Y E A S T

A News Letter for Persons Interested in Yeast

Official Publication of the
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I. Centraalbureau voor Schimmelcultures, Yeast Division, Delft, Julianalaan 67a Netherlands. Communicated by David Yarrow.

The following new species have been deposited in the CBS collection since the last issue of the News Letter. The CBS identification is given in parenthesis in those cases where the newly described species proved to be identical with an existing one.

Candida graminis CBS 6403
Candida hordei CBS 6406
L. Rodrigues de Miranda 7 H. C. Diem, Can. J. Bot. 52: 279 (1974)
Candida lipolytica var. thermotolerans CBS 6659 (Saccharomycopsis lipolytica)
Candida pseudolipolytica CBS 6660 (Saccharomycopsis lipolytica)
V. M. Blogodatskaja & A. Kockova-Kratochvilova, Biologia (Bratislava) 28:709 (1973).
Candida nouvelii CBS 6552 (C. albicans)
H. Saëz, Bull. Soc. Mycol. France 89:79 91973).
Candida parapsilosis var, tuxtlensis CBS 6557 (C. guilliermondii)
Hansenula pozolis CBS 6556 (Kluveromyces fragilis)
T. Herrera, M. Ulloa & I. Fuentes, Bol. Soc. Mex. Micol. 7:17 (1973)
Hanseniaspora occidentalis CBS 2592
M. Th. Smith, A. v. Leeuwenhoek 40:441 (1974).
Kloeckera corticis var. pulquensis CBS 6558 (K. apiculata)
M. Ulloa & T. Herrera, Bol. Soc. Mex. Micol. 7:27 (1973)
Nadsonia commutata CBS 6640
W. I. Golubev, Microbiologiya 42:1058 (1973)
Pichia methanolica CBS 6515, 6512, 6513, 6514, 6516
K. Kato, Y. Kurimura, N. Makiguchi & Y. Asai, J. Gen. Appl. Microbiol. 20:123 (1974).
Rhodotorula ulzamae CBS 6610 (R. pilimanae)
Torulopsis navarrensis CBS 6612
I. Moriyon & C. Ramirez, Proc. 4th Int. Symp. Yeasts, Vienna 1974, p. 233.
Torulopsis austromarina CBS 6179, 6588.
J. W. Fell & I. Hunter, A. v. Leeuwenhoek 40:307 (1974).
Trichosporon oryzae CBS 6678, 6679 (Candida sp.)
H. Ito, H. Iizuka & T. Sato, Agr. Biol. Chem. 38:1597 (1974).

II. National Collection of Yeast Cultures, Lyttel Hall, Nutfield, Redhill, Surrey RH1 4HY, England. Communicated by Barbara Kirsop, Curator, N.C.Y.C.

The NCYC now contains over 1050 different yeast strains. They are at present maintained in liquid MYGP medium at 4°C and subcultured every 6 months. Between 1954 and 1960 many of the strains in the collection were also freeze-dried by the method described in the Journal of the Institute of Brewing, 1955, 61, 566.

During 1972-73 all the yeasts maintained in the NCYC, apart from some recently deposited strains and some genetically marked mutants, were examined morphologically and biochemically. Appropriately significant tests were selected for each strain and results were compared with the original descriptions at the time of deposition. It was found that a significant amount of variation had taken place

in both the biochemical and morphological properties of strains that had been maintained conventionally for long periods. Equivalent freeze-dried cultures of many of the yeasts were compared with the subcultured strains and it was found that properties of the lyophilized cultures had remained stable. Although initial viability of cultures immediately after freeze-drying had been disappointing, only a very small subsequent decrease in viability has been detected after storage for between 10 and 20 years. Detailed study of brewing yeasts showed that brewing performance remained unchanged after prolonged storage in the freeze-dried state.

Since it is considered that the major function of a culture collection is to maintain cultures in such a way that they represent accurately the strains that were deposited, it is now the intention to lyophilize the majority of the strains held in the NCYC. Strains that have been shown to react poorly to lyophilization will continue to be maintained conventionally and will also be maintained experimentally on silica gel.

The results of the comparison of subcultured and freeze-dried strains will be published in the Journal of the Institute of Brewing, 1975.

A new catalogue of strains in the NCYC is the in course of preparation ad will be available in 1975. People requiring copies are asked to contact the Curator, N.C.Y.C. Brewing Industry Research Foundation, Lyttel Hall, Nutfield, Surrey, England in order that their names may be put on the mailing list. It is hoped that workers will appreciate the importance of depositing new species and strains of special interest with the Collection and will also keep the curator informed of any information obtained using strains from the Collection.

Identification of yeast cultures can be undertaken following prior consultation with the curator. A charge is made for this service.

Charges for cultures at the present time are £ 5 per culture for industrial organizations and £ 2 per culture for research and educational establishments. A charge is made for airmail postage. Yeasts may be dispatched on agar slopes or as lyophilized cultures, depending upon the method of maintenance.

Microbiology and Fermentation Laboratory of the Brewing Industry Research Foundation, Nutfield, Redhill, Surrey, England. Communicated by Barbara Kirsop.

We have been especially interested recently, in the excretion by brewing yeasts of organic acids and acetate esters and also in the effects of trace quantities of oxygen on these processes. The pattern of acid excretion is markedly strain dependent, so that individual yeasts give a characteristic finger print when a fermented, malt extract medium is examined; there is little overall difference between Sacch. cerevisiae and Sacch. uvarum, except that the latter

species, typically, excretes larger quantities of malic acid. If the fermentation is carried out using a large inoculum of cells which have been propagated semi-anaerobically, acid excretion is increased substantially. This has led us to study the enzyme content of the cells at the time of inoculation and to measure the extent to which the enzymes of the tricarboxylic acid cycle persist during a period of anaerobiosis.

Ethyl acetate and iso-amyl acetate are, quantitatively the most significant esters excreted by yeast. The extent to which they accumulate in the medium is also very susceptible to the supply of trace quantities of oxygen. This is thought to be due to the stimulating effect of oxygen on biomass formation, which reduces the availability of metabolites which are necessary for ester synthesis.

III. University of East Anglia, School of Biological Sciences, University Plain, Norwich NOR 88C. Communicated by R. J. Pankhurst and J. A. Barnett.

"A key to the yeasts is now available, in the form of a packet of 120 punched cards, quickly sorted by hand, as referred to on page 23 of A New Key to The Yeasts, J. A. Barnett and R. J. Pankhurst, North-Holland Publishing Company, 1974. This punched card key covers all the 434 species listed in the book, with 60 physiological tests. This key enables any selection of tests to be used in any order, which is a great advantage over a conventional diagnostic key. There is a sheet of instructions and a list of names of species to go with it. The cost, including postage and packing, is either (i) for the United Kingdom £10 first copy, further copies at 2 £ each or (ii) for overseas $25 (USA) [or equivalent] for the first copy, further copies $5 each. Please send cheques and all inquiries to R. J. Pankhurst, Botany Department, British Museum (Natural History), Cromwell Road, London SW7 5BD."

A New Key to The Yeasts

Should sufficient copies of this book be sold, we shall consider bringing out a second edition, once about another 100 yeast species have been described. I would be grateful for the following information. (i) Comments, favorable or unfavorable, on the book, to help decide whether it is worth bringing out again and, if so, how it should be modified; (ii) reprints of published descriptions of all yeasts not dealt with either in A New Key or in The Yeasts (Lodder 1970). James A. Barnett, School of Biological Sciences, University of East Anglia, NORWICH, England.

IV. Research Institute of Fermentation, Yamanashi Univ., Kitashin-machi, 1-13 - 1, Kofu, 400, Japan. Communicated by Shoji Goto.

Yeasts from the Pacific Ocean

We made an ecological survey of the horizontal and vertical distribution of marine yeasts along Long. 150° E from Lat. 44° N to

the equator in the Pacific Ocean during, 1967. Twenty yeast strains were isolated from sea water samples collected from 0 to 4,000 m depth in the Pacific Ocean. They were identified as strains belonging to the following seven species of four genera: 3 strains of Deb. hansenii, 1 of Candida krissii nov. sp., 5 of Rhodotorula glutinis, 5 of Rh. rubra and 3 of Rh. marina.

Seven yeasts were isolated from sea water samples (from 0.3 and 2.0 m depth) and sediment samples (from 5.5 m depth) in Aburatsubo Inlet of the Miura Peninsula, Japan, 1967. These yeasts were identified as 2 strains of Deb. hansenii, 2 of Torulopsis candida, 1 each of Rh. rubra, Rh. marina and Cryptococcus infirmo-miniatus.

S. Goto et al: Proc. IV IFS: Fermentation Techn. Tokyo, p. 805 (1972).

S. Goto et al: J. Gen. Appl. Microbiol., 20, in press.

V. Department of Biology, Chonnam National University, Chonnam, Korea. Communicated by Park, Myung Sam and Soon Bai Chun.

Studies on Yeast Isolated from Marine Substrates (II)

The following is a summary of recent results from this laboratory.

In order to examine the distribution of yeasts associated with the marine waters of bathing beaches, collections were made during July of 1973 at the bathing beach of the Bang Juk Po of Dolsan island in Korea.

With two unidentified species designated "Candida SPI" and Debaryomyces SPI", 4 taxa of yeasts were isolated: Torulopsis magnoliae, Cryptococcus infirmo-minatus, Candida beechii, and Debaryomyces hansenii.

Candida beechii and Torulopsis magnoliae have not yet been reported from marine locales. The presence of the above taxa in saline waters is probably a reflection of human excrements in the bathing beach.

Debaryomyces SPI is quite similar to Debaryomyces hansenii except for the negative assimilation of ethanol. In this regard, further study is required to determine the relationship between them.

VI. Bacteriologisch-Serologisch Laboratorium der Rijksuniversiteit, Oostersingel 59 Groningen (Holland). Communicated by N.J.W. Kreger-van Rij.

The following article has appeared recently:

N.J.W. Kreger-van Rij and M. Veenhuis, Spores and septa in the genus Dipodascus. Can. J. Bot., 52: 1335 - 1338, 1974.

The following publications are in press:

N.J.W. Kreger-van Rij, The species Dipodascopsis uninucleata (Biggs) Batra et Millner. In: Persoonia.

N.J.W. Kreger-van Rij and M. Veenhuis, The structure of hyphal septa in Cryptococcus laurentii. In: Annali di Microbiologia.

N.J.W. Kreger-van Rij, M. Veenhuis and C.J. Leemburg-van der Graaf, Ultrastructure of hyphae and ascospores in the genus Eremascus Eidam. In: Antonie van Leeuwenhoek.

VII. Janssen Research Foundation Koninklijke laan 17, 2340 Beerse, Belgium. Communicated by S. De Nollin.

Below follow abstracts of two recent papers.

S. De Nollin and M. Borgers: "The ultrastructural morphology of C. albicans after in vitro treatment with miconazole." Sabouraudia: in press.

SUMMARY

Electron microscopic examination was performed on Candida albicans yeast cells after exposure in vitro to different doses of miconazole. It appeared that this drug exerts its effect primarily on the plasmalemma and the cell wall. After exposing cultures to drug concentration of 10^{-6}M and 10^{-5}M progressive degradation of plasmalemma and cytoplasmic organelles were seen. Injured parts of cellular material were sequestered from the rest of the cytoplasm and extruded into the central vacuole. A similar degradation occurred at the cell periphery. Cell death, characterized by the loss of their normal shape, dissolution of the plasmalemma and by severe alterations of all cytoplasmic organelles, was evident at the 5.10^{-6}M and 10^{-5}M dose levels. From the morphologic point of view a clear dose relationship could be established.

The following article has appeared recently: M. Borgers and S. De Nollin: "The preservation of subcellular organelles of Candida albicans with conventional fixatives." The Journal of Cell Biology 62: 574-581, 1974.

SUMMARY

The application of conventional fixatives for the visualization of the ultrastructure of Candida albicans has been described. The problem of inadequate permeation of chemical fixatives was solved by sectioning solidified pellets of the yeast in the presence of the fixative, a procedure that yields fairly well-preserved subcellular structures.

VIII. Pharmacology Department, University of Hawaii, 1960 East West Road, Honolulu, Hawaii 96822. Communicated by James F. Lenney.

Recent Publications:

J. F. Lenney, Three yeast proteins which specifically inhibit yeast proteases A, B, and C. J. Bact. in press.

J. F. Lenney, Ph. Matile, A. Wiemken, M. Schellenberg and J. Meyer (1974) Activities and cellular localization of yeast proteases and their inhibitors. Biochem. Biophys. Res. Comm. in press.

We have found that proteases A, B, and C. of S. cerevisiae are localized in the vacuole. The corresponding inhibitors of these three enzymes are present in the extravacuolar cytosol. This compartmentation of the yeast proteases suggests that their function is primarily the intravacuolar digestion of proteins. When the yeast cell and its vacuoles are disrupted, these proteases form complexes with their inhibitors. Each inhibitor is specific for its corresponding protease. The A and B inhibitors have molecular weights of about 9000 and are very thermostable. The C inhibitor has a molecular weight of about 20,000 and is relatively unstable. The A inhibitor seems to exist primarily as a dimer. Whereas proteases A, B and C destroy the B inhibitor, only B has a pronounced destructive effect on the A inhibitor. Pepstatin is a selective inhibitor of A while chymostatin and antipain specifically inhibit B.

IX. Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Ill. 60439. Communicated by F. Schlenk.

Isolated yeast cell vacuoles of Candida utilis did not show active transport of several biochemical compounds which they concentrate in vivo (K. D. Nakamura and F. Schlenk (1974) J. Bacteriol. 118, 314-316).

Results of our collaboration with (the late) H. de Robichon-Szulmajster have been summarized: H. de Robichon-Szulmajster, K. D. Nakamura, and F. Schlenk: Active Transport of S-Adenosylmethionine in Yeast (1974) Proc. Intern. Symp. on the Biochemistry of S-Adenosylmethionine. Academia Nazionale dei Lincei, Rome (Columbia Univ. Press, 1975). In the same Symposium, the biosynthesis (by yeast) of several new derivatives of S-adenosylmethionine has been reported by F. Schlenk (Introductory Lecture). Their penetration into the cells and vacuoles has been observed by ultra-violet microscopy: Active Transport of Exogenous S-Adenosylmethionine and Related Compounds into Cells and Vacuoles of S. cerevisiae (1974) K. D. Nakamura and F. Schlenk (1974) J. Bacteriol. 120, 482-487.

Dr. K. D. Nakamura has joined the Dept. of Microbiology, U. of Colorado Med. School, Denver, Colorado 80220.

X. Department of the Army, U.S. Army Foreign Science and Technology Center, 220 Seventh Street NE., Charlottesville, Virginia 22901. Communicated by Edward Spoerl.

