Y E A S T

A News Letter for Persons Interested in Yeast

Official publication of the
International Council of Yeasts and Yeast-like Microorganisms

June 1970 Volume XVIX, Number 1

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Many thanks to those who have contributed to this issue by sending in news items and accounts of research projects. The next issue will be published in December 1970. A contribution of $1.00 from those who have not contributed for some time would be appreciated to finance future editions of the News Letter. Many thanks to those who have contributed recently.

H. J. Phaff

I Ajinomoto Co., Inc., Central Research Laboratories, Suzuki-cho, Kawasaki, Japan. Communicated by T. Nakase.

1. The following paper will appear in the June issue of J. Gen. Appl. Microbiol.

"Significance of DNA base composition in classification of yeast genera Hanseniaspora and Kloeckera."
by T. Nakase and K. Komagata

Summary: The base composition of DNA (GC content) of 31 cultures of Hanseniaspora and Kloeckera which represented 14 species was studied. The GC content of these apiculate yeasts ranged from 26.8 to 38.0%.

Thirty one cultures employed were divided into 4 groups on the basis of the GC content, and this grouping agreed with that based on biochemical criteria commonly employed in yeast taxonomy. The first group (H. osmophila-K. africana) exhibited a GC content of 37.3-38.0%, and assimilated maltose but not glycerol or Ca-2-ketogluconate. The second group (K. javanica) exhibited a GC content of 33.7-34.6%, and assimilated glycerol but not maltose or Ca-2-ketogluconate. The third group (H. guilliermondii, H. uvarum-K. apiculata) exhibited a GC content of 31.0-31.7%, and assimilated Ca-2-ketogluconate but not maltose or glycerol. The last group (H. valbyensis-K. japonica) exhibited a GC content of 26.8-27.6%, and did not assimilate maltose, glycerol and Ca-2-ketogluconate.

2. Now we have finished the analysis of the DNA base composition of the yeast genera Pichia, Debaryomyces, Torulopsis, Cryptococcus and Rhodotorula, and are preparing the manuscripts for publication.

II Microbiological Laboratory of the Institute of Chemistry of the Slovak Academy of Sciences, Bratislava, Dubravska cesta, CSSR. Communicated by Dr. A. Kocková-Kratochvílová.

Towards the end of 1969 the first volume of the Kocková-Kratochvílová A., Sǎndula J., Sedlárová L., Vojtková-Lepšíková A. and Kasmanová M.: "Taxometric study of the genus Saccharomyces(Meyen)Reess" was published by the Publishing House of the Slovak Academy of Sciences, Bratislava, in the Edition of "Biologické práce" Vol. XV/1 in English. It involves 192 pages, 76 figures and 56 tables.

The first two Volumes elaborate the industrial strains, brewing and baker's yeast. Since the present section is the first of three, it is preceded by an extensive introduction, explaining the author's attitude to taxonomy, the employed classification principle and formed conception, biochemical and phylogenetic justification for the used auxilliary classification as well as the principal questions of the used numerical method.

In the present study many characters were selected according to anticipation of their acquirement in the evolution of species or shaping of a group under natural conditions. It applies to both characters acquired under conditions of technology of fermentation and those due to the effect of natural ecological factors. Since the classification is performed in the lowest taxonomic ranks, within a large species or between groups of closely related species whose existence is at present questionable, the operational characters are often quantitative and their variability should be taken for granted, which is in conformity with evolutionary classification principles.

Numerical evaluation of results obtained in this work differs from those of various other similar studies by bestowing special attention on selection of characters and statistical analysis of quantitative characters as a basis for formation of levels of continuous characters. The present work compares various methods for evaluation of similarity coefficients, computed on the basis of coding and weighting of characters.

The previous taxonomies of yeasts build their systems on predominantly morphological and biochemical characters. In this study, apart from these two, also evaluation of other aspects such as serology, genetics and technology was introduced, which resulted in formation of a special set of characters. On the basis of this complex attitude the numerical evaluation resulted in the formation of completely natural groups of these strains or intermediary groups. In a study of 88 strains, originally determined as S. carlsbergensis, S. uvarum, S. logos and S. monacensis, S. mandshuricus etc., two groups were formed, one of which includes the original strains of S. carlsbergensis in sensu stricto, the other S. uvarum.

The second edition of the "Catalogue of cultures of the Czechoslovak collection of yeasts and yeast-like organisms" appeared at the end of 1969. It includes 14 Czechoslovak collections of bacteria, viruses, yeasts and yeast-like organisms, molds and other fungi. A separate catalogue of yeasts and yeast-like organisms is available and if requested, will be sent by Dr. A. Kocková-Kratochvílová (Institute of Chemistry of the Slovak Academy of Sciences, Bratislava, Dubravska cesta, CSSR).

Czechoslovak collections of yeasts are in the following places:

1) CCY, the main collection of the Microbiological Laboratory of ICH SASc., Bratislava, containing type-cultures, industrial strains, hybrids, mutants and other important strains for genetic studies for biochemistry and immunology.
2) RIVE, the collection of the Research Institute for Viticulture and Enology in Bratislava, comprises technologically important strains, as well as strains of various species isolated in Czechoslovakia.
3) CCPY, collection of the Laboratory for Mycological Research, Faculty of Medicine, Comenius University in Bratislava, includes pure cultures isolated from untreated patients with typical pathological processes in various forms of candidiasis.
4) RIBM, collection of the Research Institute of Brewing and Malting in Prague was restored during the last years and contains technologically important brewing yeasts.

Since in past CCY fused many times with other partial collections, it includes some strains of the same origin provided from different collections. These strains are preserved separately. Since the reidentification studies on the basis of multiple information advances only slowly, the original names of the species were left unchanged, as they were originally designated. The original designations are predominantly introduced after Lodder and Kreger-van Rij, Kudrjavcev, Boidin etc.

When publishing experimental results with pure cultures received from these collections, quotation of origin of strains is requested. Before the number of applied strains the sign of partial collections is necessary (e.g. CCY, RIVE, CCPY or RIBM).

Personal Communication: DrSc Anna Kocková-Kratochvílová is now active as Professor in the Department of Microbiology of the Ernst-Moritz-Arndt University of Greifswald (the present address: 2 Greifswald, Jahnstrasse 15, German Democratic Republic) during the summer semesters from March 15 till August 30, 1970 and from March 1 till August 30, 1971.

III Institute of Fermentation, Yamanashi University, 1 - Kitashin-machi, Kofu, Japan. Communicated by Shoji Goto.

The authors have studied the yeast flora of various natural habitats. As two of these studies, yeasts in core samples from stratigraphic drillings and in Antarctic regions were reported.

1. Mycoflora in Core Samples from Stratigraphic Drillings in Middle Japan. IV.
J. Sugiyama and S. Goto, J. Faculty of Science, Univ. of Tokyo, Sec. III, Vol. 10(7), 97-116 (1969).

The yeast flora in core samples from two stratigraphic drillings in central Japan consisted of asporogenous yeasts alone, viz., Candida, Trichosporon and Rhodotorula. Particularly, C. guilliermondii var. japonica was characteristic of the yeast flora in core samples. Their abilities to grow under extreme environmental conditions was demonstrated. An analysis of the data obtained suggests that yeasts may have survived in the deep lithosphere. Yeasts were detected in core samples taken from depths of about 800 to 2.500 meters. The deepest sample from which C. guilliermondii var. japonica was isolated was obtained at a depth of about 2.500 meters.

Identified yeast species including four new taxa were: C. guilliermondii var. japonica(var.n.), C. ishiwadae(sp.n.), C. terebra(sp.n.), C. tropicalis, Trich. cutaneum, Rh. glutinis, Rh. rubra, and Rh. terea(sp.n.).

2. A Taxonomic Study of Antarctic Yeasts.
S. Goto, J. Sugiyama and H. Iizuka, Mycologia 61, 748-774 (1969).

Yeast species isolated from samples of water, soil, algae, mosses, penguin dung, etc., from Lakes Vanda, Bonney, Miers, and Fryxell in the Dry Valley region of South Victoria Land, and from Capes Evans and Royds on Ross Island, Antarctica, were studied from the standpoint of taxonomy and ecology.

Thirty-one yeast strains were isolated at two different temperatures (10 and 25 C), using three different culture media, each with three different concentrations of NaCl (0, 30, 100 g/liter).

Species and varieties identified are as follows: Sporobolomyces antarcticus (sp. nov.), Cryptococcus albidus, Torulopsis psychrophila (sp. nov.), Candida australis (sp. nov.), C. diffluens, C. humicola, C. scottii, Trichosporon cutaneum var. antarcticum (var. nov.), Rhodotorula glutinis var. rufusa, Rh. rubra, Rh. rubra var. miersensis (var. nov.), and Rh. texensis. Of these Sp. antarcticus, Tor. psychrophila, C. diffluens, C. humicola, C. australis, Trich. cutaneum var. antarcticum, Rh. glutinis var. rufusa, and Rh. rubra var. miersensis, are recorded for the first time in the Antarctic yeast flora. Tor. psychrophila and C. scottii are obligate psychrophiles.

The marked difference in the yeast flora of Lake Vanda and Lake Bonney deserved consideration. The very unusual conditions pertaining to salinity and temperature gradations in the water of these lakes may be important factors in determining the distribution of yeast found. Very marked interspecific differences were observed among the Antarctic yeasts with respect to the growth rate at different temperatures and degrees of salinity but no conspicuous intraspecific differences were noted.

IV. Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201. Communicated by M. A. Gordon.

Below follows the summary of a paper to be published in Sabouraudia, Nov. 1970.

FILAMENTATION AND ENDOGENOUS SPORULATION IN CRYPTOCOCCUS NEOFORMANS

Morris A. Gordon and June Devine

SUMMARY

Sodium deoxycholate, a component of the antifungal antibiotic preparation Fungizone (amphotericin B, Squibb), is itself inhibitory in relatively low concentrations (0.25%) for most strains of Cryptococcus neoformans tested. Surviving cells exposed to these concentrations often give rise to morphologic variants consisting of minutely-encapsulated, oval and elongate cells with a tendency toward filamentation.

Certain strains of C. neoformans of capsule type C, including some of the above variants and their parent cultures, developed, in infected mouse tissues, very large, spherical, thick-walled, heavily-encapsulated cells, some of which, upon transfer to agar media, produced as many as eight internal spores. These often appeared to arise through free-cell formation, leaving an epiplasmic residue in many cases, but failed to take ordinary ascospore stains. There was some evidence of conjugation preceding sporulation. Filamentous variants retained pathogenicity for mice, and often appeared in tissue sections in the altered form.

V. Université de Lyon, Laboratoire de Biologie Végétale, 43, Boulevard du 11 Novembre 1918, 69 - Villeurbanne - France. Communicated by Dr. M. C. Pignal.

Below follow the latest news from our laboratory.
The following articles have been published:

- F. JACOB - Pichia tannicola, nouvelle espèce de levure isolée de liqueurs tannantes végétales. Bull. Soc. Mycol. France 85:111-115. 1969.

- F. JACOB - "Pichia" adzetii et "Pichia" abadieae, nouvelles espèces de levures isolées de liqueurs tannantes végétales. Id. 85:117-127. 1969.

- F. JACOB - Deux espèces nouvelles de levures asporogènes isolées de de liqueurs tannantes végétales. Ann. Inst. Pasteur 118:207-213. 1970.

Accepted for publication:

- S. PONCET - Le genre Hansenula H. & P. SYDOW. Application d'une méthode d'analyse factorielle a la taxonomie de ce groupe. Ann. Inst.

Pasteur.

- J. B. FIOL - α-glucosidase, α-galactosidase et β-glucosidase chez Kluyveromyces wikenii et Kl. aestuarii. Ann. Inst. Pasteur.

Submitted for publication:

- M. C. PIGNAL - A new species of yeast isolated from decaying insect-invaded wood. Antonie van Leeuwenhoek.

- M. C. PIGNAL - Quelques levures associées à des larves xylophages de Coléoptères Buprestides au Canada. Naturaliste Can.

VI Maharashtra Association for the Cultivation of Science (M.A.C.S.) Research Institute, Poona 4, India. Communicated by G. B. Deodikar, Director.

Mr. M. S. Kumbhojkar, under the supervision of Dr. R. S. Dhavlikar, has been working on comparative cyto-taxonomy and bio-chemical specification of some osmophilic yeasts. He has so far isolated about 90 primary isolates from fermented honeys. These isolates were found to grow on 60% jaggery (unrefined brown sugar made from palm sap) containing inorganic salts at pH 6.5 and temperature 30°C. Their taxonomy, cytology and biochemical and nutritional studies have indicated that they belong to:

1) Saccharomyces rouxii Boutroux
2) Saccharomyces mellis Fabian et Quinet
3) Schizosaccharomyces octosporus Beijerinck

A preliminary note on the above work has been recently reported (M. S. Kumbhojkar. 1969. "Osmophilic Yeasts from Indian Honeys", Current Science, 38(14), 347-348). The author observed that under the same species, a number of constant colony-morphotypes exist. Thus S. rouxii shows three constant colony-morphotypes and S. octosporus shows six.

