Y E A S T

A News Letter for Persons Interested in Yeast

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I. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852. Communicated by Douglas S. King.

I have recently joined the staff of the Mycology Department of the American Type Culture Collection and will be working with the yeast collection. Our current activities at the ATCC include initiating a study of the genus Trichosporon. In the course of this study we plan to use a computer evaluation of the data.

Attached is a list of yeast strains newly accessioned to the ATCC.

Candida albicans	ATCC 28461, D. Yarrow, CBS, Delft, The Netherlands
Candida albicans	ATCC 28516, J. Van Cutsem, Janssen Pharmaceuticals, Beerse, Belgium
Candida albicans	ATCC 28517, J.M.T. Hamilton-Miller, Royal Free Hosp. Sch. of Med., Univ. of London, England
Candida albicans	ATCC 28815, E. Grunberg, Hoffman-LaRoche, Inc., Nutley, New Jersey
Candida albicans	ATCC 28835, M. S. Collins, School of ATCC 28836, Medicine, University of ATCC 28837, California, Davis, California
Candida citrea	ATCC 28533, ATCC 28534, D. Yarrow, CBS, Delft, The Netherlands
Candida glaebosa	ATCC 28472, D. Yarrow, CBS, Delft, The Netherlands
Candida hydrocarbofumarica	ATCC 28532, D. Yarrow, CBS, Delft, The Netherlands
Candida lambica	ATCC 28789, C. R. Lazarus, University of Florida, Gainesville, Florida
Candida levantina	ATCC 28531, D. Yarrow, CBS, Delft, The Netherlands.
Candida parapsilosis	ATCC 28473, ATCC 28474, ATCC 28475, ATCC 28476, D. Yarrow, CBS, Delft, The Netherlands
Candida pseudotropicalis	ATCC 28838, E. Grunberg, Hoffman-LaRoche, Inc., Nutley, New Jersey
Candida sake	ATCC 28721, S. A. Meyer, American ATCC 28722, Type Culture Collection, ATCC 28723, Rockville, Maryland

Candida savonica	ATCC 28530, D. Yarrow, CBS, Delft, The Netherlands
Candida tropicalis	ATCC 28707, D. Drutz, University of Texas, San Antonio, Texas
Candida tropicalis	ATCC 28724, S. A. Meyer, American Type Culture Collection, Rockville, Maryland
Candida tropicalis	ATCC 28748, J. Lilaramani, Indian ATCC 28749, Agricultural Research ATCC 28750, Institute, New Delhi, India
Candida tropicalis	ATCC 28774, Centraalbureau voor ATCC 28775, Schimmelcultures, Delft, ATCC 28776, The Netherlands
Candida valdiviana	ATCC 28529, D. Yarrow, CBS, Delft, The Netherlands
Candida valida	ATCC 28477, ATCC 28478, ATCC 28525, D. Yarrow, CBS, Delft, The Netherlands
Candida viswanathii	ATCC 28777, Centraalbureau voor Schimmelcultures, Delft, The Netherlands
Candida sp.	ATCC 28528, D. Yarrow, CBS, Delft, The Netherlands
Cyptococcus albidus var. albidus	ATCC 28773, R. L. Brunker, Leidy Lab. Univ. of Pennsylvania, Philadelphia, Pennsylvania
Cryptococcus neoformans	ATCC 28737, S. Shadomy, Medical College of Virginia, Virginia Commonwealth Univ., Richmond, Virginia
Cryptococcus neoformans	ATCC 28738, L.J.R. Milne, Western Central Hospital, Edinburgh, U.K.
Cryptococcus neoformans	ATCC 28769, T. Kobayashi, Nagoya ATCC 28770, University School of Medicine ATCC 28771, Nagoya, Japan
Lodderomyces elongisporus	ATCC 28479, ATCC 28480, ATCC 28481, D. Yarrow, CBS, Delft, The Netherlands
Pichia fermentans	ATCC 28483, ATCC 28484, ATCC 28526, D. Yarrow, CBS, Delft, The Netherlands
Pichia kurdiavzevii	ATCC 28527, D. Yarrow, CBS, Delft, The Netherlands

Pichia onychis	ATCC 28486, D. Yarrow, CBS, Delft, The Netherlands
Pichia pastoris	ATCC 28485, D. Yarrow, CBS, Delft, The Netherlands
Pichia sp.	ATCC 28487, ATCC 28488, D. Yarrow, CBS, Delft, The Netherlands
Pichia fluxuum	ATCC 28778, Centraalbureau voor Schimmelcultures, Delft, The Netherlands
Pichia pinus	ATCC 28779, Centraalbureau voor Schimmel- ATCC 29780, cultures, Delft, The Netherlands ATCC 28781 ATCC 28782
Saccharomyces cerevisiae	ATCC 28683, H. Bussey, McGill Uni- ATCC 28684, versity, Montreal, Quebec, ATCC 28685, Canada
Saccharomyces ùvarum	ATCC 28827, National Collection of Yeast Cultures, Surrey, England
Saccharomyces cerevisiae	ATCC 28382, ATCC 28383, F. Karst, Inst. Biol. Molec. et Cell. Strassburg, France
Saccharomyces cerevisiae	ATCC 28503, ATCC 28504, ATCC 28505, ATCC 28506, ATCC 28507, ATCC 28508, S. Ulaszewski, Zool. Inst., Wroclaw Univ., Wroclaw, Poland
Saccharomyces uvarum	ATCC 28518, ATCC 28519, ATCC 28520, ATCC 28521, ATCC 28522, ATCC 28523, ATCC 28524, A.M.A. ten Berge, Van't Hoff Laboratory, St. Univ. of Utrecht, Netherlands
Torulopsis castellii	ATCC 28482, D. Yarrow, CBS, Delft, The Netherlands
Trichosporon aculeatum	ATCC 28680, Centraalbureau voor Schimmelcultures, Delft, The Netherlands
Trichosporon aquatile	ATCC 28574, Centraalbureau voor Schimmelcultures, Delft, The Netherlands
Trichosporon capitatum	ATCC 28575, Centraalbureau voor Schimmel- ATCC 28576, cultures, Delft, The Nether- ATCC 28591, lands

Trichosporon cutaneum	ATCC 28592, Centraalbureau voor Schimmelcultures, The Netherlands
Trichosporon fermentans	ATCC 28577, Centraalbureau voor ATCC 28578, Schimmelcultures Delft, The Netherlands.
Trichosporon hellenicum	ATCC 28579, Centraalbureau voor Schimmelcultures, Delft, The Netherlands.
Trichosporon melibiosaceum	ATCC 28580, Centraalbureau voor ATCC 28581, Schimmelcultures, Delft, The Netherlands.

II. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md, 20852. Communicated by Sally A. Meyer.

The following is an abstract of a paper accepted for publication in Archives for Microbiology.

S. A. Meyer, K. Anderson, R. E. Brown, M. Th. Smith, D. Yarrow, G. Mitchell and D. G. Ahearn

Abstract

Selected yeasts classified as Candida sake van Uden et Buckley were examined for their physiological, morphological and immunological properties and their DNA relatedness. Candida maltosa Komagata, Nakase et Katsuya is herein recognized as a species separate from C. sake. Candida maltosa was distinguished from C. sake and from C. tropicalis by insignificant DNA reassociation. In addition, C. maltosa was distinguished from C. sake by its higher maximal growth temperature and lower guanine plus cytosine content of its DNA and from C. tropicalis by its failure to utilize soluble starch for growth and its resistance to cycloheximide. The species C. cloacae and C. subtropicalis are placed in synonymy with C. maltosa.

III. The University of Connecticut, Marine Research Laboratory, Noank, Connecticut 06340 U.S.A.. Communicated by John D. Buck.

A paper entitled "Distribution of aquatic yeasts-Effect of incubation temperature and chloramphenicol concentration on isolation" has been accepted for publication in Mycopathologia. The summary is as follows:

Fresh (river), estuarine, and marine waters in and along the coastline of Connecticut were cultured by the membrane filter technique at 20 and 37 C on a complex medium containing 0-1000 mg/L of chloramphenicol. Using counts on medium with 500 mg/L antibiotic as a base, ratios of total and pink counts were recorded for other chloramphenicol concentrations at both temperatures for the waters sampled. Variable results were obtained; in general, both total and pink yeast counts decreased with increasing antibiotic levels, the decrease being most apparent at > 400 mg/L chloramphenicol. Medium without antibiotic and with 100 mg/L

always produced bacterial overgrowth. A total of 209 white yeasts were isolated from all platings; the genera Torulopsis, particularly T. candida, and Candida were dominant with lesser numbers of Cryptococcus, Trichosporon, sporogenous genera, and Kloeckera. Most species isolated were found on media at all chloramphenicol levels. Comparisons were made of yeast distributions in these temperate waters with reports from other areas.

Current research activities include the following:

1. Development of selective media for human-associated yeasts, particularly Candida albicans, in recreational and other waters.

2. Occurrence and distribution of human pathogenic yeasts in molluscan shellfish.

We would appreciate hearing from other yeast workers of their experiences in these areas and receiving appropriate reprints. A recent EPA-ASTM symposium included a task group to consider the possibility of using yeasts as supplemental indicators of water quality. I would be pleased to learn of any research in this aspect of mycology.

IV. Department of Biology, Chonnam National University, Chonnam, Korea. Communicated by Myung Sam Park.

"Distribution of Wild Yeasts Isolated from Several Natural Habitats in Korea."

Abstract

Various wild yeasts were isolated from natural habitats in different regions and seasons in Korea. The author found that some species were isolated regardless of both regions and seasons, while others were confined to special regions and seasons or hosts.

1. 96 strains of wild yeasts were isolated by six different collection methods from thirteen regions. Among them, were 12 strains of Saccharomyces, 1 strain of Debaryomyces, 7 strains of Hansenula, 4 strains of Pichia, and 1 strain of Kluyveromyces. Of the asporogenous yeasts there were 2 strains of Cryptococcus, 45 strains of Torulopsis, 16 strains of Candida and 3 strains of Rhodotorula.

2. Among the yeasts isolated from exudates of 7 species of trees, there were 14 species of yeast from the genus Quercus and 9 species were isolated from 6 other species of trees.

3. The frequency of yeast from exudates of trees ranged from 56.5% in spring, in which water metabolism is in great activity, to 34.7 in summer and 8.7% in autumn.

4. The species from exudates of trees are consistent with those from Drosophila.

5. 7 strains were isolated from Drosophila crops which were collected from Mt. Gyeryong and Mt. Sok-ri located in the region of 36' to 38' latitude. Fermentation of these strains was stronger than other species. Especially Saccharomyces florentinus (culture No. 205 and 207) and S. cerevisiae (culture No. 206) were strong fermenters of maltose. The kinds of yeasts vary according to the species or genus of Drosophila.

6. S. cerevisiae var. tetrasporus which is considered as ancestor of culture yeast was isolated from fruits.

7. Among 43 strains isolated from fruits or mature fruits of trees in natural habitats, 10 strains of Candida and 19 strains of Torulopsis amounted to one-third of the total numbers of yeast isolated.

8. Hansenula anomala var. anomala isolated from Zostera marina is believed to be worth utiliziang as an edible yeast because of its strong fermentation on sugars.

9. Saccharomyces ludwigii (culture No. 220-C) and Kluyveromyces veronae (culture No. 215) were rarely isolated from natural habitats and Schizosaccharomyces japonicus var. koreanus was also identified as a rare species.

It is worthy of note that these species hav not yet been isolated from natural substrates.

V. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Atlanta, Georgia 30333. Communicated by Errol Reiss.

Since being appointed as Research Microbiologist with the Mycology Division last May, a program has beem implemented by me to study the immunochemistry of yeasts and yeast-like fungi of medical importance. The objective of such work is to develop better reagents for immunodiagnosis of the mycoses.

Sequential hydrolysis of cell walls of the yeast form of Histoplasma capsulatum with β-glucanase, Pronase, and chitinase.

by

E. Reiss and L. Kaufman

Cell walls of the yeast form of Histoplasma capsulatum, isolated by mechanical disruption, were exposed to sequential digestion with microbial enzymes and the soluble products were examined for antigenicity. In the first stage, treatment with β(1→3) glucanase of Cladosporium resinae resulted in 41% solubilization of the walls of which 8% represented non-dialyzable material reactive in immunodiffusion with goat H. capsulatum antiserum. Digestion of the β-glucanase resistant walls with Pronase solubilized an additional 7.5% of the original dry weight, and a portion of the extracted material was reactive in immunodiffusion tests with anti-H. capsulatum serum. The pronase-resistant cell walls then were

extracted with chitinase from Serratia marcescens releasing 26.7% of the wall dry weight.

After the third stage a cumulative total of 75% of the cell walls was extracted. The resistant residue retained the ability to bind fluorescein-conjugated H. capsulatum antibody, but the intensity of fluorescence was markedly weaker than that of the untreated cell wall control. Studies are in progress to characterize the antigens released by enzymic hydrolysis and to correlate each stage of extraction with changes in the wall ultrastructure and antigenic behavior.

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

Lectures presented at the occasion of Professor O. Nečas' 50th birthday at the Faculty of Medicine, Brno, in April 1975:

Svodoba, A., Kopecká, M.: Protoplasts as model objects for the study of cell morphogenesis. I. Biogenesis of the cell wall in yeast protoplasts.

Gabriel, M.: Protoplasts as model objects for the study of cell morphogenesis. II. Cell wall regeneration in protoplasts of moulds, green algae and blue-green algae.

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Lectures, which will be presented at the meeting of Czechoslovak Microbiological Society in Kosice, September 1975.

Svoboda, A.: Conjugation in yeast protoplasts.

Gabriel, M.: Formation and growth of spheroplasts of the blue-green alga, Anacystis nidulans.

Kopecká, M., Horák, J.: The action of antibiotic lomofungin on yeast cells and on the synthesis of cell wall components in protoplasts.

Summaries of these lectures will be published in Folia Microbiologica.

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Ph.D. Theses completed under the guidance of Professor O. Necas:

Svoboda, A.: Cell wall regeneration in yeast protoplasts.

Havelková, M.: Ultrastructure of fresh, growing, regenerating and reverting protoplasts of yeasts.

Kopecká, M.: Biogenesis of the fibrillar wall component in protoplasts of yeasts.