I would like to mention that a paper, "Reversible permeability changes in the membrane of a yeast cell sugar compartment", authored by Spoerl, E., S. H. Benedict, S. N. Lowery, John P. Williams and J. P. Zahand, has been accepted for publication in the Journal of Membrane Biology. The summary is as follows:

Sorbose uptake by Saccharomyces cerevisiae was increased 40 to 60% by glucose and other metabolizable sugars. Neither growth nor binding accounted for the increased uptake. However, accessibility of a restrictive intracellular compartment was increased as shown by counterflow and efflux measurements. Efflux from the compartment was more than doubled by glucose. This effect was reversed by

washing and was prevented by iodoacetic acid and other inhibitors, but not by cycloheximide. No evidence was found for a facilitated transport system in the compartment membrane such as exists in the external cell membrane. It was concluded that sorbose crosses the compartment membrane by simple diffusion and that a reaction requiring the metabolism of sugars increases the permeability of the membrane. Arabinose and fucose entered and were lost from the compartment like sorbose, whereas dimethylsulfoxide was unaffected by the compartment. All three of these latter compounds were bound by the cells when glucose was available in uptake suspensions. Binding was prevented by iodoacetic acid, but not by cycloheximide.

XI. Rutgers University, Institute of Microbiology, New Brunswick, New Jersey 08903. Communicated by J. O. Lampen.

The following is a list of publications from my laboratory on yeast that have recently appeared or are in press and expected to be out in the very near future:

1. Kuo, S.-C., and J. O. Lampen. The action of antibiotics on enzyme secretion in yeasts: Studies with cytochalasin A. Anal. N.Y. Acad. Sci. 235: 137-148 (1974).

2. Lampen, J. O. Movement of extracellular enzymes across cell membranes. Symp. Soc. Exp. Biol. 28: 343-366 (1974).

3. Ghosh, B. K., Montenecourt, B., and Lampen, J. O. Abnormal cell envelope ultrastructure of a Saccharomyces mutant with invertase formation resistant to hexoses. J. Bacteriol. 116: 1412-1420 (1973).

4. Colonna, W. J., and Lampen, J. O. Structure of the mannan from Saccharomyces strain FH4C, a mutant constitutive for invertase biosynthesis. I. Significance of phosphate to the structure and refractoriness of the molecule. Biochem. 13:2741-2748 (1974). II. Protein moiety and components of the carbohydrate-peptide bonds. Biochemistry 13:2748-2753 (1974).

5. Liras, P., and Lampen, J. O. Sequence of candicidin action on yeast cells. Biochimica et Biophysica Acta (in press) (1974).

6. Liras, P., and Lampen, J. O. Protection by K^{+} and Mg^{++} of growth and macromolecule synthesis in candicidin-treated yeast. Biochimica et Biophysica Acta (BBA Report) (in press) (1974).

7. Colonna, W. J., Cano, F. R., and Lampen, J. O. Microheterogeneity in yeast invertase. Biochimica et Biophysica Acta (in press) (1974).

8. Goldstein, A., and Lampen, J. O. Beta-D-fructofuranoside fructohydrolyase from yeast. Methods in Enzymology (in press) (1974).

XII. Department of Biology, Faculty of Medicine, J. E. Purkyne, University, Brno, Czechoslovakia. Communicated by Marie Kopecká.

The following papers have been published from our institute in 1974:

1. Nečas, O., Svodoba, A.: Effect of proteases, phospholipases and polysaccharide-splitting enzymes on plasma membrane particles and on the synthesis of the fibrillar cell wall component in yeast protoplasts. Folia microbiol. 19, 81 - 87, 1974.

2. Nečas, O., Svoboda, A.: Reversion of Saccharomyces protoplasts as revealed by freeze-etching. Proc. Fourth Int. Symp. on Yeasts, Vienna, Austria 1974, Part 1, D9.

3. Svodoba, A., Nečas, O.: Morphogenesis during protoplast reversion in dimorphic yeast. Proc. Fourth Int. Symp. on Yeasts, Vienna, Austria 1974, Part 1, D13.

4. Gabriel, M.: Cell wall regeneration and reversion in protoplasts of green algae, Uronema gigas. Proc. Fourth Int. Symp. on Yeasts, Vienna, Austria 1974, Part 1, D3.

5. Havelková, M.: Regeneration of cell wall in protoplasts and spheroplasts of Nadsonia elongata. Proc. Fourth, Int. Symp. on Yeasts, Vienna, Austria 1974, Part 1, D4.

6. Kopecká, M., Phaff, H. J., Fleet, G. H.: The ultrastructure of the yeast cell wall after enzymic degradation by purified enzymes. Proc. Fourth. Int. Symp. on Yeasts, Vienna, Austria, 1974, Part 1, D8.

7. Kopecká, M., Gabriel, M., Nečas, O.: A Method of isolating anucleated yeast protoplasts unable to synthesize the glucan fibrillar component of the wall.

8. Kopecká, M., Phaff, H. J., Fleet, G. H.: Demonstration of a fibrillar component in the cell wall of the yeast Saccharomyces cerevisiae and its chemical nature. J. Cell Biol. 62, 66 - 76, 1974.

9. Kopecká, M.: Sporulation in protoplasts of the yeast, Saccharomyces cerevisiae. J. Gen. Microbiol. 83, 171 - 178, 1974.

The following papers are in the press:

10. Kopecká, M., Phaff, H. J.: On the ultrastructure of the yeast cell wall, Biologia (Bratislava).

11. Kopecká, M.: The isolation of protoplasts of the fission yeast Schizosaccharomyces by Trichoderma viride and snail enzymes. Folia microbiol.

The following are the main problems studied at present in our laboratories: In the laboratory of Dr. A. Svoboda the ability of yeast protoplasts to conjugate and to form zygotes. In the laboratory of Dr. M. Gabriel cell wall regeneration in protoplasts of the blue-green alga, Anacystis, as well as the formation of protoplasts from various blue-green and green algae. Dr. M. Kopecká is studying the effect of the antibiotic

lomofungin, an inhibitor of RNA-synthesis, on cell wall synthesis in yeast protoplasts; and in a cooperative project she studies the metabolism of anucleated yeast protoplasts (protein synthesis, RNA-synthesis, transport of monosaccharides and their utilization). Dr. M. Havelková is absent on leave.

XIII. Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Niigata University, 8050, Igarashi 2 no cho, Niigata-shi, Niigata 950-21, Japan. Communicated by Hirosato Tanaka.

Below follow the abstracts of recent works:

1. Multiple β-1 → 3 Glucanases in the Lytic Enzyme Complex of Bacillus circulans WL 12.
Yoshinori Kobayashi, Hirosato Tanaka and Nagahiro Ogasawara

Agricultural and Biological Chemistry, 38(5), 959-965, 1974.

Bacillus circulans WL 12 produces a strong cell wall lytic enzyme complex when grown on the mycelia of Piricularia orzyae P_2 as a carbon source. The lytic enzyme complex was found to have β-1 → 3 and β-1 → 6 glucanase and chitinase activities. By the use of polyacrylamide gel electrophoresis, the β-1 → 3 glucanase activity was found to be due to at least six different enzymes. The activity profile of the multiple β-1 → 3 glucanases was determined with a vertical electrophoretic apparatus equipped with a cooling device. After an electrophoretic run, the gel was sliced into slices of 2 mm in thickness. The pieces were homogenized and enzymes were extracted. The activity profiles against laminarin, oat glucan, pachyman, Phytophthora glucan, cell walls of Piricularia oryzae P_2 and Saccharomyces cerevisiae were obtained. The results indicated the presence of at least six (or possibly eight) enzymes which somehow decomposed laminarin. The enzymes were shown to have different affinities to various β-1 → 3 glucans.

2. Concerted Inductions of the Multiple β-1 → 3 Glucanases in Bacillus circulans WL 12 in Response to Three Different Substrates.

Hirosato Tanaka, Yoshinori Kobayashi and Nagahiro Ogasawara

Agricultural and Biological Chemistry 38, (5) 967-972, 1974.

Bacillus circulans WL 12 was grown on three different substrates, namely, Piricularia oryzae P_2 mycelia, Saccharomyces cerevisiae cells and pachyman (a β-1 → 3 glucan obtained from sclerotium of Poria cocos). In each case, β-1 → 3 and β-1 → 6 glucanases and chitinase were produced in the culture broth. The most conspicuous differences in the three different systems were the lytic actions toward P. oryzae P_2 cell walls. The enzyme complex induced by P. oryzae P2 cell walls was by far the most lytic against P. oryzae P_2 cell walls either with the dialyzed culture fluid or with the systems in which β-1 → 3 glucanase activity of each system was made equal. The differences might be due, in part, to the synergistic effect of chitinase. However, the experimental results were more in favor of other interpretation. We considered that the differences

in the lytic acitivity toward P. oryzae cell walls might be due to the dissimilarity in the compositions of the multiple β-1 → 3 glucanase between the enzyme complexes. As expected, profound differences were observed in the patterns of β-1 → 3 glucanases in the large scale polyacrylamide gel electrophoresis. Certain key enzymes seem to play important roles. And synergistic effects between the multiple β-1 → 3 glucanases for the further degradation of fungal and yeast cell walls and other β-1 → 3 glucans with mixed linkages are also expected. There is a concomitant production of various enzymes upon induction with cell walls or with glucans of mixed linkages. We would like to propose a term "concerted induction of enzymes" for this type of response of organisms to substrates. Presumably the organism produces small amount of constitutive enzymes which start to break down insoluble macromolecules. Various solubilized fragments start to enter into the cells and serve as inducers. Presumably it is a dynamic process during the course of growth that the pattern of enzymes are being established.

3. Purification and Properties of F-I_a, a β-1 → 3 Glucanase Which is Highly Lytic toward the Cell Walls of Piricularia oryzae P_2.

Yoshinori Kobayashi, Hirosato Tanaka and Nagahiro Ogasawara

F-I which showed the highest lytic activity toward Piricularia oryzae P_2 cell walls among the six multiple β-1 → 3 glucanases produced by Bacillus circulans WL 12 was purified to the state of homogeneity to give a single band in disc polyacrylamide gel electrophoresis at pH 9.4 and 4.0. The molecular weight was estimated to be around 48,000. F-I hydrolyzed laminarin by a random mechanism. It cleaved both β-1 → 3 and β-1 → 4 linkages adjacent to β-1 → 3 glucosidic linkages in oat glucan. The enzyme liberated glucose, oligosaccharides and a high molecular weight heteroglycan from Piricularia oryzae P_2 cell walls. Heteroglycan showed a single sedimentation peak in ultracentrifugation and contained mannose glucose and galactose.

XIV. The Research Laboratories of Kirin Brewery Co., Ltd. Takasaki, Gumma Pref., Japan. Communicated by Tatsuhiko Kaneko.

The following is the abstract of a paper presented at the First Intersectional Congress of the International Association of Microbiological Societies, Tokyo, Japan, September 1-7, 1974.

YEAST PROTOPLAST FORMATION BY A PURIFIED ENZYME, ZYMOLYASE, AND ITS REGENERATION

Kumpei Kitamura and Yasushi Yamamoto

Protoplasts were prepared from cells of Sacch. carlsbergensis by Zymolyase, which is a purified glucanase from Arthrobacter luteus and hydrolyzes β-1, 3-glucan, specifically releasing laminaripentaose as the minimum product. The yeast cells cultivated in a yeast extract medium containing glucose were treated by Zymolyase in a reaction mixture containing 0.6 M KCl and 0.1 M MgSO4 as a stabilizer at 25°C with gentle

shaking. After incubation for 10 min, destruction began to be observed sporadically over the cell wall, and then protoplasm became spherical within the perforated and worn envelope, and, at last the protoplast slipped out the remaining envelope faintly visible. When the protoplasts were incubated in a liquid medium containing 0.6 M KCl or 0.8 M sucrose at 30°C, the protoplasts began to grow within 1 hr and then they changed to a variety of forms to show increase of cellular materials. After 24 hr, regeneration of normal cells from the protoplasts was seen to occur in a medium containing sucrose.

The following article has recently been published:

T. Inoue, Y. Yamamoto, E. Kokubo and Y. Kuroiwa, Formation of acetohydroxy acids during wort fermentation by brewer's yeast. Rept. Res. Lab. Kirin Brewery Co., Ltd. No. 16, p. 11 (1973).

The following article is in the press:

T. Inoue. Influence of temperature on formation of acetohydroxy acid by brewer's yeast. Rept. Res. Lab. Kirin Brewery Co. Ltd., No. 17.

XV. Faculty of Agriculture, Kochi University, Nankoku, Kochi, Japan. Communicated by Susumu Nagasaki.

The following articles have recently been published.

Production, Purification, and Some Properties of Yeast Cell Lytic Enzyme from a Species of Fungi Imperfecti. S. Yamamoto, J. Fukuyama and S. Nagasaki; Agr. Biol. Chem., Vol. 38 (1974), pp. 329-337.

Enzymic and Structural Properties of Crystalline Yeast Cell Lytic Enzyme from a Species of Fungi Imperfecti. S. Nagasaki, J. Fukuyama, S. Yamamoto and R. Kobayashi; Agr. Biol. Chem., Vol. 38 (1974), pp. 349-357.

Purification and Properties of an Endo β-1, 6-Glucanase from Rhizopus chinensis R-69. S. Yamamoto, R. Kobayashi and S. Nagasaki; Agr. Biol. Chem., Vol. 38 (1974), pp. 1493-1500.

Physicochemical, Chemical and Enzymatic Properties of a Crystalline Yeast Cell Lytic Enzyme from a Rhizopus Mold. S. Yamamoto, R. Kobayashi and S. Nagasaki; Agr. Biol. Chem., Vol. 38 (1974), pp. 1563-1573.

Living Yeast-Degrading Systems in Some Microorganisms: Isolation of Component Enzymes, their Enzymic Properties, and their Applications to Medical and Technological Use. S. Yamamoto, R. Kobayashi, K. Takida and S. Nagasaki; Proceedings of the Fourth International Symposium on Yeasts, Vienna, Austria (1974), pp. 51-52.

XVI. The University of Dundee, DD1-4HN. Dept. of Biochemistry, Medical Sciences Institute. Communicated by R. K. Poole. (Note: Dr. Poole's address after January 1, 1975 will be Department of Microbiology, Queen Elizabeth College, Campden Hill Road, University of London, LONDON W8.

Below follows a summary of my dissertation submitted to the University of Wales for a Ph.D. Much of this work has now been published. This abstract forms a review of the work performed so far.

Structure and Function of the Fission Yeast Schizosaccharomyces pombe.

Addition of deoxyglucose (60-70µg/ml) to cultures of Schizosaccharomyces pombe 972h⁻ growing with glucose as sole carbon source resulted in the formation of abnormal cells showing cell-wall lesions. These cells showed increased susceptibility to snail digestive enzymes and changes in cell-wall composition. A procedure has been developed for the preparation and disruption of sphaeroplasts from such cells (1).