These colony-morphotypes within each species are constant and do not change according to cultural conditions. We are, therefore, differentiating these constant colony morphotypes as strains or biotypes under their respective species. We would like to know how such variants are treated by other yeast taxonomists.

Shri M. S. Kumbhojkar will present his investigations in the form of Ph.D. Thesis.

Since we have started work on osmophilic yeasts recently, we are in need of reprints on osmophilic yeasts. We will appreciate receiving reprints from other colleagues in this field.

VII Microbiology Department, Macdonald College of McGill University, Quebec, Canada. Communicated by Ronald E. Simard.

Following is a summary of my doctoral dissertation under the guidance of Prof. A. C. Blackwood.

YEASTS FROM THE ST. LAWRENCE RIVER

Samples were taken from five stations on the St. Lawrence River during the summer of 1968 and the total numbers of yeasts and their taxonomic distribution determined. The occurrence of high and low populations of yeasts was correlated with coliform count, biochemical oxygen demand and dis-

solved oxygen and other pollution indices.

Nearly all the samples (96%) contained yeasts up to a concentration of 9,500 per 100 ml. In saline waters the numbers of yeasts were similar to those found in fresh water. Rhodotorula and Candida were the most common genera isolated and Rhodotorula glutinis was the most common species encountered in the river.

The persistance of Rhodotorula glutinis in polluted water and its distribution pattern correlated with various pollution indices suggest that this organism could be used as a pollution indicator.

Salts of copper, cobalt, iron, zinc, manganese, magnesium and arsenic had a growth promoting effect on this organism when used in various concentrations. Compounds commonly found in polluted water wastes e.g. glucose, egg albumin, creatinine, ammonium sulfate, vitamin-free casamino acids, urea could be used by Rhodotorula glutinis as the sole carbon or nitrogen sources.

Papers will be published in the near future based on the thesis presentation.

III Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada. Communicated by J. F. T. Spencer.

Below follow the summaries of two papers which were recently submitted for publication.

Yeasts isolated from the South Saskachewan, a polluted river.

J. F. T. Spencer, P. A. J. Gorin and N. R. Gardner

Submitted to Canad. J. Microbiology

ABSTRACT

Minimum numbers of yeasts isolated from the Saskatchewan River in the summers of 1964 and 1965 ranged from 400 to 500 cells/ liter upstream from the city of Saskatoon, to 4600 cells/liter immediately downstream. In the summer of 1968, a period of extremely low water, the counts were 150 cells/ liter upstream from the city and 30,000 cells/liter downstream.

Proton magnetic resonance spectra of the mannose-containing polysaccharides from representative cultures of the different species isolated were used as an aid in classification. The majority of the species were asporogenous, and included representatives of the genera Candida, Trichosporon, Rhodotorula, Torulopsis and Cryptococcus. Some species of Pichia, Saccharomyces and Debaryomyces were isolated. The yeasts were mostly introduced into the river with the effluent from the Saskatoon sewage system.

OCCURRENCE OF 2-ACETAMIDO-2-DEOXYGLUCOMANNANS IN THE CELLS OF CERTAIN YEASTS

P. A. J. Gorin, J. F. T. Spencer and A. J. Finlayson

Submitted to Carbohydrate Research

ABSTRACT

Mannose-containing polysaccharides containing up to 6% of protein were obtained by extraction of several different yeasts with hot aqueous alkali followed by purification via their copper complexes. Their p.m.r. spectra contain signals with chemical shifts corresponding to N-acetyl groups which are constituents of 2-acetamido-2-deoxyglucomannans, being present in proportions up to 17%. The polysaccharides from Pichia bovis and Saccharomyces phaseolosporus contain 2-acetamido-2-deoxy-D-glucopyranosyl non-reducing end-units, as shown by methylation-fragmentation analyses. The units in each polysaccharide appear to have the α-configuration.

IX Louisiana State University, Department of Food Science and Technology, Baton Rouge, Louisiana, 70803. Communicated by S. P. Meyers.

The following two papers were presented at the recent American Society for Microbiology Meeting in Boston, Massachusetts, May, 1970.

BACTERIOLOGICAL PROCEEDINGS-1970

A26. Extracellular Proteolysis by Candida lipolytica. S. P. Meyers, J. Rhee, and D. G. Ahearn, Louisiana State University, Baton Rouge, and Georgia State University, Atlanta.

Thirty-three isolates of Candida lipolytica from diverse environments were assayed for ability to produce extracellular protease. All isolates produced an inducible enzyme in defined yeast nitrogen base medium (Difco) supplemented with gelatin, casein, or soybean protein. Addition of 0.5% Tween 80 significantly increased the rate of enzyme production, whereas increase of the glucose concentration from 0.1 to 0.5% delayed enzyme synthesis. Cell-free preparations of 18- to 96-hr culture broths of representative strains were tested for casein hydrolysis by a modified Hayashi tyrosine assay. An alkaline protease was detected in culture broths at pH 7 to 9 within 24 hr, and reached maximal concentration between 48 and 96 hr. Enzyme activity was negligible in broths from 144-hr cultures. Maximal activity observed, (0.338μM tyrosine per 0.5 ml/per 20 min) in 1.0%, isoelectric casein (Difco) pH 6.0 citrate buffer, was equivalent to approximately 10 mg/ml of an NBC proteinase preparation. These data support our earlier reports on widespread extracellular proteinase activity among certain yeasts.

BACTERIOLOGICAL PROCEEDINGS-1970

G113. Amine Compounds as Nitrogen Sources for Yeasts. S. P. Meyers, M. E. Nicholson, and D. G. Ahearn. Louisiana State University, Baton Rouge, and Georgia State University, Atlanta.

Ethylamine and methylamine were tested as sole nitrogen sources [in defined yeast carbon base medium (Difco)] for yeasts from the Spartina alterniflora marshland ecosystem in Barataria Bay, Louisiana. In sediments from this locality, the predominant yeasts, K. drosophilarum and Pichia species, occurred in concentrations of >75,000 viable cells/cm^3. Sporogenous species comprised the major portion of the indigenous yeast population.

Pichia species (119) utilized a range of primary, secondary, and tertiary amines without prior adaptation; the amines did not serve as a carbon source. Secondary and tertiary methylamines were better sources of nitrogen than the ethyl compounds. The Pichia species (119) produced excellent growth in 0.5 M trimethylamine (TMA) within 36 hr, and, following adaptation, grew in 1.0 M TMA; growth in trimethylamine oxide was weak to negligible. Strain variability was common in amine utilization by K. drosophilarum and Pichia species (86). The significant abundance of amine-utilizing yeasts in the Spartina rhizosphere suggests a role for these microorganisms in estuarine nitrogen regeneration processes.

Dr. Ahearn visited Dr. Meyers in March under auspices of the AIBS Visiting Biologists Program and the LSU Sea Grant Program. Two seminars were presented:

ROLE OF YEASTS AND YEAST-LIKE FUNGI
IN SPOILAGE OF FRANKFURTERS
AND OTHER COLD-STORED MEAT PRODUCTS

ROLE AND PHYSIOLOGY OF YEASTS
IN DECOMPOSITION OF OIL POLLUTANTS
IN FRESH AND MARINE WATERS

Drs. Meyers and Ahearn have been invited to participate in a symposium "Microbiological Aspects of Seawater Pollution" to be held in conjunction with the Society for Industrial Microbiology meeting at Kingston, Rhode Island, August 2-7, 1970. The title of their paper is "The Role of Yeasts in Decomposition of Oils in Marine Environments".

X Georgia State University, Department of Biology, 33 Gilmer Street, S.E., Atlanta, Georgia 30303. Communicated by D. G. Ahearn.

Recent Publications: Mycological studies in Barataria Bay, Louisiana, and biodegradation of oyster grass, Spartina alterniflora. Meyers, S. P., M. L. Nicholson, J. Rhee, P. Miles and D. G. Ahearn. Coastal Studies Bull. (5):111-124. 1970.

Abstracts: Physiology and ultrastructure of hydrocarbon-utilizing yeasts. D. G. Ahearn, W. E. Turner and R. R. Mohan. Bacteriol. Proc. G63 p 24.
Effects of oil pollution on populations of yeasts in fresh water. W. E. Turner and D. G. Ahearn. Bacteriol. Proc. G24 p 18.

The proceedings of the Symposium held in Plattsburgh in 1969 are now in final preparation. The literature citation has been changed from what was originally announced. The correct citation is: "Recent Trends in Yeast Research" (D. Ahearn, ed.). Spectrum, Monograph Series in Arts & Sciences. No. 1. Georgia State University (in press). Publication is expected by late summer or early fall 1970.

XI Division of Laboratories & Research, New York State Department of Health, New Scotland Avenue, Albany, New York 12201. Communicated by M. R. Edward.

As a continuation of our research on the ultrastructure and growth characteristics (especially the budding process) of pathogenic yeasts, detailed studies were made of Torulopsis glabrata and Cryptococcus neoformans. The following projects have been submitted for publication or

are in preparation.

1. Edwards, M. R. and Holt, S. C.: Surface structures in budding cells of Torulopsis glabrata. Proc. 27th Annual Meeting of the Electron Microscopy Society of America, 1969, p. 390-1.
2. Collins, D. N. and Edwards, M. R.: Filamentous forms of Blastomyces dermatitidis in mouse lung; light and electron microscopy. Sabouraudia, 1970, 7:237-40.
3. Brown, J. P. and Edwards, M. R.: Micromorphology of budding cells of Torulopsis glabrata. Submitted to Canadian Journal of Microbiology.
4. Brown, J. P.: Susceptibility of some yeasts to muralysis: a light and electron microscopic study. Submitted to Canadian Journal of Microbiology.
5. Collins, D. N., Oppenheim, I. A. and Edwards, M. R.: Cryptococcosis associated with systemic lupus erythematosus. Light and electron microscopic observations.
6. Edwards, M. R., Gordon, M. A., Ghiorse, W. C. and Brown, J. P.: Micromorphology of the budding process of Cryptococcus neoformans.
7. Edwards, M. R.: Experimental histoplasmosis in mouse liver. An electron and light microscopic study.
8. Edwards, M. R. and Collins, D. N.: Experimental blastomycosis in mice. Light and electron microscopic investigation.

XII Department of Biology, J. E. Purkyně University, Brno, Czechoslovakia. Communicated by Dr. A. Svoboda.

1) The following papers have been published since the Delft Symposium in June 1969:

Havelková, M.: The mechanism of regeneration of yeast protoplasts. VII. Electron microscopy of growing and regenerating protoplasts of Nadsonia elongata. Folia Biol. (Praha) 15:462-469, 1969.

Farkaš, V., A. Svoboda, Š. Bauer: Inhibitory effect of 2-deoxy-D-glucose on the formation of the cell wall in yeast protoplasts. J. Bacteriol. 98:744-748, 1969.

Nečas, O., M. Kopecká, J. Brichta: Interpretation of surface structures in frozen-etched protoplasts of yeasts. Exptl. Cell Res. 58:411-419, 1969.

2) Papers prepared for publication:

Farkaš, V., A. Svoboda, Š. Bauer: Glycoprotein secretion by yeast protoplasts. The effect of 2-deoxy-D-glucose and cycloheximide.

Secretion of mannan-protein into the growth medium was studied under conditions where protein synthesis was stopped by cycloheximide or polysaccharide synthesis was stopped by 2-deoxyglucose. Under these conditions 2-deoxyglucose did not interfere with protein synthesis or cycloheximide with the synthesis of mannan. Both agents together inhibited secretion of both mannan and protein. The results demonstrate that mannans as well as proteins can be released

from yeast protoplasts, but only when the synthesis of polysaccharides and proteins is not blocked and that there is some dependance between mannans and proteins during their transport through plasma membranes.

Havelková, M.: A Partial Blocking of Cell Wall Formation and Regeneration of Protoplasts of Schizosaccharomyces pombe and Nadsonia elongata.

The course of the cultivation of Schizosaccharomyces pombe and Nadsonia elongata protoplasts in media containing snail enzyme was studied by light and electron microscope. The protoplasts grew and an incomplete cell wall was being formed on their surface in the presence of snail enzyme. This cell wall was composed of very thin short fibrils masked by some amorphous substance. During the presence of snail enzyme no regeneration of the protoplasts took place. Transferring into medium free from snail enzyme led first to completion of the cell wall construction and only then to regeneration of the protoplasts. The blocking of the formation of complete cell wall was reversible and independent of the time of exposure of the protoplasts to snail enzyme. These experiments have shown that a complete cell wall represents an indispensable condition for the regeneration of protoplasts, i.e., for their division (S. pombe) or budding (N. elongata).

