recognition of the mating factor in Schizosaccharomyces pombe.
55. B. Ono, Y. Ishino, R. Fujimoto, Y. Ohno, N. Maeda and Y. Tsuchiya (Fac. Pharm. Sci., Okayama Univ.). UGA suppressor loci in Saccharomyces cerevisiae.
56. A. Toh-e (Dept. Fermentation Technology, Hiroshima University). Genetic analysis of revertants from a pho81-2 mutant.
57. M. Yamaguchi, T. Hirano, M. Araki* and K. Matsubara* (Cent. Res. Rab., Jikei Univ. Sch. Med., *Inst. Mol. Cell. Biol., Osaka Univ.). Electron microscopy of hepatitis B virus antigen-producing yeast cell.
58. K. Takeo (Res. Inst. Chemobiodynamics, Chiba Univ.). Correlation between the types of the plasma membranous particles and growth and non-growth zones of S. pombe.
59. A. Hirata (Inst. of Appl. Microbiol., Univ. of Tokyo). The fine structure of ascospore development in the fission yeast by freeze substitution - II.
60. K. Tanaka and T. Kanbe (Lab. Medical Mycol., Res. Inst. Disease Mech. Control, Nagoya Univ. Sch. Medicine). Ultrastructure of mitosis in Saccharomyces cerevisiae. Reinvestigation by freeze substitution method.
61. M. Osumi, N. Yamada, A. Taki, and N. Naito (Dept. of Biology and Laboratory of Electron Microscopy, Japan Women's University). Method for Study on the yeast cells by freeze-substitution fixation.
62. T. Tachikawa, T. Miyakawa, E. Tsuchiya and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). The role of Ca^{2+} /CaM for Mating Pheromone-Induced Sexual Differentiation in Heterobasidiomycetous Yeast.
63. K. Inokuchi, A. Nakayama, R. Nakano and F. Hishinuma (Mitsubishi-Kasei Institute of Life Sciences). Characterization of the promoter region of the MF α 1 gene in Saccharomyces cerevisiae.
64. H. Ohkura, Y. Adachi and M. Yanagida (Dept. Biophys., Kyoto Univ.). Isolation and analyses of the mutants which show abnormal spindle formation and unequal chromosome separation.

65. T. Hirano and M. Yanagida (Dept. Biophysics, Kyoto Univ.).
Identification of the NUC2 gene product in Schizosaccharomyces pombe.
66. Y. Kikuchi, M. Ando, H. Shimatake and A. Kikuchi* (Sch. Med., Toho Univ.,
*Inst. Life Sci., Mitsubishi-Kasei). Isolation of yeast mutants
defective in chromosomal segregation and characterization by gene
cloning.
5. The "Yeast Conference", organized by the Hungarian Scientific Society
for Food Industry, was held in Budapest, Hungary, 29th May, 1985.
Communicated by Peter A. Biacs, General Director of the Central Food
Research Institute, H-1022 Budapest, Herman Otto ut 15, Hungary.

List of Papers*

- L. Sagi: Manufacture of bakers' yeast in Hungary
GY Follath: Novelty in bakers' yeast production
T. Deak: Contradictions in taxonomy and identification due to new results
M. Sipiczki: Possibilities of the industrial applications of modern genetics
E. Novak: Sugar metabolism in yeasts
V. Tabajdi-Pinter: Yeasts as quality characteristics of foods
B. Matrai and A. Halasz: Study of bakers' yeast and brewers' yeast by
polyacrylamide gel electrophoresis
A. Maraz: Possibilities and results in the improvement of brewers' yeast
K. Szentpetery: Detection of wild yeasts in brewery samples
E. Farkas-Szucs: Determination of viable yeast count in fermenting brewage by
means of reducing capacity.
B. Sevelia and L. Nyeste: Trends in reactor research and technology
L. Boross, B. Szajani, ZS. Buzas, B. Polyak, K. Dallmann, P. Papp, V. Halmos,
GY. Copf and J. Zsolt: Growth and ethanol fermenting capacity of yeast
cells enclosed in gel
B. Kovacs: Up-to-date methods of alcohol fermentation
A. Asvany: General problems related to the use of wine yeasts
E. Polos: Study of psychrophilic yeasts
I. Wunsche: Possibilities of utilizing Killer yeasts in wine fermentation
P. Sarkany: The role of yeasts in champagne manufacture

*Abstracts of the papers are published in Acta Alimentaria, Vol. 16 No. 1.

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XXXII. Brief News Items

1. The following item appeared in the Alumnus Magazine of Southern
Illinois University (SIU).

Carol C. Lindegren, 89, the internationally recognized "father" of yeast
genetics and the founder of SIU's first laboratory designed solely for
research, died on Jan. 19, 1986. He was a resident of San Diego, Calif. Mr.
Lindegren retired from SIU in 1964, but continued to conduct research in the
Biological Research laboratory, which he organized after coming to SIU in
1948. He also established SIU's microbiology department and served as its
chairman until his retirement. SIU's Life Sciences I building was renamed
"Carl C. and Gertrude Lindegren Hall" in 1977. Gertrude Lindegren, Mr.
Lindegren's late wife, teamed with him as a research partner for more than 40

years. Their early discovery that yeast cells are sexed and can be cross-bred to form new strains opened up a new field of research in genetics and heredity. His wife, Zella, survives.

J.K. Bhattacharjee
Dept. of Microbiology
Miami University, Oxford,
Ohio 45056

2. The following paper will appear in a forthcoming issue of Letters in Appl. Microbiol.

"A revised method for the application of API 50 CH carbohydrate kits to yeasts".

A revised method is described for the application of the API 50 CH carbohydrate kit to yeasts. The method permits an objective assessment of positive assimilation with a high degree of reproducibility.

Renny Ison
The Lord Zuckerman Research Centre
The University
Whiteknights
PO Box 234
Reading RG6 2LA, England

* * *

3. "The Fungi of our Mouldy Earth" by Wm. Bridge Cooke, Beihefte zur Nova Hedwigia No 85, 467 pp., J. Cramer, Gebr. Borntraeger Verlagsbuchhandlung, Berlin. Stuttgart.

A number of yeast species are listed. There are three major parts in the book: (1) material from the Laboratory Guide, (2) keys to all categories, (3) a systematic list of species together with the locations from which each species had been isolated or reported.

Wm. Bridge Cooke
1135 Wilshire Ct.
Cincinnati, OH 45230

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