Increased specific activities of catalase, and the respiratory enzyme cytochrome c oxidase, NADH-cytochrome c oxidoreductase, succinate-cytochrome c oxidoreductase, malate dehydrogenase and succinate dehydrogenase were observed during glucose derepression (2). Growth on glycerol is diauxic and is characterized by higher specific activities of these enzymes (3).

Large-scale synchronous cultures were prepared by a sedimentation-velocity size-selection method in a zonal rotor (4). In such cultures, rates of oxygen uptake rose to maxima twice per cell-cycle; respiration rates at the maxima and minima showed different sensitivities to the uncoupling agent CCCP, and the inhibitors antimycin A and KCN. Heat evolution increased at a linear constant rate, but in the presence of CCCP, peaks were observed in phase with the peaks of oxygen uptake. Respiration rates of synchronous cultures grown on glycerol exhibited two steps of increase per cell-cycle, the timings of the abrupt rises being the phase with respiration maxima in glucose-grown cultures (3). The plateaux and rises of respiration rate were differentially sensitive to CCCP.

The cell-cycle of glucose-grown cells was also analysed by separation of cells into size or density classes by zonal centrifugation (2). These enzymes listed previously (except cytochrome c oxidase) all showed periodic expression of activity as two peaks in the cell-cycle of glucose-repressed cells. Cytochrome c oxidase exhibited only one peak per cycle. Some of these enzymes were also assayed in glycerol-containing synchronous cultures (3); all showed similar peak patterns except cytochrome c oxidase which exhibited two steps of increase per cell-cycle.

Cytochromes c_{548}, b_{554} and b_{560} showed broad peak patterns of synthesis during the cell-cycle of glucose-repressed cells (5). Amounts of cytochrome $a + A_3$ and b_{563} (identified as b_T by its reaction on pulsing with O_2) oscillated in phase, showing two peaks per cycle. Amounts of cytochrome "P-420" decreased during the first 3/4 of the cell-cycle, whilst that of cytochrome P-450 (undetectable in glycerol-grown cells) increased. In glycerol-grown cells, a, b, and c type cytochromes exhibit step patterns of synthesis (3).

Homogenates were prepared from sphaeroplasts of glucose-repressed cells and fractionated in sucrose gradients (6). Inclusion of EDTA resulted in decreased density and sedimentation coefficients of mitochondria compared with these fractionated in the presence of Mg^{2+}. Mitochondria were heterogeneous with respect to size and density as judged by the distributions of cytochrome c oxidase, succinate dehydrogenase, malate dehydrogenase and NADH-cytochorme c oxidoreductase. Catalase was non-sedimentable under all conditions investigated; acid p-nitrophenylphosphatase was heterogenously distributed after high-speed zonal centrifugation.

It is concluded from these observations that the intracellular levels of many mitochondrial components exhibit complex fluctuations during the cell cycle of both glucose- and glycerol-grown cells of this organism. These results constitute a starting point for further investigations of the mode of mitochondrial biogenesis during cell growth and division.

(1) Poole, R. K. & Lloyd, D. (1973) Archiv. Microbiol. 88, 257-272.

(2) Poole, R. K. & Lloyd, D. (1973) Biochem. J. 136, 195-201.

(3) Poole, R. K. & Lloyd, D. (1974) Biochem. J. 141-148.

(4) Poole, R. K., Lloyd, D. & Kemp, R. B. (1973) J. Gen. Microbiol. 77, 209-220.

(5) Poole, R. K. Lloyd, D. & Chance, B. (1974) Biochem. J. 138, 201-210.

(6) Poole, R. K. & Lloyd, D. Eur. J. Biochem., submitted for publication.

XVII. National Research Council, Division of Biological Sciences, Ottawa, Canada, K1A-OR6. Communicated by Byron F. Johnson.

Below follow two abstracts and one title of recent work from this laboratory.

Canadian Journal of Genetics and Cytology, September 1974.

Morphometric Analysis of Yeast Cells III. Size Distribution of 2-deoxyglucose-induced Lysing Schizosaccharomyces pombe cells and Their Sites of Lysis.

Byron F. Johnson, Calvin Lu and Sidney Brandwein

To cultures of Schizosaccharomyces pombe, 2-deoxyglucose (2DG) was added either as 7 µg/ml during inoculation of the cultures (low dosage), or as 250 µg/ml during the log phase (high dosage). Samples were removed from the cultures, and lysing and non-lysing cells were measured and tabulated. Addition of the high dosage was followed immediately by lysis, with over 85% of the lysing cells found in cytolysis at their primary growing ends. Lysis ensued only at the beginning of the stationary phase in the low dosage experiments; 64% of the affected cells lysed at their cell plates. Cells lysing at their primary ends (high dose experiments) were shorter than the controls; cells lysing at their cell plates (low dose experiments) were longer than the controls. The cell division

process of the last cell cycle completed in the culture is unusual in its susceptibility to the low initial dose of 2DG, suggesting that cell division metabolism is fundamentally different from wall extension metabolism in the fission yeast.

Can. Journal Biochemistry, Vol. 52, No. 11, November 1974.

DNA Synthesis in Isolated Yeast Mitochondria.

L. Zeman and C. V. Lusena

ABSTRACT

Isolated yeast (Saccharomyces cerevisiae) mitochondria incorporate radioactive precursors into mitochondrial DNA. This in vitro labelled DNA was characterized by isopycnic and sedimentation velocity centrifugation both in the native and denatured state. The profiles of isopycnic CsCl gradients obtained by centrifugation in a fixed angle rotor are skewed toward high density. The skew is neither due to the presence of in vitro labelled nuclear DNA nor due to random breaks in mitochondrial DNA which would reveal, then, its heterogeneity in base composition. The in vitro labelled DNA is reproducibly recovered as a class of molecules sedimenting at about 5-8 S, indicating a molecular weight of $1\text{-}4 \times 10^5$ dalton while the smallest in vivo labelled fragments sediment at about 13-14 S, corresponding to $1.6\text{-}2.0 \times 10^6$ dalton. After denaturation, the in vitro labelled DNA molecules sediment at about 2-5 S, corresponding to a single-strand molecular weight of $1\text{-}7 \times 10^4$ dalton which is about one hundred times less than the observed size of the denatured in vivo labelled molecules.

Vol. IX, Methods in Cell Biology, D. M. Prescott Editor, Academic Press. IN VIVO AND IN VITRO SYNTHESIS OF YEAST MITOCHONDRIAL DNA.

L. J. Zeman and C. V. Lusena

XVIII. Kyoto University, Faculty of Agriculture, Dept. of Food Science and Technology, Kyoto 606, Japan. Communicated by Akira Kimura.

1. Occurrence of free base of phytosphingosine in Candida intermedia (IFO 0761). A. Kimura, M. Kimura, H. Ozaki, T. Tochikura and K. Koshimizu. Agric. Biol. Chem. 38 (6) 1263-64, 1974.

This paper shows biochemical work on Candida intermedia from which we isolated for the first time the free base of phytosphingosine, the presence of which has so far been doubted, since many lipid chemists have failed to find this substance.

1st Intersectional Congress of the International Association of Microbiological Societies. Tokyo, September 1 - 7, 1974.

ABSTRACT

Fermentative Production of CDP-Choline and the Related Cytidine Coenzymes by Dried Cells of Yeasts.

Kimura, Y. Kariya, K. Aisaka and T. Tochikura.

CDP-choline is not only an important intermediate in the synthetic pathway of lecithin and sphingomyelin, but also an effective drug for brain damage. We succeeded in the production of CDP-choline from phosphorylcholine and CMP, which had been a discarded product of nucleotide industry. About 77% of the yeasts tested carried out this formation of CDP-choline, but only few of them utilized choline itself. Recently, however, a strain, Hansenula jadinnli, has been found, which can biosynthesize CDP-choline directly from CMP and choline. This yeast can also biosynthesize CDP-monomethylethanolamine (CDP-MMEA), CDP-dimethylethanolamine (CDP-DMEA), CDP-monoethylethanolamine (CDP-MEEA), CDP-diethylethanolamine (CDP-DEEA), respectively, in high yield. This transformation consists of three reactions; (I) Phosphorylation of CMP to CDP, further to CTP. (II) Phosporylation of aminoethanols. (III) Condensation of CTP and phosphorylaminoethanols. To carry out these reactions, the following conditions are indispensable. (1) Yeast cells should be dried until the water content become less than about 30%. (2) The optimum concentration of CMP, phosphate and glucose should be chosen. The characteristic point of our method is that the reactions are carried out by the energy (FDP, ATP) obtained through glycolysis.

XIX. Zoology Department, West Mains Road, Edinburgh University, EH9-3JT, U.K.. Communicated by J. M. Mitchison.

Our present group working on the yeast cell cycle (mainly Schizosaccharomyces pombe) includes Dr. Bruce Carter, Jim Creanor, Dr. Peter Fantes, Davor Fatori, Kim Nasymth, Dr. Paul Nurse, Dr. Chris Sissons and Bill Staatz. Dr. John May from Monash University will be here until the end of the year. Next year, we are expecting Dr. Alan Day from the University of Western Ontario, and Dr. Pierre Thuriaux and Dr. Michele Thuriaux-Minet from Bern University.

Our recent publications are:

1. Burns, R. G. (1973). ^{3}H-Colchicine binding. Failure to detect any binding to soluble proteins from various lower organisms. Exp. Cell Res. 81, 285-292.

2. Dawes, I. W. and Carter, B.L.A. (1974). The timing of mitochondrial deoxyribonucleic acid during the cell cycle in Saccharomyces cerevisiae. Biochem. Soc. Trans. 224-227.

3. Fraser, R.S.S., Creanor, J. and Mitchison, J. M. (1973). Rapid and selective inhibition of the synthesis of high molecular weight RNA in yeast by lomofungin. Nature 244, 222-224.

4. Fraser, R.S.S. and Creanor, J. (1974). Rapid and selective inhibition of RNA synthesis in yeast by 8-hydroxyquinoline. Eur. J. Biochem. 46, 67-73.

5. Mitchison, J. M. (1973). Differentiation in the cell cycle. In "The Cell Cycle in Development and Differentiation" Eds. Balls, M. and Billett, F. S. pp. 1-11. Cambridge Univ. Press. London.

6. Mitchison, J. M. (1973). The cell cycle of a eukaryote. Symp. Soc. Gen. Microbiol. 23, 189-208.

7. Mitchison, J. M., Creanor, J. and Sartirana, M. L. (1973). Enzyme synthesis, RNA synthesis and the effects of actinomycin D in protoplasts of the fission yeast Schizosaccharomyces pombe. In "Yeast, Mould, and Plant Protoplasts" Eds. Villanueva, J. R., Garcia-Acha, I., Gascon, S. & Urburu, F. pp. 229-245. Academic Press, London & New York.

8. Sissons, C. H., Mitchison, J. M. and Creanor, J. (1973). Enzyme synthesis and potential during induction synchrony in the fission yeast Schizosaccharomyces pombe. Exp. Cell Res. 82, 63-72.

9. Stebbing, N. (1972). Amino acid pool components as regulators of protein synthesis in the fission yeast, Schizosaccharomyces pombe. Exp. Cell Res. 70, 381-389.

10. Wain, W. H. and Staatz, W. D. (1973). Rates of synthesis of ribosomal protein and total ribonucleic acid through the cell cycle of the fission yeast Schizosaccharomyces pombe. Exp. Cell Res. 81, 269-278.

11. Dawes, I. W. and Carter, B.L.A. Nuclear and mitochondrial gene replication during the yeast cell cycle: evidence from nitrosoguanidine mutagenesis. Nature 250 (1974) 709-712.

Papers in press at the time of writing (November 1974)

12. Carter, B.L.A. and Dawes, I. W. Synthesis of two DNA-dependent RNA polymerases during the yeast cell cycle. Exp. Cell Res.

13. Fraser, R.S.S. and Creanor, J. Mode of action of 8-hydroxyquinoline. Eur. J. Biochem.

14. Mitchison, J. M. Sequences, pathways and timers. In "Cell Cycle Controls." Eds. Padilla, G. M. Cameron, I. L. and Zimmerman, A. M. Academic Press, New York.

15. Mitchison, J. M. and Carter, B.L.A. Cell Cycle Analysis. In "Methods in Cell Physiology" Ed. Prescott, D. M. Academic Press, New York.

16. Padilla, G. M., Creanor, J. and Fraser, R.S.S. In "Cell Cycle Controls" Eds. Padilla, G. M., Cameron, I. L. and Zimmerman, A. M. Academic Press, New York.

XX. Brandeis University, Rosenstiel Basic Medical Sciences Research Center, Waltham Massachusetts 02154. Communicated by James E. Haber.

Bisexual Mating Behavior in a Diploid of Saccharomyces cerevisiae: Evidence for Genetically Controlled Non-Random Chromosome Loss During Vegetative Growth.

James E. Haber

A diploid strain of Saccharomyces cerevisiae has been isolated which exhibits bisexual mating behavior. The strain mates with either a or α strains with a relative mating efficiency of 1 to 2%. The efficiency of mating is correlated with the frequency with which subclones of this strain revert to a single mating type. Crosses of the bisexual diploid with a/a or α/α diploids yield bisexual diploid segregants with a frequency of approximately 3%. Analysis of the segregation of the mating type alleles and other markers on chromosome III indicates that the primary event which leads to the bisexual phenotype is the loss of one homologue of chromosome III during vegetative growth to produce a monosomic (2n-1) diploid. Evidence is presented that the loss of chromosome III and possibly of other chromosomes during vegetative growth is affected by a recessive nuclear gene - her (hermaphrodite) - which is not closely linked to the mating type locus.

Methionine-dependent Synthesis of Ribosomal Ribonucleic Acid During Sporulation and Vegetative Growth of Saccharomyces cerevisiae.

Peter J. Wejksnora and James E. Haber

J. Bacteriol., December 1974

ABSTRACT

Methionine limitation during growth and sporulation of a methionine-requiring diploid of Saccharomyces cerevisiae causes two significant changes in the normal synthesis of ribonucleic acid (RNA). First, whereas 18S ribosomal RNA is produced, there is no significant accumulation of either 26S ribosomal RNA or 5.8S RNA. The effect of methionine on the accumulation of these RNA species occurs after the formation of a common 35S precursor molecule which is still observed in the absence of methionine.

During sporulation, diploid strains of S. cerevisiae produce a stable, virtually unmethylated 20S RNA which has previously been shown to be largely homologous to methylated 18S ribosomal RNA. The appearance of this species is not affected by the presence or absence of methionine from sporulation medium. However, when exponentially growing vegetative cells are starved for methionine, unmethylated 20S RNA is found. The 20S RNA, which had previously been observed only in cells undergoing sporulation accumulates at the same time as a methylated 18S RNA.