Kopecká, M., A. Svoboda and J. Brichta: Ultrastructural Picture of Shift-down in Yeast Cells.

Yeast cells of Saccharomyces cerevisiae and Schizosaccharomyces pombe from log phase of growth, cultivated in wort nutrient medium, show striking changes in their cell walls and in their proximity after shift-down into solutions of nonmetabolizable sugars alone (D-mannitol, L-rhamnose).

In the cytoplasm of these cells a greater number of vesicles appear, originating from the endoplasmic reticulum or Golgi apparatus. These vesicles are also near the cell surface and are distinctly observable inside the cell wall itself. Metabolic activity of these cells is suddenly decreased after transferring them from wort to non-metabolizable sugar solutions and the next cell division does not occur. The number of vesicles and their occurrence in the wall depends partially on the sugar concentration, time of exposure and the metabolic activity of the cells before shift-down.

Taking into account other indirect confirmations one can presume that after shift-down the processes leading to the formation of the cell wall are disconnected in some way. It is then possible to follow the single stages of the overall process (formation of vesicles, their transport to the cell surface and their incorporation into the wall) which probably takes place very rapidly in logarithmic cells.

XIII Karl-Marx-Universität, Klinik für Hautkrankheiten, Mykologische Abteilung, 701 Leipzig, Liebigstrasse 21, East Germany. Communicated by Christina Schönborn.

"Yeasts are able to reduce tellurite and to store tellurium. Earlier (Zbl. Bakt., I. Abt. Orig. 201 406 1966) reference was made to the possibility to utilize the variable ability to reduce tellurite by various kinds of yeast for diagnostic purposes. To suppress the accompanying bacterial flora on mycological nutrient media tellurite concentrations of 0.01 to 0.1% are customary. Investigations on tellurite resistence by yeasts, however, are lacking up to now.

We determined the growth of twelve different strains of yeast on corn meal agar with addition of 0.001 to a maximal of 0.18% potassium tellurite (K_2TeO_2). Test strains we used were Candida albicans, C. lipolytica, C. pelliculosa, C. guilliermondii, C. tropicalis, Torulopsis glabrata, Trichosporon cutaneum, Cryptococcus neoformans, Rhodotorula mucilaginosa, Sporobolomyces roseus, Saccharomyces cerevisiae and Sacch. fragilis.

The most sensitive organism was T. glabrata; this yeast was already strongly inhibited at a tellurite concentration of 0.03% in the nutrient medium. With Saccharomyces cerevisiae and Sacch. fragilis a very significant growth inhibition was observed with 0.08% tellurite. With 0.18% tellurite only C. lipolytica, C. guilliermondii, C. tropicalis, Cryptococcus neoformans and Rhodotorula mucilaginosa developed more or less normally. A complete suppression of yeast growth did not occur with any of the test species. When yeast colonies grown on corn meal agar with 0.18% potassium tellurite were transferred to tellurite free media it was shown that the cells of T. glabrata and S. cerevisiae had lost their viability. In further experiments it was tried to make use of the degree of tellurium storage inside the yeast cell as a measure of the enzymatic activity by photomicrography. With the aid of this method it was possible to demonstrate a decrease in reduction ability with C. albicans after the action of one or two units per ml of nystatin."

XIV Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki, Finland. Communicated by Prof. Heikki Suomalainen.

T. Nurminen, E. Oura and H. Suomalainen, The enzymic composition of the isolated cell wall and plasma membrane of baker's yeast. Biochem. J. 116 (1970) 61-69.

A study was made of the enzyme content of the isolated cell walls and of a plasma-membrane preparation obtained by centrifugation after enzymic digestion of the cell walls of baker's yeast. The isolated cell walls showed no hexokinase, alkaline phosphatase, esterase or NADH oxidase activity. It was concluded that these enzymes exist only in the interior of the cell. Further, only a negligible activity of deamidase was detectable in the cell walls. Noticeable amounts of saccharase, phosphatases hydrolysing p-nitrophenyl phosphate, ATP, ADP, thiamin pyrophosphate and PP_i, with optimum activity at pH 3-4, and an activity of Mg^{2+}-dependent adenosine triphosphatase at neutral pH, were found in the isolated cell walls. During enzymic digestion, the other activities appearing in the cell walls were mostly released into the medium, but the bulk of the Mg^{2+}-dependent adenosine triphosphatase remained in the plasma-membrane preparation.

Accordingly, it may be assumed that the enzymes released into the medium during digestion are located in the cell wall outside the plasma membrane, whereas the Mg^{2+}-dependent adenosine triphosphatase is an enzyme of the plasma membrane. This enzyme differs from the phosphatases with pH optima in the range pH 3-4 with regard to location, pH optimum, substrate specificity and different requirement of activators.

Heikki Suomalainen, Trends in physiology and biochemistry of yeasts. Paper presented at the Third International Symposium on Yeasts, Delft 1969. Symp. Proc. Antonie van Leeuwenhoek. 35 (1969) 83-111.

Erkki Oura, Die biochemische Zusammensetzung der Hefe in Abhängigkeit von der Aerobität des Mediums. Paper presented at II Internationales Symposium der Gärungsindustrie, Leipzig 1968, S. 437.

Erkki Oura, Biochemische Unterschiede bei anaerob und aerob kultivierten Hefen. Paper presented at Wissenschaftliches Kolloquium des Instituts für Gärungschemie und -technologie der Humboldt-Universität, Berlin 1967. Die Lebensmittel-Industrie 15 (1968) 150.

Heikki Suomalainen and Erkki Oura, Yeast nutrition and solute uptake. The Yeasts, Chapter of volume II. Ed. by A. H. Rose and J. S. Harrison, Academic Press, London. (In press).

A large review is given on yeast nutrients, solute uptake and outflow of compounds. 57 pages of text and 14 pages of references.

T. Nurminen and H. Suomalainen, The lipid composition of cell wall and plasma membrane of baker's yeast. Chem. Phys. Lipids. (In press).

The fatty acids and phospholipids in the isolated cell envelope, and in plasma membrane preparation obtained from the cell envelopes by enzymatic digestion, have been compared with those of the whole cells of baker's yeast. Whole cells and cell envelopes were found to have similar total fatty acid compositions. The major fatty acids were $C_{16:1}$, $C_{18:1}$ and C_{16}. $C_{16:1}$ acid occurred in most abundance in the cell envelopes, but $C_{18:1}$ predominated in the plasma membrane fraction. The main phospholipid components in whole cells and cell envelopes were phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine. The whole cells contained phosphatidylcholine in a larger proportion than did the cell envelopes, which were in turn richer in phosphatidylinositol and phosphatidylserine. The principal fatty acids in neutral lipids, and in phospholipids of the whole cells and of the cell envelopes, were $C_{16:1}$, $C_{18:1}$ and C_{16}. Phosphatidylinositol and phosphatidylserine contained proportionally less $C_{16:1}$ acid and more C_{16} acid than the other phospholipids. During enzymatic digestion, the original distribution of phospholipids in the cell envelopes remained almost unchanged, which indicates that the phospholipid composition of the plasma membrane corresponds in the main to that of the isolated cell envelopes.

Blanka Ries and Heikki Suomalainen, Interaction of phenylmercuric acetate with some enzymes and intact cells of baker's yeast. Suomen Kemistilehti. (In press).

The effects of phenylmercuric acetate (PMA) on some enzymes of baker's yeast and its penetration into intact yeast cells are investigated. Acid and alkaline phosphatase, the NADH oxidase system, hexokinase, pyruvic acid decarboxylase and fermentation of glucose were found to be inhibited by PMA. The strongest inhibition was usually achieved with a concentration of 10^{-3} M. The inhibition of acid phosphatase could be prevented by addition of cysteine. The external adenosine triphosphatase with optimum activity at pH 3.5 was not inhibited by PMA at the concentrations used. When yeast cells preincubated with PMA were disintegrated and fractionated, the bulk of their mercury content was found in the cell interior fraction but part of mercury was bound to the cell envelopes. The penetration of PMA into intact yeast cells was found to be quite a rapid process.

Erkki Oura, Timo Nurminen and Heikki Suomalainen, Release of enzymes and cofactors during digestion of the yeast cell wall. Paper presented at the 2nd International Symposium on Yeast Protoplasts, Brno 1968.

Timo Nurminen and Heikki Suomalainen, The lipolytic activities of the isolated cell envelope fractions of baker's yeast. The Biochemical Journal. (In press).

The existence of phospholipase and lipase activities in the isolated cell envelopes of baker's yeast is demonstrated. The content of phospholipase was found to be markedly higher than that of lipase.

After partial enzymic digestion of the isolated cell envelopes, the bulk of the lipolytic activities was recovered in the sedimentable preparations, which consisted of the fragments of the plasma membrane.

During repeated washings, the lipase was completely released from the cell envelopes, as were also the bulk of the lipid components and most of the Mg^{2+}-dependent adenosine triphosphatase, an enzyme connected with the plasma membrane. The phospholipase was more firmly bound to the preparation but not so firmly as the external saccharase. These results indicate that the lipolytic enzymes found in the cell envelopes are mostly located in the plasma membrane.

Erkki Oura and Heikki Suomalainen, Cytochrome contents in baker's and brewer's yeasts. Paper to be presented at the 8th International Congress of Biochemistry, Lucerne, September 1970.

Timo Nurminen and Heikki Suomalainen, The lipolytic enzymes of the cell envelope of Saccharomyces cerevisiae. Paper to be presented at the X International Congress for Microbiology, Mexico, D. F., August 1970.

Erkki Oura und Heikki Suomalainen, Das Verhalten der Bäckerhefe bei verschiedenen Belüftungsstarken in Laboratoriumsversuchen. Paper to be presented at the Fermentationskonferenz der Alkoholindustrie, Budapest, October 1970.

XV Instituto Nacional de Investigaciones Agronómicas. Sección de Bioquímica, Madrid, Spain. Communicated by J. Santa María.

The following are abstracts of five papers which have been submitted for publication in Anales del Instituto Nacional de Investigaciones Agronómicas (Madrid) 1970.

1 - "Segregación anormal del "mating type" en Saccharomyces" ("Unusual segregation of the mating type in Saccharomyces"). J. Santa María, D. Vidal.

A spontaneous mutant of Sacch. onubensis SBY 2535, provides a very high percentage of viable ascospores from the four-spored asci dissected; two of the four single-spore cultures arising from each of these asci, correspond to diploid cultures, which sporulate easily and the other two to haploid strains of alpha type. This behavior is constant; i.e., the diploid segregants give always rise to two diploid and two alpha haploid segregants. This type of segregation is designated as Hp.

Diploid hybrids were prepared from crosses between: 1) Alpha segregant of Sacch. norbensis SBY 2535 and haploid segregant mating type a (Sacch. cerevisiae X 2180 - 1A a) = Diploid D 17. 2) Ascospores from Sacch. norbensis SBY 2535 and 21) ascospores from S. onubensis SBY 2602 (homothallic) = Diploid D 19; 22) haploid segregant a I) S. gaditensis SBY 1668i-a = Diploid D 31, II) S. cerevisiae X 2180 - 1A a = Diploid D 20; 23) haploid segregant alpha (S. cerevisiae X 2180 - 1B alpha) = Diploid D 21.

Genetic analyses of the segregation of the mating type (alpha/a) were carried out only on asci in which all four spores survive. In D 17 the segregation is the normal one 2:2. In D 19 the segregation is complex. In most cases, 3 of the 4 spores from an ascus, give diploid cultures and the other one yields an haploid culture of the mating type alpha. The diploid cultures are homothallic (Ho) or have the segregation Hp. In D 31, new diploid cultures with segregation Hp have been isolated as well as homothallic and haploid segregants alpha and a. In D 20, together with the haploid segregants a and alpha and the diploid segregants Hp and Ho, a new type of sexual segregation appeared, which is the same as the segregation Hp, but in this case the haploid segregant is always of the mating type a; this type of segregation is designed as Hq. In D 21 are obtained the diploid segregants Hp, Hq and Ho and the haploid ones a and alpha. Moreover, the haploid segregant of mating type a, diploidizes and gives rise to a culture which the segregation a/alpha 2:2.

Therefore, it appears that the peculiar segregation Hp of the mating type of Sacch. norbensis SBY 2535, is transmitted through spores but not through haploid segregants.

This result is discussed, as well as the complex recombination found in the hybrids and it is suggested the existence of a gene Hp (or Hq) for diploidization. During the replication of DNA in the meiotic process a copy error determines the formation of two spores Hp

(or Hq) and two spores hp (or hq); the first ones generate the diploid cultures and the second ones the segregants of mating type alpha.