Summary of Ph.D. Thesis of M. Kopecká:

With protoplasts of Saccharomyces cerevisiae as a model, and then cultivated in liquid media, the ultrastructure of the fibrillar wall component was studied as well as its chemical nature, mechanism of biogenesis and its control, and its morphogenetic function.

In this work, protoplasts were used of both log- and stationary phase cells, glycogen-less and respiratory deficient mutants; light and electron microscopy (shadowing, negative, staining, freeze-etching, ultrathin sectioning), radioisotopes, inhibitors of protein and RNA synthesis, anucleated and sporulating protoplasts, X-ray diffraction, cytochemistry, chemical analysis, autoradiography.

Electron microscopy showed that on the surface of protoplasts only fibrillar wall components without amorphous matrix is organized in the form of a network consisting of flat bundles of microfibrils. One bundle contains 2 - 30 microfibrils of unknown length and 20 nm or 6 - 8 nm in width (after shadowing and negative staining, respectively). The length of bundles or individual microfibrils is not measurable due to the aposition or penetration of the bundles and to the absence of microfibril ends. Between the flat bundles there are wide meshes, thousands of Angstroms in width. Arrangement of the bundles in the fibrillar component is random. Fibrillar components from log-phase protoplasts reveal 1 - 2 dense areas 1 - 2 μm in size, absent in the nets from stationary phase cells.

The fibrillar component of protoplasts is composed of the polysaccharides β-(1→3)-glucan and chitin, representing 85% and 15% of the dry weight, respectively. Microfibrils are made up of β-(1→3)-glucan, linear molecules of β-(1→3)-glucan (representing 40% of the dry weight of net samples) and branched β-(1→3)-glucan (representing 45%). Both β-(1→3)-glucan and chitin are in the protoplast microfibrils in highly crystalline form in contrast to the cell wall of yeast. The presence of linear β-(1→3)-chains is explained by the partial removal of the β-(1→6)-glucan synthetases from the protoplasts during protoplast formation and by the functioning of β-(1→3)-glucan synthetase in protoplasts. The 5-times lower ratio of glucan: chitin in protoplast nets as compared to the walls of yeast, is considered to be a consequence of partial removal of glucan synthetases in protoplasts. The formation of fibrillar nets in protoplasts in liquid media and the absence of amorphous wall matrix is explained by the insolubility of glucan and chitin molecules which aggregate into microfibrils, while soluble molecules of the amorphous matrix escape from the protoplast surface.

Biogenesis of the fibrillar wall component follows 4 stages: 1st phase - biosynthesis of a sugar-nucleotide (unidentified as yet), 2nd phase - biosynthesis of glucan molecule, catalysed by glucan synthetases; 3rd phase - organization of microfibrils by self-assembly of glucan molecules; 4th phase - formation of fibrillar network, as a consequence of aggregation of microfibrils into bundles. Biogenesis of microfibrils is initiated by glucose, mannose or fructose. Anaerobic glycolysis is sufficient for microfibril formation; it also needs a functionally unimpaired plasma membrane and the presence of the cell nucleus, even though it is

independent of protein and RNA synthesis. The fibrillar wall component, made of glucan and chitin, is not able to ensure the functions of the cell wall in cell morphogenesis. It functions only as a passive morphogenetic factor unable to ensure the normal pattern of cell division and reinitiation of cell morphogenesis.

VII. Chemical Pharmaceutical Institute. Prof. Popova Street 14. Leningrad, USSR. Communicated by N. Elinov.

Preparation of biologically active yeast ^{3}H-mannan. (A. N. Shutko, N. I. Shatinina, N. V. Kirillova, N. P. Elinov, V. P. Komov).

The method of preparation of polysaccharides labelled with tritium has been described by K. Keck (Immunochem., 9, 3, 359, 1972). However, the need to modify the preparation results in partial loss of its biological activity.

We have prepared ^{3}H-extracellular mannan of Rhodotorula rubra using the modified method of hydrogenium isotopic exchange:

1.5 mg/ml mannan was incubated for four hours at 100°C in distilled water containing HTO with final specific acitivty of 100 mCu/ml. AFter incubation the preparation was purified from the admixture of HTO by lyophilization twice. The final specific activity of the mannan was 280 dpm.

We have shown in 1973 that extracellular mannan of Rh. rubra activates butyrylcholinesterase in vitro. It turned out that polysaacharide labelled with tritium has the identical activating and stabilizing effects on butyrilcholinesterase.

Thus we were able to prepare ^{3}H-mannan using the method of isotopic exchange modified by us. Biological activity of this polysaccharide did not differ from the native mannan.

We have ascertained earlier that extracellular mannan of Rh. rubra influences actively some steps of cellular metabolism (V.P. Komov, N. V. Kirillova, N. P. Elinov. Ukranian Biochemical Journal, 47, 2, 1975, in press). The use of mannan labelled with tritium helps to supply additional information about its interactions with several subcellular structures.

VIII. National Research Council of Canada, Division of Biological Sciences, Ottawa, Canada KIA OR6. Communicated by Byron F. Johnson.

Below follow abstracts of three articles which have been accepted for publication.

A New Method of Obtaining Zygotes in Saccharomyces Cerevisiae

Eng-Hong Lee, C. V. Lusena and Byron F. Johnson

Abstract

Experiments leading to a simple method of mass zygote formation in S. cerevisiae are described. This method ordinarily requires only about 3 hours of incubation, and consistently yielded 55-65% zygotes in seven different crosses among six different strains. Matings involving one strain required about 4 hours of incubation but otherwise the results were comparable.

Accepted for publication Can. J Microbiol.

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A Kinetic Analysis of Spontaneous ρ^- Mutations in Yeast

A. P. James, Byron F. Johnson, Elizabeth R. Inhaber and N. T. Gridgeman

Summary

Spontaneous mutation to the petite state at the level of the individual cell was studied in a haploid strain of yeast by the technique of pedigree analysis. Results indicated that 1) the mutability of ρ^+ cells within a population in log phase is variable; 2) ρ^+ mitotic buds are, on the average, about 50 percent more mutable than the ρ^+ cells from which they arose; 3) the mutability of a ρ^+ cell tends to decrease as it produces consecutive buds; 4) the probability that a mother cell will become ρ^- at or immediately subsequent to cell division is, on the average, one third the probability that its bud will be ρ^-; 5) most, if not all spontaneous ρ^- mutant cells contain mitochondrial DNA as judged from suppressiveness measurements. The data indicate that the spontaneous production of a mutant cell is a multi-step process. Neither a replicative advantage of defective mitochondrial DNA nor the existence of a "master" mitochondrial genome provides a satisfactory explanation of the process. Either selective dispensation of defective mitochondria to the bud at cytokinesis or normal retention by the mother cell of factors influencing the amplification or rate of induction of defective mitochondrial DNA could be involved.

Accepted, Mutation Research

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Morphometric Analysis of Yeast Cells. IV. Increase of the cylindrical Diameter of Schizosaccharomyces pombe During The Cell Cycle

Byron F. Johnson and Calvin Lu

Summary

The maximum cylindrical diameter of fission yeast cells was measured using an image-shearing eyepience with an optical microscope. The mean diameters were 3.4, 3.8, 4.2, 4.4, and 4.8 μm cells bearing one to five fission scars, respectively. These increments amount

to about 10% per generation, and correspond to 10% increases in cellular surface area during expansion. This result was found wholly comparable with areal increases of Saccharomyces adult cells (calculated from length and width data in the literature). Extant notions about growth of yeast cell walls are inadequate to explain these size increases.

Accepted, Experimental Cell Research

IX. Arbeitsgruppe Mykologie/Genetik, Fachbereich Biologie, Technische Hochschule Darmstadt, 61 Darmstadt, German Federal Republic. Communicated by F. K. Zimmermann.

Genetics of maltose and α-methylglucoside fermentation (in collaboration with P. Schreiber): A diploid strain heterozygous for MAL1 and MGL1 and/or MGL 3 but suc0 (maltose fermented fast, α-methylglucoside very slowly, sucrose not at all) was mutagenized with ultraviolet light and plated on a medium with maltose and 200 ppm 2-deoxyglucose. One resistant clone fermented α-methylglucoside and sucrose fast. Genetic analysis revealed a mutation in a gene unlinked to MAL1 and the MGL genes present. This mutation made maltase and α-methylglucosidase synthesis constitutive but in the absence of an MGL gene, α-methylglucoside could not be fermented. However, in the absence of MAL1 but in the presence of an MGL gene, synthesis of α-methylglucosidase and to a limited extent that of maltase could be induced by α-methyglucoside. The new mutation had no effect on maltase synthesis in the presence of MAL2.

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Genetics of carbon catabolite repression (in collaboration with P. Schreiber and R. Kern): A search was started for mutants affected in carbon catabolite repression. Mutants with negative effects were found by specifically inducing genic petites with nitrous acid (Schwaier et al., Molec. Gen. Genet. 102, 290, 1968). One out of 361 genic petites prevented maltose fermentation in a strain with MAL2. Tetrad analysis showed that petiteness and inhibition of maltose fermentation were caused by the same mutation. This inhibition was observed in combinations with MAL1, MAL3, MAL2-8^c (glucose sensitive constitutive maltase synthesis) and MAL2-47^c (glucose resistant constitutive maltase synthesis) (See: Zimmermann and Eaton, Molec. Gen. Genet. 134, 261, 1974). In the absence of carbon catabolite repressing conditions, specific maltase activities in a MAL2-8^c strain were only one tenth of those observed in the normal grande background. The mutant was called cat1 (for catabolite repression). Starting with a cat1 mutant strain, revertants to repiratory competence were isolated. One mutant was due to a suppressor which could be isolated. Preliminary experiments showed that this suppressor had an effect of carbon catabolite repression of constitutive maltase synthesis in a MAL2-8^c background. It is not clear yet whether this suppressor facilitates derepression of maltase synthesis after growth on glucose or whether it actually reduces carbon catabolite repression. The gene identified is called cat2.

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Effects of econazol nitrate, a new antifungal drug, on yeast cells (in collaboration with R. Kern). Econazol nitrate, 1-((2'-2,4-dichlorophenyl)-2'-(chlorobenzyloxy)-ehtyl))-imidazole nitrate, kills cells within a few minutes above a certain threshold concentration (between 15 - 30 ppm depending on the strain) but does not induce mitotic recombination. Cells are equally fast permeabilized which can be demonstrated using maltase as a strictly intracellular enzyme and p-nitrophenyl-glucopyranoside as a chromogenic substrate which is not taken up by intact yeast cells. Threshold concentrations were found also in the same concentration range. The effect on mitochondria was studied by measuring respiration in a Warburg apparatus along with fermentation. Again, above a certain threshold concentration, respiration was completely blocked whereas fermentation was inhibited at much higher doses. Econazol nitrate has to be considered as a membrane active fungicide.

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Non-random distribution of mitotic crossing over (in collaboration with Dr. B. K. Vig, on sabbatical leave from the Department of Biology, University of Nevada, Reno): The frequencies of mutagen induced mitotic recombinational events were compared to meiotic map distances between markers defining five intervals in the left arm of chromosome VII. The highest inducibility of mitotic crossing over was observed in an interval in the middle of that chromosome arm. Inducibility decreased towards the distal and proximal ends of this arm. Superimposed to this general susceptibility were patterns of mitotic recombination that were different between spontaneously occurring events, those induced with 1-nitrosoimidazolidinone-2, ethylmethane-sulfonate, carofur (constituent of a baby powder) nitrous acid and diepoxybutane. Only the latter two agents induced the same pattern. These results are interpreted as an indication of mutagen specificity expressed as induction of mitotic crossing over. Qualitatively the same results have been observed by workers studying the patterns of induced chromosome breaks in green plants and mammalian cells.

X. Division of Medical Physics, University of California, Berkeley, California 94720. Communicated by Robert K. Mortimer.

Saccharomycopsis lipolytica

We are cintinuing work with the hydrocarbon utilizing yeast, Saccharomycopsis lipolytica. Work in progress includes an investigation of the genetics of hydrocarbon utilization. We are collecting hydrocarbon-negative strains which will eventually be utilized in complementation and recombinational analyses. In addition we are continuing a program of inbreeding in order to obtain strains showing improved mating, sporulation and ascospore germination.

We have recently completed a genetic and biochemical investigation of a pigmented mutant of S. lipolytica. This mutant excretes large quantities of a pigment which causes colonies on YM agar plates to turn a blood-red color. A spectroscopic analysis of the pigment indicated it to be protoporphyrin IX. The mutant shows Mendelian segregation and is recessive to wild type. This mutant may prove

useful in the commercial production of protoporphyrin IX and/or in the production of labelled material for tracer studies. A manuscript covering this work will appear in the July issue of the Journal of Bacteriology.

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Yeast Genetics Stock Center

The Yeast Genetics Stock Center maintains a large collection of genetically marked strains of Saccharomyces cerevisiae. The Stock Center is funded by a National Science Foundation Grant and will furnish strains free of charge to qualified investigators or teachers. The Stock Center reserves the right to question what we feel to be unreasonable requests.

People who have requested strains or Stock Center Catalogues are already on our mailing list. We will soon be sending an enlarged and revised edition of our catalogue to the people currently on our mailing list. Investigators desiring a catalogue may write to:

Curator
Yeast Genetics Stock Center
Donner Laboratory
University of California
Berkeley, California 94720

XI. Department of Microbiology, Haryana Agricultural University, Hissar, India. Communicated by P. Tauro.

Summaries of work completed.

1. Sporulation in yeast: Need for Mitochondrial Protein Synthesis

Sporogenesis in S cerevisiae is not dependent on continued synthesis of proteins in the Mitochondria. Using chloramphenicol both in growth and sporulation media, it was found that proteins essential for respiration and sporulation are synthesized in the mitochondria early during growth in presporulation medium. Once the synthesis of these proteins is complete, further protein synthesis in the mitochondria is not essential for sporulation.

2. An easy and reliable technique for detecting respiratory deficient mutants in yeast.

As an alternate to tetrazoliun agar overlay technique and the use of nonfermentable carbohydrate media, we have found that use of bismuth sulfite agar provides a reliable technique for detecting respiratory deficient mutants. Cells are plated on YEPD agar containing 0.8% Bismuth Sulfite (Sterilized separately) and the plates are incubated for 3-4 days at 30°C. Respiratory deficient clones appear white while respiratory sufficient colonies appear black. Selection is unambiguous and highly reliable.