These effects on ribosomal RNA synthesis are specific for methionine limitation, and are not observed if protein synthesis is inhibited by cycloheximide or if cells are starved for a carbon source or for another

amino acid. The phenomena are not marker-specific as analogous results have been obtained for both a methionine-requiring diploid homozygous for met_{13} and a diploid homozygous for met_2. The results demonstrate that methylation of ribosomal RNA or other methionine-dependent events play a critical role in the recognition and processing of ribosomal precursor RNA to the final mature species.

Effect of N-methyl-N'-nitro-N- nitrosoguanidine During the Cell Cycle on Mutations Along One Arm of a Chromosome of Yeast.

Sim Gek Kee and James E. Haber

The relation between DNA replication and the action of the mutagen N-methyl-N'-nitro-N-nitrosoguanidine has been studied in Saccharomyces cerevisiae. The frequency of reversion to prototrophy of six auxotrophic markers located along one arm of chromosome VII was examined as a function of the vegetative cell cycle. Exponentially growing cells were treated with nitrosoguanidine and then separated by zonal rotor centrifugation into fractions equivalent to stages in the cell cycle. The frequency of reversion for five of the six markers is greatest during the period of DNA replication. Each marker has a single point of maximum reversion, approximately ten-fold greater than the frequency observed at other points in the cell cycle. For any one marker the effect of nitrosoguanidine is restricted to an interval shorter than the period of DNA replication. The two markers most distant from each other, ade5 and leu1, both have their highest reversion frequency early during DNA replication. The peak reversion frequency for lys 5 is somewhat later, while the peaks for tyr3 and trp5 occur near the end of the DNA replication. The results indicate that nitrosoguanidine acts primarily during DNA replication and that different markers appear to be affected at different intervals during the DNA biosynthetic period. If nitrosoguanidine does act at the growing point of DNA replication, these observations indicate that the initiation of DNA replication occurs at specific, non-random points. Further, there must be two points of initiation of DNA replication on one arm of chromosome VII.

A Requirement for the Expression of Genes Controlling the Synthesis of Ribosomal RNA During Sporulation of Saccharomyces cerevisiae.

James E. Haber

During sporulation of the yeast Saccharomyces cerevisiae there is extensive breakdown and synthesis of ribosomal RNA (rRNA). Whether new rRNA synthesis is required for the completion of sporulation was examined using diploid strains homozygous for temperature-sensitive (ts) alleles of genes controlling rRNA synthesis. These diploids, homozygous for ts alleles of either rna2 or rna6, grow at 25C but not at 34C. Similarly these diploids sporulate at 25C but not at 34C. Spontaneous revertants of the diploids, selected to grow at 34C and heterozygous for a single ts allele, also are able to sporulate at 34C. Protein synthesis throughout sporulation is not significantly different at the restrictive temperature

from the permissive temperature in either the ts diploids or their revertants. It was expected that rRNA synthesis during sporulation would be temperature-sensitive in the ts diploids as it is during vegetative growth. However, rRNA synthesis in sporulating cells is not temperature dependent. There is then an apparent contradiction between the sporulation results and a direct examination of rRNA synthesis.

XXI. Brandeis University, Rosenstiel Basic Medical Sciences Research Center, Waltham, Massachusetts 02154. Communicated by Harlyn O. Halvorson.

The following papers are submitted for publication or in press.

SUMMARY OF A PAPER SUBMITTED TO MOL. GEN. GEN.

Amar J. S. Klar and Harlyn O. Halvorson

Studies on the Positive Regulatory Gene, GAL4, In Regulation Of Galactose Catabolic Enzymes in Saccharomyces cerevisiae

Studies were undertaken to elucidate the role of the GAL4 locus in regulating the galactose catabolic enzymes in Saccharomyces cerevisiae. The GAL4 locus has been proposed to have a "positive" regulatory function since GAL4 mutants (gal4 strains) are pleiotropic, lacking UDP galactose epimerase, galactokinase, transferase and galactose permease activities (Douglas and Hawthorne, 1964, Genetics 49, 837-844). These enzymes simultaneously reappear in the revertants obtained from the gal4 strains. The GAL4 locus must be functioning by a diffusible substance since the genetic loci for these enzymes are unlinked to the GAL4 locus. This pleiotropic effect could be explained if the GAL4 gene codes for a structural component of the above enzyme activities. To study if the GAL4 gene codes for a structural component of the above enzymes, a temperature sensitive mutation mapping close to or within the GAL4 gene was isolated. The results suggest that the GAL4 locus does not code for a polypeptide common to the transferase, epimerase, galactokinase, and permease enzymes since these enzymes extracted from the ts mutant did not differ qualitatively in thermolability and temperature optima from those of the wild-type. By temperature shift-up experiments it was found that in the ts mutant the synthesis of the epimerase is temperature sensitive. Further, the GAL4 gene product does not have any effect on the in vivo stability of epimerase activity since the activity decreases at the same rate at both restrictive and permissive temperature in this ts mutant once the protein synthesis is inhibited by cycloheximide.

In conclusion, our results suggest that the GAL4 gene does not code for a peptide required for the activity of the galactose catabolic enzymes but is required for their synthesis.

NEW RNA POLYMERASE IN THE YEAST S. CEREVISIAE

E. M. Sajdel-Sulkowska, I. Takano and H. O. Halvorson

Yeast nuclei contain at least four RNA polymerase activities; I_A, I_B, II and III. Only polymerase II is totally sensitive to α-amanitin at 50 μm/ml; but evidence exists that one other enzyme is sensitive at higher concentraions of toxin. Enzymes I_A, I_B, II and III can be separated in nuclear extracts by elution on DEAE-Sephadex with $(NH_4)_2SO_4$, but elution with NH_4Cl resolves only I (I_B) and II activities and I_A and II are absent. Enzyme II obtained by NH_4Cl fractionation can be subsequently resolved with $(NH_4)_2SO_4$ into two activities; II sensitive to α-amanitin and III insensitive. Rerunning of mixed enzymes II and II with NH_4Cl results in a single activity. Enzyme III is more active at higher salt concentrations than either enzyme I_A, I_B or II, prefers denatured DNA as a template and is stabilized by bovine serum albumin. Results with SDS-polyacrylamide gels suggest that all four enzymes have at least two subunits of higher molecular weight in the range of 185,000 to 135,000. Experiments are underway to determine whether any of two subunits are shared by more than one enzyme. Using immuno-precipitation and inhibition of enzymatic activity the relationship between enzyme III and I_A and I_B was studied. Results suggest that enzymes I_A and I_B are immunologically distinct from enzyme III.

RIBOSOMAL DNA MAGNIFICATION IN YEAST

David B. Kaback and Harlyn O. Halvorson

Over 60% of the 140 ribosomal RNA genes (rDNA) are located on chromosome I in Saccharomyces cerevisiae. This conclusion was reached by showing that a strain monosomic (2n-1) for chromosome I contains approximately 30% less rDNA than a related diploid (2N) as shown by DNA-RNA hybridization and direct measurement of the rDNA containing satellite band. We have recently found that when two clones of the monosomic strain were repeatedly subcultured for over one year on agar plates the level of rDNA as determined by saturation DNA-RNA hybridization had increased from 1.6% to the wild type level of approximately 2.2%. The original strain which was stored on silica gel for the same period remained unchanged. The subclones which contain the increased levels of rDNA (HOH-3mag, and HOH-$3mag_2$) appear to be stable. They are still monosomic for chromosome I as evidenced by the characteristic 2:2. viable:inviable segregation upon meiosis due to segregation of the null chromosome into two nonviable spores and the hemizygosity of the ade 1 gene (a chromosome I marker). DNA-RNA hybridization experiments with several viable spores from HOH-$3mag_1$ show that the extra rDNA is segregating as a Mendelian allele unlinked to ade 1 indicating it is on a chromosome other than chromosome I. The presence of several tetrads where the rDNA exhibits second division segregation indicates that it is not tightly centromere linked. In summary, the rDNA level in a strain initially deficient in rDNA genes has been magnified to the wild type level. This phenomenon may be analogous to the reported magnification of rDNA in Drosophila melanogaster bb mutants which are deficient in these genes.

XXII. Arbeitsgruppe Mikrobengenetik, Fachbereich Biologie, J. W. Goethe-Universität, 6 Frankfurt/Main, Robert-Mayer-Straße 7-9, Federal Republic of Germany. Communicated by M. Brendel.

In order to enable a larger number of students in participating in the joys of yeast research we have moved to larger labs within the University's campus. Please note the changed address.

Since the last issue of the Yeast News Letter the following paper has been accepted for publication:

Isolation and characterization of mutants of Saccharomyces cerevisiae auxotrophic and conditionally auxotrophic for 5'-dTMP. Martin Brendel and Wolfgang W. Fäth. Z. Naturforsch., in the press.

SUMMARY

An improved method for isolation of yeast mutants auxotrophic for 5'-dTMP is presented. The procedure employs the two folic acid antagonists aminopterin and sulfanilamide (SAA). Selectiveness of the procedure depends on concentration of SAA and time of incubation.

44 mutants auxotrophic and 3 conditionally auxotrophic for 5'-dTMP were isolated. All belong to one complementation group. The corresponding gene was designated TMP1. Tetrad dissection revealed its chromosomal nature. TMP1 is not closely linked to the genes ADE2, LEU1, ARG4, ILV2, HIS5, LYS1, and the mating type locus. With the centromere-linked genes ARG4 and LEU1 gene TMP1 exhibited second division segregation frequencies of 0.42 and 0.53 respectively, indicative of centromere-linkage.

Strains auxotrophic and conditionally auxotrophic for 5'-dTMP were all respiratory deficient (petite). Genetical analysis indicates that the petite phenotype is due to loss of the rho factor in cells harbouring either tmp1 or $tmp1^{ts}$ alleles.

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

Following below are the abstracts of two recent publications from my laboratory. We successfully completed the conference on "New Genetics and the Future of Man," May 9 - 10, 1974, with the following guest speakers:

Drs. H. G. Khorana (MIT), B. D. Davis (Harvard), E.H.Y. Chu (Michigan), J. M. Gustafson (Chicago) and N. Hershey (Pittsburgh).

1. W. H. Crocker, Jr. and J. K. Bhattacharjee, Biosynthesis of Glutamic Acid in Saccharomyces: Accumulation of Tricarboxylic Acid Cycle Intermediates in a Glutamate Auxotroph. Appl. Microbiol; 26: 303-308 (1973).

Abstract:

An aconitaseless glutamic acid auxotroph MO-1-9B of Saccharomyces grew in glutamic acid-supplemented minimal medium, but failed to

grow when glutamic acid was substituted by proline, arginine, ornithine, or glutamine. This mutant was also unable to utilize lactate or glycerol as a carbon source. Under a glutamic acid-limiting condition, by using acetate-1-^{14}C as tracer, the mutant accumulated rather large amounts of ^{14}C-citric acid and ^{14}C-succinic acid when compared with the wild-type strain. Under excess glutamic acid supplementation, accumulation of citric acid and succinic acid was considerably reduced. When ^{14}C-glutamic acid-(U) was used as tracer, ^{14}C-α-ketoglutaric acid, ^{14}C-citric acid and ^{14}C-succinic acid were accumulated in the mutant. The citric acid peak was the largest, followed by α-ketoglutaric acid and succinic acid. In the wild-type strain under similar conditions, only small amounts of ^{14}C-citric acid and ^{14}C-succinic acid and no ^{14}C-α-ketoglutaric acid were accumulated.

2. G. D. Biswas and J. K. Bhattacharjee, Induction and complementation of lysine auxotrophs in Saccharomyces. Antonie van Leeuwenhoek, 40: 263-273 (1974).

Abstract:

Four chemical agents, EMS, MNNG, NA, ICR-170, as well as UV were used to induce mutations in the wild-type haploid strain X2180-1B (a) of Saccharomyces. A total of 2053 (EMS, 427; MNNG, 444: NA, 469: ICR-170, 456; UV, 257) lysine-requiring mutant clones were isolated from many independent treatments and by nystatin enrichment technique. Mutants were classified into various functional groups on the basis of complementation analysis with 14 tester strains (lys 1 to lys 15 except lys 3). Of the clones analyzed, the number of isolates unable to complement with a given tester strain ranged from 2 for lys 5 to 918 for lys 4. Three of the mutually complementing lysine loci (lys 1, lys 2, and lys 4) accounted together for over 85% of the mutant clones whereas lys 6, lys 7, lys 8, and lys 14 had less than 10 noncomplementing isolates each. Mutants for lys 4 were most frequent with all of the mutagens tested except with NA in which case the mutants for lys 2 were most frequent. A total of 56 isolates failed to complement with lys 10, lys 11, and lys 12. Similarly, 47 isolates failed to complement with lys 9 and lys 13 simultaneously. Only 44 isolates complemented with all of the tester strains used.

XXIV. Department of Genetics, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9. Communicated by Majdi M. Shahin.

A list of recent publications from the Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario and the Environmental Mutagenesis Branch, National Institutes of Health, Research Triangle Park, North Carolina.

Shahin, M. M., Gentner, N. E. and Nasim, A. (1973). The effect of liquid holding in Schizosaccharomyces pombe strains after gamma and ultraviolet irradiation. Radiat. Res., 53, 216-225.

Shahin, M. M. and Nasim, A. (1973). The effect of radiation sensitivity and cell stage on liquid holding response in Schizosaccharomyces pombe. Molec. Gen. Genetics, 122, 331-338.

Shahin, M. M. and de Serres, F. J. (1973). The effect of pH on hycanthone methanesulfonate induced inactivation and mitotic recombination in D5, a new diploid strain of Saccharomyces cerevisiae. Mutation Res., 21, 234. Abstract for the first international conference on environmental mutagens, Asilomar, Monterey, California, August 29 - September 1.

Shahin, M. M. 91974). Radiation inactivation and mutation induction in yeast in the presence of caffeine during irradiation. Microbial Genetics Bulletin, 36, 15-16.

Shahin, M. M. and Kilbey, B. J. (1974). Genetic activity of the anti-schistosomal agent SQ18,506 in yeast. Mutation Res., 26, 193-198.

Ong, T. and Shahin, M. M. (1974). Mutagenic and recombinogenic activities of the food additive furylfuramide in eukaryotes. Science, 184, 1086-1087.

Shahin, M. M. and de Serres, F. J. (1974). The effect of pH on hycanthone methanesulfonate induced inactivation and mitotic recombination in Saccharomyces. Mutation Res., 26, 377-384.

XXV. University of Strathclyde, Department of Applied Microbiology. Royal College Building, 204 George Street, Glasgow G1, 1XW, Scotland. Communicated by J. R. Johnston.

"The Department of Applied Microbiology has recently extended the work done on yeast. Professor E. O. Morris has supervised a small group studying the drying of commercial baking yeast under an industrial research contract with Distillers Company Limited.