The activity of the gene Hp (or Hq) would be similar to the gene D of homothallism and it is inferred of the results obtained that both are different expressions of a complex locus of diploidization.

In addition, it is worthwhile to mention:

1) In the hybrids D 17, D 19, D 20 and D 21, the four segregants of an ascus are melibiose + and only two sucrose +. It is possible that the assimilation of melibiose is controlled by polymeric genes.

2) The hybrids D 17, D 19, D 21 and D 31 are able to assimilate galactose, but none of the parental strains do so. The action of complementary genes is possible.

2 - "Regulación del metabolismo energético en levaduras" ("Regulation of energetic metabolisms in yeasts"). J. Santa María, A. Ruiz de Assín and Ana Guillén.

In the classification of yeasts are included different glucose fermenting ascosporogenous and asporogenous species, which are able to assimilate maltose without fermenting it. With three of those species, Sacch. fructuum, Dek. lactis and Dek. drosophilarum, it is found that the fermentation of maltose under standard conditions is effectively negative, but under anaerobiosis is positive.

With Sacch. fructuum it is found: 1) Alpha-glucosidase is present in cell free extracts. 2) After incubation with maltose under aerobic or anaerobic conditions, the residual sugar in the medium is exclusively maltose. 3) The maltose uptake is extremely slow, as compared to glucose. 4) Respiratory deficiency mutants were obtained by growing the wild-type in a liquid medium containing acriflavine. The "petit mutants" are able to ferment maltose under aerobic conditions. 5) The amount of maltose metabolized is the same under aerobic as anaerobic conditions.

It seems that the above evidence leads to the conclusion that the "Pasteur effect" is responsible for the inability of fermenting maltose under aerobic conditions by some yeasts.

The slowness of transport of maltose across the cell membrane establishes, in aerobiosis, the conditions suitable for the inhibition of the fermentation pathway by respiration, whereas in anaerobiosis a fermentation is achieved.

Therefore, this is a typical example of regulation of carbohydrate metabolism, in which only the mechanism of maltose transport is involved and not the inhibition of an enzyme.

3 - "Significación taxonómica de las propiedades fisiológicas de las especies incluídas en el género Kluyveromyces" ("Taxonomic significance of the physiological properties of the species included in the genus Kluyveromyces"). J. Santa María, Consuelo Sánchez.

A comparative study of the different physiological properties of Sacch. dobzhanskii, Sacch. lactis, Sacch. drosophilarum, Sacch. fragilis, Sacch. phaseolosporus and Sacch. wickerhamii, shows: 1) There is a close affinity amongst these 6 yeast species. 2) These six species are clearly differentiated from other Saccharomyces. 3) There is very little relationship between these six species and Kluyveromyces

polysporus (typical species of the genus Kluyveromyces).

Therefore, it seems justified to include Sacch. dobzhanskii, Sacch. lactis, Sacch. drosophilarum, Sacch. fragilis, Sacch. phaseleosporus and Sacch. wickerhamii in a genus distinct from both Saccharomyces and Kluyveromyces, which must be Dekkeromyces.

We have found that in Sacch. fragilis SBY 1769, the ascus does not rupture at maturity, and that Sacch. lactis, several strains of Sacch. fragilis, as well as its variety bulgaricus form spherical spores.

Consequently, the diagnosis of the genus is as follows: Dekkeromyces (Wickerham et Burton 1956, nomen nudum), Santa María 1970. Budding cells; asci may or may not rupture when mature; spores crescentiform, reniform, spheroidal or ovoid; spores retain weakly or not at all the ability to retain malachite green; requirement for nicotinic acid; ability to grow in presence of 1.0 µg/ml of actidione; utilization of L-hydroxyproline or L-lysine as the only source of nitrogen; utilization of a mixture of aminoacids as the only source of carbon, nitrogen and energy. Type species is Dekkeromyces lactis.

The fact that the spores retain hardly, or not at all, malachite green in the spore staining, is undoubtedly related to the chemical composition of the spores.

From the ecological point of view must be added to the habitats quoted in the literature as typical of these species: insects, frass, milk and milk products, etc., fruits such as the date, from which strains of the species fragilis can be very easily isolated.

As regards Sacch. fragilis var. bulgaricus, a clear separation can be seen in the physiological properties of the three strains described as belonging to the variety (Santa María, 1956) and the 9 strains which are typical representatives of the species fragilis isolated from such varied substrates as yogurt, cheese and dates, all of which confirms that it must be regarded as a variety within the species.

4 - "Pichia castillae, nov. spec., aislada de insectos" ("Pichia castillae, nov. spec., isolated from insects"). J. Santa María, Concepción García.

A new homothallic species, Pichia castillae, has been described. The species was recovered from frass of insects infesting Gymnocladus canadiensis.

5 - "Saccharomyces gaditensis y Saccharomyces cordubensis, dos nuevas especies de levaduras de "flor" ("Sacch. gaditensis and Sacch. cordubensis, two new species of "flor" yeasts"). J. Santa María.

Two new species of "flor" yeasts have been described. The latin diagnosis of Sacch. beticus Marcilla, is also included.

XVI Department of Food Science and Technology, University of California, Davis, Calif. 95616. Communicated by H. J. Phaff.

Dr. Mohamed S. Foda has completed his doctoral dissertation under the guidance of Prof. H. J. Phaff. A summary of the thesis "Physiology and biochemistry of amylose formation by Cryptococcus laurentii" follows below. Dr. Foda has returned to Egypt, where his current address is Microbial Chemistry Unit, National Research Center, Dukki, Giza, Egypt.

SUMMARY

Physiological and biochemical studies on amylose synthesis by Cryptococcus laurentii were carried out. These studies have revealed the following:

1. Amylose formation is not significantly affected by carbon source, nitrogen source or temperature of growth.

2. Amylose is formed only when the pH of the medium drops to values near 3 or lower. pH shift-down experiments showed that the organism can form amylose within a few hours in a complete medium after the shift. The organism was also able to form starch when suspended in glucose-1-phosphate solution at pH 2.5. When washed cells were suspended in media without nitrogen source in the presence of buffers in a pH range of 1.2-6, the cells formed starch at pH 2-3. At pH 3.5 or higher glycogen-like compounds accumulated in the cells.

3. In non-buffered medium with ammonium sulfate as nitrogen source the organism started to form starch when the pH dropped to less than 3.0. In the meantime the growth slowed down considerably and nucleic compounds were released into the medium.

4. Although the organism forms pure amylose polymers, at later stages the cells die releasing lipid compounds which combine with amylose, causing the formation of an insoluble complex which stains purple color with iodine resembling that formed with amylopectin. Pure amylose could be recovered from the complex by treatment with lipid solvents.

5. Capsuleless mutants were isolated and these were found to produce starch under conditions similar to those required by wild type. This observation, in addition to the constant composition of capsular polysaccharide for cells grown at different pH values, does not support a direct relation between possession of a capsule and the ability of the organism to produce starch.

6. Other mutants were isolated which produce very little or no starch in the medium under conditions of low pH. These starch-deficient mutants were also poor glycogen producers at high pH (as compared to the wild type). These results suggest a direct relation between glycogen and amylose synthesis.

7. A phosphorylase with high specific activity has been detected in homogenized cells of Cryptococcus laurentii. This enzyme can synthesize starch-like polymers upon incubation with glucose-1-phosphate in a pH range of 5.6-6.1. This enzyme, which is extremely labile, could be stabilized by storage in 0.1 M β-glycerophosphate solution containing 0.1 M sodium fluoride and 0.03 M 2-mercaptoethanol at pH 8-8.2. Partial purification of the enzyme could be accomplished by glycogen complexing with subsequent adsorption of the protein on hydroxy apatite followed by elution. The enzyme has an apparent Km 1.6×10^{-2} M for glucose-1-phosphate and about 0.3 mg/ml for glycogen. The pH optimum is 5.9-6.2. The activity is not stimulated by AMP. The enzyme is sensitive to various anions and heat. UDPG caused little or no inhibition, at least in concentrations up to 10^{-3} M.

8. Another enzyme, UDPG glycogen transglucosylase, could be detected in Cryptococcus laurentii. The enzyme is extremely sensitive and fresh cells had to be used throughout the study of this enzyme. Its pH optimum is about 5.8-6.0. The incorporation of glucose-1-phosphate, glucose-6-phosphate, and sodium fluoride in the reaction mixture improved the activity considerably. The enzyme has an apparent Km of about 5.9×10^{-4} M for UDPG.

9. Examination of some of the starch-deficient mutants revealed that the level of glycogen phosphorylase is reduced to about 25% of its original level in the wild type.

Although a marked reduction in the level of UDPG glycogen transglucosylase was also noted, the extreme instability of this enzyme makes it difficult to judge whether the difference represents a true difference in vivo.

10. The leakage of compounds absorbing strongly at 260 nm concomitant with starch release into the medium indicates that the low pH injures the cell membrane. The pH of about 3.0 probably marks the lowest value which the membrane of this organism can tolerate before undergoing substantial damage. The internal pH of the cell accordingly will drop but not to the same value of the medium because of the strong buffering capacity of cell proteins and solutes. Such a shift in internal pH may inhibit the action of branching enzyme resulting in the formation of non-branched amylose polymers. Although the role of glycogen phosphorylase is generally assumed to be degradative, low pH favors the reaction in the direction of synthesis through its differential effects on the ionic forms of glucose-1-phosphate and orthophosphoric acid. The glycogen phosphorylase of Cryptococcus laurentii has an unusually high specific activity as compared to those of other microorganisms. This property may support its proposed role in synthesis of amylose polymers under favorable conditions.

We are continuing our work on the base composition and homology of yeast DNA. Coworkers in this project are Dr. A. Martini, Miss Sally A. Meyer and Mr. Chester W. Price. Dr. Martini has worked out a much improved method for extraction of highly polymerized DNA from yeasts which previously yielded little or no spoolable DNA. Full details will be given in the fall News Letter. Publications:

J. W. Fell, A. C. Hatzell, I. L. Hunter and H. J. Phaff. Leucosporidium, gen. n., the heterobasidiomycetous stage of several yeasts of the genus Candida. Antonie van Leeuwenhoek 35, 433-462, 1969.

M. S. Foda and H. J. Phaff. Starch Synthesis by Cryptococcus laurentii. Antonie van Leeuwenhoek 35, Supplement: Yeast Symposium 1969.

Ahmed T. H. Abd-El-Al and H. J. Phaff. Purification and properties of endo-β-glucanase in the yeast Hanseniaspora valbyensis. Canadian J. of Micro. 15, 697-701, 1969.

R. Storck, C. J. Alexopoulos, and H. J. Phaff. Nucleotide Composition of Deoxyribonucleic Acid of Some Species of Cryptococcus, Rhodotorula, and Sporobolomyces, Journal of Bacteriology 98, 1069-1072, 1969.

C. L. Atkin, J. B. Neilands, and H. J. Phaff. Rhodotorulic acid. II. Production of hydroxamic acids by yeasts: Rhodotorulic acid from species of Leucosporidium, Rhodosporidium, Rhodotorula, Sporidiobolus, and Sporobolomyces, and a new alanine-containing ferrichrome from Cryptococcus melibiosum. Journal of Bacteriol. (in press).

XVII The University of New South Wales, School of Biological Technology, Box 1, Post Office, Kensington, 2033. Communicated by Pamela A. D. Rickard.

The following are abstracts of papers recently presented to the Australian Society for Microbiology.

QUANTITATIVE STUDIES OF CYTOCHROME AND MITOCHONDRIAL MEMBRANE DEVELOPMENT IN TRANSIENT AND STEADY STATE CULTURES OF SACCHAROMYCES CEREVISIAE

By. M. Ferdouse, F. J. Moss, Pamela A. D. Rickard and H. W. Blanch.

The data outlined below provide evidence for asynchronous development of mitochondrial cristae and cytochromes.

S. cerevisiae was cultured in steady state (turbidostat) continuous culture with 112 mM glucose and 40 μM dissolved oxygen; cytochrome concentration, mitochondrial volume and cristal area were all low, but measurable.

A step change was made in the glucose feed concentration such that its complete utilization was ensured and its concentration in the culture declined to zero. Dissolved oxygen was maintained at 40 μM. The decrease in glucose resulted in parallel increases in mitochondrial volume and total cristal area per unit cell volume, with disproportionate increase in cytochrome concentration. Initial decreases in the concentrations of A-, B- and C-type cytochromes per unit cristal area were followed by increases in these ratios; in the case of A-type cytochrome (but not B- and C-type) the ratio finally exceeded the original steady state value.

Although they became more clearly defined with time, at no stage was there any statistically significant variation in the cristal area per unit mitochondrial volume.