XII. National Institutes of Health, Bethesda, Maryland 20014. Communicated by Reed B. Wickner.

The following is an abstract of a talk recently given at the 1975 Squaw Valley Plasmid Meeting.

Genetics of a Double-Stranded RNA Plasmid: The Killer of Saccharomyces cerevisiae

Reed B. Wickner and Michael Leibowitz

"Killer" yeast strains carry a cytoplasmic double-stranded RNA (killer plasmid) and secrete a toxin which is lethal to strains not carrying this plasmid.

I. Pleiotropic Effects of Chromosomal Mutations Affecting Plasmid Replication or Expression. Eight chromosomal genes have been described which affect either the maintenance (mak1, mak2, mak3, M, pets) or expression (kex1, kex2, rex1) of the killer plasmid (Bevan and Somers; Vodkin and Fink; Wickner). The kex2 gene product, for example, is needed for expression of killing ability, but is not needed for maintenance or replication of the killer plasmid. Indeed, we find that strains carrying the kex2 mutation have the same double-stranded RNA species as do kex2+ strains. We have located the kex2 gene on chromosome XIV using a strain disomic for this chromosome, but it is not linked to the centromere or to any of the three other markers previously assigned to chromosome XIV. The three independently isolated mutants assigned to kex2 by complementation show reduced sporulation ability (> 200-fold) in homozygous diploids. Kex2 haploids also show an α-specific mating defect, i.e., α kex2 strains mate with a strains at a very low frequency, but a kex2 strains mate with α strains normally. We have also recently isolated a new class of chromosomal mutants in which mak defects segregate in meiosis with defects in temperature-sensitivity for growth.

II. Plasmid Mutants Dependent on Host Diploidy. Mutants of the killer plasmid have been isolated which have become dependent upon chromosomal diploidy for the expression of plasmid functions and for replication or maintenance of the plasmid itself. These mutants show no evidence of defects in any chromosomal gene needed for expression or replication of the killer plasmid.

Haploids carrying these mutant plasmids (called d for diploid-dependent) may be unable to kill or unable to resist being killed or both and show frequent loss of the plasmid. The wild type phenotype is restored by mating the d plasmid-carrying strain with either (a) a wild type sensitive strain which apparently has no killer plasmid; (b) a strain which has been cured of the killer plasmid by growth at elevated temperature; (c) a strain which has been cured of the plasmid by growth in the presence of cycloheximide; (d) a strain which has lost the plasmid because it carried a mutation in a chromosomal mak gene; or (e) another strain which carries the same d plasmid and has the same defective phenotype.

The "selfing" experiment (e) above, along with other data, indicates that the restoration of the normal phenotype is not due to recombination between plasmid genomes or complementation of plasmid or chromosomal genes.

Sporulation of the K^+R^+ diploids formed in d x wild type nonkiller crosses yields tetrads, all four of whose spores are defective for killing or resistance or maintenance of the plasmid or a combination of these. The pattern of defectiveness depends on the particular d strain used and the particular cross. These defective segregants resume the normal killer phenotype in the diploids formed when a second round of mating is performed.

XIII. Gesellschaft für Strahlen-und Umweltforschung MBH München, Institut für Biologie, Division of Biophysikalische Strahlenforschung, 6000 Frankfurt/Main, Paul-Ehrlich-Str. 20, West Germany. Communicated by S. C. Purohit.

Below follow the summaries of two recently completed papers.

Influence of Energy Metabolism on the Repair of X-Ray Damage in Living Cells. IV. Effects of 2- deoxy-D-glucose on the repair phenomena during fractionated irradiation of yeast. V. K. Jain, W. Pohlit and S. C. Purohit.

SUMMARY

Inhibition of repair of sublethal and potentially lethal damage was observed in respiratory-deficient mutants of Yeast during fractionated x-irradiation in the presence of equimolar concentrations of 2-deoxy-D-glucose and glucose in the growth medium. In the wild-type cells, on the other hand, an enhancement of repair of potentially lethal damage was observed under similar conditions.

These results suggest, by analogy, that in higher organisms also, 2-deoxy-D-glucose may differentially inhibit the repair of radiation damage in hypoxic tumour cells while enhancement of repair processes could be expected in normal tissues. (In press - Biophysik, 1975)

Recovery and Repair in Yeast Cells After Irradiation with Densely Ionizing Particles. W. E. Pohlit and M. Schafer.

ABSTRACT

A cybernetic model has been developed for radiation effects in living cells. This model includes quantitative changes in radiation sensitivity with absorbed dose, recovery (split-dose reactiviation) and repair (liquid-holding reactivation). The model has been tested extensively with sparsely ionizing radiations (x-ray and electron) using diploid and haploid yeast cells. Experiments are described in which the model is used for analyzing irradiation with alpha particles from an americium source. These particles are representative for densely ionizing particles with known LET distribution of absorbed dose. It can be shown quantitatively how much the irrepairable fraction of the radiation damage is increased in comparison with sparsely ionizing radiations. The quantitative data can be used to predict reactions of fast and slow neutrons of various energy distributions.

The consequence of these results for radiation therapy with fast neutrons and for radiation protection are discussed. (International Atomic Energy Agency Vienna, 1974 - IAEA-SM- 179/ 23).

XIV. Edinboro State College, Department of Biology, Edinboro, Pennsylvania 16444. Communicated by Frank T. Bayliss.

1. I am moving to the Department of Microbiology at San Francisco State University beginning July 1, 1975.

2. I am working on the isolation and characterization of ribosomal mutants in Saccharomyces cerevisiae. I have recently isolated mutants resistant to blastocidin S (at least 2 alleles). Blastocidin S inhibits protein synthesis at the level of the ribosome. That is, ribosomes from the sensitive wild type are inhibited in excess of 60% at 10^{-5} molar blastocidin S. Ellis Kline and I, with Larry Skogerson at Columbia are in the process of analyzing the nature of resistance of the 2 classes of mutants (spontaneous resistant mutants).

3. I am also trying to locate all known ribosomal mutants in i.e., trichodermin, cryptopleurine, blastocidin, cycloheximide, streptomycin and neomycin in order to clarify the number of alleles and to attempt to map those which have not been mapped. Blastocidin S resistance is not centromere linked and has not been otherwise located on the chromosome map. The character does segregate in the typical nuclear gene pattern (2:2).

4. I have found linkage between two loci which are not contromere linked, nor linked to any of some 50 markers tested to date. Streptomycin sensitivity (strl) is linked to a cycloheximide resistance (cyh-x)-low-temperature sensitive (lts2) lesion isolated by Arjun Singh.

Cross:

Tetrad Analysis

Cross	Test for Linkage	PD*	NPD+	T
AA-13 (α, strl)	strl & cyh-x	10	1	1
X	strl & lts2	10	1	1
XA-6-86B (a, lys2, cyh-x, lts2)	lts2 & cyh-x	12	0	0

* Parental ditype
\+ Non-parental ditype
. Tetratype

XV. Tokyo University of Agriculture, 1-1 Sakuragaoka 1-Chome, Setagayaku, Tokyo. Communicated by Michio Kozaki.

Below follow summaries of our recent studies concerning yeast immunology.

1. Serological relation between Parent cells and non-foaming mutant cells of sake yeast strains Kyokai No. 7.

By Kentaro Kodama, Michio Kozaki and Kakuo Kitahara.

L. Soc. Brew. Japan, 1974, Vol. 69(8) 535-536

Serological characteristics were examined on parent cells and non-foaming mutant cells of a sake yeast strain Kyokai No. 7 in an attempt to discriminate between antigenicities of yeast cell wall proteins. Using both the agglutination method and cross-absorption method, no serological difference was found between parent cells and mutant cells. From this result, the so-called hydrophobic proteins of parent cells was known to have no antigenicities and the mutant cell wall mannan, masking the hydrophobic groups, was suggested to have the same chemical structure as the parental one. In the present study, we found that it was impossible to apply our serological techniques for the control of sake mash, detecting an infectious yeast in the main fermenting yeast.

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2. Serological Specificities of Sake Yeast.

By Kentaro Kodama, Michio Kozaki and Kakuo Kitahara

A comparative serological study was carried out using 22 strains of Saccharomyces cerevisiae in which 9 strains of sake yeast were included and 16 strains of related species.

Test tube and slide agglutination methods and the double diffusion method were applied by a combination of 8 antisera, 81 absorbed sera and 5 monospecific sera. As a result, S. cerevisiae was divided into the following three groups by the composition of unit antigen: i) eight of nine strains of sake yeasts and one strain of brewer's yeast with unit antigens 1, 2 and 4, ii) two strains of brewer's yeast with antigens 1 and 3), iii) the remaining 11 strains, including one strain of sake yeast, with antigenic composition 1, 2, 4 and 5.

Strains of other Saccharomyces species were known to be generally of the same antigenic composition as the third group of S. cerevisiae.

Double diffusion and paper chromatography revealed that the serological differences between yeasts was caused chiefly by cell-wall mannans.

(In press).

XVI. Faculty of Arts and Sciences, Department of Biochemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15261. Communicated by Terrance Cooper.

Sumrada, Roberta and Terrance Cooper. Biochemical and genetic characteristics of urea uptake in Saccharomyces cerevisiae.---Urea transport in Saccharomyces occurs by 2 pathways: (1) an energy dependent, inducible and repressible active transport system (K_m = 0.014 mM) and (2) an energy independent facilitated diffusion system which operates at external urea concentrations in excess of 0.5 mM. Experiments characterizing the urea active transport system indicate that: (1) formamide and acetamide are competitive inhibitors of urea uptake increasing the apparent K_m value of urea by 156- and 20-fold, respectively, (2) the rate of urea accumulation is maximum at pH 3.3 and 80% inhibited at pH 6.0, (3) urea transport occurs 50% more slowly in ρ- strains than in ρ+ strains and (4) transport is sensitive to KCN only in ρ+ strains while it is sensitive to DNP and CCCP in both ρ+ and ρ- strains. Mutants were isolated which are unable to utilize 0.25 mM urea as sole nitrogen source. These strains possess all of the urea degradative enzymes and have doubling times similar to those of the wild type strains when using ammonia, allantoin or arginine as sole nitrogen source. However, mutant strains multiply three times more slowly than the wild type on medium containing 10 mM urea and have totally lost the ability to accumulate ^{14}C-urea (0.25 mM) from the medium. Mutants of this phenotype cluster in a locus, designated as dur 3, approximately 20 cM from a centromere whose identity is not as yet known. (Supported by NIH grants GM-19386 and GM-20693.)

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Sequence of Molecular Events Involved in Induction of Allophanate Hydrolase. J. Bossinger and T. G. Cooper.

Addition of urea to an uninduced culture of Saccharomyces at 22° results in appearance of allophanate hydrolyse activity after a lag of 12 minutes. Lawther and Cooper demonstrated that both RNA and protein synthesis are needed for this induction to occur. To elucidate the time interval occupied by each process involved in induction, temperature sensitive mutants, reported to be defective in mRNA transport from nucleus to cytoplasm (ts-136) and in protein synthesis initiation (ts-187), were employed along with the RNA polymerase inhibitor, lomofungin, in experiments which measure cumulative synthetic capacity to produce allophanate hydrolase (Kepes, Biochim. Biophys. Acta, 76:293). These measurements identify the time within the lag period at which each process is completed. We observed that RNA synthesis, ts-136 gene product function and protein synthesis initiation are completed at 1, 4, and 9-10 minutes, respectively. These data imply either that mutant ts-136 is defective in some process prior to mRNA transport into the cytoplasm or that mRNA spends 5 minutes (42% of the allophanate hydrolase induction lag time) in the cytoplasm prior to initiation of protein synthesis. This work was supported by NIH grants: GM 19386 and GM 20693.

XVII. Department of Biology, Brooklyn College of the City Univeristy of New York, Brooklyn, New York 11210. Communicated by Nasim A. Khan.

The following are the Summaries of two recent papers that appeared in Molec. Gen. Genetics, and the Summary of a paper submitted by Richard A. Hackel who is from my laboratory.

1. Constitutive Alpha-methylglucosidase synthesis in Yeast. Nasim A. Khan. Molec. Gen. Genetics 133: 363-365 (1974).

SUMMARY

The isolation in Saccharomyces cerevisiae of a mutant partially constitutive for alpha-methylglucosidase is described. The factor responsible for constitutive alpha-methylglucosidase synthesis in either allelic to the MGL_1 gene or closely linked to it, and is recessive in nature.

2. Genetic Control of Maltase Formation in Yeast. III. Isolation and Characterization of Temperature-sensitive Mutants Affecting Maltase Induction and Maltose Utilization. Nasim A. Khan. Molec. Gen. Genetics 136: 55-61 (1975).

SUMMARY

Temperature-sensitive mutants affecting maltose utilization in the yeast Saccharomyces cerevisiae have been isolated. Two such mutants although failing to ferment maltose at the restrictive temperature, have normal induced level of maltase. The third mutant (UNT-37) not only failed to ferment maltose but has 5-6 fold less induced level of maltase at the restrictive temperature than the parental strain. The genetic control mechanisms of maltase induction and maltose utilization have been discussed.

3. Genetic Control of Invertase Formation in Saccharomyces cerevisiae I. Isolation and Characterization of Mutants affecting Sucrose Utilization. Richard A.Hackel (Submitted for publication).

SUMMARY

Nine sucrose non-fermenting mutants have been isolated from yeast strain EK-6B, carrying the tightly linked SUC3 and MAL3 genes. Genetic studies have shown that these mutants are allelic to the SUC3 gene, and they are recessive in nature. Biochemical studies have shown that none of these mutants has detectable levels of either internal or external invertase. A single point mutation leading to the loss of both invertases suggest that either SUC3 is a control gene or it codes for a polypeptide which is shared by both invertases.

XVIII. Laboratoire d'Enzymologie, CNRS, 91 Gif-sur-Yvette-France. Communicated by Y. Surdin-Kerjan.

Below follow abstracts of four papers from our laboratory (received too late for inclusion in the fall issue of 1974 ed.).