Dr. David Berry has assisted in supervision of this work and also heads a group active in the regulation of meiosis and sporulation. His wife, Dr. Elizabeth Berry, has recently joined our staff and a new research group is beginning work on double-stranded RNA 'particles.'

My own work in Genetics has continued to be concentrated on continuous culture population genetics, genetic analysis of commercial brewing and baking yeasts, and genetics of polyene antibiotic-resistance.

Dr. C. Lewis and Dr. V. Karunakaran have recently obtained their Ph.D. degrees and summaries of these are as follows (enclosed on separate sheets).

My articles in press at the moment include:

'The genetics of flocculation in Saccharomyces and genetic analysis of commercial baker yeasts', an invited paper presented at the Second International Symposium on the Genetics of Industrial Microorganisms in Sheffield in August and 'Strain Improvement and strain stability in the filamentous fungi', a chapter of 'Industrial

Mycology', edited by J. Smith and D. Berry to be published in January, 1975."

SUMMARY

Some physiological and genetic aspects of various nystatin-resistant mutants of Saccharomyces cerevisiae were studied. The acquisition of resistance was observed to be accompanied by a decrease in the growth rates of the strains examined. Growth rates of the mutants were also affected by the concentration of the antibiotic in the culture medium; generally, there was a proportional decrease in growth rate with increase of nystatin in the medium. When stored at 4°C in MYGP broth some nystatin-resistant mutants were observed to die at a much faster rate than the sensitive wild-type. The significance of these results on the effective binding of nystatin to the cell membrane is discussed.

Genetic analysis of nystatin mutants revealed the presence of primary and secondary factors responsible for conferring resistance to nystatin. The primary genes, NysA, nysB, nysC, NysD, NysE and NysF were defined. These genes were responsible for conferring resistance in the range 30-80 units/ml. The genes nysB and nysC were recessive while the others were dominant. Tetrad analysis showed linkage between NysD and NysF, and also between genes of the group NysA, NysE, nysB, nysC, and indicated that all six genes are probably on the same chromosome. In addition, it was shown that genes, NysA, NysE, and nysB form a closely-linked cluster suggesting possible functional grouping. The gene NysA is centromere linked and is apparently located on a chromosome other than I, III, IV, VI, VII, VIII or XVI. A recessive modifier gene, mod, that enhanced the resistance conferred by NysA by about 10-20 units/ml was isolated. The data obtained also indicated the presence of a dominant modifier gene responsible for increasing the level of resistance conferred by gene NysF by approximately 60 units/ml. The gene NysA gave additive levels of resistance when in combination with any of the genes NysB, nysC, or NysE; however, no phenotypic effect with respect to resistance levels was observed with some of the other possible gene combinations. Three resistant mutants, N57, N65, and P11, with resistance levels between 40-80 units/ml, yielded very few resistant segregants when crossed with nystatin-sensitive strains, thus indicating the non-nuclear origin of resistance factors in these strains. Mutant N57, when crossed with a high resistant mutant, N95-K1 which carries gene NysA and secondary resistance factors, suppressed the phenotypic expression of all secondary levels of resistance in ascospore progeny.

The ease by which higher resistance mutants were obtained by recurrent culturing of mutants in increasing concentrations of nystatin, and the results of interactions between mutants suggest a polygenic model of quantitative inheritance for nystatin resistance. It is postulated that: (a) the quantitative effect of various nuclear and cytoplasmic factors and also their various combinations determine the resistance level of a particular mutant and (b) resistance is mediated in a step-wise manner with each increase dependent on the previous level of resistance and thus dependent on the factors responsible for that level.

SUMMARY

(1) The flocculation of both brewing and laboratory-bred strains of Saccharomyces cerevisiae has been investigated, principally from the genetical aspect.

(2) A reasonably rapid and reliable method for the quantitative measurement of degrees of flocculation was devised which also differentiated between truly flocculent strains and those which merely form clumps by non-separation of budding cells.

(3) Strain ABX, a recombinant hybrid derived from a commercial brewing yeast, was demonstrated to be most likely a diploid by cell volume determinations, DNA content estimations, and most accurately, by tetrad analyses of crosses between ABX segregants and haploids carrying gene markers on 11 different chromosomes, with the obtention of 2: 2 segregations indicating these 11 chromosomes to be disomic in ABX itself.

(4) Two dominant genes (Flo 1 and Flo 2) and one recessive gene (flo 3) for flocculence have been identified, the presence of only one of which is necessary for the flocculation phenotype to be expressed, thus providing an example of polymeric genes in yeast. The dominant genes are linked and separated on a chromosome by a distance of 8 centimorgans. Both are unlinked to the recessive gene, all 3 genes are non-centromere-linked but unlinked to the following 9 non-centromere-linked marker genes present on 6 different chromosomes: - met 8 (Chromosome II), hom 2 and trp 4(IV), trp 2 (V), ade 3 and ade 5 (VII), ura 1 (XI), met 4 and lys 10 (XVII) and, in the case of flo 3, hom 3 (V). Gene Flo 1 is present in the homozygous condition in the brewery-derived ABX strain, while Flo 2 is carried by 5 separate haploid and diploid strains, and flo 3 by a haploid. This indicates that among laboratory-bred strains at least, Flo 2 is the most prevalent of the 3 genes.

(5) Quantitatively, the presence of each gene separately resulted in approximately the same intensity of flocculation, and there was no observable additive effect of the genes in combination with each other. Nevertheless, certain strains of above average flocculence were produced in small numbers, possibly owing to the influence of additional modifier genes or cytoplasmic genetic factors.

(6) The apparent spontaneous mutation rates of the 3 flocculence genes were extremely high - 2.15×10^{-3} per cell per generation for Flo 1, 5.51×10^{-4} for Flo 2, and 1.72×10^{-2} for flo 3. Mutations in the reverse direction were much less frequent, the rate being less than 8.29×10^{-5}.

(7) No significant differences were apparent in the cell wall mannan and glucan contents among 4 haploids bearing the genes Flo 1 flo 2 Flo 3, flo 1 flo 2 Flo 3, flo 1 Flo 2 Flo 3, and flo 1 flo 2 Flo 3 respectively, although possible biochemical mechanisms for the manifestation of flocculation were discussed.

(8) Unsuccessful attempts were made to discover a faster fermenting, earlier flocculating strain than the commercial yeast AB1 among recombinants of hybrid ABX.

XXVI. Genetics of Induction and Catabolite Repression of Maltase Synthesis in Saccharomyces cerevisiae. Mykologie/Genetik, Technische Hochschule, 61 Darmstadt, SchnittspahnstraBe 10, German Federal Republic. Communicated by F. K. Zimmermann.

Genetic analysis of the function of the MAL2-gene of Saccharomyces cerevisiae has provided data to suggest that MAL2 is a positive regulatory gene (non-inducible mutant alleles, mal2, are recessive; constitutive alleles are dominant over MAL2 and mal2). The activity of the gene MAL2 does not only regulate the synthesis of maltase, but concomitantly also that of isomaltase (determined as a hydrolytic enzyme for alpha-methyl-D-glucoside). MAL2 is also involved in catabolite repression of induction of maltase synthesis (there are mutant alleles of MAL2 that make maltase synthesis not only constitutive but also largely resistant to catabolite repression). This resistance to catabolite repression extends to the synthesis of isomaltase but has no effect on catabolite repression of invertase. The inhibitory effect of catabolite repression is probably not exerted at the transcriptional level preventing the formation of the required MAL2-gene product but rather by inhibiting the function of the MAL2 gene product. This is revealed by the variable degree of dominance of glucose resistant alleles in crosses with MAL2 and mal2-mutant alleles. The situation can be described as negative complementation. A manuscript has been accepted for publication in Molec. Gen. Genet.: F. K. Zimmermann and N. R. Eaton, Genetics of induction and catabolite repression of maltase synthesis in Saccharomyces cerevisiae.

In order to test whether there are more genes involved in the regulation of maltase synthesis, another approach had to be used. It was observed that on maltose media, MAL2 strains were more sensitive to 2-deoxyglucose than constitutive MAL2 mutants. Starting out with a MAL2-strain, 2-deoxyglucose-resistant mutants were isolated on maltose medium. Some of these mutants were indeed constitutive to variable extents. Upon genetic analysis, one mutant turned out to be affected in the MAL2-gene itself, three more mutants were mutated elsewhere in the genome. The latter type was recessive as judged from the ability to ferment sucrose (this is a criterion for constitutive maltase synthesis in strains unable to form invertase).

Constitituve mutant alleles of MAL2 which are still sensitive to glucose repression were used to select mutants resistant to catabolite repression. Mutants resistant to catabolite repression of maltase synthesis can be scored for on sucrose medium with 2-deoxyglucose. This analogue represses synthesis of maltase which is required for sucrose utilization. A number of resistant mutants was obtained and, upon genetic analysis, fell into one class with a mutation in the MAL2-gene, and another class affected elsewhere in the genome. The latter class of mutants was recessive.

Identification of the still unknown structural gene(s) for maltase was attempted by searching for maltase-free mutants in strains carrying MAL2-7^c and MAL4; both genes cause a constitutive and largely glucose-

resistant synthesis of maltase. Non-fermenters were isolated from such haploid strains, but all of 21 mutants still made high levels of alpha-glucosidases (maltase plus isomaltase).

Genetic tinker toys: A strain D8 was constructed which is diploid and has the following genotype

Chromosome VII

Genetromere	leu1	trp5	cyh2	met13	tyr3	lys5	ade5
Centromere	LEU	TRP	CYH	MET	TYR	LYS	ADE

Chromosome XV

Centromere ade2-40

Centromere ade2-40

This strain can be used to study the location of mitotic crossing over as a consequence of treatment with various mutagens to see whether there are - as in the induction of chromosome breaks - agent-specific hot spots of susceptibility to undergo mitotic crossing over. D8 forms red colonies because of the block created by ade2-40. However, if ade5 becomes homozygous, the block in adenine biosynthesis caused by ade6 prevents accumulation of the precursor before the ade2-block so that the colonies will be white. All that has to be done is to isolate white sectors and test for requirements for lysine, tryosine, methionine, resistance to cycloheximide, requirements for tryptophan or leucine. Mitotic crossing over can thus be followed in seven interval of chromosome VII.

XXVII. University of Amsterdam, Section for Medical Enzymology, Laboratory of Biochemistry, Eerste Constantijn Huygens Straat 20, Amsterdam, The Netherlands. Communicated by G.S.P. Groot.

SEQUENCE HOMOLOGY OF NUCLEAR AND MITOCHONDRIAL DNAs OF DIFFERENT YEASTS.

G.S.P. Groot, R. A. Flavell, and J.P.M. Sanders

Biochimica et Biophysica Acta, in press

SUMMARY

1. Both nuclear and mtDNA of four different yeasts show approximately 10% homology as measured by DNA-DNA filter hybridization. These homologous sequences are mainly attributable to the ribosomal cistrons.

2. Melting curve analysis shows that the heterologous mitochondrial DNA-DNA hybrids contain several times more mismatching than the nuclear DNA-DNA hybrids.

3. DNA-rRNA hybridization shows that the sequences of the ribosomal cistrons in both the nuclear and the mitochondrial genome have been conserved during evolution.

4. However, melting curve analysis of the DNA-rDNA hybrids shows that the sequence of the nuclear ribosomal cistrons have undergone considerably fewer nucleotide substitutions than their mitochondrial counterparts.

5. The results suggest that the mitochondrial ribosomal cistrons have evolved more rapidly than the nuclear cistrons. This is discussed in the light of theories on the rate of molecular evolution.

ABSENCE OF POLY(A) CONTAINING RNA IN YEAST MITOCHONDRIAL RNA.

R. A. Flavell, G.S.P. Groot, G. J. Van Omten and L. A. Grivell

Nature, in press

Chromatography of mitochondrial RNA (mtRNA) from pulse labelled yeast protoplasts on oligo (dt) cellulose or poly (U) sepharose indicates that yeast mtRNA does not contain a poly (A) tract on its 3' terminus in contrast to the cell-sap mRNAs and the mtRNAs from animal cells. Control experiments showed that the negative results were not due to trivial artefacts. Furthermore we were able to confirm that yeast cell-sap RNA and mtRNA from pulse labelled chick fibroplasts contained large amounts of poly (A).

PROPERTIES OF MITOCHONDRIAL DNA FROM *Kluyveromyces lactis*.

J.P.M. Sanders, P.J. Weijers, G.S.P. Groot and P. Borst

Biochim. Biophys. Acta

SUMMARY

1. We have isolated a closed circular DNA fraction from mitochondria purified from *Kluyveromyces lactis* by centrifuging a mitochondrial lysate to equilibrium in CsCl containing ethidium bromide. Electron micrographs of appropriate gradient fractions show predominantly circular duplex DNA with an average contour length (± S.D.) of 11.4 (± 0.5) μm. The circular DNA has the same buoyant density as mtDNA in NaI gradients, but represented only up to 6% of total mtDNA.

2. Denatured total mtDNA renatures with a kinetic complexity of about 20×10^6, a value consistent with the length observed in the electron microscope.

3. mtDNA from *K. lactis* hybridizes about twice as much mitochondrial rRNA isolated from *Saccharomyces carlsbergensis* than the equal amount of *S. carlsbergensis* mtDNA (contour length 25 μm).

4. We conclude that intact mtDNA of *K. lactis* consists of a circular molecule with a contour length of 11.4 μm and a complexity equivalent to this size.

XXVIII. Central Research Laboratory, Mitsubishi Chemical Industries Limited, 1000 Kamoshida-cho, Midori-ku, Yokohama 227, Japan. Communicated by Norio Gunge.

I have moved from Dai-Nippon Sugar Mfg., Co. to the Central Research Laboratory, Mitsubishi Chemical Industries Ltd., 1000 Kamoshida-cho, Midori-ku, Yokohama 227, Japan. The following is a summary of work done recently in this laboratory.

The presence of the mitochondrial sexual factor, omega, was demonstrated in yeast collection in this laboratory. Transmission and recombination of the mitochondrial genes, C^R/C^S, E^R/E^S and O^R/O^S which control the resistance/sensitivity to chloramphenicol, erythromycin and oligomycin, respectively, were non-polar in homosexual crosses and polar in heterosexual crosses. An unusual erythromycin resistant mutant 706E11 ($C^S E^R O^S$) was isolated. This strain was omega$^-$ with respect to the mitochondrial sex and showed no recombination polarity for gene pairs of C-E, C^R-O^R and E-O when crossed to omega$^-$ tester strains having the genotype $C^R E^S O^R$, but there was a highly biased transmission, as if they were heterosexual crosses; that is, the alleles from 706E11, C^S, E^R and O^S, were preferentially transmitted.

The effect of 706E11 was also observed in determining the % of the suppressiveness of petite mutants, i.e., when crossed to ρ^- mutants, 706E11 transmitted the ρ^+ factor into the zygote progenies with higher frequency than other ρ^+ strains did, giving rise to the lower degree of suppressiveness. Thus, there was seen a correlation between the frequency of transmission of drug resistance genes and the degree of suppressiveness. The genetic analysis showed that the nuclear ploidy of 706E11 was diploid and that the frequent transmission of drug resistance genes and the ρ factor from 706E11 was due to the increased amounts of mitochondrial genomes. The omega$^+$ / omega$^-$ standard strains were kindly sent from Dr. Dujon, Gif sur Yvette, France.

Deatils of the study will be submitted for publication shortly.

XXIX. Institute of Physics, College of General Education, University of Tokyo, Komaba, Tokyo 153 Japan. Communicated by Takashi Ito.

The following is a summary of a recent paper.

"An acridine probe into the physiological state of the cell." Takashi Ito and Katsumi Kobayashi. Biochimica Biophysica Acta (1974) (in press).

SUMMARY

Acridine orange was used as a probe to look into the physiological state of the yeast cell, particularly as regards the change in the propperties of the membrane (which acts as a barrier against the incoming acridine orange) and the availability of binding sites for acridine orange in chromosomal DNA during growth. After acridine orange had been introduced into the cell, the genetic change at a specific locus with incubation time was measured photodynamically. A three-fold increase in the rate of

penetration of acridine orange into the cell was observed, for instance, in going from the resting phase to the dividing phase. A five-fold increase was observed in the number of binding sites in chromosomal DNA under the same transition of the cell. These two parameters may be useful as a measure of the physiological changes in the cell. Some environmental factors such as pH and temperature were also demonstrated to affect the parameters.

XXX. Carlsberg Foundation Biological Institute, DK-2200 Copenhagen, N 16 Tagensvej, Denmark. Communicated by Erik Zeuthen.

The following papers have been published or are in preparation:

1. Leif Rasmussen: Yeast Cells; Growth stimulating effects of particulate material in dilute nutrient media. Compt. Rend. Trav. Lab. Carlsberg 40, 107-112 (1974):

Saccharomyces cerevisiae and Schizosaccharomyces pombe have been suspended in subminimal nutrient media in which cell multiplication was suppressed. Addition of particulate material (washed polystyrene beads or heat coagulated egg albumin) resulted in stimulation of cell growth and multiplication. Presumably, mechanical action of particles on the cell surface has led to increased uptake of nutrients.

2. Birte Kramhoft and Erik Zeuthen: Synchronization of the fission yeast Schizosaccharomyces pombe, using heat shocks. In: Methods in Cell Biology, Vol. 12. Ed. by D. M. Prescott. Academic Press 1975. In Preparation:

Cultures of the fission yeast Schizosaccharomyces pombe can be synchronized when treated with 5 - 7 shocks during each of which the temperature is raised for 1/2 hour to 41°C. The interval between successive shocks is 110 minutes, and the intershock temperature is optimal for growth and division (32°C). After this treatment cell division as well as DNA synthesis and the synthesis of the step enzyme aspartatetranscarbamylase have been synchronized.

XXXI. The Finnish State Alcohol Monopoly, Alko, Box 350 SF-00101, Helsinki 10, Finland. Communicated by Heikki Suomalainen.

The following publications have appeared since the last communication. The abstracts of reports have been given in Yeast News Letter 22:2, 61, 1974 and 23:1 14-18, 1974.

Parkkinen, E., Oura, E. and Suomalainen, H., Effect of storage on the nucleic acid composition of baker's yeast. J. Inst. Brew. 80, 271-277, 1974.

Londesborough, J. C., Partial purification and characterization of an adenosine 3':5' -cyclic monophosphate phosphodiesterase from Saccharomyces cerevisiae. Biochem. Soc. Trans. 2, 398-400, 1974.

Tyorinoja, K., Nurminen, T. and Suomalainen, H., The cell-envelope glycolipids of baker's yeast. Biochem. J. 141, 133-139, 1974.

Oura, E. and Silla, K., Peat as substrate for yeast cultivation. 15. Nordiska Kemistmotet, Tampere, Finland 1974, Resumeer, p. 197 (in Swedish).

Haarasilta, S. and Oura, E., Phosphoenolpyruvate carboxykinase activity and its dependence on catabolite repression in baker's yeast. 15 Nordiska Kemistmotet, Tampere, Finland 1974, Resumeer, p. 152 (in Swedish).

Taskinen, L. and Haarasilta, S., Localization of pyruvate carboxylase and phosphoenolypyruvate carboxykinase in baker's yeast. 15. Nordiska Kemistmotet, Tampere, Finland 1974, Resumeer, p. 151 (in Swedish).

Below follow several recent abstracts.

AMMONIA FORMATION IN CULTURES OF BAKER'S YEAST GROWN ON UREA

by E. Parkkinen and E. Oura

Proc. 4 Int. Symp. Yeasts, Vienna, Austria 1974, Part I, pp. 35-36.

In cultivation of baker's yeast using urea or ammonium salts as the only source of nitrogen, the growth rate with urea was slower although the yield was the same. It seemed plausible that the action of urea amidolyase was the rate-limiting step during growth. If so, no ammonia should be detected in the medium during the growth, as has been claimed earlier.

While the urea concentration in the medium was below 0.1% (w/v), no free ammonia appeared during the growth. Changes in the pH and ammonia concentration of the medium occurred with 0.1% urea. The pH fell during the log. growth phase and there was no measurable free ammonia in the medium. Ammonia appeared during the inter-log phase. At the end of the second log. phase the pH and ammonia had again declined, while during the stationary phase, the pH increased to pH 8 and the NH_3 concentration to 5% of the total nitrogen content. In the experiments with 0.5% urea, there was steady increase in free NH_3, which could reach 10% of the total nitrogen. Ammonia formation was also observed in non-proliferating yeast suspended in 2% aqueous urea.

The results suggest that when the ratio urea/glucose is high and an ammonia accumulation also appears during the rapid growth phase, ammonia liberation is not the limiting process, but that some other factor limiting the growth rate, perhaps arising from urea itself, must be operating.

CHANGES IN COMMERCIAL BAKER'S YEAST DURING ITS RIPENING PERIOD

by E. Oura, H. Suomalainen and E. Parkkinen

Proc. 4. Int. Symp. Yeasts, Vienna, Austria 1974, Part I, 125-126.

The ripening stage primarily affects the storage qualities of baker's yeast. During this period cells reach a uniform single cell stage, and it has been shown that the storage qualities of these cells is clearly better than that of budding cells.

During the ripening period yeast takes up considerable quantities of phosphorus from the medium, but little nitrogen. A diminution in the total and acid soluble nitrogen was observed in the yeast cells, whereas the carbohydrate content (especially of glycogen and trehalose) increased. The RNA was shown to fall by 10-20%, while the amount of acid soluble nucleotides changed little.

RNA extracts of yeast samples taken before, during and after the period were fractionated by MAK column chromatography. The evolution profiles contained six fractions: nucleotides, tRNA, 5S RNA, DNA, rRNA and mRNA. In two series of fractionation we observed that, except for the mRNA, the amounts of RNA species decreased in the same way.

The changes in the amount of mRNA and the extent of incorporation of ^{14}C-uracil into the mRNA fraction were followed. The mRNA content in samples of ripened yeast was about 60% of that in unripened yeast. After 20 min's incubation in a glucose-uracil-buffer solution, the ripened and unripened yeasts were shown to have the same mRNA level. The incorporation of labelled uracil into mRNA was stronger in ripened than in unripened yeast, by more than a factor of two after 20 min incubation. This implies that the finished yeast had a deficiency of mRNA, which was quickly synthesized during this incubation.

The incorporation of labelled alanine and valine into the amino acid pool and protein in yeast was also shown to be faster for ripened yeast than for unripened yeast, although the extent of the incorporation under the same conditions was much lower than that of uracil.

When, during the ripening period, there was no longer an excess of nutrients in the medium, the synthesis of mRNA and the other RNA species was reduced leading to a reduced rate of protein synthesis. When these cells obtain favourable environmental conditions, the yeast responds by rapidly increasing the content of mRNA and can thus soon complete the mechanism of polymer synthesis. It can be inferred that a decreased capacity for RNA and protein synthesis is of importance for the stability of commercial baker's yeast.

LEVELS OF ANAPLEROTIC AND GLUCONEOGENIC ENZYMES DURING THE BIPHASIC GROWTH OF BAKER'S YEAST

by S. Harrasilta and E. Oura

Proc. 4. Int. Symp. Yeasts, Vienna, Austria 1974, Part I, pp. 11-12

The anaplerotic and gluconeogenic metabolism of baker's yeast was studied at the enzymatic level during glucose-ethanol diauxic growth in the presence and absence of aspartate. Of the two possible anaplerotic systems, only the pyruvate carboxylase by-pass was present during the whole growth process. The second system, the glyoxylate by-pass (isocitrate lyase as the indicator), like the specific enzymes of the gluconeogenic metabolism, phosphoenolpyruvate e carboxykinase and hexosediphosphatase began to appear only after the glucose had been consumed. The addition of glucose during the growth phase based on ethanol effected a rapid disappearance of phosphoenolpyruvate carboxykinase and hexosediphosphatase activities. The activity of pyruvate carboxylase decreased when the growth medium was supplied with aspartate. The presence of aspartate had no effect on the activities of the other enzyme studied.

REGULATION OF ANAPLEROTIC AND GLUCONEOGENIC ENZYMES IN BAKER'S YEAST BY THE ENVIRONMENTAL CONDITIONS

by E. Oura and S. Haarasilta

Fed. Eur. Biochem. Soc., Budapest 1974, 9. Meeting, Abstr. Commun., p. 107

The effects have been studied of aeration intensity and carbon source on the activities of anaplerotic and gluconeogenic enzymes in continuous cultivations of baker's yeast limited with carbon source.

Under anaerobic conditions with glucose as carbon source, pyruvate carboxylase (PC) was the only anaplerotic system present. With increased aeration the activity of PC decreased somewhat and that of isocitrate lyase (ICL), indicating the operation of the glyoxylate by-pass, increased, but not so as to exceed the PC activity. The PC activity was greatly reduced when the yeast grew on glucose in the presence of aspartate. PC activity was present also in yeast grown on ethanol, even though the ICL was here very active. Thus PC resembles a constitutive enzyme in yeast. Significant activities of the enzymes specific for gluconeogenesis, phosphoenolpyruvate carboxykinase and frunctose-1.6-diphosphatase, could be observed only when gluconeogenesis was necessary, i.e., in yeast grown on pyruvate or ethanol.

ANALYSIS OF YEAST PLASMA MEMBRANE PROTEINS

by J. Londesborough

Fed. Eur. Biochem. Soc., Budapest 1974, 9. Meeting, Abstr. Commun., p. 219.

Baker's yeast suspended in 5 mM K phosphate pH 7.0-1 mM $MgCL_2$-0.3 mM EDTA was disintegrated with a Gifford-Wood Minimill. Cell envelopes were obtained by centrifugation for 10 min x 1000g, unbroken cells removed, and the envelopes washed by repeated centrifugation until they contained less than 1.5% of the total cell content of NADH-oxidase. The envelopes were suspended and shaken with glass beads for 60 min in a 'Mickle' disintegrator. Cell walls were removed at 3000g and a membrane fraction obtained by centrifugation for 50 min x 34000g. This contained 0.7 mg protein/g fresh cells and only 0.1% of the total NADH-oxidase. Presumably it consists mainly of the plasma membrane, in about 7% yield. The 3400g supernatant (S) contained 0.3 mg protein/g cells, derived from the walls, the membrane, or the space between these two. S, and extracts of the membrane with the nonionic detergent Lubrol PX, were subjected to isoelectric focussing on 6% polyacrylamide gels containing 1% ampholine pH 3-10 and 15 mg/ml Lubrol. S yielded a major protein band at pH 6.5, another at pH 5.0, and five more between pH 5.4 and 4.6. About 20% of the membrane protein was soluble in 20 mg/ml Lubrol at 0°C, and yielded discrete bands at pH 7.8, 7.1, 6.9, 6.7, 6.5, 4.5 and 4.2 as well as two badly focussed and overlapping bands at pH 6.0 and 5.0. A further 30% of the membrane protein was soluble in 48 mg/ml Lubrol containing 10 mM NaOH. This material focussed poorly, but 7 discrete bands between pH 6.4 and 4.6 could be observed against the background.

NEUTRAL LIPIDS IN THE CELLS AND ENVELOPE FRACTIONS OF AEROBIC BAKER'S YEAST AND ANAEROBIC BREWER'S YEAST

by T. Nurimen, K. Konttinen and H. Suomalainen

Chem. Phys. Lipids (in press).

The neutral lipids from whole cells and cell envelops of aerobic Saccharomyces cerevisiae and anaerobic Sacch. carlsbergensis and the cell walls isolated from the cell envelopes were analysed. The effect of anaerobiosis was particularly clear on the neutral lipid composition of the plasma membrane. Compared to the anaerobic membrane, the aerobic membrane contained more $C_{16:1}$, $C_{18:1}$ and other unsaturated fatty acids, more total sterol, more than ten times as much ergosterol and less than one tenth as much squalene, reflecting differences between the aerobic and anaerobic whole cell. The main sterol in the aerobic membrane, ergosterol, was mainly in the free form, whereas zymosterol, 24(28) dehydroergosterol, epi- or fecosterol and lanosterol were predominantly esterified. In contrast, the anerobic membrane contained small amounts of biosynthetic sterol precursors of ergosterol (mainly esterified), and was clearly richer in saturated fatty acids having a greater variation in chain length and in $C_{18:2}$ acid. Both plasma membranes

contained a considerable amount of triacyglycerols, while the amount of lower acylglycerols was clearly higher in the anaerobic plasma membrane. The lipid compositions of both cell walls were relatively similar, consisting mainly of triacylglycerols and lower acylglycerols.

SOME ASPECTS CONCERNING THE COMPOSITION AND CONSUMPTION OF ALCOHOLIC BEVERAGES

by H. Suomalainen, L. Nykanen and K. Eriksson

Submitted for publication in Amer. J. Enol. Viticult.

This review primarily emphasizes the central role of yeast in the production of the volatile aroma compounds of alcoholic beverages, such as the fusel alcohols, carbonyl compounds, fatty acids, esters, etc. The small quantities of lactones and phenolic compounds extracted from wood during aging also contribute to the aroma. Animal experiments have shown that the aroma compounds in alcoholic beverages are not harmful to health. The paper shows the importance of acetaldehyde metabolism and the relationship between genetic factors and alcohol consumption, and presents some of the current ideas suggesting that serotonin and other bigenic amines may be involved in alcohol addiction.

XXXII. Mikrobiologisches Institut, Swiss Federal Institute of Technology, Weinbergstrasse 38, CH-8006, Zurich, Switzerland. Communicated by A. Fiechter.

Recent publications from this Institute follow below:

A. Einsele, H. Schneider and A. Fiechter: Studies on n-Alkane Transport in Candida tropicalis. Proc. 4th Internat. Symp. Yeasts (Vienna), Part I. pp. 91-92 (1974).

J. R. Pringle, M. Friedman, and A. Fiechter: "Genetic Elucidation of the Biological Roles of Reserve Carbohydrates in Yeast," Proc. 4th Internat. Symp. Yeasts (Vienna), Part I, pp. 37-38 (1974).

In Press

A. Fiechter: Continuous Cultivation of Yeasts. Methods in Cell Biology, XI, (D. M. Prescott, ed.), in press.

J. R. Pringle: "Chairman's Report on the Panel Discussion "The Yeast Proteases: Technical Problems in Yeast Biochemistry, and Puzzles in Yeast Physiology" ", Proc. 4th, Internat. Symp. Yeasts (Vienna) Part II, in press.

J. R. Pringle: "Chairman's Report on the Panel Discussion "The Yeast Cell Cycle" ". Proc. 4th Internat. Symp. Yeasts (Vienna) Part II, in press.

J. R. Pringle: "Methods for Avoiding Proteolytic Artifacts in Studies of Enzymes and Other Proteins from Yeast," Methods in Cell Biol. XI, (D. M. Prescott, ed.), in press.

J. R. Pringle and J.-R. Mor: "Methods for Monitoring the Growth of Yeast Cultures and for Dealing with the Clumping Problem," Methods in Cell Biology, XI, (D. M. Prescott, ed.), in press.

L. E. Wilkinson and J. R. Pringle: "Transient G1 Arrest of S. cerevisiae Cells of Mating Type α by a Factor Produced by Cells of Mating Type a", Exper. Cell Res., in press.

XXXIII. Kyoto University, Department of Industrial Chemistry, Faculty of Engineering, Yosida, Kyoto, Japan. Communicated by Teijiro Kamihara.

Below follow abstracts of several recent papers from our laboratory.

Vol. 59, No. 2, 1974. Biochemical and Biophysical Research Communications. p. 778 - 780.

EFFECTS OF THIAMINE AND PYRIDOXINE ON THE COMPOSITION OF FATTY ACIDS IN SACCHAROMYCES CARLSBERGENSIS 4228.

Yoshiki Nichikawa, Ichiro Nakamura, Teijiro Kamihara and Saburo Fukui.

SUMMARY

The cells of Saccharomyces carlsbergensis 4228 growing aerobically in the presence of thiamine and absence of pyridoxine, which were in a deficient state of respiratory activity, showed a marked decrease in the content of unsaturated fatty acids. Addition of pyridoxine to the medium prevented completely this effect of thiamine as observed in the case of respiratory activity.

Vol. 59, No. 2, 1974. Biochemical and Biophysical Research Communications.

RESPIRATORY DEFICIENCY IN SACCHAROMYCES CARLSBERGENSIS 4228 CAUSED BY THIAMINE AND ITS PREVENTION BY PYRIDOXINE.

Ichiro Nakamura, Yoshiki Nishikawa, Teijiro Kamihara and Saburo Fukui.

SUMMARY

The cells of Saccharomyces carlsbergensis 4228 (ATCC 9080), growing at a limited rate after a long lag period in the presence of thiamine and absence of pyridoxine, exhibited a markedly low respiration rate, despite the fact that the cultivation was carried out under aerobic conditions. The cytochrome oxidase activity of the cells was also negligible. The characteristic spectra of cytochrome pigments were not detected. Addition of pyridoxine to

the medium prevented the growth inhibition and eliminated these effects of thiamine.

EFFECTS OF THIAMINE AND PYRIDOXINE ON THE CONTENT AND COMPOSITION OF STEROLS IN SACCHAROMYCES CARLSBERGENSIS 4228

Jun Nagai and Hirohiko Katsuki. Department of Chemistry, Faculty of Science, Kyoto University, Kyoto, Japan, and Yoshiki Nishikawa, Ichiro Nakamura, Teijiro Kamihara and Saburo Fukui.

Biochem. Biophys. Res. Commun. 60 No. 2, p. 555 - 560 (1974).

SUMMARY

The level of sterols in S. carlsbergensis 4228 cells grown aerobically in a synthetic medium fortified with thiamine was significantly low compared with that in the control cells. The levels of free and esterified sterols in the thiamine-cells were 60% and 10% of the corresponding sterol levels in the control cells, respectively. Analysis by gas-liquid chromatography of non-saponifiable lipids extracted from the cells revealed that the amounts of squalene, lanosterol and two unidentified sterols were higher than those in the control cells and that ergosterol and zymosterol, major sterols in the control cells, were not present. These effects of thiamine on the content and composition of sterols were abolished by the addition of pyridoxine to the medium.

XXXIV. Department of Biology, McMaster University, Hamilton, Ontario, Canada. Communicated by J. J. Miller.

A convenient method of preserving yeast strains for teaching and research has been in use in this laboratory since 1969. It is especially effective with sporulated yeast or with non-sporulated cells that have been exposed to sporulation medium.

1. The yeast is separated from acetate sporulation medium by filtration on Whatman No. 50 paper and washed by passing sterilized water through the filter. The yeast layer is sucked "dry", i.e., until cracks begin to appear.

2. The yeast is removed from the filter and mixed with its own weight of anhydrous glucose. The mixture liquifies as cell water is removed osmotically.

3. Additional anhydrous glucose to a total of 4X the weight of the yeast is added to the liquid with continual mixing. A dry, granular preparation is thus obtained which is further dried over anhydrous silica gel for several days.

4. The yeast-sugar mixture is stored at 5°C, preferably in a closed container with anhydrous silica gel.

Survival of spores and cells of the order of 20-40% after three years storage has been obtained with some yeast strains. The process is simple, requires no expensive equipment, and could be scaled up to large quantities.

XXXV. Research Institute for Viticulture and Enology, 865 15 Bratislava, Matuskova 21, Czechoslovakia. Communicated by E. Minarik.

E. Minarik - A. Navara: Effect of sulphate and sulphur amino acid levels on sulphite and sulphide formation by wine yeasts. Annali di Microbiologia/special issue, 1974 - in press/. Summary.

Increased sulphate concentration in both synthetic medium and grape juice prior to alcoholic fermentation considerably elevate sulphite formation by SO_2-forming yeasts. Sulphite formation may be substantially reduced by the sulphur amino acids methionine and/or cysteine. Combined methionine and cysteine additions to the medium are particularly effective in their inhibitory influence. Sulphur dioxide levels of the must have no significant effect on sulphate uptake or sulphite formation. Methionine and cysteine show a rather stimulating effect on sulphide production in both SO_2-forming and normal yeasts. Extremely high sulphide formation is achieved by increased cysteine and combined methionine and cysteine levels. As a rule SO_2-forming yeasts form less sulphide compared with normal yeasts in media with or without added sulphur amino acids.

A. Navara - E. Minarik: Determination of sulphur dioxide and hydrogen sulphide in the presence of sulphur amino acids. Die Wein-Wissenschaft 29, Nr. 4, pp. 208-215, 1974. Summary.

Results on the influence of the sulphur amino acids methionine or cysteine in concentrations up to 1,500 ppm in water model solutions on the iodometric sulphite and sulphide determination are given. Both methods, the direct iodometric titration and the distillation procedure, showed satisfactory results. Cysteine influenced the results of sulphite determination by the iodometric titration method in the iodine reduction. The results of the distillation method were not influenced at all. Methionine did not affect the results of either determination method. The exactness of sulphide determination as CdS is satisfactory. The presence of 1 mg cysteine/20 ml model solution caused an increase of the result by 0.85 µg. 1 mg methionine/20 ml solution increased the result by 0.56 µg only. This insignificant influence during sulphide determination in grape juice, grape wine and synthetic medium may thus be practically neglected.

XXXVI. Amoco Foods Company, Amoco Research Center, Naperville, Illinois 60540, U.S.A. Communicated by C. Akin and R. J. Flannery

Torula Yeast from Ethanol: The First Large Scale Production of Food From A Non-Agricultural Source:

Standard Oil Company of Indiana, after a nine-year multi-million dollar research program, has announced a new process to grow Torula Yeast on ethyl alcohol. Standard's subsidiary, Amoco Foods Company, is building at Hutchinson, Minn., its first commercial plant with a capacity of more than 10 million pounds of yeast a year. The Amoco Torula Yeast (Candida utilis) is grown in an aseptic continuous fermentor, and harvested under sanitary conditions. This food yeast is over 50 percent protein and contains vitamins and minerals, and meets Food and Drug Administration regulations.

Amoco Foods will market the yeast to independent food processors who will incorporate the high protein ingredient in their food products in order to improve nutrition, flavor, cost or other properties of their processed foods.

XXXVII. Institut de Technologie Agricole C.P. 70, Saint Hyacinthe, Quebec, Canada. Communicated by L. O. Emard.

Below follows the abstract of a paper presently in press.

"Etudes des pommes et des levures de plusieurs vergers du Quebec en vue de la fabrication de cidres de bonne qualite" by L. O. Emard.

Agro-Alimentaire 1: 8-14, 1974 (in press).

SUMMARY

In the course of experiments conducted to study the effect of mixed cultures of yeasts on apple juice and on the flavor and other properties of the resulting cider, it was observed that a yeast identified as Lipomyces starkeyi caused a marked decrease in the acid content of the cider and imparted a rather pronounced earthy taste. This fact could possibly account for the earthy flavors which are sometimes found in certain wines which had probably been made in the presence of indigenous yeasts of such types. This strain of yeast was identified as L. starkeyi on the basis of its sporulation, morphological and physiological characteristics according to the methods described in The Yeasts (Lodder J. 1970). Among the substances it utilizes as sole source of carbon are erythritol, citric, succinic, l-malic and d-malic acids; the latter very slowly however.

XXXVIII. National and International Meetings

1. Speech at the Opening ceremony of the Fourth International Symposium on Yeasts at Vienna, Austria, July 8-12, 1974.

T. O. Wiken, Honorary Chairman of the Organizing Committee, Chairman of the Commission on Yeasts and Yeast-like Microorganisms of the International Association of Microbiological Societies (IAMS) and the International Union of Biological Sciences (IUBS).

In accordance with the Resolution adopted by the participants of the Third International Symposium on Yeasts, held at Delft and The Hague in 1969, three specialized symposia should be organized, namely in Czech-

slovakia in 1971, in Japan in 1972 and in Finland in 1973. These symposia have now taken place in Smolenice, in Kyoto and Tokyo, and in Otaniemi, respectively, and the corresponding Proceedings were printed as well as those of the general symposium at Delft and The Hague.

In accordance with the Resolution mentioned, a fourth symposium namely a general one, should be held either in Austria, the USA, the USSR, or elsewhere. And now we have come together here in Vienna to participate in this last symposium organized by a Committee under the Chairmanship of Professor Dr. H. Kaulshofer and under the auspices of the Austrian Society of Microbiology, Hygiene and Preventive Medicine, the Austrian Association of Food and Fermentation Technologists and the Experimental Station for Fermentation Industry at Vienna.

I am sure that it is not necessary to tell you that I was very happy when Professor Dr. H. Klaushofer at the State University of Agriculture in Vienna was found to be willing to organize this symposium. I knew him personally as an excellent organizer capable of finding outstanding co-workers and of delegating the different duties to the right persons. In addition, Austria and Vienna have, as already mentioned by Colleague Klaushofer, a valuable tradition in the fields of Yeast Science and Technology, and nowadays too research of highest class is performed here in the fields mentioned. The definitive programme shows conclusively that our expectations as regards the quality of the symposium will be fulfilled in all respects. I would like to express in advance the most sincere thanks to you, Professor Klaushofer, and to all Members of the Organizing Committee, particularly the Secretary, Dr. Uwe Sleytr, for what you have done and still will do for us, Participants of the Fourth International Symposium on Yeasts.

According to the Resolution adopted at the general symposium in Delft and The Hague in 1969, the Chairman and the Secretary of the symposium mentioned should remain in office as Chairman and Secretary of the IAMS Commission on Yeasts and Yeast-like Microorganisms until the general symposium to be held in 1974. In view of this it was proposed at the meeting of our Commission in Otaniemi in 1973 that in conformity to the tradition I and Dr. L. Rodrigues de Miranda here in Vienna should be replaced by Professor Dr. H. Klaushofer and Dr. U. Sleytr as Chairman and Secretary, respectively, of the Commission. All members of the Commission present at the Otaniemi Meeting agreed upon such an arrangement, and in a letter dated July 2, 1973, Professor Klaushofer definitely accepted the unanimous nomination as Chairman, which also implies that Dr. U. Sleytr automatically will take over the duty as a Secretary of the Commission.

Hence, it is today the last time I have the honor and the pleasure to speak to you as a Chairman of the IAMS Commission on Yeasts and Yeast-like Microorganisms. I would then like to say that I have got the impression that our symposia may be considered as valuable contributions to the development of General and Applied Yeast Science as well as to the development of the Personal Contacts between the scientists concerned. As a Chairman of the Commission I have experienced an extraordinarily positive response from the Scientists and Authorities in the countries, where the symposia have been organized, namely The Netherlands, Czechoslovakia, Japan, Finland and Austria. I also know that the Participants in the symposia from countries all around the world have done their utmost to overcome numerous obstacles in the form of political and economical barriers, bureaucracy, student riots

etc. etc. in order to be able to present the valuable results of their research efforts. My sincere thanks to all persons who have contributed so effectively to the success of the symposia which have taken place during my time as a Chairman of the Commission. I hope that you, Dear Colleague Klaushofer, will meet with the same positive understanding and friendship as I have had the favor to experience from 1969 until now when I leave the Chairmanship of the IAMS Commission on Yeasts and Yeast-like Microorganisms. These expectations include all of you, Dear Participants of the Fourth International Symposium on Yeasts. My best wishes for your future and, once more, my sincere thanks.

At the closing of the Symposium, Professor H. J. Phaff, speaking for the participants, expressed his appreciation and gratitude to Professor Klaushofer and all the members of his organizing committee for all they

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2. The following resolution, proposed by the IAMS Commission on Yeasts and Yeast-like Microorganisms, was adopted by the participants of the Fourth International Symposium on Yeasts, held at Vienna July 8-12, 1974:

The participants wish to thank the Austrian hosts most sincerely for a very well organized symposium, interesting from a scientific point of view and pleasant from a social point of view.

Professor H. Klaushofer and Dr. U Sleytr have succeeded Professor T. O. Wiken and Dr. L. Rodrigues de Miranda as Chairman and Secreatry, respectively, of the IAMS Commission on Yeast and Yeast-like Microorganisms. The Commission expresses sincere thanks to Professor Wiken and Dr. Rodrigues de Miranda for their excellent work in the period of 1969-1974.

The following persons will be Members of the Commission from 1974 on:

Austria - H. Klaushofer , U. Sleytr; Belgium - H. Verachtert; Brazil - A. Panek; BRD - S. Windisch; Canada - C. F. Robinow; Czechoslovakia - Kockova-Kratochvilova, A. Kotyk, E. Minarik; Denmark - A. Stenderup; DDR - H. Koch, P. Lietz, W. Nordheim; Egypt - A. S. ElNawawy; England - J. Spencer; Finland - H. Suomalainen; France - P. Galzy; Hungary - E. Novak; Ireland - R. B. Gilliland; Israel - C. Shalitin; Japan - Y. Fukazawa, Iwata, S. Nagai; Netherlands - J. C. Hoogerheide, L. Rodrigues de Miranda, T. Wiken; Poland - J. Jakubowska. B. Bachman (observer); Portugal - L. do Carmo-Sousa; Scotland - E. O. Morris; South Africa - J. P. van der Walt; Spain - J. Santa Maria; Sweden - K. Jarl; Switzerland - A. Fiechter. Ph. Matile; U.S.A - H. J. Phaff; USSR - N. Elinov, M. N. Meissel, Shavlovsky; Yugoslovia - V. Johanides.

4. The Commission proposes the following specialized Symposia;

a. In 1976 in Berlin on Yeasts in Industrial Use.

b. In 1977 either in Denmark (Aarhus) or in Hungary, France or elsewhere, on Systematics of Yeasts, including Taxonomy, Immunology, Serology and Ecology.

c. In 1978 in Poland on Genetics and Metabolism of Yeasts.

5. In 1979 a general Symposium should be held in the U.S.S.R.

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3. International Association of Microbiological Societies. (The Division of Microbiology of the International Union of Biological Sciences). Mycology Section Council Meeting, September 4, 1974.

The Mycology Section Council convened at the Imperial Hotel, Tokyo, Japan, at 1900 hours, 4 September, 1974.

The meeting was called to order by the chairman Dr. E. S. Beneke, with eight (8) delegates in attendance. Dr. Beneke noted that a quorum was not in attendance; therefore, no official business could be transacted. He also declared that an official meeting of the Mycology Section, IAMS will be held in Tokyo, Japan in 1975, concurrently with the sixth Congress of ISHAM. A slate of officiers will be elected at the time.

Dr. Beneke presented to the delegates the major developments in the Mycology Section since 1970. These were as follows:

Developments in the Mycology Section of IAMS, E. S. Beneke, Chairman, September 4, 1974.

The Mycology Section of the International Association of Microbiological Societies was established in 1970 at the Xth International Congress for Microbiology held in Mexico City, August, 1970. A business meeting for discussion was held in Exeter, England, September 19, 1971. The objectives of the IAMS Mycology Section are to serve as an assembly of medical societies, and other interested societies or commissions for an exchange of information at meetings and by publications, to represent these groups of specialists at IAMS, to maintain contact with other Sections of IAMS, to maintain committees in IAMS, and to encourage high standards of research and training throughout the world.

In April, 1972, Mail ballots were sent out to the official representatives of the medical mycology societies or mycology sections of the microbiology societies. A majority of the ballots were returned. All of these indicated approval of the proposal to organize the Mycology Section in IAMS. This was presented and approved at the Executive Board meeting of IAMS on October 9, 1972 in Marseille, France.

Two organizations, the International Society of Human and Animal Mycology and the International Commission on Yeasts and Yeast-like organisms have entered in association with this section. Other interested societies are invited to join the Mycology Section.

In the selection of names for the Advisory Council and the Statutes Committee advice was sought from a number of individuals in both the International Society of Human and Animal Mycology and the Medical Mycology Society of the Americas. This has been done through correspondence and personal contact during the annual meeting of the Medical Mycology Society of the Americas. The names on both committees have been finalized

with a few suggested modifications from Dr. Ajello, President of ISHAM in his letter dated July 23, 1973. Letters were sent to the individuals selected and all have indicated interest in serving on either the Advisory Council or the Statutes Committee.

Since the Mycology Section has been organized with functional committees, we plan to have the Statutes Committee have a final draft of the proposed statutes for June, 1975. We are planning to hold a Mycology Section business meeting during the Sixth Congress of ISHAM in Tokyo, between June 29 and July 4, 1975. This would be for dicussion and approval of the statutes, and election of new officers.

The Advisory Council and the Section officers along with the ISHAM Committee need to explore the possibilities of developments for future congresses in medical mycology and industrial mycology as well as with the yeast group. Even though these are very diverse groups, as the industrial mycologists are usually in industrial microbiology societies, there are potentials for greater future developments in this section.

Dr. Victor Skerman, Delegate from Australia called the Council's attention to the World Federation of Culture Collection (WFCC) and its wish to represent mycologists of the IAMS. Dr. Skerman pointed out that he, Dr. Carmichael, Ainsworth and others had worked hard to make this an interdisciplinary body working to reduce synonomy and called for the Mycology Section to cooperate with the WFCC.

Dr. Norman Gibbons urged the Mycology Section to encompass wide aspects of mycology, notably Industrial Mycologists. He also suggested eventually integrating the Yeast Commission meetings with the Mycology Section into a Congress.

The representative of the Commission on yeasts and yeast-like organisms commented that the commission had symposia planned to 1978, with a symposium held in Vienna in July, 1974, and future symposia in West Berlin in 1976 with the 5th International Fermentation Symposium; in Hungary or Denmark in 1977 a symposium on the Taxonomy of yeast; in 1978 a symposium on the Genetics of yeast to be held in Poland, and in 1979 a general symposium in the USSR.

Dr. Beneke reported on the EBIAMS meeting in Israel. The points discussed relative to the Mycology Sections were:

a. IAMS statutes are being revised.
b. An ad hoc committee is being formed to study the merits of seeking Union status for IAMS.
C. The Section on Bacteriology will hold its next Congress in Munich on September 3-9, 1978. It is the desire of the IAMS to hold all three Sectional Meetings simultaneously in the same country or neighboring countries in order to facilitate a more convenient and less expensive meeting of the IAMS Delegates to carry on the business of the organization at an intersectional meeting.

Dr. Wundt and Dr. Seeliger have indicated that facilities are available in Munich for a meeting of the Mycology Section, providing the council of ISHAM agrees to meet with the other IAMS Sections.

The proposed statutes for the Mycology Sections were distributed to the delegates. Dr. Beneke pointed out that there is a statutes committee working on statutes to be voted on at the 1975 Section Meeting.

Dr. Beneke also noted that a member of the Commission on Yeast and Yeast-like organisms is on the Mycology Section Advisory Committee.

Dr. Kazuo Iwata reported on plans for the 6th Congress of ISHAM in the Mycology Section of IAMA. The meeting will be held on June 29, to July 4, 1975 at the Tokyo Prince Hotel, Tokyo, Japan. Second notices of the meeting have been mailed to all ISHAM members. The meeting will consist of general paper sessions, 4 symposia and 2 panel discussions. A loan for $4,000 from IUBS has been requested for initial operation of the congress.

Tentative plans for future meetings were discussed. A tentative decision was reached to meet in Munich in 1978. A definite decision will await polling delegates from member organizations.

It was pointed out that because of space problems, a site for the 1982 meeting should be determined soon.

New business discussed:

The site and time of the next Mycology Section meeting will be in June, 1975 during the 6th Congress of ISHAM in the Mycology Section of IAMS. A specific date will be announced.

Dr. Beneke expressed the sincere thanks of members of the Section for the excellent program and facilities for the Congress and the hospitality shown by our Japanese hosts.

Dr. W. Clark moved for adjournment. The Council was adjourned at 2005.

4. Sixth annual meeting of the Commission for Yeasts of the Czechoslovak-Microbiological Society, held in Smolenice, 23 - 25th January 1974. Communicated by A. Kocková-Kratchvílová.

Scientific program:

Section 1: Applied aspects of Yeasts:

O. Bendová: The comparison of technologically important features of selected brewing yeast strains.

F. Malík: The production of pure cultures of wine yeasts in a laboratory fermentor.

L. Švorcová: Yeast contamination of sweetened mineral waters.

B. Králová: The influence of antivitamins on the biosynthesis of vitamins in yeasts.

L. Hillová: Bronchopneumonia caused by yeast-like microorganism.

Section 2: Collections, strains and their protection:

A. Kocková-Kratochvílová: Numerical taxonomy of the genera Hansenula and Pichia.

E. Sláviková: Numerical taxonomy of the genus Cryptococcus.

Z. Cirman: The legal protection of industrial microorganisms in CSSR.

Section 3: Genetics of yeasts:

L. Šilhánková: Induced mutants of Saccharomyces cerevisiae unable to ferment glucose.

P. Šmigaň: The synthesis of macromolecules and lipids in op$_1$ yeast mutants during the mutagenesis by ethidium bromide.

J. Šubík, J. Kolarov, H. Fečíková, L. Kováč: The vital function of mitochondria in growing eucaryotic cells.

Section 4: Cytology:

Y. Kochová: The effect of some cell poisons on the nuclear division in yeasts.

D. Vraná: The cytomorphological comparison of daughter and mother cells of Candida utilis.

V. Snějdar, J. Vořišek: The ultrastructure of cells of Trigonopsis variabilis.

Section 5: Immunology and informatorics:

A. Tomšíková: A comparison of the effectiveness of some serological methods in the diagnostics of candidiasis.

J. Šandula, L. Kuniak: The isolation of specific antibodies of pathogenic yeasts by affinity chromatography.

Z. Kohnová, K. Lešická: Modern methods of the informatorics

Section 6: Minisymposium on cellular transport:

A. Kotyk: The structure and function of the plasmalemma.

S. Janda, D. Michaljaničová: The transport of sugars.

L. Říhová, M. Opekarová: The transport of amino acids.

K. Siegler: The transport of cations.

J. Horák: The isolation of carrier proteins.

Section 7: Minisymposium on cell walls:

O. Nečas: The freeze-etching of regenerating yeast protoplasts of Saccharomyces cerevisiae.

A. Svoboda: The morphogenesis in Trigonopsis variabilis.

M. Kopecká: The method of isolation of nucleusless yeast protoplasts unable to synthesize glucan fibrils of the cell wall.

M. Gabriel: The regeneration of cell walls in protoplasts of Trichosporon pullans.

Z. Krátky: The influence of 2-deoxy-D-glucose on the formation of cell wall components in protoplasts of Saccharomyces cerevisiae.

P. Biely: Synthesis of the cell wall during the life cycle of Saccharomyces cerevisiae.

V. Farkaš: The study of yeast mannan-synthetase.

M. Vršanská: The lysis of yeasts under the influence of extracellular enzymes, produced by Anthrobacter strain GJM-1.

E. Streiblová: The fluorescence method for studying copulations in some heterozygotic yeasts.

XXIX. Brief News Items

1. Jeremy Thorner has just been appointed as an Assistant Professor in the Department of Bacteriology and Immunology at the University of California at Berkeley. His research interests center on the biochemical basis of mating in Saccharomyces cerevisiae, including the mode of action of the mating-type pheromones and the role of microtubules in plasmogamy and karyogamy.

2. University of Puget Sound, Tacoma, Washington, 98416. Dr. John G. Kleyn writes: During the 1973-74 academic year I had the privilege of being a guest scientist in Professor Wiken's laboratory at the Technical University in Delft. My research topic while there related to the effect of antifolate compounds on yeast growth and metabolism. I expect to have some of this material ready to submit for publication in the forthcoming year and would welcome receiving any related publications.

3. Carl C. Lindegren, Professor Emeritus of Southern Illinois University, Department of Microbiology, Carbondale, Illinois, writes: We have copies of "Modifications of the Yeast Cell Produced by Different Substances" available for distribution. This is a booklet of electron microscope pictures showing the effects of various poisons and chemicals on the internal structure of the yeast cell.

As long as our supply lasts, we will be happy to send a copy upon request.

4. Zentralinstitut für Mikrobiologie und experimentelle Therapie, German Democratic Republic, 69 Jena, Beutenbergstr. 11. Dr. H. Weber writes: The films listed below (several of which were prepared by the late Rudolf Müller) may be purchased or borrowed from "Institut für Film, Bild und Ton, DDR 108 Berlin, Krausenstr. 9/ 9/10, East Germany."

Cytomorphologie der Hefen I: Die Vakuole und die granulären und fädigen Zelleinschlüsse (Dr. Müller), 1958, T-HF 111.

Cytomorphologie der Hefen II: Der Zellkern und seine Teilung (Dr. Muller), 1958, T-HF 112.

Brechungsindex-Variationen zur Verbesserung der Phasenkontrastmikroscopie der Mikroorganismen (Dr. Müller), 1956, T-HF 184.

Basidiomyceten I: Das vegetative Mycel von Polystictus versicolor, 1957, T-HF 200.

Basidiomyceten II: Das Chondriom von Polystictus versicolor, 1957, T-HF 201.

Basidiomyceten III: Der Zellkern von Polystictus versicolor, 1958, T-HF 202.

Basidiomuceten IV: Die Kernteilung von Polystictus versicolor, 1959, T-HF 281.

Ascusentwicklung und Bildung von Ascosporen bei Schizosaccharomyces (Dr. Müller), 1962, T-HF 427.

Das Verhalten endoplasmatischer Membranen in der Zelle von Polystictus versicolor, 1967 T-HF 552.

Protoplasten-Enstehung bei Polystict. versic., 1965, T-HF 598.

Die Entstehing entwicklungsfähiger Protoplastan aus Hefezellen und ihre Reversion (Dr. Muller), 1965, T-HF 659.

5. Istituto di Igiene, della Universita di Milano, Via Francesco Sforza 35, Italy. The following paper will appear in the forthcoming issue of the Bulletin de la Société française de Mycologie Médicale:

Viviani, M. A., Tortorano, A. M., 1974. Millieu selectif pour l'isolement de Cryptococcus neoformans des expectorations à flore levuriforme mixte.

6. I would like to announce to all the friends whom I met during my research career that I have retired from research since last August from the Laboratory of Mycology of the Faculty of Chemistry of the University of Montevideo. My present address is: R. C. Artagaveytia-Allende, Salto 1184, Montevideo-URUGUAY.

7. Dr. Marjorie Crandall has accepted a position as an assistant professor in the School of Biological Sciences, University of Kentucky, Lexington, Kentucky 40506. Her previous addresses were: Department of Biology, Brooklyn College CNNY, Brooklyn, N.Y. 11210, and Department of Microbiology, Indiana University, Bloomington, Indiana 47401.

8. The following book has been published recently: Ten Years of Activity in Czechoslovak Yeast Research. Publ. House of the Slovak Academy of Sciences, Bratislava, 1974. (A. Kockova-Kratochvilova ed.) 186 pp.

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