CYTOCHROME AND ADENOSINE PHOSPHATE LEVELS IN TRANSIENT AND STEADY STATE CULTURES OF SACCHAROMYCES CEREVISIAE

By M. Akbar, Pamela A. D. Rickard and F. J. Moss

Whether induced by a high glucose concentration or anaerobic conditions, domination of ethanolic fermentation over respiration was shown to be associated with elevated concentrations of ATP, ADP and AMP.

When a step change from 3 μM to zero dissolved oxygen was made, without change in glucose concentration from 0.13 mM, a 15 hr. adaptation period was noted before a significant increase in ethanolic fermentation occurred. During this period AMP and glucose-6-phosphate increased at the expense of ADP, whilst ATP and A-type cytochrome remained constant. After 15 hr. the time course of increase in ATP concentration reflected that of decrease in A-type cytochrome so clearly that a tight coupling of the two processes was indicated.

XVIII University of California, Berkeley, Calif., Dept. of Genetics. Communicated by S. Fogel and coworkers.

Below follow several reports on projects going on in this laboratory.

Unsaturated fatty acid (UFA) auxotrophs of yeast are being employed to ascertain the role of UFA in biomembrane structure, function, and biosynthesis. Fatty acids (FA) differing in chain length, degree of unsaturation, branching, substitution groups, and steric configuration are tested for efficiency in restoring wild type growth. The effect of varying the concentration of the supplemented FA and also the carbon source (e.g., 2% glucose vs. 1% sodium lactate) is being explored.

Five FA were reported as growth-supporting: myristoleic, palmitoleic, oleic, linoleic, and linolenic. More recently, growth was attained on gamma-linoleate ($18:3\Delta^{6,9,12}$ all cis) and on ricinoleate ($18:1\Delta^{9}$ cis 12 hydroxy). All have one thing in common. They have a cis double-bond between the 9th and 10th carbon atoms. Currently, a few FA which appear to give intermediate growth are being analyzed.

The value of an UFA auxotroph is considerable. Having membranes composed of one UFA component is a significant aid to evaluating physical studies such as ESR and NMR. The common denominator of all growth-supporting FA should provide a clue to the role of FA in biomembranes. The observed specificity of the FA requirement seems to imply that the hydrophobic portions of the FA elements are doing more than providing hydrocarbon-rich areas, with the degree of unsaturation being important merely for phase transitions or fluidity. Thus, the steric conformation, position of unsaturation, and degree of polarity of the FA molecule may be more directly related to proper membrane structure and function; for example, in determining the conformation of membrane-associated proteins and consequently influencing protein-protein interactions. Membrane growth and function may really depend on having a "proper fitting" FA available.

Bernadine J. Wisnieski
Alec D. Keith

Nitroxide-free radicals have been incorporated into phospholipids and sterol esters by yeast in our laboratory. Spin-labeling is a powerful technique which allows many types of information to be obtained about biological membrane structure and the physical properties of several different kinds of molecular species. The membranes of yeast offer the opportunity to study a specific type of membrane in relatively good enrichment under in vivo conditions. It is possible to obtain information: The defined type of molecular motion of a specific lipid or protein element within a biological membrane; the polarity of the local environment to the spin-label portion of the molecule; the physical state of binding of spin-label lipid elements; and many aspects of membrane permeability processes. These studies are in progress at the present.

Dick Kiyomoto, Rolf Mehlhorn and Alec Keith

Experiments have been carried out with the object of obtaining fully deuterated yeast cells. So far we have obtained growth on the three following sets of conditions. First, 99.8% D_2O and 2% acetic acid. Second, water and 2% of 99% deuterated acetate. Third, a combination effort of 99.8% D_2O, 2% deuterated sodium acetate and .08% sodium acetate. Deuteration must be carried out by gradually increasing the percentage deuterium in the medium. Our procedure is to start with 50% D_2O and sequentially decrease the amount of water present. An increase on deuterated acetate is carried out in the same way. Growth

is very slow and is on the order of 16 hours per division. We believe it is possible to obtain fully deuterated yeast cells. In an enrichment culture we have obtained growth of an aspergillus strain on 99.8% D_2O and 99.5% deuterated acetate. We intend to carry out studies dealing with mutagenesis and to use deuterated lipids and deuterated proteins in physical studies dealing with membranes.

Julie Welch, Alec D. Keith

Fogel, Seymour, Donald D. Hurst, and Robert K. Mortimer, Brooklyn College of the City University of New York, Brooklyn, and University of California, Berkeley: Multisite conversion in yeast. -- Meiotic gene conversion was studied in unselected tetrads derived from four diploids. These represent 1) three and four-point crosses involving the arg-4 (argininosuccinate lyase) locus - 4171 tetrads; 2) crosses involving heterozygous sites in the adjacent though independently segregating gene pairs trp-1, gal-3, and SUC-1, MAL-1 - 1569 tetrads. In the arg-4 multipoint system conversional events embracing one, two, three, and four sites as well as reciprocal recombinants between mutant alleles were detected. However, the most common events (72%) were represented by the category of symmetrical co-conversions in which two, three or four sites on the same genetic strand were converted simultaneously. On the basis of an X-ray genetic fine structure map we estimate that the modal length of the informational segment transferred from one parental strand to the other during conversion is of the order of some hundreds of nucleotides. This view is further supported by the finding that symmetrical co-conversion may extend to include heterozygous sites in adjacent cistrons encoding different polypeptides. Thus the terminals of the converted interval need not correspond with the initiation and termination sites of single genetic units. -- Supported by PHS Grant RG-06979 and AEC.

Hurst, Donald D., Seymour Fogel, and Robert K. Mortimer, Brooklyn College of the City University of New York, Brooklyn, and University of California, Berkeley: Conversion-associated recombination in yeast. -- In a sample of approximately 10,000 unselected tetrads, gene conversion and associated outside marker recombination were studied at five different loci: his-2, SUP-6, arg-4, thr-3, and his-1. A total of 832 conversions were detected. Of these 402 (48.3%) were recombined for the immediately adjacent outside markers. This relationship of 2 conversions/crossover is independent of the genetic map length between the flanking markers over the range from less than 1 (intracistronic) to 20 centimorgans. In crosses involving 3 or 4 heterozygous sites within arg-4, 14 of 22 conversions for centrally located sites were reciprocally exchanged for the flanking sites situated within the same cistron. These findings suggest that all meiotic recombination in yeast is accompanied by a conversional event between the reciprocally recombined markers. On the assumption that the converted segment is approximately 1000 nucleotides in length, that the average frequency of conversion per meiosis is 1%, and that for each crossover (50 map units) there are two conversions, then conversion-associated exchange is sufficient to account for the total genetic length

of the yeast genome. -- Supported by PHS Grant RG-06979 and AEC.

IX Institute für Biophysik, Freie Universität, Habelschwerdter Allee 30, Berlin 33, Germany. Communicated by Uwe Reichert and Magrit Winter.

The effects of different doses of a gua-1 mutant in polyploid Saccharomyces cells.

In the course of our investigations on gene dosage effects in Saccharomyces, we have studied the influence of distinct wild type (+) doses against a gua-1 mutant (o) on exponential growth rate, the affected enzyme (GMP synthetase = GMP-S) and its precursor in the conversion of inosinic to guanylic acid (IMP dehydrogenase = IMP-DH). The gua-1 mutant, induced in a haploid prototroph by treatment with N-methyl-N'-nitro-N-nitroso-guanidine, is not able to multiply in media without guanine or containing guanine in the presence of excessive adenine. Using this mutant and the corresponding wild type, tri- and tetraploid strains, most isogenous and homozygous in genetic background, were prepared as described for diverse ade2 series in U.R.'s Thesis (Freie Univ., Berlin 1968, to be published). Growth rates were calculated by recording the optical density of liquid cultures in media unsuitable for the pure mutant. GMP-S activities were estimated in dialyzed crude extracts of cells, exponentially grown in a yeast extract-peptone-medium plus adenine, following B. MAGASANIK in "Methods in Enzymology" (1963, Vol. VI, p. 111-114) in principle, but including an ATP-regenerating system (phosphoenolpyruvate + pyruvate kinase), since ATP is very quickly consumed by other activities of the cell extracts. At pH 8.8 and 42°C specific wild type activities of 7.5 mU were measured by using glutamine as amino-N-donor (ammonium sulphate is less effective). The same crude extracts served for estimating IMP-DH activities, nearly according to MAGASANIK (ibid., p. 107-111). Specific wild type activities were 1.4 mU at pH 8.4 and 30°C.

genotype	+ dosage	wild type related activities of GMP-S	wild type related activities of IMP-DH	relative growth rate
++++,+++	1	1	1	1
+++o	0.75	0.76	1.8	0.95
++o	0.67	0.66	1.9	0.96
++oo	0.50	0.50	2.8	0.90
+oo	0.33	0.33	2.8	0.74
+ooo	0.25	0.30	3.2	0.63
oooo,ooo	0	(o)	---	0

As becomes evident from the table there is a linear relationship between wild type dosage and GMP-S activity, whereas IMP-DH is derepressed successively. The relative response of growth rate does not alter with diverse media, causing different doubling times in homozygous wild type. As was shown by measuring wild type activities of cells grown in synthetic media with and without guanine, GMP-S is produced constitutively, whereas IMP-DH is repressed below a 50% level. Further details will be communicated in Molec. Gen. Genetics.

XX Biological Laboratories, National Women's University, Nara 630, Japan. Communicated by S. Nagai.

Production of respiration-deficient (petite) mutants by two carcinogenic compounds, 4-nitroquinoline 1-oxide (4NQO) and nitrosoguanidine (NTG) and also the influence of nutritional deficiency of the media have been investigated. The carcinogens produced petites in remarkably high frequencies, sometimes 100 percent, in Saccharomyces cerevisiae (ref. 1), but produced some intermediate, unstable, and reversible forms of respiratory deficiency when applied to S. logos and S. rosei (ref. 2). Two strains of S. cerevisiae var. ellipsoideus gave rise to 100 percent petites when they were grown, without addition of any particular mutagen, in Schopfer's medium (glucose, 3%; asparagine, 0.1%; KH_2PO_4, 0.15%; $MgSO_4 \cdot 7H_2O$, 0.05%). Addition of yeast extract, pantothenate, and biotin, respectively, to this medium completely reversed the production of petites (ref. 3). The petites also appeared in high frequencies when a few other strains were grown in high dilutions (x50, 100, 200, and 400) on Lindegren's medium containing yeast extract and peptone in abundance (unpublished result).

Publications:

1. Production of respiration-deficient mutants in yeast by a carcinogen, 4-nitroquinoline 1-oxide. Mutation Res. 7(1969):333-337.
2. Unstable and reversible forms of hereditary respiration deficiency in Saccharomyces logos and S. rosei. Antonie van Leeuwenhoek, Supplement Yeast Symposium (1969), C 1-2.
3. High-frequency production of respiratory mutants in yeast under nutritional deficiencies. Mutation Res. 8(1969):557-564.

XXI Groupe Euratom de L'Université de Louvain, Laboratoire D'Enzymologie, de Croylaan 46, 3030 Heverlee, Belgium. Communicated by A. Goffeau.

Below follow the abstracts of two papers which will be published soon. This work is the result of a collaboration between the Laboratory of Génétique Biochimique of Dr. Heslot, INA, Paris and our Laboratory of Enzymology, University of Louvain, Belgium.

Respiratory metabolism of a petite-negative yeast Schizosaccharomyces pombe 972h$^-$.

H. HESLOT, A. GOFFEAU and C. LOUIS

Abstract:

The respiratory metabolism of Schizosaccharomyces pombe 972h$^-$, a haploid, "petite negative" fission yeast, has been studied. The only non-fermentable substrate on which aerobic growth could be obtained was glycerol. Limited growth was observed under anaerobic conditions. Addition of 0.3% KNO_3 to a rich medium partially relieves the oxygen-requirement. Tampered repression of glucose respiration was observed. The properties of a mitochondrial fraction were studied. Several substrates such as ethanol,

α-ketoglutarate, malate, pyruvate were not oxidized. Insensitivity to rotenone of NADH oxidation by a mitochondrial fraction, suggests the absence of site I phosphorylation. Site II and site III are present. The use of several mitochondrial inhibitors permits the conclusion that the requirement of oxygen for growth is not related to the functioning of the respiratory electron transport chain, to the formation of respiratory energy or to the functioning of the mitochondrial protein synthesis system. It was concluded that the apparent correlations between the non-viability of "vegetative petite" mutants, the absence of CRABTREE effect and the incapacity to grow under anaerobic condition, are not strictly valid under our experimental conditions.

Segregational respiratory-deficient mutants of a petite-negative yeast Schizosaccharomyces pombe 972h$^-$.

H. HESLOT, C. LOUIS and A. GOFFEAU

Abstract:

No viable respiratory-deficient mutants of Schizosaccharomyces pombe 972h$^-$ could be obtained by acriflavine and ethidium bromide treatments. These mutagens induce 15 to 70% of microcolonies that, after a growth-lag of a few days, further develop into normal respiratory-competent colonies. These results suggest that lethal, unstable vegetative petites were induced.