1. tRNAs Undermethylation in a Met-regulatory mutant of Saccharomyces cerevisiae. Colette Fesneau and Huguette de Robichon-Szulmajster. Anny Fradin and Horst Feldmann (Institut für physiologische Chemie und Physikalische Biochemie der Universität 8000 München 2, Goethestrasse 33, West Germany). (Submiteed to Biochimie).

A study in vivo and in vitro methylation of tRNAs in regulatory mutants affected in methionine-mediated repression (eth2, eth3, eth10) has led to the following results:

1) The eth2-2 carrying strain presents a great undermethylation of its tRNAs, of the same order of magnitude as the one observed during methionine starvation of methionine auxotrophs.

2) This undermethylation leads to a shift of the $tRNA^{met}_{III}$ peak on a BD cellulose column, while $tRNA^{met}_{I}$ peak is unchanged.

3) The study of a double mutant strain carrying eth2 and met2 mutations has shown that this undermethylation is a consequence of the high internal pool of methionine.

4) Undermethylation unequally affects the different bases and the different tRNA species.

2. Biochemical and Regulatory Effects of Methionine Analogues in Saccharomyces cerevisiae. Françoise Colombani, Hélène Cherest and Huguette de Robichon-Szulmajster (Submitted to J. Bacteriol.)

The effect of three methionine analogues, ethionine, seleno-methionine and trifluoromethionine, on the biosynthesis of methionine in Saccharomyces cerevisiae has been investigated. We have found that:

1) a sharp decrease in the endogenous methionine concentration occurs following the addition of any one of these analogues to growing cells;

2) all of them can be charged on the $tRNA^{met}$ in vitro as well as in vivo with as a consequence their incorporation into proteins;

3) ethionine and seleno-methionine can be activated as homologues of S-adenosylmethionine while trifluoromethionine cannot;

4) all of them can act as repressors of the methionine biosynthetic pathway. This has been shown by measuring the de novo rate of synthesis of methionine in a culture made in the presence of any one of the three analogues.

3) Existence of two levels of repression in the biosynthesis of methionine in Saccharomyces cerevisiae. Effect of Lomofungin on enzyme synthesis. Yolande Surdin-Kerjan and Huguette de Robichon-Szulmajster. (Submitted to J. Bacteriol.).

Derepression of a methionine biosynthetic enzyme (homocysteine synthetase) has been studied after repression either by exogenous methionine, or by exogenous S-adenosylmethionine (SAM). Lomofungin, which inhibits the synthesis of ribosomal precursor and messenger ribonucleic acid but not of protein in Saccharomyces cerevisiae has been used in this system. It has been shown that the addition of this antibiotic prevents the derepression of homocysteine synthetase after repression by exogenous methionine but not after repression by exogenous SAM. This, and the kinetics of repression after addition of methionine or SAM to the growth medium provide evidences that methionine (perhaps via methionyl-$tRNA^{met}$) would act at the transcription level, while SAM would act at the translation level.

4. Methionine Biosynthesis in Saccharomyces cerevisiae. I. Genetical Analysis of Auxotrophic Mutants. Monique Masselot and Huguette de Robichon-Szulmajster. (Manuscript in preparation).

In order to know how many structural genes are implicated in the specific steps of the biosynthesis of methionine in Saccharomyces cerevisiae, a hundred mutants were studied by complementation. 23 groups were defined named MET1 to MET25. Each time it was studied, no recombination between independent mutants of the same complementation group and no linkage between different groups was found. Preliminary to biochemical studies, mutants of each complementation groups were tested for capacity to utilize various precursors of methionine.

XIX. Laboratoire d'Enzymologie de Louvain, 1348 Louvain-la-Neuve, Belgium. Communicated by A. Goffeau.

Below are listed recent publications from our laboratory.

Foury, F and Goffeau, A. Stimulation of Yeast RNA Synthesis by Cycloheximide and 3', 5'-Cyclic AMP. Nature New Biology, 245, 44-47 (1973).

Foury, F. and Goffeau, A. Combination of 2-deoxyglucose and snailgut enzyme Treatment for Preparing Sphaeroplasts of Schizosaccharomyces pombe. Journal of General Microbiology 75, 227-229 (1973).

Goffeau, A., Landry, Y., Foury, F. and Briquet, M. Oligomycin Resistance of Mitochondrial Adenine Triphosphatase in a Pleiotropic Chromosomal Mutant of a "petite-negative" yeast, Schizosaccharomyces pombe. J. Biol. Chem. 248, 7097-7105 (1973).

Colson, A. M., Colson, C. and Goffeau, A. Systems for membrane alteration: genetic pertubation of mitochondra in a "petite-negative" yeast. Methods in Enzymology, 32/B, Chapter 81, 838-843 (1974).

Goffeau, A., Colson, A. M., Landry, Y., Foury, F., and Briquet, M. Stable Pleiotropic Chromosomal Mutations with Modified Mitochondrial ATPase and Cytochromes aa_3 and b in Schizosaccharomyces pombe. In Biomembrane, Architecture, Biogenesis, Bioenergetics and Differentiation, edited by L. Packer, 35-48 (1974) Acad. Press.

Goffeau, A., Colson, A. M., Delhez, J., Foury, F., Labaille, F., Landry, Y., Mohar, O. and Mrena, E. In Membrane Biogenesis: Mitochondria Chloroplasts and Bacteria, ed. by A. Tzagoloff, Plenum Publishing Corporation (In press).

Foury, F. and Goffeau, A. Stimulation of active uptakes of nucleosides and amino acids by cyclic adenosine 3', 5',-monophosphate in yeast. J. Biol. Chem (In press).

Landry, Y. and Goffeau, A. Physiological and genetic modification of the expression of the yeast mitochondrial adenosine triphosphatase inhibitor. Biochim. Biophys. Acta (In press).

Colson, A. M., Goffeau, A., Briquet, M., Weigel, P., and Mattoon, J. R. Nucleo-cytoplasmic interaction between oligomycin-resistant mutations in Saccharomyces cerevisiae. Molecular and General Genetics (In press).

Dissertations

Souchary, A. Stimulation des oxydations mitochondriales par les cations alcalino-terreux chez la levure Saccharomyces cerevisiae. Thèse de Docteur Ingénieur en Biochimie présentée à l'Université de Paris VII. (1974).

Foury, F. Répression par le glucose et dérépression respiratoire chez la levure Schizosaccharomyces pombe. Thèse de DOcteur 3ème Cycle en Physiologie végétale présentée à l'Université de Paris-Sud, Centre d'Orsay (1973).

Landry, Y. Relations entre l'activité et la structure quaternaire de l'ATPase mitochondriale. Thèse de Doctorat d'Etat en Pharmacie, présentée a l'Université de Nancy I (1973).

Odaert, S. Etude comparative de l'action de 2 dithiocarbamates sur l'oxydation du succinate par des mitochondries de la levure Saccharomyces cerevisiae et par des mitochondries de foie de rat. Mémoire de Licence en Sciences Zoologiques présenté à l'Université de Louvain (1974).

Convent, B. Yeast mitochondrial response to herbicides. Mémoire de Licence en Sciences Naturelles Appliquées présenté à l'Université de Louvain (1974).

XX. Takeda Chemical Industries Ltd., Alol-Cho 1-5, Takasago, Japan. Communicated by Takashi Suzuki.

The following two abstracts deal with recent work on yeast aconitase from our laboratory.

Induction and Stabilization of Yeast Aconitase Activity by Fluorocitrate

Takashi Suzuki, Shun-ichi Akiyama, Osamu Yamazaki, Kiyoshi Nara
Yoshio Nakao and Hideo Fukuda

Agr. Biol. Chem., 39(1) 97-103, 1975

Both fluorocitrate and fluoroacetate acted on yeast aconitase as an inudcer and a stabilizer of the enzyme. Fluoroacetate appeared to function after conversion to fluorocitrate in the cells. Inhibitors of protein synthesis and terminal respiratory system showed a strong inhibitory effect on the inductive formation of aconitase. In addition, these fluorocompounds were also found to induce various microbial aconitases.

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The Aconitase of Yeast

II. Crystallization and General Properties of Yeast Aconitase

J. Biochem., 77, 367-372 (1975)

Takashi Suzuki, Osamu Yamazaki Kiyoshi Nara,
Shun-ichi Akiyama, Yoshio Nakao and Hideo Fukuda

Yeast aconitase [citrate (isocitrate) hydro-lyase, EC. 4.2, 1.3], inductively formed by Candida lipolytica in the presence of fluoroacetate, was purified approximately 100-fold by Sephadex G-100 gel filtration and DEAE-Sephadex column chromatography, yielding dark-brown needle crystals. The crystalline aconitase was homogeneous as judged by polyacrylamide gel electrophoresis and sedimentation by ultracentrifugation. The enzyme showed maximal activity at pH 8.0 and at 55°. It has an $S_{20,w}$ of 5.04S, a molecular weight of 68,500 and an isoelectric point of pH 4.2. The presence of 2.10 moles of iron per mole of the enzyme was demonstrated by atomic absorption spectroscopy.

XXI. Laboratoire de Structure et Fonction des Biomembranes, Département de Biologie, U.E.R. de Luminy, 70, Route Léon Lachamp, 13288, Marseille. Communicated by Edgard Azoulay.

Localisation de L'alcool Deshydrogenase Mitochondriale de Candida Tropicalis

Michel Gallo, Bernadette Roche-Penverne et Edgard Azoulay

Hydrocarbons induce an alcohol dehydrogenase of mitochondrial origin, which has been specifically localized on the external membrane of Candida tropicalis mitochondria.

Classical techniques were used to separate and identify these external membranes from purified mitochondria. These external membranes contain antimycin A-insensitive NADH and NADPH cytochorme c reductases but no L-kynurenine hydroxylase activity.

The presence of alcohol dehydrogenase on the external membrane explains why the oxidation of higher alcohols is not coupled to oxidative phosphorylation.

Published in FEBS Letters, 1974, Volume 46, 1.

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Métabolisme énergétique chez Candida tropicalis
Oxydation phosphorylante chez Candida tropicalis cultive sur alcanes

Michel Gallo et Edgard Azoulay

Summary. Mitochondria of Candida tropicalis grown in n-alkanes have been isolated from protoplasts lysed. The obtention of intact mitochondria is proved by the respiratory controls and electron microscopic examination. Regardless of the substrate and the growth phase, these mitochondria possess three functional sites of phosphorylation. The oxidation of the substrates is stimulated by the presence of ADP and the P/O has a value of about 3 and 2 respectively for NAD linked and FAD-linked oxidation.

As for other yeasts, mitochondria of C. tropicalis are permeable to NADH which is oxidized at site II. This oxidation is especially antimycin-resistant and demonstrates more similarity between C. tropicalis mitochondria and those of antimycin-resistant mutants isolated from Candida utilis.

Exogenous NADH is strongly oxidized. The stoichiometry ratio is between 1 and 1-4 and the apparent K_m is 10^{-5} M and 3.10^{-5} M, respectively for mitochondria isolated from acetate and tetradecane grown cells. With intact mitochondria, this NADH oxidase is inhibited by excess substrate. This property disappears when submitochondrial particles are used.

A NADH oxidase respiratory shunt, nonphosphorylative, which represents about 30 p. cent of NADH oxidase linked to phosphorylation has been demonstrated in the case of mitochondria isolated from acetate or glycerol

grown cells. With "Tetradecane mitochondria" the activity of the respiratory shunt is minimal (5 p. cent).

Mitochondrial oxidation of the NADH produced during dehydrogenation of higher alcohols and aldehydes is closely coupled to oxygen, cyanide insensitive and non-phosphorylative.

Published in BIOCHIMIE, 1974, 56. 1129-1143.

XXII. Laboratory of Microbiology and Laboratory of Biochemistry and Biophysics, Technische, Hoogeschool, Delft, The Netherlands. Communicated by J. C. Hoogerheide.

Below follows a summary of my recent studies at the Laboratory of Microbiology and the Laboratory of Biochemistry and Biophysics. It summarizes a recent publication entitled: "Studies on the Energy Metabolism during anaerobic fermentation of glucose by baker's yeast" published in Rad. and Environm. Biophys. 11.295.1975., and a recent paper I presented March 25, 1975 at the 2nd International Symposium on Microcalorimetry, at Bedford College, University of London.

Heat development during anaerobic and aerobic metabolism of baker's yeast

by

J. C. Hoogerheide

Summary

Microcalorimetry in combination with conventional methods of determining metabolic activity opens the possibility to study the economy of cell metabolism.

Modern biochemistry has demonstrated conclusively the intimate interrelation of cell metabolism and ADP phosphorylation (ATP production) both under anaerobic and aerobic conditions. However, very little is known about the actual fate of the ATP, once it is produced.

Using commercial baker's yeast as test organism with glucose, ethanol, acetic- and lactic acids as substrates, the fate of the work-energy (ATP) produced by both of the dissimilatory processes was studied by determination of heat development in combination with CO_2 development during anaerobic fermentation and O_2 utilization for determination of the corresponding respiratory activity.

Heat development was measured by means of the LKB Flow-Microcalorimeter and corresponding metabolic activity by means of the Warburg technique. From these data one can calculate heat development (expressed in μ calories) per mm^3 CO_2 produced, or heat development (in μ calories) per mm^3 O_2 consumed. Based on the inevitable ATP production, its full or partial participation in energy requiring synthetic reactions, one can calculate the expected heat ranges.

Substrate	metabolism	ATP produced per mole substrate metabolized	Expected heat production range
glucose	fermentation	2	670-1220 μ cal/mm^3 CO_2
glucose	respiration	38	1710-5100 μ cal/mm^3 O_2
ethanol	respiration	18	1670-4880 μ cal/mm^3 O_2
lactic acid	respiration	18	1640-4840 μ cal/mm^3 O_2
acetic acid	respiration	11	1700-4670 μ cal/mm^3 O_2

By circumventing the inherent dangers of misinterpretation of the heat curves experimentally obtained, it was found that during glucose fermentation by baker's yeast, at least at the start of the fermentation, the ATP produced was quite economically utilized by the yeast cell for synthetic processes both by "resting" cells and by cells in the presence of an assimilable N-source. Under aerobic conditions, however, practically all ATP produced was wasted as heat production, with all substrates tested.