Segregational respiratory-deficient mutants resistant to cobalt sulphate were isolated. Some of them are deficient in cytochrome a + a_3 and do not respire. The morphology of their mitochondrial membranes is modified: either the cristae disappear or they show aberrant concentrical or tubular structures. Segregational mutants resistant to the respiratory inhibitors: dinitrophenol and decamethylene diguanidine were obtained. Their mitochondrial structure or functions did not seem to be modified.

A segregational mutant resistant to benzimidazole does not grow on glycerol although growth on glucose and respiration does not seem to be affected.

XXII Department of Genetics, University of California, Davis, Calif. 95616. Communicated by S. R. Snow.

Since the last communication from this laboratory, three students have completed their dissertations. Miss Beverly Reno submitted a Master's thesis titled "Genetic Studies of Radiation Sensitive Mutants of Saccharomyces cerevisiae". Jonathan C. Kuhn submitted a Ph.D. dissertation titled "Genetic and Biochemical Studies of Acid Phosphatase Mutants in Saccharomyces cerevisiae", and Christopher T. Korch submitted his Ph.D. dissertation titled "Genetical and Biochemical Studies of Complementation in the First Gene for Histidine Biosynthesis in Saccharomyces cerevisiae". Summaries from these dissertations are reproduced here.

Reno, Beverly D.: Genetic Studies of Radiation Sensitive Mutants of Saccharomyces cerevisiae. Two MMS-sensitive strains, M-1 and M-19, and one X-Ray sensitive strain, XS-3, were isolated. These haploid mutants were tested for their cross sensitivity to MMS, X-rays, UV light and nitrous acid. The sensitive allele, mms-1, of the mutant M-1 was recessive, not centromere linked and appeared to be linked to markers on chromosome V. In a diploid homozygous for the mms-1 allele, sporulation was prevented. Mutants M-19 and XS-2 were not further studied.

The induction of intra- and intergenic recombination by treatment with MMS, X-irradition and UV irradiation in the diploid strain X1599, homozygous

for the mms-1 allele, was significantly reduced. Photoreactivation of UV-induced lethality and gene conversion in strain Z1599 is comparable to that of the wild type strain. These characteristics of the mms-1 allele are discussed with reference to mutants of yeast, bacteria and Ustilago.

The effect of photoreactivation upon UV-induced gene conversion in six uvs diploid strains made by Snow (1967 and 1968) was tested. Photoreactivation can reverse gene conversion in these UV-sensitive strains, and therefore, pyrimidine dimers must be important in the induction of gene conversion.

The effect of liquid holding in the dark after UV irradiation was determined for six uvs diploid strains made by Snow (1967 and 1968). With the exception of the diploids homozygous for the alleles uvs-4 and uvs-5, liquid holding increased survival.

The effect of liquid holding in the dark and the effect of photoreactivation on survival was determined for haploid uvs mutants after UV irradiation. The ability of photoreactivation to further enhance survival after liquid holding was tested. Three mutants lost viability during the period of liquid holding, and for only two of these, did photoreactivation subsequent to liquid holding substantially increase viability above the survival level present after liquid holding. One mutant which was selected as a UV-sensitive mutant was also found to be photoreactivation negative.

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Kuhn, Jonathan C.: Genetic and Biochemical Studies of Acid Phosphatase Mutants in Saccharomyces cerevisiae. One of the proteins found in the cell wall of Saccharomyces cerevisiae is the enzyme acid phosphatase (E.C.3.1.3.2). In the present investigation, mutants (acp^-) were isolated that lacked this enzyme by in vivo determinations. The acp_1 locus was found to be unlinked to a centromere and leu_1. All the mutations isolated in this study were due to changes in a single gene which seems to be the structural gene for acid phosphatase. The examination of cell-free extracts from mutant strains demonstrated that all the acid phosphatase activity in S. cerevisiae is the result of a single enzyme. Attempts to isolate mutants constitutive for acid phosphatase were unsuccessful.

The present investigation also showed that S. cerevisiae is capable of utilizing a number of organic phosphomonoesters as phosphate sources. Strains lacking acid phosphatase are unable to utilize such compounds to fulfill their phosphate requirements when the orthophosphate of the medium is depleted. Acid phosphatase is synthesized in large amounts when inorganic phosphate becomes limiting. Under conditions where orthophosphate is not limiting, there is no synthesis of acid phosphatase and any enzyme present decreases by simple dilution. The synthesis of acid phosphatase allows S. cerevisiae to obtain usable phosphate from compounds that contain phosphate in a form that cannot be used when this enzyme is absent.

The K_m of acid phosphatase was estimated to be 5.7×10^{-4} M with p-nitrophenyl phosphate as the substrate by both in vivo and in vitro determinations. Orthophosphate and inorganic arsenate were shown to be competitive inhibitors of acid phosphatase. The K_i'1 for phosphate and arsenate are about 3×10^{-4} M and 1.3×10^{-4} M, respectively, by both in vivo and in vitro measurements.

These results imply that acid phosphatase is in direct contact with its substrate in living cells. It was estimated that each cell possesses about 122,000 molecules of acid phosphatase. Attempts to elute this enzyme from living cells using various substances were only slightly successful. Glucose and cysteine can effect a slight elution of acid phosphatase.

Another class of mutants was isolated in this study. These mutants (bgp^S) are sensitive to β-glycerol phosphate. This phenotype is the result of mutation at a single gene which is unlinked to a centromere and to arsenate resistance (ar). The growth of normal cells (bgp^r) is partially inhibited by β-glycerol phosphate. This suggests that the mutant phenotype is due to an increase in sensitivity to the type of inhibition found in normal cells.

It was demonstrated that double adenine requiring mutants can utilize 2'-, 3'- and 5'-adenylic acid to satisfy their requirements. Acid phosphatase is not apparently involved in this utilization.

The PO_4 requirement per cell was estimated as 2.7×10^{-7} μg. This estimate represents a maximum value.

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Korch, Christopher T.: Genetic and Biochemical Studies of Complementation in the First Gene for Histidine Biosynthesis in Saccharomyces cerevisiae. Intragenic complementation is a genetic phenomenon that may provide information about the tertiary structure of the subunits polypeptide chains in a multimeric enzyme with identical subunits. In this study a system has been constructed from which this information may be obtained. Sixty-five independent mutations of the his1 gene in Saccharomyces cerevisiae were studied. This gene codes for the first enzyme of the histidine biosynthetic pathway, N-1-(5'-phosphoribosyl)-adenosine triphosphate:pyrophosphate phosphoribosyl transferase (PR-ATP transferase). This study correlates findings from 1) mutant characterization, 2) genetic mapping, and 3) complementation mapping with cluster analysis of the his1 gene to 1) molecular weight, 2) number of subunits, and 3) observations based on electron microscopy of the enzyme.

The 65 his1 mutants were tested for reversion by ethyl methanesulfonate, ICR-170, and nitrous acid; and several mutants were reverted by these mutagens. Three mutants are able to revert and simultaneously gain the ability to excrete histidine, i.e. revert to resistance to feedback inhibition. Forty-five of the mutants tested complement and 18 do not. None of the 52 his1 mutants tested were suppressible by the nonsense suppressor, SUP11. This suggests that another function, vital to the cell, is associated with the his1 gene product such that a nonsense mutation would destroy the activities of both the PR-ATP transferase and this second vital function.

Sixty-five his1 alleles were ordered on the genetic map by allelic mapping methods utilizing gamma rays, X-rays, and MMS; the three maps agree well in order and have been oriented on chromosome V. The 18 noncomplementing mutants map only in the distal half of the genetic map, whereas, the complementing mutants are distributed throughout. The mutants which revert to feedback resistance map in the proximal end of the gene. Also in this end of the gene are the mutations having a very high X-ray or MMS induced homoallelic reversion rate. This suggests that several missense mutations can occur in this region of the gene which result in amino acid substitutions in the polypeptide that result in the wild-type phenotype.

The 45 complementing mutants fall into 31 complementation groups which can be used to construct a complex complementation map with 18 complementation units. Cluster analysis by either visual inspection or computer analysis shows two very definite clusters.

The PR-ATP transferase has been partially purified (about 300-fold). The apparent K_m for ATP and PRPP concentrations are 3.18×10^{-4} M and 3.69×10^{-4} M, respectively. This enzyme is inhibited by L-histidine and L-histidine methylester. By sucrose gradient analysis the transferase has a sedimentation coefficient of 8.55 s and a molecular weight of about 1.7×10^5.

By gel filtration the molecular weight is $(2.38 \pm 0.09) \times 10^5$, and the Stokes' radius is 58 ± 9 Å. The discrepancy between these two estimates of molecular weight implies the PR-ATP transferase molecule is asymmetrical. In vitro complementation between crude extracts of hisl mutants occurs. Electron microscopy of the yeast and Salmonella PR-ATP transferases indicates that the subunits are arranged in a hexagonal ring with a radius of about 60 Å.

A model of PR-ATP transferase was made from the genetical and biochemical data which has the following attributes: (1) the six subunits are isologously associated in a hexagonal ring with two points of contact per subunit, (2) the folding of the polypeptide chain in the subunit is such that certain regions defined on the basis of genetic fine structure mapping are associated with the points of contact, and (3) certain other genetically defined regions of the folded polypeptide chains in the active molecular are associated with the catalytic and feedback inhibition sites.

A paper is scheduled to appear shortly in Molecular and General Genetics which describes the use of methylmethanesulfonate in fine-structure genetic mapping. The summary of the paper is as follows:

> Snow, R. and C. T. Korch: Alkylation Induced Gene Conversion in Yeast: Use in Fine Structure Mapping. Methylmethanesulfonate (MMS) causes gene conversions in heteroallelic diploids of Saccharomyces cerevisiae. The frequency of production of prototrophic convertants is linearly proportional to the square of the time of MMS treatment, and the regression of prototrophs on dose varies depending upon the particular pair of alleles present in the diploid. The regressions show an additivity relationship, in that when a triad of heteroallelic diploids of the type m_1/m_2, m_2/m_3, m_1/m_3 is considered, two of the regressions add up approximately to the third. MMS can, therefore, be used in fine structure mitotic mapping of genes. Good agreement was found both in relative order and spacing of alleles at the histidine 1 locus of yeast when the fine structure map based on the X-ray mapping method was compared with that based on MMS.

XXIII The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, Texas Medical Center, Houston, Texas 77025. Communicated by Dr. Miguel Flores da Cunha.

Below follows the summary of a paper currently in press in Genetical Research, Vol. 15, 1970. My present address is Section of Medical Genetics, Biology Department, The University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025.

Mitotic mapping of Schizosaccharomyces pombe

By Miguel Flores da Cunha

SUMMARY

Genetic mapping by means of mitotic haploidization (induced by para-fluorophenylalanine) and mitotic crossing-over was carried out with the fission yeast Schizosaccharomyces pombe. Thirty-two different genetic markers were involved in this investigation; some meiotic linkage relationships had been previously reported (Leupold, Megnet) for 16 of these loci. Mitotic haploidization experiments resulted in the genetic identification of six chromosomes in the haploid complement.

Furthermore, in an attempt to study the mechanism of action of para-fluorophenylalanine (pFPA) on mitotic haploidization, pedigree analyses were performed by micromanipulation of diploid cells growing in the presence of pFPA. Haploid cells were detected after 40 hours of contact with the analogue and many lethal pedigree branches were observed. These observations seem to agree with Kafer's (1961) and Lhoa's (1968) suggestion that mitotic haploidization in Fungi is achieved by progressive loss of chromosomes throughout cell divisions.

XXIV The University of Texas at Dallas, Division of Biology (Formerly South West Center for Advanced Studies). Communicated by H. Gutz and coworkers (P. Angehrn, M. Flores da Cunha, S. Goldman, H.-J. Treichler).

Meiotic and mitotic gene conversion. As reported previously, one ade 6 mutant (M26) of Schizosaccharomyces pombe is remarkable in showing high frequencies of gene conversion in meiosis but not in mitosis. The results obtained with ade 6-M26 seem to reveal some hitherto unknown aspects of meiosis and genetic recombination. A working hypothesis has been developed in which it is assumed that different alleles are differently "preconditioned" prior to meiosis: e.g., the alleles might be present with different frequencies in incompletely replicated replicons (see the conversion model of Taylor, 1967). The conversion behavior of ade 6-M26 can be explained by assuming that the site of M26 is near the end of a replicon, and that presence of this site results in the replicon being nearly always incompletely replicated prior to meiotic pairing. This effect could result if the M26 site is part of an amber codon. [Mosig et al. (1968) obtained evidence that amber mutations slow DNA replication locally].

In addition to the data at hand, further two- and three-factor crosses involving nonidentical ade 6 mutations are being analyzed to obtain further genetic evidence for or against this hypothesis. If ade 6-M26 is indeed part of an amber codon, the mutation should be suppressible by "supersuppressors" [equivalent to amber and ochre suppressors in bacterial systems (Manney, 1968)]. It was found that reversions of M26 to prototrophy are partially due to suppressors which seem to be supersuppressors. Experiments are underway to determine the effect of these suppressors on other mutations of S. pombe known to respond to supersuppressors (Barben, 1966). Other experiments are in progress to test which of the supersuppressors found by Barben affect ade 6-M26. These experiments will hopefully allow a description of the properties of ade 6-M26 in molecular terms.

Studies on the mating type of S. pombe. The mating-type locus of S. pombe consists of two subunits with distinct functions (Leupold, 1958). This locus influences the copulation behavior of haploid and diploid cells as well as the ability of diploid cells to undergo meiosis. The mating-type locus is therefore of special interest with respect to regulation mechanisms in eukaryotic organisms. In continuation of our work on twin meiosis (Gutz, 1967), as well as on mitotic crossing-over and lethal mutations in the mating-type locus (Angehrn and Gutz, 1968; Gutz and Angehrn, 1968), we have started experiments on the mating types of six S. pombe strains isolated from different parts of the world. This work is expected to give further insight on the genetic structure of the mating-type locus and its regulatory functions in the life cycle of S. pombe. The work is done in cooperation with Dr. Frank Doe of The University of Dallas.

Mapping of genes by haploidization. Except for the original mapping studies of Leupold (1958), which resulted in one linkage group, no other mapping of S. pombe has been carried out. Using 32 markers, we have done extensive haploidization experiments to determine linkage groups (Flores

da Cunha, 1969). We have genetic evidence for at least six different chromosomes in S. pombe: Chromosome I with 12 different genes, chromosome II with 15 genes (including the mating-type linkage group of Leupold, 1958), chromosome III with two genes, and chromosomes IV and VI with one gene each.

XXV Van 't Hoff Laboratory, State University of Utrecht, Sterrenbos 19, Utrecht, The Netherlands. Communicated by A. M. A. ten Berge.

Genetic studies on maltose- and α-D-methylglucoside fermentation in Saccharomyces carlsbergensis.

In our laboratory we have been studying the regulation of α-glucosidase synthesis in the inducible strain Saccharomyces carlsbergensis N.C.Y.C. 74 (V. V. Koningsberger, 1967; R. Hartlief and V. V. Koningsberger, 1968; R. van Wijk, J. Ouwehand, T. van den Bos and V. V. Koningsberger 1969).

A genetical approach was started in 1968 in the "Forstbotanical Institute", Freiburg, Germany, under guidance of Dr. F. K. Zimmermann and continued in Utrecht from 1969.

So far, the results are briefly as follows:

1) S. carlsbergensis N.C.Y.C. 74 is diploid and heterothallic. Tetrad analysis was not feasible because of low spore viability. 89 haploid spores were obtained by random spore isolation. Hybrids from intercrosses of these spores yielded 70% viable spores, allowing tetrad analyses.
By mutagenesis with 1-Nitroso-Imidazolidone auxotrophic mutants were obtained, some of which could be classified by means of complementation tests with S. cerevisiae mutants (Seattle Stock): ade-1; ade-2; ade-4; ade-5; ade-6; ade-7; lys-1; trp-5; trp-2; his-4; his-5; ura-2; ura-3.
2) The 89 spores (of which 48 were "a" and 41 "α"), were all maltose fermenters, but only 27 fermented α-D-methyl-glucoside (abbreviation: αDMG).
Spore 6 and spore 10 complemented each other for αDMG fermentation; tetrad analysis of the hybrid indicated the heterozygosity for not more than 2 complementary genes, preliminarily called MGL_6 and MGL_{10}, respectively.
The αDMG non-fermenters among the 89 original spores were tested for complementation with an "a" or "α" MGL_6 strain and with an "a" or "α" MGL_{10} strain, giving the following result:

αDMG fermenters	αDMG non-fermenters (62 strains)		
	Complementary to MGL_6	Complementary to MGL_{10}	Complementary to neither MGL_6 nor MGL_{10}
27	24	18	20

This result can be explained by assuming that the strain is heterozygous for not more than 2 complementary MGL genes.
Strains lacking both MGL_6 and MGL_{10} were still inducible by maltose for α-D-methylglucosidase activity in the cell extract, suggesting that MGL_6 and MGL_{10} are not structural genes for α-D-methylglucosidase. A further elucidation of the function of these genes and the isolation of a structural mutation for α-D-methylglucosidase are in progress.

3) 10 of the 89 spores of S. carlsbergensis were crossed with mal⁻ tester strains ("a" ade-4 suc mal gal and "α" ade-8 trp ilv SUC mal gal, Seattle Stock) giving 2+ : 2- segregation for maltose fermentation in the tetrads. Intercrosses of the 10 spores yielded only maltose fermenting progeny. Thus, in our strain the fermentative ability towards maltose is dependent upon one gene, when compared with the mal⁻ tester strains.
Crosses with strains, containing MAL1 through MAL5 (all strains obtained from Dr. N. Eaton, New York), showed that our gene is not identical with MAL1 through MAL5.
The cross with a MAL6 strain is in progress.

4) By mutagenesis with 1-Nitroso-imidazolidone 15 recessive mal⁻ mutants were obtained, which could not ferment maltose and which were inducible by maltose for neither maltase nor α-D-methyl glucosidase activity in the extract, but which had about the same basal activity for both enzymes as the wild strain after growth on galactose.
After crossing the mutants with an "a" MAL ade-1 trp-5 strain, from which crosses they were regained in "a" trp-5 and "α" ade-1 strains, the mutants were brought into a complementation experiment. None of the mutants complemented one of the others for maltose fermentation.
These data suggest that the mal mutants are all regulatory mutants.
A further investigation of the properties of the mutants is in progress.

References.

V. V. Koningsberger 1967: The regulation of inducible α-glucosidase synthesis in yeast. In: Regulation of nucleic acid and and protein biosynthesis. V. V. Koningsberger and L. Bosch (editors). Elsevier Publishing Company, Amsterdam 1967 p. 310-330.

R. Hartlief and V. V. Koningsberger 1968: Characterization of messenger RNA in protoplasts of Saccharomyces carlsbergensis. Biochim. Biophys. Acta 166 (1968) 512-531.

R. van Wijk, J. Ouwehand, T. van den Bos and V. V. Koningsberger 1969. Induction and catabolite repression of α-glucosidase synthesis in protoplasts of Saccharomyces carlsbergensis. Biochim. Biophys. Acta 186 (1969) 178-191.

XXVI Laboratorio di Mutagenesi e Differenziamento C.N.R. c/o Istituto di Genetica, viale Matteotti 1/A, 56100 - Pisa, Italy. Communicated by Dr. N. Loprieno.

From May 29-31, 1970 this Laboratory organized an informal meeting on "Repair and mutations in microorganisms"; 37 scientists from different European countries discussed their latest results. Topics regarding yeast genetics were as follows:

F. FABRE (Orsay, France): Effect of UV light during the mitotic and meiotic division in Sch. pombe.

A. ABBONDANDOLO (Pisa, Italy): Mutations induced by radiations in G1 and G2 stages of Sch. pombe.

R. EGEL (Freiburg, Germany): Mutations affecting meiosis and their influence on commitment to meiosis in fission yeast.

S. BONATTI (Pisa, Italy): Temperature-sensitive mutations in Sch. pombe.

U. LEUPOLD (Bern, Switzerland): Studies on mutations and recombination in nonsense suppressors of Sch. pombe.

M. SCHUPBACH (Bern, Switzerland): UV-sensitive mutants of Sch. pombe.

N. LOPRIENO (Pisa, Italy): Spontaneous mutations and the effect of mutator genes in Sch. pombe.

P. K. ZIMMERMANN (Freiburg, Germany): The hybrid enzyme concept in mutagenesis of diploid cells.

B. KILBEY (Edinburgh, Scotland): Interaction results with yeast.

B. COX (Oxford, England): Gene conversion and repair.

E. MOUSTACCHI (Orsay, France): Repair of cytoplasmic genetic damage, a new class of mutants.

D. WILKIE (London, England): Mutations affecting the mitochondrial system in S. cerevisiae.

D. H. WILLIAMSON (London, England): Observation on the mechanism of the cytoplasmic petite mutation in yeast.

N. NASHED (Freiburg, Germany): Studies on phospholipid metabolism in S. cerevisiae under conditions associated with respiratory deficient mutations.

XXVII Institut für Biochemie der Universität Würzburg, 87 Würzburg, Germany. Communicated by E. Schweizer.

We started work on the genetics of the yeast fatty acid synthetase multienzyme complex. The following is the summary of a manuscript in preparation:

Mutants have been isolated from S. cerevisiae which grow only in the presence of myristic, palmitic, stearic or oleic acid, added in emulsified form to the normal growth medium. So far, one of these mutants has been investigated in more detail. The mutant allele exhibits a 2:2 Mendelian segregation pattern and manifests itself in "petite" as well as in "grande" cells. The defect has been atributed to the synthesis of a non-functional fatty acid synthetase complex. By comparison of the purified multienzyme complexes from wild type and from mutant cells, it could be shown that one of the seven component enzymes involved in over-all fatty acid biosynthesis, was completely inactive in the mutant. The specific activities of the other six component enzymes were identical in the mutant and in the wild type complexes. The reaction step affected was, in the mutant investigated, the condensation of acetate and malonate to form acetoacetate and CO_2. Sedimentation in the analytical ultracentrifuge revealed the same molecular weight for both, the mutant and the wild type multienzyme complexes. The biosynthesis of fatty acid synthetase in yeast is constitutive and not subject to repression by long chain fatty acids.

XXVIII Brewing Industry Research Foundation, Lyttel Hall, Nutfield, Redhill, Surrey, England. Communicated by A. H. Cook.

The research topics at the Foundation are largely related to the problems arising in the fermentation of brewing malt wort by Saccharomyces cerevisiae. The current topics, which are especially important with regard to the rate and extent of fermentation, are:

1) The quantitative relationship between the anaerobic utilization of sugar and the production of yeast mass, as influenced by the quantity and nature of the source of carbohydrate and nitrogen and of other nutrients. The growth yield per mol of carbohydrate used varies from 11.0 - 13.8 g dry weight of yeast. Mass increase continues after cell multiplication ceases and the weight of individual cells may treble in this period.

2) The differences between strains of S. cerevisiae with regard to adaptation to maltose fermentation after prior utilization of glucose from a mixture of these two sugars. Some strains fail to adapt unless cell multiplication occurs, while others have no such restriction.

3) Factors which influence the fermentation of maltotriose, with special reference to cation and pH effects. Some results of this work are in the press (K. Visuri & B. H. Kirsop, J. Inst. Brew.,). Maltotriose fermentation was shown to be markedly influenced by pH, with maximum utilization occurring at pH 5.0 - 5.5. In contrast to the situation with glucose no significant activity occurs at alkaline pH. At values below 5.0 the degree of inhibition is reduced by the presence of potassium though, again unlike the position with glucose, substantial inhibition is noted at pH 3.0 - 3.5. Divalent metals such as magnesium and zinc stimulate maltotriose utilization. The effects of pH on potassium and the divalent metals were shown to be exerted on the maltotriose uptake process rather than on later metabolic events.

4) The α-glucosidase content of cells at different stages of fermentation.

5) The relationship between cell size and carbohydrate source; glucose and maltose are equally useful as a source of yeast mass but glucose gives a greater number of cells.

6) The role of divalent cations in yeast flocculation, with special reference to the extent of cation binding and the influence of different cations on cell clumping.

7) The chemical properties of yeast cell walls as revealed by degradation with specific enzymes.

8) The nature and properties of the large quantities of extracellular simple peptides and polypeptides produced by yeast during fermentation.

A new catalogue of the National Collection of Yeast Cultures is now being printed.

XXIX Research Institute for Viticulture and Enology, Bratislava, Czechoslovakia. Communicated by E. Minárik.

In May 1970 the following handbook will be published:

L. Laho, E. Minárik, A. Navara: Enology/appr. 350 pages, in Slovak/, Príroda, Bratislava 1970.

Part 2: E. Minárik: Microbiology of Wine. A comprehensive survey of microbiological problems connected with winemaking. The life cycle, systematics,

cytology, genetics, morphology, physiology, biochemistry, identification, classification and description of yeasts and yeast-like microorganisms, bacteria and molds most frequently occurring on primary and secondary habitats in the vineyard, press-house, wine cellar etc., are described. Factors influencing the alcoholic and malo-lactic fermentation, the most important activators and inhibitors of fermentation, the role of biological stabilizors, e.g. sulphur dioxide, sorbic acid, diethylpyrocarbonate etc., are discussed. Microbiological examinations in the wine-microbiological laboratory, rules of sampling and analysis of wine material, as well as aspects of control in the wineries are given. The most frequent wine defects and diseases caused by various microorganisms, are described. The particular processes and problems, the description of important microorganisms involved in this process of wine making, are accompanied by synoptical tables and many illustrations.