As a result, addition of 2-4 dinitrophenol, a powerful "uncoupler" of phosphorylation from the respiration process, did not result in an appreciable increase in heat development aerobically.

Even in the presence of an assimilable N-source, allowing unrestricted growth only a very small percentage of the ATP produced is utilized for synthetic processes, at least during the lag period. A gradual improvement of this poor economic utilization of ATP was noted during the pre-logarithmic growth phase. This wastefull aerobic metabolism of baker's yeast and its apparent restricted ability to utilize ATP for synthetic processes may be caused by the exceptionally low content of messenger RNA, typical of baker's yeast subjected to a ripening process before harvesting in order to improve its keeping stability (Oura et al., Proceedings 4th Int. Symposium Yeasts, 1974, p. 125).

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

Below follow two abstracts based on work from our laboratory. One article was recently published and the second one is in press.

Phosphorylation of CMP by the Released CMP Kinase System from a Yeast: Saccharomyces carlsbergensis

Akira Kimura, Makoto Morita and Kosaku Murata

Agric. Biol. Chem. 39, 621-625. 1975

During our studies on fermentative production of CDP-choline by dried cells of yeasts, a CMP kinase system was found to be released into the supernatant of the reaction mixture, while pyrophosphorylase remained bound to cells. The supernatant phosphorylated CMP to CDP and further to CTP. The phosphorylation was inhibited by glucose. However, the inhibition was reversed by addition of small amount of ATP. Addition of inorganic orthophosphate accelerated the phosphorylation by the supernatants of early stages of the reaction, for inorganic phosphate was deficient in the supernatants.

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Fermentative Formation of CDP-Choline by Intact Cells of Yeast: Saccharomyces carlsbergensis (IFO 0641) Treated with a Detergent: Triton X-100

Akira Kimura and Makoto Morita

Agric. Biol.Chem. 39 (1975), in press

Formation of cytidine-5'-diphosphate-choline from cytidine-5'-monophosphate consists of two reactions; (1) phosphorylation of CMP to CTP catalized by the CMP kinase system, (2) condensation of CTP and phosphorylcholine catalyzed by pyrophosphorylase. The formation of CDP-choline has been achieved only by dried cells of yeasts, but not by intact cells. However, in this paper it is revealed that intact cells of Saccharomyces carlsbergensis carried out the above two reactions upon treatment with certain detergents: Triton X-100, Nonion B, Ampholytic Detergent A, and Anion C. Out of these detergents, Triton X-100 was most effective for the formation of CDP-choline. From the effect of Triton X-100 on these two reactions a difference in localization of the two types of enzymes is discussed.

XXIV. Biological Institute of the Carlsberg Foundation 16, Tagensvej, DK-2200 Copenhagen, Denmark. Communicated by Eric Zeuthen.

The following work is nearly finished and ready for publication:

Kirsten Hamburger, Birte Kramhoft, Susanne B. Nissen and Erik Zeuthen

Rates of gaseous exchanges (CO_2 and O_2) through the synchronized and the normal cell cycle of Schizosaccharomyces pombe.

Summary

The uptake of O_2 and the production of CO_2 was measured on synchronous and logarithmic cultures using the Gradient Diver technique (1) and the Warburg technique. Cells were synchronized by heat shocks (2) or by selection (3). The media were complex (yeast extract with 3% glucose) or defined (EMM 2 with 0.13% glucose (4)). Our results show that the rate of CO_2 production increased linearly between successive divisions in both synchronous systems. The rate of increase doubles during the division period. The oxygen uptake is low in both media. RQ is 1 in the complex medium and about 6 in the defined medium, suggesting some degree of glucose

repression. The mode of growth of the rate of oxygen uptake through the cell cycle may follow an overall exponential course but the picture is blurred by a tendency towards cyclicity.

References:

1. B. Andersen Nexø, K. Hamburger and E. Zeuthen: Compt. Rend. Trav. Lab. Carlsberg 39, 33 (1972).

2. B. Kramhøft and E. Zeuthen: Compt. Rend. Trav. Lab. Carlsberg 38, 351 (1971).

3. J. M. Mitchison and W. S. Vincent: Nature 205, 987 (1965).

4. J. M. Mitchison and P. R. Gross: Exptl. Cell Res. 37, 259 (1965).

XXV. Laboratory of Industrial Biochemistry, Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Yoshida, Kyoto, Japan. Communicated by Saburo Fukui and Atsuo Tanaka.

Summaries of recent work follow:

Catalase Activities of Hydrocarbon-utilizing Candida Yeasts

Yutaka Teranishi, Atsuo Tanaka, Masako Osumi and Saburo Fukui

Agr. Biol. Chem., 38(6), 1213-1220, 1974

The catalase activities of the Candida cells grown on hydrocarbons were generally much higher than those of the cells grown on lauryl alcohol, glucose or ethanol. Km values for hydrogen peroxide of the enzymes from the glucose-and the hydrocarbon-grown cells of Candida tropicalis were the same level. The enzyme activities of the yeasts were higher at the exponential growth phase, especially of the hydrocarbon-grown cells, than at the stationary phase. Profuse appearance of microbodies having honogeneous matrix surrounded by a single-layer membrane has also been observed electronmicroscopically in the hydrocarbon-grown cells of several Candida yeasts. Cytochemical studies using 3,3'-diaminobenzidine (DAB) revealed that the catalase activity was located in microbodies. These facts suggest that the catalase activities would be related to the hydrocarbon metabolism in the yeasts.

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Induction of Catalase Activity by Hydrocarbons in Candida tropicalis pK 233

Yutaka Teranishi, Susumu Kawamoto, Atsuo Tanaka, Masako Osumi and Saburo Fukui

Agr. Biol. Chem., 36(6), 1221-1225, 1974

1. The catalase activity of Candida tropicalis pK 233 was induced by hydrocarbons but not by glucose, galactose, ethanol, acetate or lauryl alcohol.

2. The induction of the catalase activity depending upon hydrocarbons was sensitive to cycloheximide but not chloramphenicol.

3. Glucose repressed strongly the induction of the catalase activity by hydrocarbons but galactose did not affect seriously.

4. When C. tropicalis was incubated with hydrocarbons, the appearance of microbodies was observed electronmicroscopically.

Comparative Studies on Respiratory Activity and Cytochrome Content of Candida tropicalis pK 233 Grown on Hydrocarbon and on Glucose

Agric. Biol. Chem. 38, 1581-1587 (1974).

Some differences in the respiratory activity as well as in cytochrome content were observed between glucose-grown (G-cells) and hydrocarbon-grown cells (H-cells) of Candida tropicalis pK 233. The Q_{O_2} values of H-cells measured with n-alkanes were markedly high when compared with those measured with glucose during the whole growth phase. On the other hand, the values of G-cells measured with glucose were significantly higher than those measured with n-alkanes in the exponential growth phase, but there was little difference between both values in the stationary phase. Although a significant difference in quality was not recognized in the cytochrome patterns between G-cells and H-cells, the contents of a-, b- and c-type of cytochromes in H-cells were twice as much as those in G-cells.

In general, H-cells were more sensitive to respiratory inhibitors than G-cells. When glucose was used as oxidation substrate, KCN did not exert any inhibitory effect, but rather stimulated the respiration of G-cells. On the other hand, respiratory activity of H-cells measured with n-alkanes was remarkably lowered by KCN.

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Some Specific Features of the Respiration of Hydrocarbon-utilizable Candida Yeasts

Yutaka Teranishi, Atsuo Tanaka and Saburo Fukui

Agr. Biol. Chem., 38(10), 1779-1783, 1974

The respiration of both glucose-grown and hydrocarbon-grown cells of Candida tropicalis pK 233 harvested in the stationary phases was not inhibited by cyanide when glucose was used as oxidation substrate, but the former was rather stimulated in the presence of cyanide. When n-alkanes were used as oxidation substrate, cyanide lowered the respiratory activities of both cells to about 50%. With respect to the susceptibility to cyanide, the younger cells growing on n-alkanes were less sensitive

in hydrocarbon oxidizing ability than the older cells, whereas the older cells growing on glucose or n-alkanes were more resistant in glucose oxidizing ability than the younger cells. Acetate was oxidized by both glucose-grown and hydrocarbon-grown cells of the yeast. Laurate was oxidized by hydrocarbon-grown cells, but not by glucose-grown cells. The respiration on laurate was inhibited completely by 3.3 mM of cyanide. In general, hydrocarbon-grown cells of Candida tropicalis pK 233 were more sensitive to various respiratory inhibitors than glucose-grown cells, although the oxidation substrates had a significant effect.

The respiration of both glucose-grown and hydrocarbon-grown cells of C albicans, C. guilliermondii and C. lipolytica harvested in the stationary phases was also resistant to cyanide when glucose was used as oxidation sunstrate. But the respiration on n-alkanes of these cells was inhibited significantly by 3.3 mM of cyanide except for C. albicans.

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Ultrastructure of Candida Yeasts Grown on n-Alkanes

Appearance of Microbodies and Its Relationship to High Catalase Activity

Masako Osumi, Naoto Miwa, Yutaka Teranishi, Atsuo Tanaka, and Saburo Fukui

Arch. Microbiol. 99, 181-201 (1974)

Catalase activities of the cells growing on n-alkanes of various strains of Candida yeasts were markedly higher than those of the cells growing on glucose, ethanol or acetate. In connection with this, electron-microscopical studies revealed abundant appearance of specific microbodies having homogeneous matrix surrounded by single unit membrane in the hydrocarbon-growing cells. Localization of catalase activity in the microbodies, in addition to the mitochondria, was confirmed by cytochemical treatment of the cells with 3, 3'-diaminobenzidine reagent.

XXVI. University of East Anglia, School of Biological Sciences, University Plain, Norwich NOR 88C, England. Communicated by James A. Barnett.

The following have been published recently:

Payne, R. W., Walton, E. & Barnett, J. A. (1974). A New Way of Representing Diagnostic Keys. Journal of General Microbiology 83, 413-414.

Matthewson, D. K., and Barnett, J. A. (1974). The Effects of Different Carbon Sources and Changes in the Growth Medium on the shape of Cells of the Yeast Trigonopsis variabilis. Journal of General Microbiology 83, 427-430.

Barnett, J. A., Bascomb, S. and Gower, J. C. (1975). A Maximal Predictive Classification of Klebsielleae and of the Yeasts. Journal of General Microbiology, 86, 93-102.

Barnett, J. A. (1975). The Entry of D-ribose into Yeasts of the Genus Pichia. Journal of General Microbiology (in the press).

Barnett, J. A. (1975). The Utilization of Sugars by Yeasts. Advances in Carbohydrate Chemistry and Biochemistry 32, (in the press).

XXVII. Janssen Pharmaceutica, Research Laboratoria, 2340 Beerse, Belgium. Communicated by Sonja De Nollin.

The following two abstract represents recent work from this laboratory.

Electron Microscopic Structure of Yeast Cells and Changes After In Vitro Treatment with Miconazole

Vlaams Diergeneeskundig Tijdschrift: 44, 77-91 (1975).

Summary

Electron microscopic examination was performed on different yeast species (Candida albicans, Saccharomyces cerevisiae, Pityrosporum canis, Cryptococcus neoformans), using a new method for the ultrastructural preservation of subcellular organelles consisting in sectioning the cells (7.5 μ) prior to fixation.

Apart from this descriptive morphology of untreated cells, the ultrastructural changes in Candida albicans, after exposure to different doses of miconazole were investigated.

After exposure to fungistatic dose levels the earliest morphologic alterations are displayed at the cell wall and plasmalemma. After treatment with fungicidal doses, cytoplasmic remnants are segregated from the rest of the cytoplasm and sequestered into the central vacuole. A similar process of discharge of injured cell parts is seen at the cell periphery.

Progressive degradation of the plasmalemma and all other cell organelles resulting in complete cell necrosis has been observed.

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Scanning Electron Microscopy of C. albicans after In vitro Treatment with Miconazole. Antimicrobial Agents and Chemotherapy:

Abstract

A study was made on the in vitro action of the antimycotic miconazole on Candida albicans yeast cells with scanning electron microscopy and the effects were compared with those seen on the yeast cells by means of transmission electron microscopy. It was found that cells exposed to fungistatic and minimal fungicidal doses of miconazole (10^{-7} M and 10^{-6} M), presented rough surfaces and had multiple, desoriented buds and bud scars. Whereas in control cultures the cells were well separated, the

treated ones formed small clusters of interconnected cells.

After exposure to a fungicidal concentration (10^{-4}M) of the drug, most of the remaining cells showed smooth surfaces and were covered with numerous vesicular structures probably representing cytoplasmic remnants derived from broken cells. This has been substantiated by the presence of abundant fragments of cell walls and confirmed by examination of similarly treated cultures in the transmission electron microscope. Moreover, the cells with an apparently intact surface with scanning electron microscopy, were shown upon transmission electron-microscopical examination to be completely necrotic inside.

XXVIII. Department of Biochemistry, University of Sydney, Sydney, N.S.W., 2006, Australia. Communicated by Audrey M. Bersten.

The following is a Summary of a paper presented by Audrey M. Bersten and Nicolle H. Packer at the Australian Biochemical Society's Annual Meeting held in May 1975. Ab abstract of the paper has been published in the Proceedings of the Australian Biochemical Society, Vol. 8 (1975).

The utilization of the methyl group of methionine in lipid synthesis by Trigonopsis variabilis, strain CBS 1040, was followed during the cultivation of cells in batch cultures. Maximum triangular form development occurred during exponential growth. The specific growth rate at the time of maximum triangular cell formation and the maximum number of triangular cells in the population were inversely related with respect to temperature. Growth at 35° completely inhibited triangular cell formation but growth between 15°C and 25°C favoured it.

Lipid synthesized during the period of maximum triangular cell formation, as a function of label incorporated into the various lipid classes of total cellular lipid, is characterized by a high sterol to phospholipid ratio, phospholipids rich in linoleic acid ($C_{18:2}$) at the expense of oleic acid ($C_{18:1}$) and triglycerides enriched with myristic acid also at the expense of oleic acid. Trigonopsis variabilis apparently possesses a regulatory mechanism for the control of the degree of unsaturation of membrane lipids while maintaining adequate fluidity in the triglyceride droplets. The probable involvement of sterols in the morphogenic character was confirmed by growth in the presence of 30 µg Nystatin/ml on peptone-yeast extract- glucose agar, equivalen to that of untreated control cultures exhibiting one hundred percent ellipsoidal character and the development at this concentration of Nystatin of predominantly triangular shaped cells.