The following manuscript has been submitted for publication to Biologické práce SAV (Biological Works SAV) in Bratislava: E. Minárik - P. Rágala: "Influence of fungicides on the yeast flora of the vine". This work will be published in German, approx. 110 pages in the Publishing House of the Slovak Academy of Sciences in 1971. Brief summaries: In the course of seven years 33 inorganic and organic fungicides have been tested on their influence on yeasts and yeast-like microorganisms, and on the alcoholic fermentation of grape juice in laboratory and field scale tests. Copper preparations practically do not influence the composition of the yeast flora, nor do they affect the fermentation, provided normal concentrations have been applied. Fungicides, based on carbamates, e.g. Novozir N 50, Kasebon, Manzate D, Dithane M 45 and Antracol, usually had no influence on the development of dominant yeast species. Combinations of Novozir N 50 with copper fungicides cause a suppression of Saccharomyces sp. in higher concentrations. Fungicides based on phtalimides (Captan, Phaltan, Difolatan and some others) show a strong inhibition of yeast activity; the natural composition of the yeast flora of grapes is significantly changed. Saccharomyces species are most affected, the development of some less active species in fermentation, e.g. Torulopsis bacillaris, as well as the fungus Hyalodendron sp. being stimulated. Thiuram fungicides (e.g. Polyram, Polyram-Combi, Basfungin, Kupfer-Polyram) do not slow down the development of yeasts. TMTD, on the other hand, inhibited sporogenous yeast species similarly to some phtalimid fungicides. This preparation stimulates the development of Torulopsis bacillaris in the yeast flora of the fermenting grape juice. Sulphur in combination with Captan and Phaltan shows a reduced inhibitory action on yeasts; in combination with carbamate fungicides this action is however strengthened. Some seldom occurring yeasts isolated for the first time in Czechoslovakia are described and problems of vine protection and wine technology connected with the application of organic fungicides are briefly discussed.

XXX Meetings

1. Chairman T. O. Wikén of the Commission on Yeasts and Yeast-like Microorganisms (Council for International Co-operation in Yeast Science) has called a meeting of the Commission on August 7, 1970 at 10 AM in Mexico City, just prior to the start of the International Congress of Microbiology. The place for the meeting will be announced later. Among other things, the organization of the Fourth International Yeast Symposium in 1974 will be discussed. Members of the Commission who cannot attend in person are kindly requested to write Dr. Wikén their ideas or views on the next International Symposium.

2. The Fifth International Conference on Yeast Genetics - Canada - 1970.

The Fifth International Conference on Yeast Genetics will be held at the Chalk River Nuclear Laboratories of Atomic Energy of Canada Limited, Chalk River, Ontario. The dates are September 29 through October 2, 1970.

The success of these meetings in the past has been derived in part from their informal nature. This feature will be maintained in the coming conference and all who attend will be able to participate in the discussions. Ample opportunity will also be provided for more relaxed exchanges.

The number of participants must be limited and the local committee would appreciate knowing your plans as soon as possible. A form is included for this purpose.

Further information, together with a detailed program, will be mailed as soon as possible to all who have notified the committee that they plan to attend.

Chalk River is situated on the Ottawa River about 130 miles from Ottawa and group transportation from Ottawa will be provided. The conference is to be held when Canadian weather is at its best and Canadian scenery at its most colorful. We hope you will be able to come.

Local Committee: J. G. Kaplan and A. P. James, Co-chairmen, R. J. Doyle, R. H. Haynes, H. Heick, C. Lusena, M. A. Nasim, and C. Robinow.

3. Y E A S T S Y M P O S I U M 1 9 6 9

The Proceedings of the Third International Symposium on Yeasts held at Delft and The Hague, 2 - 6, June, 1969

Contents:

Resolution adopted by the Participants of the Symposium
List of Participants, with complete addresses, and portraits
Opening address by T. O. Wikén, Chairman of the Organizing Committee
M. INGRAM. Yeast science today and tomorrow
L. J. WICKERHAM. Yeast taxonomy in relation to ecology, genetics and phylogeny
PH. MATILE. Prospects of yeast cytology
J. C. HOOGERHEIDE. Horizons in industrial application of yeasts
HEIKKI SUOMALAINEN. Trends in physiology and biochemistry of yeasts
C. W. EMMONS. Pathogenic yeasts
Abstracts of 162 papers on Taxonomy, Cytology, Genetics, Ecology, Pathology and Immunology, Technology, Metabolism, Biosyntheses, and Enzymology. These are two-page abstracts, many with illustrations, graphs and tables.
Author index, Reference index, Subject index

Total number of pages: 520

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For lists of contents of Vols. 29 - 35 apply to the Editors, c/o Dr. A. A. Houwink, Julianalaan 67A, Delft, The Netherlands.

XXXI Brief News Items

1. The Editor announces with regret the death of Dr. M. P. Scarr, who was killed in a road accident last October. She worked in the laboratories of Tate and Lyle, Ltd., Keston, Kent, England.

2. Dr. Yasuo Umeda writes: I have the pleasure of informing you that effective Sept. 25, 1969 I assumed my new responsibility as managing Director of Kirin Brewery Co. Ltd. at our head office located at Kashiwabara Bld., 1-4, Kyobashi, Chuoh-ku, Tokyo.

3. My interest in yeast is especially its use in various food products and, of course the question arises as to the possible hazards from the intake of either large or limited amounts of RNA. It seems to me that this is an extremely important question. I suggest that anyone interested in the RNA problem communicate with me or others through the Yeast News Letter.

   L. K. Riggs
   Gar-Baker Laboratories Inc.
   110 West 18th Street
   New York, New York 10011

4. A method has been developed for rapid and easy measurement of the viscosity of the yeast cell interior. The fluorescence polarization of fluorescein in yeast cells is directly related to viscosity and is easily measured in a fluorometer. The interior viscosity of a haploid strain of Saccharomyces cerevisiae was found to be 14 centipoise. Exposure of this strain to 0.5% phenethyl alcohol increases intracellular viscosity and increases calcium content fivefold. The method opens the possibility of detecting viscosity changes in yeast in response to environmental agents and possible species differences. [A preliminary note has appeared in Biochem. Biophys. Res. Commun., Dec. 1969]

   Victor W. Burns
   University of California
   Davis, California 95616

5. Recent Publications:

   a. Maxwell, W. A. and Edward Spoerl, 1969. Uptake of mannitol by Saccharomyces cerevisiae. J. Cell Biol. 43, 87.

   b. Maxwell, W. A. and Edward Spoerl, 1970. Mannitol uptake and retention by Saccharomyces cerevisiae. Bacteriol. Proc. p. 145.

   c. Spoerl, Edward, 1970. Enhanced CO_2 production by yeast exposed to elevated temperature. J. Gen. Microbiol. 61 (in press).

   Recent work in our laboratory has involved further studies on mannitol transport which was described in part in the last issue of the

News Letter. A report will soon be forthcoming on this work. We have also been examining the effects of uranyl nitrate on transport systems. It has been found that uranyl nitrate does not inhibit sugar transport specifically, as is often indicated in the literature, but rather that it inhibits a variety of transport systems. Besides inhibiting sugar transport, various amino acid and vitamin transport systems are equally affected.

W. A. Maxwell

As of July 17, 1970 my address will be: Life Sciences Division, Stanford Research Institute, Menlo Park, California.
(Former address: Medical Research Laboratory, Fort Knox, Ky. 40121)

6. Two Ph.D. Theses will be submitted shortly at the University of Orsay, Science Faculty, by H. CHEREST, entitled:
"Biosynthèse de la méthionine et sa régulation chez Saccharomyces cerevisiae"

and by A. MIRE, entitled:
"Contribution à l'étude de la thréonine désaminase de Saccharomyces cerevisiae: souches mutantes haploides et diploides".

Dr. H. de ROBICHON-SZULMAJSTER
Centre National de la Recherche Scientifique
Laboratoire D'Enzymologie
91 Gif-sur-yvette, France

7. Titles of two papers which we have published recently are as follows:

1. The pigmentation of bovine serum albumin by the black yeast Phialophora jeanselmei.
2. The genetically determined binding of alcian blue by a minor fraction of yeast cell walls.

Paul Ottolenghi
Carlsberg Laboratorium
Physiological Department
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Copenhagen, Valby, Denmark

8. The following are recent publications from our laboratory.

N. J. W. Kreger-van Rij and M. Veenhuis, Septal pores in Endomycopsis platypodis and Endomycopsis monospora. J. Gen. Microbiol., 57:91-96, 1969.

N. J. W. Kreger-van Rij and M. Veenhuis, A study of vegetative reproduction in Endomycopsis platypodis by electron microscopy. J. Gen. Microbiol., 58:341-346, 1969.

N. J. W. Kreger-van Rij
Laboratory of Bacteriology and Serology
Oostersingel 59
Groningen - Netherlands

9. The following publications have appeared.

- BIZEAU C. - Etude de l'influence de l'aération sur la sélection de mutants "colonie lisse" chez Saccharomyces cerevisiae HANSEN. C. R. Soc. Biol. 163, n°3, p. 692, 1969.

- BIZEAU C., GALZY P., BASTIDE J. M., BASTIDE Madeleine - Mise en évidence d'une différence antigénique contrôlée par un gène de la série pl et localisée dans la paroi chez Saccharomyces cerevisiae HANSEN. C. R. Acad. Sci. Paris 269, 1632-1634, 1969.

- GALZIN M., GALZY P., BRET G. - Etude de la flore de la levure dans le fromage de Roquefort. Revue Générale des question laitières. T. L., n°, 491-492, p. 1-37, 1970.

- VEZINHET Françoise, ARNAUD A., GALZY P. - Note technique. Remarques sur une technique permettant une sporulation rapide des zygotes chez la levure. Ann. Technol. Agric. INRA. (Sous presse).

The following publications will be published shortly.

- ARNAUD A., VEZINHET Françoise, GALZY P. Caractéristiques d'un mutant homothallique de Saccharomyces cerevisiae HANSEN.

- GUIRAUD J. P., VEZINHET Françoise, ALBERT J., GALZY P. - Influence de la nutrition des cellules de levure sur leur aptitude à métaboliser certains substrats.

P. Galzy
Institut Nacional de la Recherche Agronomique
Montpelliers (Herault), France

10. The following publications have appeared recently:

Gezelius, K. and Norkrans, B. (1970). Ultrastructure of Debaryomyces hansenii. Arch. Mikrobiol. 70, 14-25.

Norkrans, B. (1969). Hydroxylamine as the sole nitrogen source for growth of some Candida spp. Acta Chem. Scand. 23, 1457-1459.

Norkrans, B. (1969). The sodium and potassium contents of yeasts differing in halotolerance, at various NaCl concentrations in the media. Antonie van Leeuwenhoek, 35 (supplement) G31.

Norkrans, B. and Kylin, A. (1969). Regulation of the potassium to sodium ratio and of the osmotic potential in relation to salt tolerance in yeasts. J. Bacteriol. 100, 836-845.

Brigitta Norkrans
Marine Botanical Institute
University of Goteborg
41319, Goteborg, Sweden

11. Dr. Jitka Lieblová writes that Dr. K. Beran, Institute of Microbiology, Czechoslovak Academy of Science, Prague, has been seriously ill for three months already. We extend to him our best wishes for a speedy recovery.

The following publications have appeared:

Beran, K. and Zemanová, J. (1969). Cell growth and population activity of Sacch. cerevisiae in two-stage continuous cultivation. Biotechn. Bioengin. 11, 853-862.

Beran, K. (1969). Analysis of growth and multiplication of yeast cells Sacch. cerevisiae in continuous culture from the aspect of relative age of the cells. From "Continuous cultivation of microorganisms" Proc. of the 4th Symposium, Prague, June 17-21, 1968, p. 87-103.

Beran, K. (1968). Budding of yeast cells, their scars and ageing. Adv. Microbiol. Physiol. 143-171.

12. From Kazushige Sumino and coworkers the following two publications were received, reprinted from J. Fermentation Technol. 48, 84-90 and 91-97, 1969 (with English summaries): Physiological and biochemical studies on Sacch. sake. XI. Comparison of contents of various components of cells grown anaerobically with or without lactic acid. XII. Uptake and metabolism of exogenous lactic acid by Sacch. sake cultivated statically in a medium containing lactic acid. (From the Laboratory of Industrial Biochemistry, Faculty of Engineering, Kyoto University, Kyoto, Japan).