XXIX. Institut für Biochemie der Universität Würzburg, Röntgenring 11, 87 Würzburg, West Germany. Communicated by E. Schweizer.

Below follow summaries of three articles recently submitted for publication from this laboratory.

Malonyl and Palmityl Transferase-less Mutants of the Yeast Fatty Acid Synthetase Complex. A. Knobling, D. Schiffmann, H. -D. Sicklinger and E. Schweizer.

146 independently isolated mutants of the fatty acid synthetase gene locus fas 1 were subdivided into six different complementation groups. Three of these groups, Va, Vb and Vd, have not yet been described before. The mutant fatty acid synthetase isolated from representatives of complementation group Vb were specifically deficient in two component enzymes at the same time, the malonyl and palmityl transferases. Among more than 180 fas 1 and fas 2-mutants systematically screened for malonyl and plamityl transferase activities no mutant was found affected in only one of these two fatty acid synthetase component enzymes. From this it is concluded that both transfer reactions are catalyzed by the same enzyme. In any malonyl transferase-less fatty acid synthetase, none of the two known malonyl binding sites - neither enzyme bound pantetheine nor the non-thiol binding site - accepts malonate. This indicates that malonate is transferred to both groups by the same enzyme. So far, no acetyl transferase-less fas-mutants have been characterized. On the other hand, the mutants of two fas 1-complementation groups, Va and Vd, though negative in overall fatty acid synthetase activity had no deficiency in any of the known component enzymes, testable in vitro. A possible interrelationship between both findings is discussed.

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Temperature-Sensitive Mutants of the Yeast Fatty acid Synthetase Complex. A Knobling and E. Schweizer.

By genetic complementation analysis, 88 independently isolated temperature-sensitive fatty acid synthetase mutants have been assigned to the six different fas-complementation groups II (fas1), III (fas1), VB (fas1), VI (fas2), VIII (fas2) and IX (fas2). The complementation groups Va, Vc, Vd, IV and VII observed among nonconditional fas-mutants have not been found among the temperature-sensitive strains studied. From the failure to detect pantetheine-deficient conditional fas-mutants it is concluded that the yeast acyl carrier protein has an exceptionally stable tertiary structure. Furthermore, the lack of temperature-sensitive mutants of complementation group IV possibly indicates that this group specifically represents only nonsense and frameshift mutations. Almost half of the temperature-sensitive fas1 and fas2 mutants studied exhibited noncomplementing characteristics. These results confirm the existence of noncomplementing fas1 and fas2 missense mutations. From this it is concluded that both fatty acid synthetase loci encode multifunctional polypeptide chains rather than several monofunctional component enzymes. The possible existence of an independent acyl carrier protein as suggested by the genetic data reported in this study is discussed. With 10 different temperature-sensitive fas1 and fas2 mutants the dependence of cellular growth rates on growth temperature and fatty acid supplementation was determined. With all mutants studied fatty acid-independent growth was completely suppressed at nonpermissive temperatures (34-37°C). In fatty acid-supplemented media, however, these mutants exhibited the same growth characteristics as wild type yeast cells. In contrast to this, wild type yeast growth was found to be fatty acid-independent at all temperatures studied. Other than in vivo, the purified fatty acid

synthetase isolated from five different temperature-sensitive fas1 and fas2 mutants exhibited in vitro no increased thermolability compared to the wild type enzyme. From this it is concluded that the specific conformation of fatty acid synthetase subunits either froms only at the ribosomal level during translation, or that this conformation is stabilized by the assembly of subunits into the multienzyme complex structure.

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Control of Fatty Acid Synthetase Biosynthesis in Saccharomyces cerevisiae. G. Dietlein and E. Schweizer.

143 out of 308 fas1-mutants (47 percent) and 139 out of the 443 fas2-mutants (32 percent) genetically studied in this laboratory fail to complement with any other fas-mutant of the same gene locus. From these non-complementing fas-mutants no mutant fatty acid synthetase can be isolated using the wild type enzyme purification procedure. Furthermore the noncomplementing fas-mutants generally contain no material immunologically crossreacting with a specific fatty acid synthetase antiserum. However, subunits obtained after dissociation of the complex with sodium dodecyl sulfate still crosss react with this antiserum. Therefore, it is concluded that noncomplementing fas-mutants contain no fatty acid synthetase component proteins, though one of the two fas-loci is mutantionally unaffected. This conclusion was further confirmed by ^{14}C-amino acid incorporation studies which indicated that in noncomplementing fas-mutants, other than in wild type and complementing fas-mutant cells, no label was incorporated into fatty acid synthetase subunits or precursor proteins. At nonpermissive temperature, the same biochemical and immunological characteristics were observed with temperature-sensitive noncomplementing fas-mutants. These results suggest that noncomplementing fas-mutants either represent regulatory mutants unable to induce the mutationally unaffected other fas-gene locus or that they are association-defective mutants. In both cases the resulting individual subunits of the complex may be rapidly degraded by intracellular proteases.

XXX. Department of Biological Sciences, Madurai University, Madurai, India 625021. Communicated by J. Jayaraman.

The Biochemistry Group in the above department has been interested in studying various aspects of mitochondriogenesis in the yeast, Saccharomyces cerevisiae, for the past four years. Some of the highlights of the work are listed below.

a. The now classical glucose repression phenomenon which controls mitochondriogenesis in this organism, can be reproduced in spheroplast suspensions.

b. Using the antibiotics cycloheximide and chloramphenicol, which inhibit protein synthesis in the cytoplasm and mitochondria, respectively, it has been shown, that as far as the organelle is concerned, the two systems need not be closely interdependent. In other words, products of the mitochondrial protein synthesis accumulate in presence of cycloheximide and cytoplasmic proteins (needed for the mitochondrial assembly) accumulate in presence of chloramphenicol.

c. A direct proof for the above has been demonstrated as follows: one set of spheroplasts was incubated with cycloheximide and the other set with chloramphenicol. Particles isolated from both sets showed highly reduced (40-50% of control) cytochrome oxidase activity. But on mixing the two particulate fractions, the cytochrome oxidase activity reached the control level; thus there has occurred a complementation between the two independently accumulated partial products.

d. A similar result has been obtained with the enzyme ATPase also. With cycloheximide incubation, the enzyme activity is lost. With chloramphenicol treatment, the activity is not lost but it is present exclusively in the supernatant. At that state, it is cold sensitive and oligomycin insensitive. If to this supernatant are added particles isolated from spheroplasts treated with cycloheximide, then the entire enzyme activity becomes particulate, cold insensitive and oligomycin sensitive. Thus there is clear evidence that the OSCP is contributed by mitochondria for the complementation.

e. By varying the time of incubation with chloramphenicol we have found that the appearance of OSCP or the cytochrome oxidase subunits made by mitochondria, can be timed. Thus perhaps a temporal sequencing can be achieved.

f. Techniques have been standardized to study in a similar way (unfortunately with whole cells) the time of appearance of lipid components like cardiolipin, ubiquinone and phospholipids.

g. Other studies are going on with synchronous cultures and these will be reported subsequently.

An interesting sideline of this work has been the use of gut juice from locally available snails. It is made to a powder form and found to be stable up to one month at room temperature (in South India!). Those who are interested in trying out this inexpensive product may contact Dr. Jayaraman.

XXXI. Ecole Nationale Supérieure Agronomique de Montpellier, Laboratoire de Recherches de la Chaire de Génétique, I.N.R.A., Montpellier, France. Communicated by Pr Galzy.

The articles below have appeared recently or will soon be published.

MOULIN, G., GALZY, P., JOUX, J. L. - International Congress of Food Sciences and technology - Madrid - Spain-23-27 September 1974. Remarks on the production of single cell yeast on whey: some remarks are made on the metabolism of Kluyveromyces fragilis. The consequences of these are presented in the utilization of this strain for the production of single cell protein on whey.

The characteristics of K. fragilis allow to make a continuous culture on deproteinized whey with nutrient supplements. It is easy to get a product without lactose and ethanol at the end of the process. It is possible to work with high concentrations of lactose, because the glucose effect does not exist in K. fragilis.

PELLECUER, M., VEZINHER, F., PASERO, J., GALZY, P. - Genetics of industrial microorganisms - Second International Symposium. Sheffield, England - 25-31 August 1974. Remarks on the mating type of Saccharomyces cerevisiae.

VEZINHET, F., PELLECUER, M., GALZY, P., AND PASERO, J. - Heredity (In press). Study of some anomalies of copulation in Saccharomyces cerevisiae HANSEN: A method for detecting mating-type mutations revealed the following phenomena in addition to point mutations for mating-type: (i) lethal deletions in the mating type chromosome. (ii) illegitimate copulation between cells of the same mating-type and (iii) plasmogamy without karyogamy.

BIZEAU, C., BASTIDE, M., GALZY, P., BASTIDE, J. M. - Abstract of the 4th Symposium on yeast - Vienna, Austria - 1974 - Part I. Study of morphological mutants of Saccharomyces cerevisiae HANSEN by means of immunofluorescence.

BASTIDE, M., BIZEAU, C., GALZY, P., BASTIDE, J. M. - C. R. Acad. Sc. Paris series D. 279 1135-1138.

Etude de l'évolution des determinants immunologiques de quelques souches de Saccharomyces cerevisiae HANSEN au cours de la croissance.

Strains different in twos by one or two genes are studied by immunofluorescence technique. An evolution of antigenic determinants during the growth was observed.

BIZEAU, C., GALZY, P., BASTIDE, M., BASTIDE, J. M. - C. R. Acad. Sc. Paris Serie D 279 1955-1958.

Etude de mutants morphologiques de Saccharomyces cerevisiae HANSEN au moyen de l'immunofluorescence.

"Smooth colony" mutants are compared with the wild strains. All the mutants have lost antigenic determinants.

Two theses (Thèse de spécialité de biochimie) have been completed in the laboratory.

R. RATOMAHENINA: Etude et selection de souches de levures en vue de la culture sur lactosérum.

Different characters of the strains are studied: Fatty acid, proteins and nitrogen composition. The fermentative and oxidative metabolism are compared.

D. BOUDIER: Quelques effects du gène du signe au cours du cycle biologique de Saccharomyces cerevisiae HANSEN.

Two strains a/α and α/α were compared during the vegetative growth. During the exponential phase the strains are quite similar but during the stationary phase, there are some differences especially in the biochemical composition of the cells.

The modifications of the cell wall of the two strains were studied during incubation in sporulation medium.

XXXII. Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. Communicated by Arnold L. Demain.

Below follow summaries of recent work in our laboratory.

Production of Tryptophan by DL-5 Fluorotryptophan-Resistant (5-FT^r) Mutant Strains of a Methanol Utilizing Yeast, Hansenula polymorpha DL-1

Emmanuel Denenu and Arnold Demain

A methanol-utilizing yeast strain, Hansenula polymorpha DL-1, was mutagenized with ethyl methanesulfonate (EMS) and mutants resistant to 5-fluorotryptophan were selected. Mutant strain 1-22, which was resistant to 500 mg/l 5-FT produced 25 mg/l of tryptophan in mineral salts-methanol medium supplemented with biotin and thiamine as opposed to the wild type strain which produced 3.5 mg/l. Mutant 2-7 (a 2000 mg/l 5-ft^r isolate obtained from mutagenesis of strain 1-22) was able to produce 60 mg/l of tryptophan in the same medium. Addition of 250 mg/l of yeast extract to the mineral salts-methanol medium resulted in a slight increase in cell yield and a marked increase in tryptophan production (240 mg/l) by the 2-7 strain.

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Effect of Temperature and Growth Rate on Viability Cell Size, and Cell Yield on Methanol by Hansenula polymorpha

C. L. Cooney, N. Makiguchi and M. Montgomery

Hansenula polymorpha DL-1 is a thermotolerant yeast which can utilize methanol as a sole carbon-energy source and has been examined for use in the production of Single-Cell Protein. We have examined the effect of both growth temperature and growth rate on the viability, cell size and cellular yield on methanol at steady state in methanol-limited chemostat culture. The viability of the cells decreased with increasing temperature over the range of 30 to 43°C and with increasing growth rate over the range of 0.05 to 0.125 hr^{-1}. The cell size was strongly affected by growth temperature and increased with increasing temperature. The cellular yield on methanol decreased with increasing temperature and was maximum at a certain growth rate.

XXXIII. Allied Breweries (Production) Limited, The Brewery, Station Street, Burton-on-Trent DE14 1BZ, England. Communicated by P. A. Martin.

The following is a summary of the Ph.D. thesis of Elizabeth Anderson, a member of the Research Department staff, submitted to the University of Bath. The work was carried out under the guidance of Prof. A. H. Rose.

Sporulation and Hybridization of Industrial Strains of Saccharomyces cerevisiae and Saccharomyces carlsbergensis

A study was made of the sporulating behavior of selected strains of Saccharomyces cerevisiae and Saccharomyces carlsbergensis and the fermentation properties of these 'parent' yeasts and the strains derived from them by hybridization of mating isolates. Brewing strains sporulated less readily than baking, distilling and other strains, and Sacch. carlsbergensis had poorer overall sporulating ability than Sacch. cerevisiae. Examination of the inheritance patterns among hybrid strains for flocculence, head-forming ability, duration of lag phase of growth, and attenuative ability indicated that all these properties are probably controlled by multiple gene systems. More detailed respirometric experiments demonstrated that polymeric gene systems control fermentation of saccharides in wort, and that the rate at which a yeast can ferment these sugars is related to its protein content, phase of growth and the composition of the wort.

Arising from the work for this thesis the following paper will be published shortly:

The Sporulation and Mating of Brewing Yeasts

Elizabeth Anderson, P. A. Martin

J. Inst. Brew., 1975, 81, (May/June)

A study has been made of the sporulating behavior of 20 selected brewing strains of yeast, and the mating activity of the products of sporulation. "Lager" yeasts (strains of Saccharomyces carlsbergensis) in general sporulated to a lesser degree and more slowly than 'ale' yeasts (strains of Saccharomyces cerevisiae) and produced 1- or 2-spored asci compared with 2- or 3- spored asci for the latter yeasts. Most of the parent strains of Sacch. cerevisiae were shown to be heterozygous for mating type and they were all probably either triploid or aneuploid. Two of the strains of Sacch. carlsbergensis were apparently homozygous for mating type and also triploid or aneuploid. The compatibility system favors outbreeding of yeasts, "ale" yeasts being more compatible with "lager" yeasts than with other "ale" yeasts.

The following is an abstract of a paper read at the European Brewery Convention Congress, May 1975.

Ethanol Tolerance of Brewing Yeasts

A. Day, Elizabeth Anderson, P. A. Martin

Strains from several Saccharomyces species (sake yeasts, brewing yeasts, Sacch. diastaticus and osmophilic strains of Sacch. rouxii and Sacch. bailii) have been examined for their ability to grow and survive in a range of ethanol concentrations (5-15%). Strains of sake yeast were found to be highly ethanol-tolerant whereas sugar-tolerant Saccharomyces strains are generally ethanol intolerant. Most brewing strains are intermediate in their ethanol tolerance levels. An alcohol-tolerant strain of Sacch. diastaticus was hybridized with mating strains from a poorly ethanol-tolerant Sacch. cerevisiae brewing yeast. The resulting hybrids showed a range in their ethanol tolerance levels but in contrast to the brewing strain were non head-forming and poorly flocculent. Among brewing yeasts a correlation has been found between growth rate, ability to grow and survive in the presence of alcohol and RNA content. The mechanism and inheritance of alcohol tolerance is discussed in relation to cell composition.

XXXIV. Miller Brewing Company, Milwaukee, Wisconsin 53201. Communicated by Michael C. Barney.

Below follows the abstract of a paper we presented at the annual meeting of the American Society of Brewing Chemists, May 4-8, 1975, in New York.

The use of lyophilization as a means of long-term storage of brewer's yeast (Saccharomyces uvarum).

by M. C. Barney and J. R. Helbert

Lyophilization was compared with slant culturing and with storage under oil as a means of preserving Saccharomyces uvarum. Several types of suspending media were tested, and mist desiccans, horse serum, and skimmilk were used for a long term study. A range of shelling temperatures was investigated and -30°C was found optimal in terms of viability. Samples were revived every three months for an 18-month period. Mutation rates, assimilation and fermentation characterisitcs, growth requirements, and gas-liquid chromatographic profiles from CS_2 extracts of experimental fermentations, all indicated that lyophilization was comparable to the other storage methods. Although vaiability decreased significantly over the test period, sufficient normal, viable cells survived to maintain a reliable stock.

XXXV. Research Institute for Viticulture and Enology, 886 15 Bratislava, Matuškova 21, Czechoslovakia. Communicated by E. Minárik.

E. Minárik - P. Rágala: The selective effect of vine protective agents on the microflora of grapes. 4th International symposium on

Enology, Valencia, May 1975 (in press).

The influence of different fungicides on the yeast flora of grapes and fermenting grape juice has been investigated for several years. Fungicides may be classifed into three groups according to their effect on yeasts: 1) fungicides showing strong inhibition effect, e.g. fungicides such as captafol, captan, folpet, dichlofluanid, suppressing totally or partly first of all ascosporogenous yeast species, 2) fungicides with moderate or weak inhibition, e.g. fungicides such as thiram, and 3) indifferent fungicides, e.g. systemic fungicides such as benomyl, dichlozoline, methylthiophanate, etc., 4) fungicides such as dithiocarbamate, influencing neither growth nor fermentation activity of yeasts. Fungicides of the first group and partly also of the second one often cause a shift in the representation of dominantly occurring yeast species in spontaneously fermenting musts in favour of anascosporogenous yeasts displaying low fermentation activity. For modern wineries two aspects are of special importance: 1) the reduction of pesticide residues in grape juice prior to fermentation by settling and (or) separation, 2) the application of selected pure yeast starters resistant to pesticides. Ecological, biochemical and technological aspects of the secondary effect of pesticides on yeasts are briefly discussed.

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E. Minárik: Sulphite- and sulphide formation by wine yeasts in the course of grape juice fermentation. Die Wein Wissenschaft 1975 (in press).

SO_2- and H_2S-formation in fermenting grape juice depends more or less on the composition of the must and on the yeast strain applied. SO_2 forming yeast strains produce high quantities of SO_2; their sulphate uptake is very important, too. In the presence of cysteine large quantities of hydrogen sulphide are formed, presumably by desulphydration of cysteine. Sulphur amino acids (methionine, cystein) decrease sulphite formation from sulphate in all yeasts, especially in SO_2-forming strains. Elemental sulphur increased H_2S-formation in spontaneously fermenting grape juice and, in the presence of methionine, also in selected pure yeast cultures. Copper-ions did not cause a significant increase in hydrogen sulphide formation in fermenting must as should have been expected. Methionine and pantothenate generally decreased H_2S-formation. The importance of pure yeast starters with low SO_2-and H_2S-forming ability for modern wineries is underlined.

XXXVI. United States Department of Agriculture, Northern Regional Research Laboratory, Peoria, Illinois 61604. Communicated by C. P. Kurtzman.

The following are titles and abstracts of recent work from this laboratory.

Wickerham, L. J., and C. P. Kurtzman. 1975. Synergistic color variants of Aureobasidium pullulans. Mycologia 67: 342-361.

Abstract

Color variants of Aureobasidium pullulans were isolated from materials collected in tropical and subtropical regions of the world. Three different types of variants produce colonies that are either yellow, red, or purple. When the variants are either mixed with or grown near many different species of yeasts and other microfungi, the rapidity and intensity with which pigment is produced are markedly enhanced. Yellow variants are moderately unstable and change to red. The red variants are highly unstable, changing to yellow. Reversion to the normal wild type is seldom observed. Addition of acid or base causes the pigments to function as pH indicators. Pigment formation is temperature sensitive and mixtures of variants and elicitor strains are colorless at 35 C, but show typical enhancement of pigmentation when removed to 25 C. Variants incubated alone at 35 C show temporary intense pigmentation upon removal to 25 C.

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Kurtzman, C. P., F. L. Baker and M. J. Smiley. 1974. Specimen holder to critical-point dry microorganisms for scanning electron microscopy. Appl. Microbiol., 28:708-712.

Abstract

Critical-point drying of microorganisms for scanning electron microscopy can be rapidly and effectively accomplished by use of a newly described specimen holder. Up to eight different samples of spores or vegetative cells are placed between polycarbonate membrane filters in the holder and processed through solvent dehydration and critical-point drying using carbon dioxide without loss or cross contamination of micro-organisms. Yeasts, molds, bacteria, and actinomycetes have been successfully processed.

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Taylor, N. W. and W. L. Orton. 1975. Calcium in flocculence of Saccharomyces cerevisiae. J. Inst. Brew. 81:53-57.

Abstract

A quantitative method was adopted for measuring flocculation intensity of yeast photometrically. In three strongly flocculent strains of Saccharomyces cerevisiae examined with this method, flocculation intensity depended on ionic strength of the medium as well as on Ca concentration, and was maximum at about 0.01 ionic strength. At this optimum ionic strength, when free Ca concentration was varied in stabilized complexing systems, a transition occurred at about 10^{-8} M Ca between flocculent and nonflocculent states. At higher Ca concentrations, flocculation intensity was nearly constant. The observed transition is at much lower Ca levels than other effects noted in the literature.

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Costello, A. J. R., T. Glonek, M. E. Slodki, and F. R. Seymour. Phosphorus-31 nuclear magnetic resonance spectroscopy of extracellular yeast O-phosphonohexoglycans. Carbohydr. Res. In Press.

Abstract

^{31}P nuclear magnetic resonance spectra of a number of purified yeast O-phosphonohexoglycans were recorded. The data therefrom were correlated with established chemical aspects of individual and collective polymer structure, permitting (a) conclusions to be drawn regarding the chemical environment of the phosphate groups of these polymers, and (b) assignment of anomeric configurations to the hexosyl phosphate residues.

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Kurtzman, C. P. and M. J. Smiley. 1974. A taxonomic reevaluation of the round-spored species of Pichia. Proc. Fourth Int. Symp. Yeasts. Part I, pp. 231-232, Vienna.

XXXVII. National and International Meetings.

1. Dear Colleagues:

I have the pleasure to inform you that under the auspices of the International Commission on Yeasts and Yeast-like Organisms (IAMS) a specialized symposium on "Yeasts in Industrial Use" will be held at the Congress Hall in Berlin from June 28th - July 3rd, 1976, as part of the :Fifth International Fermentation Symposium" (5th IFT).

An organizing committee with Dr. S. Windisch as Chairman will organize the Yeast Symposium.

Documents can be received from:

Prof. Dr. D. Windisch
Institut fur Garungsgewerbe
und Biotechnologie
Seestrasse 13
1 Berlin 65
West Germany

2. Seventh annual meeting of the Commission for Yeasts of the Czechoslovak Microbiological Society held in Smolenice-Castle, 5-7 February 1975. Communicated by E. Minarik.

Scientific program:

Section 1: Minisymposium on cell wall enzymes:

V. Farkas: General aspects of yeast cell wall biosynthesis.

P. Biely: Regulation of biosynthesis and secretion of yeast cell wall enzymes.

A. Kosinova: Multiple forms of yeast invertase and their mutual relation.

Z. Kratky: Metabolic stability of wall mannan and its secretion into the grwoth medium of yeasts.

M. Vrsanska: Utilization of lytic enzymes produced by Arthrobacter strain GJM-1 for the study of yeast wall formation.

J. Vorisek: Cytochemical detection of polysaccharides on membrane surface of the endoplasmatic reticulum and plasmalemma in Saccharomyces cerevisiae and Trigonopsis variabilis.

A. Kockova-Kratochvilova-Z. Holan: Cytological and chemical changes in cell wall of Rhodotorula gracilis. Characteristics and life cycle.

E. Streiblová: Taxonomic singificance of the cell wall in lower fungi.

Section 2: Minisymposium on contaminating yeasts of food:

L. Svorcova: Yeast-like microorganisms as contaminants of sweetened mineral waters.

J. Svrckova: The problem of yeasts in soda-water manufacture.

O. Bendova-V. Kurzova: Extraneous yeasts in beer production.

J. Savel - Yeasts, contaminants of beer.

H. Cihova: Yeasts as negative factor in food hygiene.

M. Polster: Problem of toxicity in yeasts.

L. Svorcova: Utilization of artificial sweeteners by yeasts.

Section 3: Cytology-genetics-immunology-biochemistry:

Y. Kochova: Candida albicans - evidence of intranuclear spindle.

E. Slvaikova: Giant colonies of yeasts.

D. Vrana: The role of daughter cells in physiological state formation of the population of Candida utilis.

A. Navara-E. Minarik: Determination of H_2S and SO_2 in fermenting broth in the presence of sulphur amino acids.

Y. Gbelska et al.: Investigation on a defective yeast mutant in choline synthesis.

S. Cerna-A. Sukenikova: Study on phospholipids of a yeast mutant defective in adenine nucleotide transfer through mitochondrial membrane during mutagenesis by ethidiumbromide.

A. Tomsikova: Contribution to the immunology of Cryptococcus sp.

D. Sikl et al.: Structural and immunochemical investigation of polysaccharides of Candida lipolytica.

J. Subik: Influence of mucidine on growth and metabolism of Saccharomyces cerevisiae.

M. Vidova: Influence of nalidixic acid on the growth of Saccharomyces cerevisiae under different conditions of cultivation.

O. Volfova: Oxidation of methanol by protoplasts of Candida boidinii 11 Bh.

P. Pilat: Influence of methanol on the growth of Candida boidinii 11 Bh.

3. The Seventh Annual Meeting of Yeast Genetics Conference-Japan.

The Seventh Annual Meeting of the Yeast Genetics Conference-Japan was held on October 5 and 6, 1974 at the Department of Microbiology, Shizuoka College of Pharmacy, Shizuoka City, Japan. Around seventy yeast researchers met, and the following topics were presented and discussed.

Session 1. Mutation and Radiation Effects - Chairmen: T. Takahashi T. Ito.

1. T. Ito and K. Kobayashi (Univ. Tokyo) - Photodynamic probe into the physiological state of the cell.

2. T. Ito and K. Kobayashi (Univ. Tokyo) - Cell growth and photodynamic sensitivity of S. cerevisiae.

3. T. Saeki, I. Machida and S. Nakai (Natl. Inst. Radiol. Sci.) Induction of genetic recombination by split-dose irradiation.

4. T. Takahashi (Suita Lab., Brew. Sci. Res. Inst.) - Mitomycin C induced chromosomal aberrations during mitosis.

Session 2. Regulation and Metabolism - Chairmen: H. Tamaki, I. Takano.

5. S. Harahsima and Y. Oshima (Osaka Univ.) Genetic mapping of homothallism genes in Saccharomyces yeasts.

6. a Toh-e and Y. Oshima (Osaka Univ.) Genetic regulation of acid phosphatase synthesis.

7. H. Tamaki (Doshisha Women's College) Glucoamylase genes in Saccharomyces diastaticus.

8. I. Nakamura, Y. Nishikawa, T. Kamihara, S. Fukui, J. Nagai and H. Katsuki (Kyoto Univ.) Effects of thiamine and pyridoxine on the respiratory activities and the lipid composition in S. carlsbergensis.

Session 3. Sexuality and Its Genetic Control - Chairmen: T. Takahashi, Y. Oshiama.

9. C. Shimoda (Osaka City Univ.), N. Yanagishima (Nagoya Univ.), A. Sakurai and S. Tamura (Inst. Phys. Chem. Res.) Sex specific protein-like substances responsible for the sexual aggutination in S. cerevisiae.

10. Y. Kawanabe, M. Hagiya (Osaka City Univ.), K. Yoshida and N. Yanagishima (Nagoya Univ.) Microscopic observation of sexual aggutination in yeast.

11. Y. Matsushima, C. shimoda (Osaka City Univ.) and N. Yanagishima (Nagoya Univ.) Mutations affecting sexual cell agglutination and zygote formation.

12. T. Takahashi and K. Sakai (Suita Lab., Brew. Sci. Res. Inst.) Isolation of temperature sensitive mutants on mating reaction and genetic studies of the mutants.

13. M. Tsuboi (Osaka City Univ.) and N. Yanagishima (Nagoya Univ.) The role of RNase in the sporulation of S. cerevisiae.

14. T. Yamazaki and Y. Ohara (Yamanashi Univ.) Low frequency segregation of the tetratype tetrads in Saccharomycodes ludwigii.

15. Y. Nakatomi (Oriental yeast co. Ltd.) Sporogenous a and α mater cultures segregated from Baker's yeast.

16. H. Mori (Noda Inst. Sci. Res.) and S. Windisch (Inst. Garungsgwerbe, Berlin, Germany) Homothallism in Saccharomyces rouxii.

Session 4. Cytoplasmic Inheritance and Drug Resistance - Chairmen: T. Morita, N. Gunge

17. N. Gunge (Cent. Res. Lab., Mitsubishi Chem. Ind.) Effect of ploidy on the transmission polarity of mitochondrial genes.

18. K. Suda (Nara Univ. Eud.) and A. Uchida (Kobe Univ.) A mutant of yeast with temperature sensitive respiration: High sensitivity to chloramphenicol at high temperature.

19. H. Tohoyama and T. Murayama (Ehime Univ.) Copper and Cadmium resistance in yeast.

20. S. Nagai (Nara Women's Univ.) Sensitivity and resistance to an antifungal antibiotic, Blasticidin in Saccharomyces yeasts.

21. K. Wakabayashi and S. Kamei (Univ. Tokyo) On the double mutant of oligomycin resistance.

22. T. Morita, and I, Mifuchi (Shizuoka Coll. Pharm.) Cytochrome-a less mutant of yeast induced by 4-NQO.

Session 5. Cytology and Function of Cell Organelles - Chairmen: T. Hirano, M. Osumi.

23. J. Ishiguro and Y. Arakatsu (Konan Univ.) Electrophoretic study on the protein of yeast mitochondrial ribosome.

24. S. Tsuyumu (Shizuoka Univ.) and B. G. Adams (Univ. Hawaii, U.S.A.) Mechanism of the induction of galactose utilizing enzymes in yeast.

25. M. Osumi (Japan Women's Univ.) Enzyme activities of microbody in Candida tropicalis.

26. K. Takeo (Kyoto Univ.) Ultrastructural comparison of the yeast-like form of Mucor with those of normal Ascomycetes yeasts.

27. T. Hirano (Tokyo Metropol. Isotope Res. Inst.) Application of energy non-dispersive X-ray analysis on the transmission electron microscope in phosphorus metabolism of yeast cells.

Session 6. Reports from International Meetings - Chairman: N. Yanagishima.

28. S. Nagai (Nara Women's Univ.) Topics in the International Symposium on Yeasts and Yeast-like Organisms held in Austria.

29. I. Takano (Cent. Res. Inst., Suntory Ltd.,) Topics in the International Conference on Yeast Genetics and Molecular Biology held in England.

The next annual meeting of the Yeast Genetic Conference-Japan will be held at Nara or Osaka in August, 1975.

Toshiaki Takahashi

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4. August 17 - 22, 26th Annual Meeting, Society for Industrial Microbiology concurrently with the Third International Biodegradation Symposium at the University of Rhode Island, Kingston, R. I. Information -- Mrs. Ann Kulback, SIM, 1401 Wilson Blvd., Arlington, Va. 22209 or (for IBS) Dr. R. W. Traxler, 231 Woodward Hall, U. of Rhode Island, Kingston, R. I. 02881.

The preliminary SIM program includes symposia on Interpretation of New Federal Regulations for Product Registration, Fermentation Development and Optimization, Microbiology of the Eye, Single Cell Protein and Products from Microbes, also a Round Table on Production of Energy or Enzymes by Fermentation. Contributed papers will cover a wider variety of topics.

The IBS program is truly international with speakers from both Eastern and Western Europe, North America, Japan, India, Africa, and New Zealand. The topics are equally varied from marine fouling and petroleum degradation in aquatic environments to the deterioration of food crops by insects and fungi. The program extends to August 23 with 4 sessions including a symposium Microbial Transformation of Metals.

Emily M. Owen, Chairman
1975 Publicity Committee
Society for Industrial Microbiology

5. A Colloquium on Regulatory Biology will be held on the Columbus campus of the Ohio State University September 4-6, 1975. It is the second annual Colloquium of the College of Biological Sciences. Speakers will include: W. Szybalski (U. Wisconsin), J. King (M.I.T.), M. Cashal, (N.I.H.), S. Bourgeois (Salk Inst.), R. Goldberger (N.I.H.), J. C. Copeland (Ohio State U.), E. Zeuthen (Carlsberg Foundation), G. Marzluf (Ohio State U.), T. Humphreys (U. Hawaii), H. F. Lodish (M.I.T.). Contributed presentations will also be included. The proceedings of the Colloquium will be published by the Ohio State University Press. For further information write to: Colloquium, College aof Biological Sciences, The Ohio State University, 484 W. 12th Ave., Columbus, Ohio 43210.

George Marzluf
James C. Copeland

6. United Nations Educational, Scientific and Cultural Oragnization, United Nations Environment Programme, The Government aof Malayasia and International Cell Research Organization Panel on Microbiology.

The Fifth International Conference on the Global Impacts of Applied Microbiology (GIAM V) will be held at the University of Malaya, Kuala Lumpur, Malayasia during 22-27 March 1976, and will be a follow-up of the Conference held in Stockholm (Sweden) 1963, Addis Ababa (Ethiopia) 1967, Bombay (India) 1969 and Sao Paulo (Brazil) 1973.

The Conference will be organized by the Panel on Microbiology of the International Cell Research Organization (ICRO) and the Government of Malaysia, under the auspices of UNESCO and the United Nationas Environment Programme (UNEP), in consultation with the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the United Nations Industrial Development Organization (UNIDO) and the International Atomic Energy Agency (IAEA). A number of other international and national organizations, industries and firms are expected to join as co-sponsors of the Conference.

For further information write:

Professor W. R. Stanton
Chairman, GIAM V Programme Sub-Committee
P. O. Box 2612
Kuala Lumpur
Malaysia

7. The 7th Stadler Genetics Symposium was held April 18-19, 1975 -- Columbia, Mo.

E. Chargaff: Impact of Biochemistry on Genetics.

R. B. Helling: Eukaryotic Genes in Prokaryotic Cells.

O. L. Gamborg: New Advances in Somatic Cell Hybridization in Plants.

R. Flavell: Genome Organization in Higher Plants.

N. Sueoka: Chromosome Replication and Gene Expression in Bacillus subtilis.

C. D. Miles: Genetic Analysis of Photosynthesis.

A. C. Wilson: Relative Rates of Evolution of Organisms and Genes.

G. B. Johnson: Enzyme Polymorphism and Adaptation.

D. E. Metter: Natural Selection and Adaptive Resemblances.

Proceedings of this and previous Symposia are available from Stadler Genetics Symposia, 117 Curtis Hall, University of Missouri, Columbia, Missouri, 65201. Vols. 3-5 $4.00, vols. 6 and 7 $4.50, each. For postage and handling $.50 is due per order.

XXXVIII. Obituaries

1. I have the painful task to announce to you the death of Huguette de Robichon-Szulmajster, in April 1974. It is still hard to believe since she showed such an enthusiasm for life, for science and since she did so much for all of us. Y. Surdin-Kerjan, Laboratoire d'Enzymologie, CNRS, 91 Gif-sur-Yvette (France).

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2. Professor Teijiro Uemura, the late President of the National Iwate University, Vice Chairman of the Organizing Committee of the 1st Intersectional Congress of the IAMS, a member of the Yeast Commission of IAMS representing Japan, died from heart failure on April 13, 1974 at the age of sixty-five.

At the first anniversary of his death, we should like to do honor to the memories of his scientific life.

He graduated from the University of Tokyo in 1932 and took office in 1948 as professor of agricultural microbiology in the

Faculty of Agriculture, Tohoku University, Sendai, after he had worked as a research fellow at the University of Tokyo and as chief researcher at the Institute for Fermentation, Osaka. In 1944, he was granted the degree of Doctor of Agriculture from the University of Tokyo. In 1963, he was transferred to the University of Tokyo as professor of microbial physiology at the Institute of Applied Microbiology. In 1968, he retired because of the age limit policy of Japanese universities. In 1973, he was inaugurated as President of National Iwate University.

His scientific achievement was mainly concerned with microbial physiology which covered many kinds of microorganisms, such as molds, yeasts and bacteria.

His scientific life may be divided into three periods.

The first period (1932-1944) may be considered as the life in the University of Tokyo and in the Institute for Fermentation, Osaka (1940-1948), where he studied mainly the decomposition of amino acids by fungi and bacteria.

In the second period (1944-1963), he envisaged vividly his own concept on the microbial metabolism through the studies of his group. In this period, his attention was mainly directed on the analysis of the various fermentation and brewing processes, such as the fermentations of penicillin and other antibiotics, the brewing of Sake and Shoyu (soy sauce), rumen, fermentation, and amino acid fermentation, etc. Through these studies, he emphasized the variability of the microbial metabolic activities with special reference to the environmental factors, including nutritional factors.

He never forgot to reflect on the microbial life in nature when he was carrying out these types of researches, and furthermore, he was interested in the microbial life in natural environments.

In Sake brewing, yeasts are not always in the pure culture state in the sense of the techniques in general microbiology. The metabolic activities of the yeasts in this process vary due to the variations in the environmental conditions during the progress of fermentation and they were compared with the activities of microorganisms cultured under the laboratory conditions. One of the important contributions is that the mechanism of succinic acid formation under anaerobic conditions was clarified. Furthermore, the mechanism of alcohol tolerance was established in relation to the growth of yeasts and the environmental changes, among which the concentrations of carbohydrate and nitrogenous compounds were especially considered. He also analyzed the behavior of nutritionally different yeasts in this process when they are cultured together. Another one was the spoilage of brewing processes.

Other contribution to yeast science include the relation between nutrition and metabolism under the condition of pantothenate and biotin deficiency, the problem of salt tolerance of Shoyu yeasts and its relation to their metabolism, the role of yeasts in the maturing process of ensilage, the physiological significance of nucleotide excretion, etc.

The third period of his studies (1963-) took place at the University of Tokyo. Proceding this period, a unique type of fermentation was developing in Japan to produce amino acids and nucleotides by fermentation processes. Several of these compounds are familiar seasoning agents. With his excellent insight, he made important contributions to the understanding of this complicated fermentation process. It is easily understood that his attention was directed mainly to the cellular regulation mechanism in the third period of his scientific life.

In addition to these research activities, he wrote many excellent books or monographs important to microbiological research. For example, "The Enzymes (1940)" was the only orthodox textbook on enzymology available at that time in Japan. "The Enzymes and Penicillin (1948)", and "An Aspect of Amino Acid Fermentation (1959)" were monographs or articles expressing his strong and deep insight concerning dynamic aspect of microbial metabolism.

As a professor, he trained many excellent Japanese microbiologists who are playing active roles in various fields.

Since about 1960, he was a member of various national committees and councils, and made many contributions to the organization of microbiological research activities in Japan.

Indeed, he was a good teacher and a good leader, and also a good friend for us. He always guided us seriously not only our scientific performance but also private circumstances as well as possible.

He is only a memory to us now, but his instructions are still fresh and will live in our minds.

Masaya HAYASHIBE, Faculty of Science
Osaka City University

Hiroshi KURAISHI, Institute of Applied Microbiology, the University of Tokyo

Yasushi YAMAMOTO, Kirin Brewery Co., Sendai

XXXIX. Brief News Items

1. N.J.W. Kreger- van Rij and M. Veenhuis, Electron microscopy of ascus formation in the yeast Debaryomyces hansenii. J. gen. Microbiol. (accepted for publication). N.J.W. Kreger- van Rij, Groningen (Holland), Oostersingel 59, Netherlands.

2. The following articles have recently been published: Golubev, W. I., Okunev, O. N., Vdovina, N.W., 1974. The assimilation of i-inositol by yeasts as a diagnostic critertion. Mikrobiologia, 43, N6, 1047-1051.

Golubev, W. I., 1974. The yeasts in forest ant-hills. Priroda, N 11, 96-97. W. I. Golubev, Institute of Biochemistry, USSR Academy of Sciences, Pustchino, Moscow Region 142 292, USSR.

3. My colleague Jean Shennan and I have recently published a review in Progress in Industrial Microbiology, 13, 1-57 (1974). Its title is "The Growth of Yeasts on Hydrocarbons." I am very sorry to say that we have now run out of reprints. J. D. Levi, B. P. Proteins, Ltd. Orangemouth Division, P. O. Box No. 33, Orangemouth, Stirlingshire, FK3 9XN, England.

4. An invitation paper entitled "Cellular Organization in Relation to Cancer" by Carl C. Lindegren has appeared in the Jubilee issue of Annali di Microbiologia, celebrating Professor Onorato Verona's 60th birthday. Carl C. Lindegren, Southern Illinois University at Carbondale, Carbondale, Illinois 62901.

5. The "Laboratoire d'Enzymologie de l'Universite Catholique de Louvain" has been moved to a new campus at Louvain-la-Neuve. Our new address is:

Laboratoire d'Enzymologie
Universite de Louvain
Place Croix du Sud, 1
Sciences 14, Tour b
1348 Louvain-la-Neuve
Belgium

We study the structure and biogenesis of the mitochondrial and plasmic membranes of Schizosaccharomyces pombe and Saccharomyces cerevisiae. A. Goffeau.

6. Dr. Cyril Rainbow, Bass Production Limited, Research Laboratory, High Street, Burton-on-Trent, England, will retire on July 31 of this year. I am sure our readers will joine me in wishing him well.

7. Locations of biochemical mutants of Saccharomyces cerevisiae and Schizosaccharomyces pombe in adenylate biosynthesis.

In the yeast Saccharomyces cerevisiae and Schizosaccharomyces pombe several mutants have been isolated which are deficient in different enzymatic steps in the de novo adenine pathway. We are presently reviewing the biochemical and genetical aspects of the de novo adenine pathway in both yeast organisms.

Majdi M. Shahin and N. H. el Kouni, Department of Genetics, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada.