Y E A S T

A News Letter for Persons Interested in Yeast

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I. Editorial. The Editor wishes to extend to all readers of the Yeast News Letter a prosperous and scientifically rewarding new year.

You will note that an additional Associate Editor, who will represent Japan, has been added to the board. After some discussion with knowledgeable yeast workers in Japan it appeared convenient and advantageous to have a single person on the editorial board who would be responsible for collecting news items and the subscription fee (1.50 U.S. dollars) and transmit these as a single item to the editor in Davis. Therefore Japanese subscribers are requested to send news items (in November and in April) and subscription dues ($1.50 in Japanese yen in November) to Dr. Nagai who will forward these items to the Editor in Davis. The new policy will start with the dues statement accompanying the fall issue.

Another innovation is the start of a new rubric (under Brief News Items) dealing with employment sought or offered. Readers are encouraged to send items pertaining to employment for future issues.

H. J. Phaff

II. Centraal Bureau voor Schimmelcultures, Yeast Division, Julianalaan 67A, Delft, The Netherlands. Communicated by D. Yarrow.

The following new species have been deposited in the CBS collection since the last issue of the News Letter.

Ambrosiozyma philentoma CBS 6276 (Type), 6279
J. P. van der Walt, Mycopath. Mycol. Appl. 46:305 (1972).

Candida tsukubaensis CBS 6389 (Type)
H. Onishi, A. v. Leeuwenhoek 38:365 (1972).

Cryptococcus diffluens var. uruguaiensis CBS 6436 (Type)
R. C. Artagaveytia-Allende & L.A. de Queiroz, Publ. No. 663, Inst. Micol. Univ. Fed. Pernambuco, 1970.

Kluyveromyces waltii nom. nud.
K. Kodama, Proc. 4th Int. Ferment. Symp. Kyoto, p. 291.

Pichia melissophila CBS 6344 (Type)
J. P. van der Walt & W. C. van der Klift, A. v. Leeuwenhoek 38:361 (1972).

Pichia naganishii nom. nud. CBS 6429
K. Kodama, Proc. 4th Int. Ferment. Symp. Kyoto, p. 291, 1972.

Torulopsis kruisii CBS 6451 (Type)

Torulopsis schatavii CBS 6452 (Type)
A. Kocková-Kratochvilová & D. Ondrusová, Biologia (Bratislava) 26:485 (1971).

The 28th edition of the List of Cultures, 1972, has now been published and may be obtained from the Centraalbureau voor Schimmelcultures, Oosterstraat 1, Baarn.

III. Louisiana State University and Agricultural and Mechanical College, Department of Food Science, Baton Rouge, Louisiana, 70803, U.S.A. Communicated by Samuel P. Meyers.

The following notice of a forthcoming Workshop may be of interest to the readers of the Yeast News Letter. Various of the papers pertain to fungal degradation of oil, several of which specifically involve yeasts. These are listed as follows:

1. THE IMPACT OF OIL ON MICROBIAL MARSHLAND ECOSYSTEMS
   D. G. Ahearn, S. P. Meyers, S. Crow and N. Berner
   Department of Biology, Georgia State University, Atlanta and Department of Food Science, Louisiana State University, Baton Rouge.

2. PRODUCTION AND CHARACTERIZATION OF EMULSIFYING FACTORS FROM HYDROCARBONOCLASTIC YEAST AND BACTERIA
   P. E. Guire, J. D. Friede and R. K. Gholson
   Department of Biochemistry, Oklahoma State University, Stillwater.

3. THE DEGRADATION OF CRUDE OIL BY YEASTS AND ITS EFFECT ON LESBISTES RETICULATUS
   W. L. Cook, J. K. Massey and D. G. Ahearn
   Department of Biology, Georgia State University, Atlanta.

I attended the recent (September 11-16, 1972) International Symposium in Marine Mycology in Bremerhaven Germany, where I presented the following two papers:

IMPLICATIONS OF YEASTS AND YEAST-LIKE FUNGI IN MARINE PROCESSES
S. P. Meyers and D. G. Ahearn

CONTRIBUTION OF FUNGI TO BIODEGRADATION OF SPARTINA AND OTHER BRACKISH MARSHLAND VEGETATION
S. P. Meyers

All of the contributions, together with the discussions will be published in a special volume on the "Veroffentlichungen des Instituts für Meeresforschung, Bremerhaven", to be published early in 1973.

Our research efforts are continuing in analyses of hydrocarbonoclastic yeasts as well as physiological and enzymatic studies of the striking marshland yeast biota.

We always welcome hearing from yeast workers in ecological and related subject areas.

THE MICROBIAL DEGRADATION OF OIL POLLUTANTS

A Workshop Sponsored
by
The Office of Naval Research
The United States Coast Guard
The Environmental Protection Agency
and
Georgia State University
December 4-6, 1972

Workshop Chairman: Donald G. Ahearn

Program Coordinator: Warren L. Cook

Co-Editor: Samuel P. Meyers

WORKSHOP GOALS

The objectives of this workshop are: (1) to determine the present status of knowledge concerning the use of microorganisms in facilitating oil biodegradation, (2) to assess future areas of investigation, (3) to promote cooperative research projects, and (4) to promote communication and exchange of information. To attain these goals, the workshop has been developed for the informal presentation of information. Panel chairmen

will direct small discussion groups. Members as well as observers of the panel will be given the opportunity to offer information of interest to the workshop.

PANEL MEETINGS

Bacterial degradation of oil, the range and mechanisms of enzymatic activity. Dr. P. H. Pritchard, Chairman.

Fungal degradation of oil, the range and mechanisms of enzymatic activity. Dr. J. J. Cooney, Chairman.

Toxicity and pathogenicity of hydrocarbonoclastic microorganisms. Dr. C. W. Hendricks, Chairman.

Environmental considerations in microbial degradation of oil. Dr. J. D. Friede, Chairman.

PRESENTED LECTURES

December 4 - IMPACT OF OIL ON MARSHLAND MICROBIAL ECOSYSTEMS. Donald G. Ahearn

December 5 - MICROBES AND PETROLEUM, A REAPPRAISAL OF DYNAMIC INTERRELATIONSHIPS. W. R. Finnerty

December 6 - STATUS AND FUTURE PROSPECTUS FOR CONTROLLED BIODEGRADATION OF OIL. C. E. ZoBell

The final publication of the proceedings of the workshop will be through the cooperation of the Center for Wetland Resources, Louisiana State University, Baton Rouge, Louisiana.

IV. Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pustchino, Moscow region, USSR. Communicated by W. I. Golubev.

The following articles have recently been published:

Golubev, W. I. 1972. Conditions of production of extracellular polysaccharides by yeast. Nautch. dokl. vissch. schkoli (Biol. nauki), N 1, 89-91.

Guzev, V. S., Golubev, W. I., Zviagintzev, D. G. 1972. Detection of microcapsules in microorganisms and the control of their complete decapsulation by microelectrophoresis. Mikrobiologia 41, 115-120.

Golubev, W. I., Bab'eva, I. P. 1972. Yeasts of the genus Debaryomyces Klöck. in nests of ants of the group Formica rufa L. Ekologia, N 1, 78-82.

Aksenov, S. I., Bab'eva, I. P., Golubev, W. I. 1972. Studies on the processes of moistening and drying of capsulated and noncapsulated forms of Cryptococcus albidus var. diffluens by NMR-spin echo method. Izv. Akad. Nauk SSSR, ser. biol., N 4, 545-558.

Golubev, W. I., Bab'eva, I. P. 1972. Debaryomyces formicarius sp. n. and Debaryomyces cantarellii associated with ants of the group Formica rufa L. J. Gen. Appl. Microbiol. 18, 249-54.

The following articles are in the press:

Golubev, W. I., Bab'eva, I. P. Taxonomic criteria of the genus Cryptococcus Kütz. Mikologia i Phytopathologia.

Golubev, W. I. Nadsonia commutata nov. sp. Mikrobiologia.

Work in progress:

A taxonomic study of yeasts occurring on islands in the southern part of the Atlantic Ocean.

V. Universita di Pisa, Istituto di Microbiologia Agraria e Tecnica, Italy. Communicated by O. Verona.

O. Verona - Yeast in poultry faeces. Annali di Microbiologia ed Enzimologia (in press).

It is well known that various yeast species are present in animal faeces and that the yeasts inhabiting the gastro-enteric cavity of warm-blooded animals have a thermophilic behavior. The author observed statistically on 100 samples the yeasts present in his country in the poultry faeces. He found that Sacc. telluris (or Torulopsis bovina) was present in 94%. This is a truly resident species. The following other species are probably occasionally present: Pichia kudriawzevii (Candida krusei) 10%; Pichia farinosa 2%; Candida albicans 34%; C. lusitaniae 2%; C. guillermondii 2%; C. silvicola 2%; Tor. candida 2%; T. glabrata 4%.

VI. Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02881, U.S.A. Communicated by Raja Seshadri.

Completed Ph.D. Thesis: (October, 1972)
"Seaweeds as a habitat for yeasts"

Recent Publications:

Seshadri, R. and J. McN. Sieburth. 1971. Cultural estimation of yeasts on seaweeds. Appl. Microbiol. 22:507-512.

Seshadri, R. and J. McN. Sieburth. (in press) Taxonomy of yeasts from seaweeds and adjacent waters: Emphasis on a key and computer comparison of the genus Candida Berkhout. J. Bact.

Seshadri, R. and J. McN. Sieburth. (in press) Seaweeds as a habitat and source of yeasts in inshore waters. Mar. Biol.

VII. Lehrstuhl für Mikrobiologie der Technischen Universität Berlin und Forschungsinstitut für Mikrobiologie im Institut für Gärungsgewerbe und Biotechnologie, Seestr. 13, 1 Berlin 65 (West), Germany. Communicated by S. Windisch.

S. Windisch and V. Goslich: Sind Kohlensäureflaschen Keimträger? Das Erfrischungsgetränk 23(7), 121-122. 1970.

Summary:

In order to control survival of yeasts (Saccharomyces carlsbergensis, S. cerevisiae, S. diastaticus, C. krusei, C. utilis and Rhodotorula glutinis) 100 ml suspensions of each strain in beer wort containing 6.5 x 10^6 cells/ml were added to gas-bottles with 8 kg CO_2. The same experiments were done with Lactobacillus brevis and Pediococcus cerevisiae suspensions in a glucose peptone yeast extract medium.

CO_2 escaping the pressure-reducing valve of upright standing bottles did not carry along any microbes. Suspended yeast cells on the bottom of the flask were not found to be alive after 24 hours, but there were living cells of the bacteria after 24 hours. The quality of beer or of soft drinks will therefore not be impaired as long as the CO_2 bottle is free of non-gas liquids.

G. Koppensteiner and S. Windisch: Der osmotische Wert als begrenzender Faktor für das Wachstum und die Gärung von Hefen. Archiv. f. Microbiologie 80, 300-314. 1971.

Summary: The Osmotic Pressure as Limiting Factor for Growth and Fermentation of Yeasts.

The limit values of osmotic pressure were investigated which ten osmotolerant and two nonosmototolerant yeasts supported in solutions of sugars

(fructose, maltose, xylose) and salts (lutrol, sodium chloride, potassium chloride, ammonium chloride, ammonium sulphate and lithium chloride) during fermentation and growth by cryoscopy and isothermal destillation. Osmotolerant and nonosmotolerant yeasts did not show any different reaction when growing on agar media containing 2% resp. 50% glucose. The limit values for fermentation of osmotolerant yeasts in fructose solutions were found to be from 238 atm (Saccharomyces rosei) up to 620 atm (S. mellis and S. heterogenicus). The corresponding values of nonosmotolerant yeasts were 160 atm with S. cerevisiae and 124 atm with S. carlsbergensis. The limit values for growth were mostly lower than for fermentation. The highest osmotic values tolerated were found in solutions of fructose. The salt tolerance increased in accordance with the rows of Hofmeister. The low compatibility of concentrated solutions of sodium chloride could be raised by small amounts of fructose. The same effect was caused by lactose and galactose, two sugar types which the yeasts under study do not ferment. Yeast's different tolerance of concentrated solutions of various substances is discussed. The cause is supposed to be due to variability in permeability and a different effect of the dissolved substances on the cytoplasm.

G. Koppensteiner and S. Windisch: Über die Ursachen der Osmotoleranz bei Hefen. Archiv f. Mikrobiologie 83, 193-202. 1972.
Summary: About the Causes of Osmotolerance in Yeasts.
The question, if the yeast cell volume depends on the osmotic pressure and on the solutes of the cell, has been investigated. In concentrated solutions all yeasts investigated reduced their volume. A smaller reduction was found in osmotolerant yeasts. Osmotolerant yeasts begin to reduce their volume at higher osmotic pressures than nonosmotolerant ones. In concentrated nutrient media the cells of osmotolerant and nonosmotolerant yeasts grow in their normal size. The cell size depends only in the range between ± 0.7 to 2% and 10% NaCl on the osmotic pressure of the surrounding medium. Below 2% there is no osmotic reaction and above 10% the cell size remains unchanged. The osmotic value in the cells of both yeast types is about the same. Causes of osmotolerance in yeasts may be a modified structure of cytoplasm and an increased content of bound water.

S. Windisch: Ergebnisse der Forschung an Hemiascomyceten um und seit Wilhelm Henneberg. Kieler Milchwirtschaftl. Forschungsberichte 23, 139-148. 1971. (a review)

I. Neumann: Biotaxonomische und systematische Untersuchungen an einigen Hefen der Gattung Saccharomyces. (Thesis, Technical University Berlin). Beihefte zur NOVA HEDWIGIA Nr. 40, I-V, 1-79. 1972.
Summary:
1. The aim of this work is a systematic investigation of the yeasts of the genus Saccharomyces, following from a review of the literature on classical and modern concepts of species and definition of species in the system of yeasts. Recent publications in this field are evaluated and discussed.
2. For genetic analysis, the method of EMEIS and GUTZ (1958) for mass spore isolation was used in addition to standard taxonomic tests.
3. 151 yeast strains of different origin, belonging to 19 species and 2 varieties of the genus Saccharomyces were studied; 42 of these strains did not sporulate and could not be subjected to genetical control.
4. Determination of yeasts on the basis of critical evaluation of morphological characteristics and results of genetical studies, showed that 130 strains were related to S. carlsbergensis and S. cerevisiae. The remaining 21 yeasts, with a small number of strains, could be divided into 8 other species. Their properties are not clearly defined. The

question of maintaining these species is discussed.

5. Out of 109 strains studied genetically, 59 were homothallic, 50 were heterothallic, but only 6 of these 50 were diploid. The remaining yeasts included 3 triploids, 15 tetraploids, 9 pentaploids and 6 hexaploids. Eleven yeasts were polyploid, but their make-up could not be determined exactly.
6. Study of 30 carbon sources for assimilation and 11 for fermentation demonstrated the absence of properties differentiating one species from another. This fact, together with the establishment of polyploidy, is discussed and its significance for ecology considered.
7. It is proposed that the strains studied be regarded as populations of one species, which on the basis of priority should be named S. cerevisiae HANSEN. Whether the "biological species concept" as described by MAYR (1967) may be used in the case of yeasts is also discussed.

S. Windisch and Ingrid Neumann: Yeast breeding and taxonomy of Saccharomyces. Proc. 2nd Internat. Specialized Yeast Symposium Kyoto. 1972. (in press).

S. Windisch: Zur Züchtung von Saccharomyces-Hefen. Monatsschrift für Brauerei 25(8), 201-203. 1972.

Summary:
After a short survey about history, principles and methods a procedure for the last deciding steps of breeding Saccharomyces yeasts for technical purposes is described. One has to start from euploid heterothallic haploid strains. However, such yeasts are not easily obtained, because most strains in industry and nature are polyploid and aneuploid. The usual crossing work should result in heterothallic diploid yeasts which come as near as possible to the breeding goal. They serve as breeding strains (1st step). The hybrids (2nd step) obtained by crossing single-spore cultures with each other are homozygous for all important genes. After selection for technical fitness they lead to production strains with optimal performance (3rd step).

VIII. Brooklyn College, Department of Biology, Yeast Genetics Group, New York. Communicated by N. R. Eaton.

Activities of N. R. Eaton, R. A. Hackel and F. K. Zimmermann:

Genetics of alpha-methylglucoside fermentation.

The following complementing gene pairs have been reported to confer on cells the ability to ferment alpha-methylglucoside: MGL1 MGL2 and MGL3 MGL2 (Hawthorne, Heredity, 1958). Since MGL2 is tightly linked to MAL3 (Hawthorne and Mortimer in: The Yeasts, 1969), functional distinction between the two can be made only using a strain which has lost the MAL3 function but can still complement MGL1 or MGL3. We have shown that MAL4, or a closely linked gene, also complements MGL1 and MGL3 for alpha-methylglucoside fermentation, but MAL1, MAL2, MAL6 or genes closely linked to these maltose genes do not. Since data have been presented (Okada and Halvorson: Biochim. Biophys. Acta, 1964) which suggest that MGL2 may control the production of an alpha-glucoside permease and since it might be expected that permease genes are linked to each of the maltase genes, a likely hypothesis to explain complementation leading to alpha-methylglucoside fermentation is the requirement for an unlinked permease gene (MGL2-MAL3 or MGLX-MAL4) as well as the structural gene (MGL1 or MGL3) (Khan and Haynes: Molec. Gen. Genetics, 1972) for the

hydrolytic enzyme. If each MAL gene is, indeed, associated with an alpha-glucoside permease gene, the lack of complementation of MGL1 or MGL3 by MAL1, MAL2 or MAL6 requires explanation. The following experimental results show that, while MAL3 and the partially constitutive MAL4 are induced by alpha-methylglucoside, MAL1, MAL2 and MAL6 are not. This suggests that this lack of induction may be the basis of non-complementation of MGL1 and MGL3: In MALX MGL1 or MALX MGL1 MGL2 diploids (where X = 1, 2, 3, 4, or 6) growth in 0.3% glucose + 2.0% α-methylglucoside induces α-methylglucosidase in all strains, but maltase only in those strains carrying MAL3 or MAL4. Maltose induces both enzymes in all of these strains, and, once the cells are induced by maltose, they are able to utilize α-methylglucoside. Functional maltase, or the ability to form maltase, is not required for α-methylglucosidase induction, since the maltase-negative MGL2 MGL1 can ferment α-methylglucoside. These results are consistent with the idea that α-methylglucoside fermentation requires not only MGL1 or MGL3, but also a functional permease linked to each of the MAL genes, and that only those associated with MAL3 or MAL4 can be induced by α-methylglucoside. It should be mentioned, however, that the results are equally consistent with the possibility that α-methylglucoside fermentation requires interaction of the inducer with regulatory genes closely linked to MAL3 and MAL4.

Disaccharide fermentation mutants.

In the preceding issue of this News Letter we have described mutants that prevent fermentation of maltose and sucrose even in the presence of constitutive maltase. We have isolated eleven more mutants of that "dsf" type which turned out to be genetically stable in crosses. Complementation tests were performed by crossing these mutants to the previously described ones. The results indicated that one gene is represented by five, another by four and one more by two mutant alleles. Three more genes are defined by only one mutant each. Five more mutants complemented all previously known mutants and represent at least one more gene. This indicates that disaccharide fermentation mediated by constitutive maltase requires a minimum of seven genes.

New sucrose genes.

In the preceding News Letter we have described two unlinked genes which allow for sucrose fermentation. These genes were induced by UV in a sucrose nonfermenting strain. Enzymatic analysis was performed with crude extracts of segregants. Invertase activity was drastically lower than in a strain with a classical SUC gene. However, the invertases found were typical invertases as judged from determinations of K_m, pH optimum, sensitivity to p-mercurybenzoate and heat sensitivity.

Sucrose non-fermenting mutants.

We have tried to isolate sucrose non-fermenting mutants from strains carrying either SUC2 or SUC3. Slow fermenters obtain very frequently, but mutants with an absolute block in sucrose fermentation are very hard to get or to detect. Only one completely blocked mutant was obtained from EK-6B which carries the MAL3 SUC3 cluster. This mutant fermented maltose like the parent, moreover, when crossed against a maltose sucrose non-fermenting strain, half of the segregants fermented maltose but none sucrose.

IX. Department of Biology, Brooklyn College of the City University of New York and the Department of Biology, York University, Toronto, Ontario, Canada. Communicated by N. A. Khan and R. H. Haynes.

GENETIC REDUNDANCY IN YEAST: NON-IDENTICAL PRODUCT IN A POLYMERIC GENE SYSTEM

Molec. Gen. Genetics 118:279-285

Summary

The alpha-methylglucoside fermenting enzymes produced in response to the two complementing gene pairs (MGL_1 MGL_2) and (MGL_3 MGL_2) were purified and certain of their physical properties were compared. Although both enzymes have the same molecular weight, substrate and serological specificities, they differ with respect to their specific activity, Michaelis constant and heat stability. On the basis of these findings we argue that the loci MGL_1 and MGL_3 are the structural genes responsible for the production of the alpha-methylglucosidases in yeast and that the loci controlling maltase and alpha-methylglucosidase production arose from a common ancestor by gene duplication.

X. Ecole Nationale Supérieure Agronomique de Montpellier, Laboratoire de Recherches de la Chaire de Génétique, Montpellier, France. Communicated by P. Galzy.

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

Lumaret, R. and P. Galzy. Remarques sur le contrôle génétique de la résistance au cuivre chez Saccharomyces cerevisiae HANSEN. Canad. J. of Genetics and Cytol. (in press).
A strain of Saccharomyces cerevisiae HANSEN mutant for copper resistance and colony morphology was studied. The control of "smooth colony" character is monogenic and the control of copper resistance is digenic: the wild strain was mutant for one of the two genes.

Pellecuer, M., P. Galzy and J. Pasero. Selection of mutants for the sexual sign of Saccharomyces cerevisiae HANSEN. 4th International Fermentation Symposium, Kyoto, March 19-25, 1972.
This paper deals with a method making possible the isolation of mutants for the sexual sign.

Chassang-Douillet, A., J. Ladet, H. Boze and P. Galzy. Remarques sur le métabolisme respiratoire de Kluyveromyces fragilis VAN DER WALT. Zeitschrift für Allgemeine Mikrobiologie (in press).
Fermentative and respiratory metabolism of Kluyveromyces fragilis are studied. Kluyveromyces fragilis is not sensitive to glucose effect but gives a Pasteur reaction. The respiratory activity (Q_{O_2}) is more important than the fermentative activity ($Q^{N2}_{CO_2}$).

XI. Laboratoire D'enzymologie, Université de Louvain, Kardinaal Mercierlaan 92, 3030 Heverlee, Belgium. Communicated by A. Goffeau.

The laboratories of Enzymology and Cytogenetics of the "Université de Louvain" have recently published the following papers concerning the functions and the biogenesis of a "petite-negative" species: Schizosaccharomyces pombe.

Modification of mitochondrial ATPase in chromosomal respiratory-deficient mutants of a "petite-negative" yeast: Schizosaccharomyces pombe 972h⁻. A. Goffeau, A. M. Colson, Y. Landry and F. Foury. Biochem. Biophys. Res. Comm. 48, 1448-1454, 1972.

Glucose superrepressed and derepressed respiratory mutants in a "petite-negative" yeast: Schizosaccharomyces pombe 972h⁻. F. Foury and A. Goffeau. Biochem. Biophys. Res. Comm. 48, 153-160, 1972.

Combination of 2-deoxyglucose and snail gut enzyme treatments for spheroplast preparation in Schizosaccharomyces pombe. F. Foury and A. Goffeau. J. Gen. Microbiol. 74, 1973 (in press).

The following dissertations have also been completed:

Obtention par radiations ionisantes de mutants respiratoires modifiés dans l'ATPase mitochondriale chez la levure: Schizosaccharomyces pombe. By M. Devroede, Licence en Sciences botaniques, 1971 (promoteur A. Goffeau).

Analyse spectrophotométrique de la chaîne respiratoire de la levure Schizosaccharomyces pombe 972h⁻. By H. Taeter, Ingénieur chimiste et des Industries agricoles, 1972 (promoteur A. Goffeau).

Isolement et caractérisation de mutants thermosensibles en l'absence de protection osmotique chez la levure Schizosaccharomyces pombe. By P. Shuffart, Licence en Sciences botaniques, 1972 (promoteurs A. M et C. Colson).

XII. Suita Laboratory, Brewing Science Research Institute, Asahi Breweries Ltd., Deguchi-cho 5-3, Suita, Japan. Communicated by Toshiaki Takahashi.

ABNORMAL MITOSIS INDUCED BY ACRIFLAVINE IN SACCHAROMYCES CEREVISIAE

Genetic effects of acriflavine for the following heterozygote of Saccharomyces cerevisiae were tested during mitosis.

ADE1	α	TRP1	URA3	LEU1	arg4	his6	lys1	met3
ade1	a	trp1	ura3	leu1	ARG4	HIS6	LYS1	MET3

The yeast strain was inoculated in YEPD medium containing different concentrations of acriflavine. After two days at 25C, growing cells were spread onto YEPD agar plates and incubated at 28C. When colonies appeared, they were replica plated onto YPE-glycerol agar medium and minimal agar medium. After one night of incubation at 28C, induced ρ- mutants and auxotrophs were scored.

Concentration of acriflavine	Number of cells/ml	Frequency ρ- mutants	auxotroph/ρ-	auxotroph/ρ+
0 ppm	3.0×10^{8}	1.7×10^{-3}	0	7.0×10^{-5}
1	3.0	8.4×10^{-3}	0	0
5	1.5	6.2×10^{-1}	2.5×10^{-3}	7.1×10^{-3}
10	1.1	6.7×10^{-1}	4.9×10^{-3}	7.4×10^{-3}
15	0.9	5.3×10^{-1}	1.6×10^{-2}	3.6×10^{-2}
20	0.7	5.1×10^{-1}	1.1×10^{-2}	1.9×10^{-2}

Among the respiratory sufficient colonies, twenty auxotrophs were isolated and analyzed genetically. Seven of these auxotrophs could not sporulate and could not be mated and thus genetic analysis of them was impossible. But the simultaneous occurrence of auxotroph induction by mitotic recombination and loss of sexuality by some other genetic defects on sex controlling systems may be assumed. One auxotroph could sporulate, but all ascospores did not germinate, and some chromosomal aberrations with lethal effect should be considered. Two auxotrophs showed normal sporulation and normal

ascospore germination. From the segregation data of the tetrads from auxotroph (ade) and auxotroph (lys), they were concluded as mitotic recombinants. One auxotroph (ura3 arg4 lys1) showed mating ability with α-haploid, and the resulting hybrid showed 2:2 segregation on all genetic markers. Therefore it was determined as haploid. By microscopic observation, the haploidization was not based on the sporulation induced by acriflavine treatment. Three auxotrophs could mate with a- or α-haploid, but the hybrids did not sporulate. They were assumed to be aneuploids having an intermediate chromosome number between haploid and diploid. The remaining five auxotrophs could sporulate, but the maximum germinating ascospores in each ascus were two. From this fact and the segregation data, they were assumed as monosomics. Thus, haploidization, reduction of chromosome number, mitotic recombination and some other chromosomal aberrations induced by acriflavine in diploid strain of S. cerevisiae were established beside the ρ- induction.

The second division segregation rates of used markers, estimated by the genetic analysis of assumed monosomics, were as follows:

ade1: 0%; mating type: 40.5%; trp1: 0%; ura3: 4.8%; leu1: 7.1%; arg4: 22.6%; his6: 31.0%; lys1: 57.2% and met3: 7.1%

Some other ρ- inducers have shown the similar effect in yeast mitosis, the details will be published.

XIII. Institut für Biochemie, Universität Würzburg, Rontgenring 11, 87 Würzburg, Germany. Communicated by Eckhart Schweizer.

The following is a summary of an article which is now in press in Molec. Gen. Genetics:

MAPPING OF A COMPLEX GENE LOCUS CODING FOR PART OF THE SACCHAROMYCES CEREVISIAE FATTY ACID SYNTHETASE MULTIENZYME COMPLEX. G. Burkl, H. Castorph and E. Schweizer.

Using two different approaches - UV induced mitotic recombination and meiotic segregation - a fatty acid synthetase gene locus has been mapped on chromosome Fragment V of the Saccharomyces cerevisiae genetic map. This locus has been tentatively designated fas 1AB since it is a complex locus coding for at least two different fatty acid synthetase component enzymes, namely the β-hydroxyacid dehydratase and the enoyl reductase. According to the meiotic segregation patterns obtained, fas 1AB is 41.6 centimorgans from ura1 and 35.7 centimorgans from trp3. Furthermore, the same criteria of mitotic sectoring and meiotic segregation indicate that the second known fatty acid synthetase gene cluster in yeast is genetically unlinked to fas 1AB or to any other of the known genetic loci on chromosome Fragment V.

XIV. Abteilung für Biophysikalische Strahlenforschung, Paul-Ehrlich-Strasse 20, 6 Frankfurt/Main, West Germany. Communicated by V. K. Jain.

Below follow the abstracts of two recent papers:

INFLUENCE OF ENERGY METABOLISM ON THE REPAIR OF X-RAY DAMAGE IN LIVING CELLS. I. Effect of respiratory inhibitors and glucose on the liquid-holding reactivation in yeast. V. K. Jain and W. Pohlit. Biophysik (in press).

Summary: Liquid-holding reactivation in the yeast S. cerevisiae after X-irradiation was observed to be inhibited in the presence of 2,4-dinitrophenol, antimycin A and KCN. However, this inhibition could be reversed by glucose. Radiation resistance and reactivation capacity of a respiratory-

deficient mutant was also observed to increase by pre-irradiation incubation in glucose.

It is concluded that (i) post-irradiation reactivation is an ATP-dependent process, (ii) ATP required can be supplied by the fermentative or the respiratory pathways and (iii) the intracellular energy storage system(s) can profoundly influence the cellular radiation response.

Significance of these results for the radio-therapy of tumours is discussed.

REGULATION OF ENERGY FLOW AND CONTROL OF THE CELL CYCLE IN SACCHAROMYCES CEREVISIAE. V. K. Jain. Biophysik 8:133-140, 1972.

Summary: Measurements of glucose utilization and ethanol production by a respiratory-deficient mutant of S. cerevisiae in a batch culture show that during the phase of acceleration, the glucose utilization per newly formed cell, k, is higher than the average value and the fermentation $F < 1$. In the phase of retardation, on the other hand, k is lower and $F > 1$. Correlating with the changes in the physiological state of the cell population, these results indicate that a considerable fraction of the total glucose consumed is utilized for the synthesis of polysaccharides in the G_1-phase, whereas reserve carbohydrates are catabolised during ($S + G_2 + M$)-phases of the cell cycle.

A cybernetic model for the regulation of the energy (ATP) flow during the cell cycle is presented. It is postulated that the coupling between the energy-yielding and energy-consuming processes is provided by (i) a feedback regulation of the rate of energy production by the energy level and (II) formation and breakdown of an intracellular energy storage system with a control function in the $G_1 \rightarrow S$ transition during the cell cycle.

XV. Institute of General Genetics, University of Oslo, Post Office Box 1031, Blindern, Norway. Communicated by Øistein Strømnaes.

THE CONSEQUENCES OBSERVED IN HETEROZYGOUS ade2/+ DIPLOID CELLS OF SACCHAROMYCES CEREVISIAE FOLLOWING MUTAGENIC TREATMENT OF THE HAPLOID PARENTS. B. A. Siddiqi.

A portion of sectored red and whole red colonies appears when white diploid cells heterozygous at the ade2 locus are treated with EMS and UV. This may be due to the delayed effect of these mutagens. The principal mechanisms which cause this change are mitotic crossing over and/or gene conversion.

The purpose of this investigation was to determine whether or not such a phenotypic effect also can arise in other ways. In other words: Will red sectors appear in clones of diploid cells resulting from the mating of haploid parental cells in which either or both have been treated with EMS and UV. If so, do these mutagens differ in their efficiency?

The haploid parental strains were a ade2 trp2 and α arg9. Cells were suspended in 1% EMS solution for 2 hr, or UV-irradiated for 2 min at 40 cm below a Hanovia model II mercury discharge tube. The survival value of the two parental strains differed after EMS treatment. In the case of the a ade2 trp2 haploid, only 27.12% survived, whereas, treatment of the α arg9 haploid resulted in 57.13% of surviving cells. With UV treatment the survival was 24-26% of both strains. All treatments were carried out prior to mating.

Suspensions of each haploid culture containing 1×10^8 cells/ml were prepared. Crosses were made by transferring 1 ml aliquots of each parental culture to 5 ml of complete liquid medium and incubated for 5 1/2 hr at 28C. After incubation the cells were washed and plated on minimal medium +

adenine. Only diploid culture could grow on this medium.

The following results were obtained.

In the control, that is heterozygous diploid cells (ade2/+) formed from untreated parents, only two red clones were found among 12743 studied (0.015%). While treatment of either of the parent with EMS and UV resulted in a significant increase above control in the number of whole red and sectored red colonies among the resulting diploids.

When only one parent was treated, the percentages of whole red and sectored red colonies were always higher after treatment of the a ade2 trp2 parent than after treatment of the α arg9 parent. This was true whether the treatment was with EMS or UV (Table 1).

Table 1. The percentages of sectored and whole red colonies among ade2 diploid cells when either or both of the haploid parents were treated with EMS and UV

Type of cross	EMS	UV
Treated a ade2 trp2 X Untreated α arg9	1.42	2.25
Untreated a ade2 trp2 X Treated α arg9	0.79	1.79
Treated a ade2 trp2 X Treated α arg9	1.57	2.49

When both haploid parents were treated with either EMS or UV, the percentages of whole red and sectored red colonies recovered were not significantly different from those percentages recovered after treatment of only the a ade2 trp2 parent (Table 1).

The relative percentages of whole red and sectored red colonies following UV treatment of either or both parents, do not differ significantly. In each case approximately 70-75% of the colonies are sectored red and 25-30% are wholly red.

A similar relationship was observed after EMS treatment. In the cross where only α arg9 was treated 94% of the red colonies recovered were sectored, whereas in the other two cases 90% are sectored.

The treatment with UV results in a higher percentage of whole reds among total red colonies than does the treatment with EMS. These results are analogous to the results obtained after treating diploid cells with the same mutagens (Siddiqi, B. A., Hereditas, 69, 305, 1971).

The conclusion that can be drawn is that the mechanism(s) leading to the change from heterozygosity to homozygosity at the ade2 locus can be triggered one cell generation before the change is actually observed. The change from ADE/ade to ade/ade can be initiated in either the adenine dependent or the adenine independent parent.

XVI. Department of Microbiology, Indiana University, Bloomington, Indiana 47401, U.S.A. Communicated by Marjorie Crandall.

In the last issue I reported the discovery of conditions which derepress the synthesis of haploid agglutination or mating factors in the diploid of Hansenula wingei. These derepressed diploids have been found to mate with a high frequency producing triploids. The conditions which derepress the

synthesis of 5-factor (the glycoprotein agglutination factor from mating type 5) in the diploid are to grow cells to stationary phase in Yeast Nitrogen Base (Difco) + 3% glucose broth containing 0.63% YE or containing 0.4×10^{-3} M each of $NaVO_3$ and $NaMoO_4$. The condition which derepresses the synthesis of 21-factor (the glycoprotein agglutination factor from mating type 21) in the diploid is to grow cells to exponential phase in Yeast Nitrogen Base + 3% glucose broth containing 0.63% YE + 10^{-3} M Na_2EDTA. To prepare a triploid, a lysine-requiring diploid of genetic constitution 5 ac ly eb_1eb_2 x 21 ac ly eb_1eb_2 was derepressed for 5-factor synthesis and then mixed together with an auxotrophic strain 21 of genetic constitution 21 ad hi. Prototrophic triploids were purified and tested for DNA content per cell. Values in terms of μg DNA x 10^9 per cell for two haploid strains were 2.6 and 2.8; for two diploid strains were 4.1 and 4.3; and for six triploid isolates were 6.1, 5.8, 6.9, 7.7, 5.7 and 5.7. Each value is the average of determinations of DNA content and cell concentration from two different cultures. Because there are large errors associated with both the extraction of DNA and cell counts it is conceivable that the haploid value is too high. According to the average DNA content in diploids (4.2) and in triploids (6.3) the haploid value should be 2.1 but was found to average 2.7. Despite these errors it can be stated with certainty that triploids have been isolated. Other criteria besides the DNA content indicating that these prototrophs are triploids are that the frequency of prototrophs resulting from the mixture of a derepressed diploid + haploid was increased over the uninduced diploid controls, 2) the triploids have a larger cell size and lower final cell concentration in late stationary phase and 3) they are nonagglutinative as would be expected. It was interesting to note that even the uninduced diploid will mate with a haploid but with a much lower frequency.

XVII. Laboratoire D'enzymologie, C.N.R.S., 91-Gif-Sur-Yvette, France. Communicated by H. de Robichon-Szulmajster.

Summaries of two manuscripts recently submitted for publication are presented below:

RELATIONSHIP BETWEEN METHIONYL TRANSFER RIBONUCLEIC ACID CELLULAR CONTENT AND SYNTHESIS OF METHIONINE ENZYMES IN SACCHAROMYCES CEREVISIAE. Y. Surdin-Kerjan, H. Cherest and H. de Robichon-Szulmajster.

Abstract: Derepression of some methionine biosynthetic enzymes (methionine group I enzymes) obtained in methionine limitation has been found accompanied by an important lack of in vivo charging of bulk $tRNA^{met}$, and, in addition, by a decreased rate of synthesis of all tRNAs. Under the same conditions, methionyl-tRNA synthetase (MTS) is derepressed rather than repressed. These results are in very good agreement with those previously published based on studies of a mutant with an impaired MTS (5) and reinforce the idea that the rate of synthesis of methionine group I enzymes can be related to the total content of met-$tRNA^{met}$ per cell. They also render unlikely that MTS could be a constituent of the regulatory signal.

S-ADENOSYL METHIONINE MEDIATED REPRESSION OF METHIONINE BIOSYNTHETIC ENZYMES IN SACCHAROMYCES CEREVISIAE. H. Cherest, Y. Surdin-Kerjan, J. Antoniewski and H. de Robichon-Szulmajster.

Abstract: SAM has been shown to provoke repression of methionine group I enzymes in wild type cells. Repressive effects of SAM could not be due to its transformation into free methionine since the intracellular pool of this amino acid remains low and constant in repressive and non-repressive growth conditions. In addition, derepression in methionine limitation is accompanied by a severe decrease in SAM pool size, although methionine adenosyl trans-

ferase is slightly derepressed. Different hypotheses have been considered in order to account for the previously reported implication of met-tRNA^{met} (5, Y. Surdin-Kerjan et al., submitted for publication) and presently reported SAM effects in this regulatory process.

XVIII. Laboratory of Cell Biology, Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558, Japan. Communicated by N. Yanagishima.

N. Yanagishima, C. Shimoda and M. Tsuboi. We have been studying problems of genetic and physiological regulation of the life cycle in Saccharomyces cerevisiae. We are also interested in morphological mutants and artificial induction of changes in ploidy. S. Doi, S. Kitano and M. Hagiya are also working at our laboratory as graduate students.

The following articles have been published recently:

Sakai, K. and N. Yanagishima: Mating reaction in Saccharomyces cerevisiae. I. Cell agglutination related to mating. Arch. Mikrobiol. 75, 260-265, 1971.

Shimoda, C. and N. Yanagishima: Role of cell wall degrading enzymes in auxin-induced cell expansion in yeast. Physiol. Plant. 24, 46-50, 1971.

Yanagishima, N.: Induced production of a sexual hormone in yeast. Physiol. Plant. 24, 260-263, 1971.

Shimoda, C., M. Osumi and N. Yanagishima: Responsiveness of yeast cells to auxin in relation to cell age. Plant and Cell Physiol. 12, 581-592, 1971.

Tsuboi, M., S. Kamisaka and N. Yanagishima: Effect of cyclic 3',5'-adenosine monophosphate on the sporulation of Saccharomyces cerevisiae. Plant and Cell Physiol. 13, 585-588, 1972.

Sakai, K. and N. Yanagishima: Mating reaction in Saccharomyces cerevisiae. II. Hormonal regulation of agglutinability of a type cells. Arch. Mikrobiol. 84, 191-198, 1972.

Tsuboi, M., T. Takahashi and N. Yanagishima: Effect of the homothallism-controlling gene, D on the sporulation in Saccharomyces cerevisiae. Japan. J. Genetics 47, 185-192, 1972.

Shimoda, C. and N. Yanagishima: Mating reaction in Saccharomyces cerevisiae. III. Changes in autolytic activity. Arch. Mikrobiol. 85, 310-318, 1972.

We are working on the following problems using Saccharomyces cerevisiae:

Specific inhibition of DNA synthesis during mating reaction. We have found that there is a possibility that a type cells produce a specific inhibitor of DNA synthesis of opposite mating type cells, like α type cells do.

Biochemical and morphological changes in cell wall during mating reaction. Enhancement of both synthesis and degradation of cell wall polysaccharides during mating reaction has been found. Changes in cell wall fine structure and nuclear behavior during mating reaction have been studied in collaboration with Dr. M. Osumi, Japan Women's University, Tokyo.

Studies on hormone like substances and agglutination factors in mating reaction. We are trying to clarify the physiological action and biochemical nature of the regulating substances and agglutination factors involved in the mating reaction in collaboration with Drs. S. Tamura and A. Sakurai, Institute of Physical and Chemical Research, Tokyo.

Effect of cyclic AMP on sporulation. We are obtaining evidences for the involvement of cyclic AMP in the regulation of the change from mitotic division to the meiotic one.

Genetic and physiological regulation of yeast cell form. Artificial induction and physiological and genetic characterization of morphological mutants are being studied in collaboration with Dr. T. Takahashi, Suita Lab., Brewing Science Research Institute, Suita. Auxin has been known to induce diploidization in a haploid strain.

XIX. Eidgenössische Technische Hochschule, Mikrobiologisches Institut, Zürich, Switzerland. Communicated by R. Hütter.

Below are presented the summaries of three dissertations from our Institute.

Dissertation No. 4862. A. Schürch.

Summary: A transport and a regulatory mutant were isolated from a 5-methyltryptophan (5MT) sensitive double mutant strain of Saccharomyces cerevisiae.

The transport mutation leads to altered uptake activities for tryptophan and several other amino acids in a NH_4^+ containing medium. The mutation affects different amino acid permeases to various extents. It allows maximum transport activity and alters the excretion of transport substrate. In cells taken from the stationary phase of growth a decrease in tryptophan uptake was found in NH_4^+-containing medium but not in proline-containing medium if compared with log-phase cells. Furthermore, the incorporation of tryptophan into protein of wild type cells was greater in the NH_4^+ containing medium than in proline containing medium. This suggests that the general amino acid permease serves mainly a catabolic role while the specific amino acid permeases serve anabolic and regulatory functions. Altered uptake activity for 5MT is followed by altered 5MT-sensitivity.

The regulatory mutation leads to a loss in the ability to be derepressed of cells growing in a medium with 5MT or 3-amino-1,2,4-triazole (3AT). It is proposed that 5MT causes false feedback inhibition of anthranylate synthetase and therefrom a deficiency of tryptophan results. This deficiency is followed by derepression of the tryptophan biosynthetic enzymes; therefore wild type cells can partially overcome the 5MT inhibition. The 5MT-sensitive regulatory mutant lacks this ability to be derepressed for up to now unknown reasons. Two analyzed 5MT-resistant mutants are feedback-resistant and therefore not 5MT-inhibited. 3AT leads to derepression of wild type-enzymes of tryptophan, histidine and arginine biosynthesis. These data suggest a superordinate center of regulation of various pathways of amino acid biosynthesis.

The aggregation of anthranylate synthetase and indole-3-glycerol phosphate synthetase as well as the active fractions after incomplete rupture of the latter were examined. Several kinetic parameters were measured with partially (83-fold) purified anthranylate synthetase.

Present address: Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27706, U.S.A.

Dissertation No. 4825. H. Henke.

Summary: The biosynthesis of tryptophan in Coprinus radiatus is controlled by four genetic loci: trp-1 controls the Trp.-Sase, trp-3 controls the AA-Sase, trp-4 controls the PRA-Isom./InGP-Sase and trp-6 controls the PR-Tase. A new modification of gene-enzyme relationships in tryptophan biosynthesis has been demonstrated.

AA-Sase and PRA-Isom./InGP-Sase are found in a complex with a $s_{20.w}$ = $10.5 \cdot 10^{-13}$ sec; in this complex the activities are stabilized by each other. The AA-Sase may be active separated from this complex. This new kind of gene-enzyme relationship expresses a typical sedimentation pattern in sucrose gradients; AA-Sase activity is found in a band with $s_{20.w} = 10.5 \cdot 10^{-13}$ sec and in another band with $s_{20.w}$ = $4.6 \cdot 10^{-13}$ sec.

The zone centrifugation pattern of Coprinus radiatus has been demonstrated also in some other basidiomycetes which belongs to the Agaricales. Some other basidiomycetes show a different sedimentation pattern. The distribution of the various pattern amongst the basidiomycetes was examined. The results are discussed in a phylogenetic point of view.

AA-Sase from two strains of Coprinus lagopus has been isolated and partially purified. The pH optima for enzyme stability and activity is at pH 7.5 with L-glutamine as substrate. The K_m values are $7.5 \pm 0.4 \cdot 10^{-6}$ M for chorismate and $1.5 \pm 0.3 \cdot 10^{-3}$ M for L-glutamine for AA-Sase with $s_{20.w} = 4.6 \cdot 10^{-13}$ sec (pH 7.5, 37°C).

Present address: Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan 48104, U.S.A.

Dissertation. Yvonne Schuerch-Rathgeb.

Summary: The physiological and enzymatic characteristics of 69 trp3 mutants of Saccharomyces cerevisiae were used to group these in three classes A, B and C. The mutants of the first class (trp3A) do not have an active ASase nor an active InGPSase. They do not grow in medium that contains anthranilic acid and will not accumulate this intermediate product. The trp3B mutants are inactive in the InGPSase, but active in the ASase; they accumulate anthranilic acid, but do not grow on it. Trp3C possess an active InGPSase but an inactive ASase. They do not accumulate anthranilic acid but will grow on medium containing it. The results of the in vivo experiments agree with those in vitro.

The size of the ASase and the InGPSase of the trp3B and trp3C mutants were ascertained by the determination of the sedimentation constant on a sucrose gradient. Values of 6,9 S, 4,5 S and 3,6 S were obtained for the InGPSase, while the ASase gave values of 6,9 S and 5,4 S. The wild type aggregate had a sedimentation constant of 6,9.

The trp3 gene can be divided in two regions, one determining the activity of the InGPSase, the other, the formation of the enzyme aggregate. The region determining the activity of the InGPSase lies on the N-terminal side, while the aggregate formation is determined by the C-terminal part of the gene.

Interallelic complementation was analyzed with 16 mutants. In all cases, including trp3B x trp3C combinations the formation of hybrid enzymes seems to be the mechanism of complementation. No evidence for cross-feeding could be found.

Of the 33 trp3 mutants examined, 8 were suppressible by the ochre-suppressor S11. Of these, 5 mutants belonged to the trp3A class and 3 to the trp3C; of the 15 trp3B mutants analyzed, none were suppressible.

Present address: Division of Immunology, Duke University Medical Center, Durham, North Carolina 27706, U.S.A.

XX. Laboratory of Microbiology, Gulbenkian Institute of Science, Oeiras, Portugal. Communicated by N. van Uden.

Since our last contribution to the Yeast News Letter, some years ago, our laboratory has undergone profound changes in its structure and activities. Here follows a short outline.

Head of the laboratory: N. van Uden, Dr. med. univ. (Univ. Vienna)

Senior Researcher: L. J. Archer, Ph.D. (Georgetown Univ.), Dr. Biol. & "agregado" (Univ. Oporto)

Research Associates:
M. Vidal Leiria, Lic. Biol. (Univ. Lisbon), M.A. Microbiology (Univ. California)
R. Castelo Branco, Lic. Vet. Med. (Univ. Lisbon)
A. Madeira Lopes, Lic. Biol. (Univ. Lisbon), M.Sc. Genetics (Univ. Washington)
M. J. Marinho, Lic. Pharm. (Univ. Oporto)
M. C. Cabeça Silva, Lic. Biol. (Univ. Lisbon)

Graduate Students:
J. M. Cardoso Duarte, Chem. Eng. (Univ. Lisbon)
M. C. Loureiro Dias, Chem. Eng. (Univ. Lisbon)
J. M. do Carmo, Lic. Biol. (Univ. Lisbon)
J. Martinez Peinado, Lic. Biol. (Univ. Navarra)
J. D. Arrabaça, B.Sc. (Univ. Lisbon)

Recent publications (1971-72):

Van Uden, N. (1971) Kinetics and energetics of yeast growth, in A. H. Rose and J. S. Harrison (eds), The Yeasts, Vol. 2, Physiology and Biochemistry of Yeasts, Academic Press, London and New York.

Oliveira-Baptista, A. and N. van Uden (1971) Occurrence of two maximum temperatures for growth in yeasts, Zeitschrift für Allgemeine Mikrobiologie 11, 59-60.

Van Uden, N. (1971) Thermodynamics of the chemostat, Zeitschrift für Allgemeine Mikrobiologie 11, 541-550.

Gómez Vinarás, F. and L. J. Archer (1972) Thermal sensitivity of Bacillus subtilis in synchronous culture (in press).

Archer, L. J. (1972) Heat sensitivity of competent and pre-competent cells of Bacillus subtilis (in press).

Vidal-Leiria, M. and N. van Uden (1972) Inositol dehydrogenase from the yeast Cryptococcus melibiosum, Biochimica et Biophysica Acta (in press).

Current projects:

I M. Vidal Leiria and N. van Uden: Isokinetic temperature ("compensation temperature") of thermal death in yeasts and its correlation with the maximum temperature for growth

II L. J. Archer: Heat sensitivity of competent cells of B. subtilis

III J. M. do Carmo and L. J. Archer: Genetic transformation for thermal death rates and maximum temperature for growth

IV A. Madeira Lopes and N. van Uden: Inheritance of the maximum temperature for growth in yeasts

V J. M. Cardoso Duarte, M. J. Marinho and N. van Uden: Flow calorimetry and thermodynamics of continuous yeast cultures

VI J. D. Arrabaça, M. C. Loureiro Dias and N. van Uden: Flow calorimetry and thermodynamics of yeast growth in batch cultures

VII J. Martinez Peinado and N. van Uden: Maintenance metabolism in sodium chloride adapted yeasts

XXI. Departamente de Microbiología, CSIC y Facultad de Ciencias, Universidad de Salamanca, Spain. Communicated by J. R. Villanueva.

Our Department organized the Third International Symposium on Yeast Protoplasts which took place in Salamanca (October 2-5, 1972). The proceedings of the Symposium are going to be published by Academic Press with the title "Yeast, mould and plant protoplasts".

The following papers have been or are going to be published:

INFLUENCE OF GLUCOSE CONCENTRATION OF THE MEDIUM ON THE INVERTASE CONTENT OF A STRAIN OF SACCHAROMYCES BEARING THE SUC2 GENE. S. Gascón and P. Ottolenghi (Compt. Rend. Trav. Carlsberg 39, 15, 1972).

The cellular content of invertase (β-D-fructofuranoside fructohydrolase, Ec. 3.2.1.26) under the control of the SUC2 gene of Saccharomyces was studied in continuous culture. The invertase content of the cells was found to increase as the glucose concentration in the culture supernatant decreases. With the strain used, and at pH 5.25, a fifty-five fold difference in the amount of cellular invertase could be obtained by varying the glucose concentration in the culture. The pH of the culture also influenced the amount of invertase per unit weight of cells: the enzyme content was lowest at pH 5.2-5.5 and markedly higher both at lower and at higher pH (4 and 6, respectively). (The experimental work described in this paper was done at the Carlsberg Laboratories in Copenhagen.)

BIOSYNTHESIS OF MANNAN IN SACCHAROMYCES CEREVISIAE. ISOLATION OF A LIPID INTERMEDIATE AND ITS IDENTIFICATION AS MANNOSYL-1-PHOSPHORYL POLYPRENOL. R. Sentandreu and J. O. Lampen (FEBS Letters 27, 331, 1972).

We have been able to isolate by combination of mild alkaline treatment with silicic acid, DEAE-cellulose and Sephadex LH-20 columns, a mannolipid that participates in the synthesis of yeast wall mannoproteins.

The purified lipid intermediate is homogeneous by chromatography on Silica Gel G plates, resistant to alkaline ethanolysis and acid-labile. Mild acid hydrolysis (0.1 N HCl, 100C, 20 minutes) rendered all the radioactive mannose water soluble and the ratio of total mannose to total phosphorus in the aqueous phase after mild hydrolysis was 1.27:1.0.

These results together with the olive-green color given by this lipid with p-anisaldehyde reagent indicate that the lipid intermediate that participates in the synthesis of yeast mannoproteins is a phosphodiester of mannose and an isoprenoid alcohol. (The experimental work described in this paper was carried out while R. Sentandreu was a Visiting Fellow at the Institute of Microbiology, Rutgers University.)

MORPHOGENETIC AND NUTRITIONAL STUDIES OF GEOTRICHUM LACTIS CELLS. Angel Durán, Federico Uruburu and Julio R. Villanueva (Archiv für Mikrobiologie, in press).

Geotrichum lactis was grown in culture media with different carbon and nitrogen sources. Biotin is needed as growth factor. The distinct morphologic patterns of the cells through the culture media show the presence of a new growing form we have called yeastlike form. This thick-walled form emerges from mature arthrospore cells developed through fragmentation of the mycelium. The arthrospore cells and the yeastlike cells have walls with different chemical and structural composition, as the electron microscope and their resistance to some lytic enzymatic extracts show.

XXII. The Research Laboratories of Kirin Brewery Co., Ltd., Takasaki, Gumma Pref., Japan. Communicated by Yasushi Yamamoto.

Mr. Kumpei Kitamura, Mr. Tatsuhiko Kaneko and I have engaged in research of yeast cell wall lytic enzymes produced by a strain of Arthrobacter luteus

for several years, and have published or presented the following papers.

T. Kaneko, K. Kitamura and Y. Yamamoto. Arthrobacter luteus nov. sp. Isolated from Brewery Sewage. J. Gen. Appl. Microbiol. 15, 317 (1969)

K. Kitamura, T. Kaneko and Y. Yamamoto. Lysis of Viable Yeast Cells by Enzymes of Arthrobacter luteus. Arch. Biochem. Biophys. 145, 402 (1971)

K. Kitamura, T. Kaneko and Y. Yamamoto. Lysis of Viable Yeast Cells by Enzymes of Arthrobacter luteus. I. Isolation of Lytic Strain and Studies on its Lytic Activity. J. Gen. Appl. Microbiol. 18, 57 (1972)

K. Kitamura, T. Kaneko and Y. Yamamoto. Lysis of Viable Yeast Cells by an Enzyme "Zymolyase" of Arthrobacter luteus. IVth Intern. Ferm. Symp., 1972, Kyoto, Japan.

K. Kitamura and Y. Yamamoto. Purification and Properties of an Enzyme, Zymolyase, Which Lyses Viable Yeast Cells. Arch. Biochem. Biophys. in press.

XXIII. Department of General Biology, Medical Faculty J. E. Purkyně University, Brno, Czechoslovakia. Communicated by Marie Kopecká.

In our laboratory we are mainly concerned with the study of the formation of the fibrillar wall component by yeast protoplasts. Protoplasts of Saccharomyces cerevisiae are our main model object, even though in some experiments we also use protoplasts of Schizosaccharomyces pombe, Nadsonia fulvescens and of other yeast species. We would like to obtain detailed information on the chemical composition and structure of the fibrillar wall component, about which till now we have information only from X-ray diffraction diagrams made in cooperation with Dr. D. R. Kreger. We also know the solubility properties of fibrillar walls and we tried some specific enzymes kindly provided by Prof. H. J. Phaff. We would welcome cooperation with experts in polysaccharide chemistry, who would be interested in the study of the structure of native glucans which are present in the fibrillar nets of protoplasts. We would be able to prepare for them samples of the fibrillar walls of protoplasts in sufficient quantity and purity. Further, we are interested in the study of external conditions and cell processes which lead to the formation of glucan microfibrils by yeast protoplasts, the localization of formation of glucan macromolecules and microfibrils, in the mechanism involved in the formation of glucan microfibrils from macromolecules, in the structure of glucan microfibrils, and regulation of formation of glucan microfibrils by yeast protoplasts. "The formation of the fibrillar wall component by yeast protoplasts" constitutes the subject for Ph.D. work of Marie Kopecká.

The following papers recently appeared or are in press or prepared for press:

Kopecká, M. Dictyosomes in the yeast Schizosaccharomyces pombe. Antonie van Leeuwenhoek 38, 27-31, 1971.

Kopecká, M., A. Svoboda and J. Brichta. Structure of yeast cells in various osmotic conditions. Symp. Yeasts as Models in Science and Technology, Smolenice, Czechosl. 1971, Slov. Acad. Sci. 1972, p. 183-184.

Kreger, D. R. and M. Kopecká. On the nature of fibrillar nets formed by Saccharomyces cerevisiae protoplasts in liquid media. III. Int. symp. on yeast protopl. Salamanca, 1972, Academic Press, in press.

Kopecká, M. Reaggregation of fibrillar wall components of yeast proto-

plasts. Folia microbiol., in press.

Kopecká, M., M. Gabriel and O. Nečas. A method of isolating nucleiless yeast protoplasts and their cytological properties. In preparation.

Kopecká, M., H. J. Phaff and H. Tanaka. Demonstration of the fibrillar component in the cell wall of yeast. In preparation.

The following lectures were presented in 1972:

Kopecká, M. and D. R. Kreger. An electron microscope study of the solubility properties of the fibrillar nets formed by protoplasts of Saccharomyces cerevisiae in liquid media. III. Int. symp. on yeast protoplasts, Salamanca, 1972.

Kreger, D. R., M. Kopecká. On the nature of fibrillar nets formed by protoplasts of Saccharomyces cerevisiae in liquid media. III. Int. symp. on yeast protoplasts, Salamanca, 1972.

Kopecká, M. Reaggregation of fibrillar wall components of yeast protoplasts. Ann. meet. Czech. Microbiol. Soc., Brno, 1972.

Kopecká, M. Formation of fibrillar glucan by yeast protoplasts. Vth ann. meet. Czechosl. yeast group, Smolenice, 1972.

XXIV. Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, U.S.A. Communicated by F. Schlenk.

The final results of post-doctoral studies of K. A. Killick have been published: Modification of pigmentation and cell wall structure of Rhodotorula aurantiaca by culture in presence of sulfur amino acids. Canadian J. Microbiol. 18, 423-427 (1972).

Two papers were presented at the Third International Symposium on Yeasts Protoplasts, October 2-5, 1972, Salamanca, Spain: G. Svihla, K. D. Nakamura, J. L. Dainko and F. Schlenk: Abnormal Growth and Budding of Candida utilis after lethal X Irradiation. G. Svihla and F. Schlenk: Ultraviolet Microscopy of Yeast. Samples of the UV photomicrographs of this laboratory were awarded the Second Prize in the photographic exhibit of the Symposium. The techniques used are summarized by G. Svihla, in Encyclopedia of Microscopy, P. Gray, ed., Van Nostrand Reinhold, New York (in press).

XXV. Department of Biology, University of California, Los Angeles, Calif. 90024, U.S.A. Communicated by J. R. Wells and T. W. James.

CELL CYCLE ANALYSIS BY CULTURE FRACTIONATION OF SCHIZOSACCHAROMYCES POMBE

A method has been developed for the isolation of age-related cell size classes from cultures of the yeast Schizosaccharomyces pombe. This has been done by the use of a reorienting gradient zonal centrifuge rotor, the SZ 14 (Sorvall). The method employed rate-sedimentation of the yeast cells in a 15-30% W/W sucrose gradient. The separation was carried out in a volume of 1,300 ml. at 1,500 rpm. A cell suspension of 4×10^9 cells in 15 ml. was dynamically loaded in a few seconds yielding a narrow starting zone. The sedimentation required 5 1/2 minutes and deceleration was carried out under controlled conditions. The reoriented, distributed cells were unloaded and isolated into specific fractions for biochemical assays and photographs. An ad hoc method of isolation was used, namely the cells were fixed while in the logarithmic phase of growth prior to their isolation. A post hoc method is also possible with this rapid method. The 20 fractions of different cell size represented cells in balanced growth and are without distortions which normally accompany methods for inducing cell synchrony. Time-lapse photomicrography studies were done on growing microcolonies of

the yeast to obtain data relating the cell length to age. Elongation stops at about .75 units of the cell cycle. However, it was discovered that cells beyond this length distributed themselves over a volume difference of 200 mls. in the gradient. To establish that these were distributed according to age, a set of cell characteristics was used, the most important of which was the presence of division septa. In the heaviest fraction, 55% of the cells show well developed cell plates. With these data it was possible to characterize the age of cells in various fractions.

Two types of experiments were carried out. The first was to discover the best centrifugation conditions which would isolate the yeast cells over the widest range of cell fractions. The second was to estimate the time of synthesis of DNA and compare it to the synthetic period obtained by other means, i.e., induced synchrony and selection synchrony. The first experiments established the conditions given above. Furthermore, they established that cell fractions could be obtained which were easily related to age, and this was apparently governed by combination of two parameters, size and density. The second set of experiments established that cells at the beginning of the cell cycle contained 0.032 μ grams DNA/10^6 cells. The average age for the middle of the DNA S period from three separate experiments was found to be 123 minutes or 0.83 of the cell cycle.

This value for the time of the DNA S period agrees well with the work of Bostock on strain 132, although the strain used in the present experiments was 972h$^-$. Other work on 972h$^-$ S period suggests it is closer to cell fission. Changes in the time of the S period have, however, been noted in old strains 132, and our work would indicate that the same may occur with strain 972h$^-$.

These techniques are presently being employed in an attempt to resolve the contradictory data on the timing of the synthesis of the mitochondrial DNA in Saccharomyces cerevisiae. The work on S. pombe summarized above is in press in Experimental Cell Research.

XXVI. Department of Bacteriology and Immunology, University of Western Ontario, London, Canada. Communicated by Carl Robinow.

Mitosis in three heterobasidiomycetous "yeasts", the former Candida scottii, Rhodotorula glutinis and Sporobolomyces salmonicolor has been studied by E. Kathleen McCully and C. F. Robinow.

The course of mitosis has been established on the basis of three types of observation: on living cells by phase contrast microscopy, on cells stained for chromatin and studied by ordinary microscopy and on glutaraldehyde fixed cells studied with the electron microscope.

Nuclear division in these yeasts involves migration of all of the chromatinic portion of the nucleus into the bud, dissolution of the nucleolus in the cytoplasm of the mother cell, partial breakdown of the envelope around that portion of the nucleus that enters the bud, contraction of the chromatin (chromosomes) and the development of a mitotic spindle apparatus. One of the two daughter nuclei produced by this process of "mitosis in the bud" returns to the mother cell.

We believe that our observations are of interest not only to students of mitosis but also to Yeast taxonomists. The differences between mitosis in ascomycetous yeasts and heterobasidiomycetous yeasts are so striking that future studies of mitosis in asporogenous yeasts should reveal their taxonomic affinities to either ascomycetes or basidiomycetes.

McCully, E. K. and Robinow, C. F. (1972) Mitosis in heterobasidiomycetous yeasts. I. Leucosporidium scottii (Candida scottii). J. Cell Sci.

10, 857-881.

McCully, E. K. and Robinow, C. F. (1972) Mitosis in heterobasidiomycetous yeasts. II. Rhodosporidium sp. (Rhodotorula glutinis) and Aessosporon salmonicolor (Sporobolomyces salmonicolor). J. Cell Sci. 11, in press.

XXVII. Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki, Finland. Communicated by H. Suomalainen.

FORMATION OF AROMA COMPOUNDS IN ALCOHOLIC BEVERAGES

H. Suomalainen and L. Nykänen

Paper presented at Suntory Ltd., Osaka, Japan 1972 and at the 3rd Nordic Aroma Symposium, Aulanko, Finland 1972. Submitted for publication in Wallerstein Lab. Commun.

The volatile aroma in alcoholic beverages is built up by numerous components; the aroma of whisky comprises over 200. These components include carbonyl compounds, fusel alcohols, fatty acids and their esters, compounds containing sulfur and nitrogen, phenolic compounds and lactones. On investigating the formation of the aroma components, it is noted that the identified compounds mostly occur in fermentation media and, moreover, that the yeast produces the bulk of the aroma. The phenolic compounds and the lactones in whisky are found to originate mainly from the wooden barrels. In addition to the aromatic properties, the physiological effects of the aroma components have been considered.

STRUCTURE AND FUNCTION OF THE YEAST CELL ENVELOPES

H. Suomalainen and T. Nurminen

3rd Int. Symp. on Yeast Protoplasts, Salamanca 1972, abstracts, p. 38.

Cell envelopes consisting of the cell walls proper and fragments of the plasma membrane, but free from cytoplasmic contamination, have been isolated from baker's yeast (Saccharomyces cerevisiae). After enzymatic removal of most of the carbohydrates, the plasma membrane fraction was purified by centrifugation. After enzymatic digestion, saccharase and various acid phosphatases were soluble, but most of Mg^{2+}-dependent ATPase, phospholipase and lipase was still sedimentable. Accordingly, the solubilized enzymes were mainly situated externally, but the three latter enzymes in the plasma membrane.

Neutral lipids, principally triglycerides, sterol esters and free sterols, amounted to 50% of the envelope lipids. Phospholipids, mainly phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol, were only present in the plasma membrane. The major fatty acids were $C_{16:1}$ and $C_{18:1}$ acids. 2-Hydroxy-$C_{26:0}$ and $C_{26:0}$ acids predominated among very long-chain fatty acids, and largely originated from a glycosphingolipid containing inositol, phosphorus and mannose, present in both the wall and the plasma membrane. Small amounts of other glycolipids were detected.

THE EFFECT OF AERATION ON THE GROWTH ENERGETICS AND BIOCHEMICAL COMPOSITION OF BAKER'S YEAST

E. Oura

Thesis, Helsinki University, 1972, 124 pp.

A continuous cultivation method was developed to study the effects of aeration intensity on yeast in conditions where the influences of changes in growth rate and of catabolite repression were eliminated. Further a chemically defined medium with the substrate as the only carbon and energy source was used.

The observed material changes occurring during the growth of yeast were treated using growth equations divided to: (a) the assimilation of substrate to form yeast material and (b) the energy-yielding catabolic reactions. This kind of model allowed the actual ratio for oxidative phosphoryla-

tion during growth conditions to be estimated by two different methods. The values obtained suggest that the P/O ratio in growing baker's yeast is 1.9. Further the results indicated that the Y_{ATP} value is not constant during growth under different intensities of aeration. The energy required for different functions varies with the growth conditions. The energy used for the formation of secondary and tertiary structure of polymers and for the organization of cell structure is the major energy requirement of yeast. The aerobic cell, having a more complicated cell structure than the anaerobic cell, requires 40% more energy for the formation of cell structure than the anaerobic cell.

Changes in the biochemical composition of the following factors under different intensities of aeration were determined: adenosine nucleotides, NAD-$NADH_2$-system, flavine nucleotides, activity to ferment glucose, activities to oxidize glucose or ethanol and the activities of 12 enzymes functioning in the glycolytic pathway, TCA-cycle, HMP-pathway, glyoxylate pathway, electron transport chain and reactions from glutamic acid. Oxygen influences the activity of the enzymes under conditions where catabolite repression plays no part. Yeast has a potential for oxidative metabolism even under oxygen-limited growth. This is shown by maxima in the amounts of cytochromes and the activities of other respiratory enzymes at aeration intensities where the availability of oxygen limits the oxidative potentiality. The observed maximal activity of respiration correlates with the activity of the two oxidoreductases measured. At higher intensities of aeration the metabolism is disturbed. These observations are discussed in terms of several possible regulatory mechanisms.

One of the references in the thesis was the following, unpublished report (available from the author):

REACTIONS LEADING TO THE FORMATION OF YEAST CELL MATERIAL FROM GLUCOSE AND ETHANOL
E. Oura
Research Laboratories of State Alcohol Monopoly (Alko), Report 8078, 34 pp.

The starting point was to resolve the extent to which yeast contains carbohydrates, proteins, nucleic acids, neutral lipids and phospholipids and the monomer composition of these main components.

The reaction sequence leading to the formation of each monomer, hexose amino acid, nucleotide, glycerol, acetyl-CoA and phosphoryl compound have been derived. The subsequent polymerization reactions leading from these monomers to the polymers have been determined. Considerable attention was given to the changes in the adenosine phosphate and respiratory nucleotide systems.

Consequently, the equations for the material and energy changes occurring during the assimilation of either glucose or ethanol to form cell material are presented. The equation: glucose → cell material indicates that no ATP is produced during the assimilation of glucose when the formation of oxaloacetate was taken to occur via the carboxylase reaction. Moreover, the production of reduced respiratory nucleotides is quite small. The equation: ethanol → cell material, shows that the energy changes during the assimilation of ethanol are much greater than those with glucose. The most prominent feature is the high ATP consumption during this process. Changes in the content of carbohydrates, proteins and nucleic acids in yeast do not much alter these estimations. In contrast, the estimations can be greatly altered by changes in the lipid content.

The thesis and the report 8078 cover the following abstracts:

ENERGETICS OF YEAST GROWTH UNDER DIFFERENT INTENSITIES OF AERATION
E. Oura
1st International Symposium on Advances in Microbial Engineering, Marianske Lazne, Czechoslovakia 1972, abstracts, pp. 42-43.

THE PRODUCTION OF ENERGY DURING AEROBIC GROWTH OF BAKER'S YEAST ON GLUCOSE AND ETHANOL
E. Oura
2nd Congress of Yogoslav Microbiologists with International Participation, Opatija 1972, summaries, p. 83.

THE ABILITY OF STORED BAKER'S YEAST TO SYNTHESIZE RIBONUCLEIC ACID AND PROTEIN
E. Parkkinen, S. Grba, E. Oura and H. Suomalainen
2nd Congress of Yugoslav Microbiologists with International Participation, Opatija 1972, summaries, pp. 110-111.

The changes in the uptake and incorporation of RNA precursor and amino acids into RNA and protein during storage of a baker's yeast cake at 35°C have been studied. Uracil-2-^{14}C was used as a low molecular weight RNA precursor and L-methionine-(methyl-C^{14}) and L-valine-C^{14} were used as labelled amino acids. After two days of storage the ability to incorporate uracil into RNA and amino acids into protein is lost after this time, but the uptake of these compounds into total cold acid-soluble pool decreases more slowly until the fourth day, but thereafter it decreases very rapidly. The incorporation of uracil into nucleotides and RNA decreases more rapidly than that of amino acids into protein.

The results show the relation between the time of storage and its ability to synthesize RNA and protein, as assessed by measuring the incorporation of labelled precursors.

ASPECTS OF CYTOLOGY AND METABOLISM OF YEAST
H. Suomalainen, T. Nurminen and E. Oura
in Progress in Industrial Microbiology, vol. 12, ed. by D. J. D. Hockenhull, J. & A. Churchill, London (in press).

A large review is given on cytology and growth metabolism of yeast. Morphology, chemical and enzymic composition, and the functions of cell wall and plasma membrane are considered. Further the review includes the following aspects of yeast growth: phases of growth in aerobic glucose medium, catabolite repression and role of oxygen in adaption to respiration and in formation of mitochondria. 13 tables, 14 figures and 282 references.

A MANGANESE-DEPENDENT ADENYL CYCLASE IN BAKER'S YEAST, SACCHAROMYCES CEREVISIAE
J. C. Londesborough and T. Nurminen
Acta Chem. Scand. (in press).

Commercial yeast homogenates synthesized 8-^{14}C-3'5' cyclic AMP from 8-^{14}C-ATP at a rate of 4.4 nmole/min/g fresh yeast at 30°C and pH 6.8, in the presence of 5 mM $MnCl_2$ plus 1 mM $MgCl_2$. With the 10 min x 1000 g sediment the rate in 5 mM $MgCl_2$ alone was only 11% of that in 5 mM $MnCl_2$ plus 0.4 mM $MgCl_2$. About 75% of the total activity sedimented in 60 min at 140000 g, and 12% was retained by well washed cell envelopes free from mitochondrial and cytoplasmic markers. For cell envelopes in the presence of 0.4 mM cyclic AMP the apparent Km for ATP was 2.9 mM and the pH optimum was 5.7. It was suggested that much of the activity was native to the plasma membrane, but additional subcellular sites could not be excluded.

The following publication has appeared since the last communication. The abstract of the report has been given in the Yeast News Letter in June 1972.

H. Suomalainen, J. Dettwiler and E. Sinda. α-Glucosidase and leavening of baker's yeast. Process Biochem. 7 (1972), 16-19.

XXVIII. Mikrobiologisches Institut, Swiss Federal Institute of Technology, Weinbergstrasse 38, Zürich, Switzerland. Communicated by A. Fiechter.

Recent publications:

H. P. Knöpfel. Zum Crabtree-Effekt bei Saccharomyces cerevisiae und Candida tropicalis. Thesis. Juris Druck + Verlag, Zurich, 1972, 83 pp.

A. Fiechter and H. Schatzmann. Glucose uptake rate of Saccharomyces cerevisiae in presence and absence of oxygen. Proc. 4th Int. Ferm. Symp., Kyoto, 1972.

M. T. Küenzi and A. Fiechter. Regulation of Carbohydrate Composition of Saccharomyces cerevisiae under Growth Limitation. Arch. Mikrobiol. 84, 254-265, 1972.

In press:

H. Hug and A. Fiechter. Assimilation of aliphatic hydrocarbons by Candida tropicalis. I. Analytical problems in hexadecane batch experiments. (Accepted for publication in Arch. Mikrobiol.)

H. Hug and A. Fiechter. Assimilation of aliphatic hydrocarbons by Candida tropicalis. II. Fatty acid profiles from cells grown on substrates of different chain lengths. (Accepted for publication in Arch. Mikrobiol.)

A. Einsele, H. Blanch and A. Fiechter. Agitation and aeration in hydrocarbon fermentations. (Accepted for publication in Biotechnol. Bioeng. Symp. Nr. 4)

XXIX. Macdonald College of McGill University, Quebec, Canada. Communicated by M. A. Lachance.

The following material summarizes the work being done on Yeast in the Department of Microbiology at Macdonald College.

The papers below were presented at the Annual Congress of A.C.F.A.S. (French Canadian Association for the Advancement of Science), on October 13, 1972 in Ottawa:

PRODUCTION DE LIPIDES PAR RHODOTORULA GLUTINIS EN CULTURE CONTINUE
J. Goulet and A. C. Blackwood
Abstract:

Considering the actual development of metabolites and biomass by processes of microbial fermentation, it seems that lipid production by microorganisms could be of particular interest in the coming years. Therefore, we have undertaken research on biosynthesis of lipids by the yeast Rhodotorula glutinis. To achieve this purpose, we have used a two-stage continuous fermentor system, and we have devised a rapid method of determination of optimal pH and temperature, in a continuous process. The composition of lipids produced by this yeast has turned out to be interesting, especially as far as the high level of long-chain unsaturated fatty acids is concerned.

ETUDE DE LA CROISANCE D'ENDOMYCOPSIS FIBULIGERA SUR DES RÉSIDUS DE PATES ALIMENTAIRES
M. A. Lachance and A. C. Blackwood
Abstract:

An ascosporogenous yeast, Encomycopsis fibuligera, has been isolated by enrichment techniques from the endogenous flora of residues of commercial

pasta, in order to study production of microbial protein from the starch contained in these industrial wastes. The rate of starch degradation has been observed in relationship to the rate of growth of the culture. The different components of pasta have been evaluated as to their contribution to the growth of the microbe. We have found that these materials constitute an almost complete medium for this yeast; it seems that such a method of bio-recycling could become quite useful in the world of nutrition.

XXX. Université Laval, Faculté D'Agriculture, Cité Universitaire, Québec 10, Canada. Communicated by R. E. Simard.

The main activities of our Laboratories are:

A - Alcoholic Fermentation

We are actively engaged in wine research mainly fruit wines from blueberries and apples. We are developing blueberry table wines as well as blueberry Vermouth and blueberry liquors. The blueberries used are the wild type (Vaccinium angustifolium) and is particularly predominant in cold countries like Canada, Denmark, Finland, Russia, etc. These products will be on the canadian market in the near future.

In order to develop those products, Dr. R. E. Simard and Dr. M. Boulet toured the French wine industry last September and also visited Prof. Suomalainen's laboratories and a few fruit wine factories in Finland.

Also, our laboratories are engaged in cider making and several research projects are underway in order to produce hard cider from McKintosh apples.

Two reports are available on those subjects:

"Manuel sur les techniques de fabrication du cidre"
Dr. Ronald E. Simard, 250 pp. (1972)

"Produits Alcoolisés du Bleuet"
Dr. Ronald E. Simard, 125 pp. (1972).

B - Biological Treatment of Waste Water

Research on biological treatment of domestic waste water is very active and a few papers have been accepted for publications.

BIOLOGICAL TREATMENT OF DOMESTIC SEWAGE BY YEASTS
Thanh, N. C. and R. E. Simard. 1972.

A strain of Rhodotorula glutinis Y-3772 when inoculated in domestic sewage reduced the ammoniacal nitrogen, D.C.O., phosphates and total nitrogen in the following proportions: 65% - 65% - 99.9% and 75%. Physical conditions affecting the growth of this yeast of the medium have been studied (pH, temperature, aeration, etc.). The residue contained more than 50% protein and the amino acid pattern has been determined. This protein has been suggested to be used as animal feed.

BIOLOGICAL TREATMENT OF DOMESTIC SEWAGE BY FUNGI
Thanh, N. C. and R. E. Simard. 1972.

Trichothecium roseum was selected among seventeen strains of fungi, occurring in domestic waste water and giving the highest reduction in pollutants. Optimum conditions for their reduction has been obtained.

TRAITEMENT BIOLOGIQUE DES EAUX USÉES DE CROUSTILLES
Simard, R. E., G. Busque and R. R. Riel. 1972.

XXXI. Yeast Genetics Stock Center

Addition to Culture List I (see previous issue Yeast News Letter).

The Yeast Genetics Stock Center has been established through a grant (GB-28955) from the National Science Foundation for the purpose of maintaining and making available strains of Saccharomyces cerevisiae containing the most commonly studied genetic loci. We invite correspondence concerning the deposition of stocks that you believe should be available through the Stock Center. Requests for copies of the complete stock list and culture requests should be addressed to: Dr. John Bassel, Yeast Genetic Stock Center, Donner Laboratory, University of California, Berkeley, California 94720.

4. Fermentation Markers:

a. Galactose:

Locus	Strain	Genotype	Ref
gal1	G1-a	a gal1 ade6	1
	G1-α	α gal1 ade6 pet	
gal2	X3163-4C	a gal2 met1 trp3 arg1 ade5	2,3
	X3163-2A	α gal2 met1 trp3 arg1 ade5	
gal3	110-2A	a gal3 trp1 his1	---
	110-2B	α gal3 trp1 met1 ura1	
gal4	G4-a	a gal4 met1 ura1 ade6	4
	G4-α	α gal4 ade6	
gal5	156-5C	a gal5 his1	5
	140-1D	α gal5 ura1 trp1 ade6	
gal7	G7-a	a gal7 his1 ade6	5
	*G7-α	α gal7 trp1 met1 ade6 leu	
gal10	G10-a	a gal10 ade6	6
	*G10-α	α gal10 trp5 arg4 his5 lys1 met ade ura	
i^-	108-3A	a i^- his1 ade6 thr1 trp1	7
	106-3D	α i^- ura1 his1	

b. Sucrose, maltose, melibiose, α-methyl glucoside (ref. 8):

1315-2E	α SUC1 MAL1 trp1 ura1 ade8
X2180-1A	a SUC2 gal2 CUP1
1453-3A	a MAL2 MEL1 his4 leu2
1412-4D	a SUC3 MAL3 MEL1 MGL2 MGL3 ade2
1403-7A	a MAL4 MEL1 MGL3 gal3 gal4 trp1 ura3

* Contain auxotrophic requirements not specified genetically

REFERENCES:

1. de Robichon-Szulmajster. 1958. Science 127, 28.

2. de Robichon-Szulmajster. 1961. Ann. Tech. Agricole 10, 81.

3. Douglas and Condie. 1954. J. Bacteriol. 68, 662.

4. Douglas and Hawthorne. 1964. Genetics 49, 837.

5. Douglas. 1961. Biochim. Biophys. Acta 52, 209.

6. Douglas and Pelroy. 1963. Biochim. Biophys. Acta 68, 155.

7. Douglas and Hawthorne. 1966. Genetics 54, 911.

8. Mortimer and Hawthorne. 1971. "The Yeasts", Vol. 1, Academic Press.

Note: Lee Hartwell has decided to change the designation of his cell lysis ts mutants from pop 1-8 to cly 1-8. This decision was made because Fred Sherman has published on some porphyrin accumulating mutants which were also designated pop.

XXXII. National and International Yeast Meetings

1. A symposium will be held on May 17-18, 1973 in Arc et Senans France, on the anniversary of the hundred and fiftieth birthday of Louis Pasteur.

   The central topic will be the development of Pasteur's research in the field of oenology. One special session of the Symposium will be concerned with yeasts.

   More precise information will be available at the following address: Fondation C. N. Ledoux, 25620 Arc et Senans, France.

   Communicated by P. Dupuy

2. The Fifth Annual Meeting on Yeast Research in Czechoslovakia. November 22-24, 1972 at the Home of Scientific Workers of the Slovak Academy of Sciences, Smolenice Castle, Czechoslovakia. Communicated by Dr. A. Kocková-Kratochvílová.

   a) Genetics and life cycles

   Kováčová, V. Yeast cytoplasmic heredity (a review)
   Šilhánková, L. Suppressor mutation in yeasts (a review)
   Kováčová, V. and Vlčková, V. Tetrade analysis of the heredity of Saccharomyces cerevisiae cytochrome b
   Nešvera, J. Mutagenese as a method for the study yeast chromosome structure
   Kocková-Kratochvílová, A. The biosynthesis of carothenoids in Rhodotorula and Rhodosporidium species
   Jemek, J. Accumulated substances during the cultivation in the presence of deoxy-sugars.

   b) Yeast immunology

   Šandula, J. and Kocková-Kratochvílová, A. The relationship between the immunological activity of polysaccharides and the yeast taxonomy (a review)

Tomšíková, A. and Pokorný, M. The anticorporal response in skin candidoses.

c) Protoplasts and yeast cell wall

Nečas, O. Principles of the yeast cell wall morphogenesis (a review)

Kopecká, M. The formation of fibrilar glucan on yeast protoplasts

Svoboda, A. Conditions for the organization of complete cell wall on yeast protoplasts

Havelková, M. The relationship of membrane structures to the cell wall formation

Gabriel, M. Principles of the cell wall regeneration in protoplasts of lower plants

Farkaš, V. The biosynthesis of yeast mannan and chitin

Biely, P. The interference of hexose analogs with the cell wall biosynthesis

Streiblová, E. The yeast cell wall synthesis during the yeast cell division

Masler, M. The biochemical analysis of cell walls of various Cryptococcus species

Voříšek, J. Biosynthetical principles of the study of binding proteins from Saccharomyces cerevisiae membranes.

d) Processes on mitochondria

Greksák, M., M. Hanicová and K. Nejedlý. The activity of mitochondrial enzymes in synchronic cultures of aerobically and anaerobically growing yeasts Saccharomyces cerevisiae

Kolarov, J., J. Šubík, H. Fečíková and L. Kováč. The transport of ions through the mitochondrial membrane

Šubík, J., J. Kolarov, Š. Kužela and L. Kováč. Yeast mutants in the study of the mitochondrial membrane biogenesis.

e) Growth and division of yeast cells

Vraná, D. The growth of yeast cell by division - the division forms assumptions for the growth (a review)

Lieblová, J. and K. Beran. The growth of single Saccharomyces cerevisiae cells with respect to their relative age

Vraná, D. Features of Candida utilis "hypertrophically" growing in excess substrate during twostep continual cultivation

Holan, Z. and K. Beran. The role of chitin in the budding process of Saccharomyces cerevisiae

Šnejdar, V., K. Beran and Z. Holan. Changes in chemical composition of Rhodotorula glutinis cell walls and their structural response during the batch cultivation

Šnejdar, V., Z. Holan and J. Ludvík. Studies of the ultrastructure of Rhodotorula sp., cultivated under various conditions in onestep cultivation.

f) Technology

Volfová, O. The cell growth and the formation of oxidative products during the fermentation of Candida lipolytica in gas oil pattern

Vernerová, J. Possibilities and conditions of yeast biomass production on n-alkanes

Pilát, P. The growth of Candida tropicalis in mineral oils

Minárik, E. The reduction of sulphate to sulphite by wine yeasts

Kurzová, V. and M. Kahler. The participation of brewing yeasts

in the continual production of beer

Bendová, O. and G. Basařová. The assimilation of amino acids of brewing yeast from wort

Bendová, O. and B. Pardonová. The selection of brewing yeast strains from the viewpoint of production applicability

Moštek, J. and T. Tichá. The contribution to the problem of microbial determination of inosite in brewing

Moštek, J. and H. Čížková. Some new knowledge on metabolical and technological features of spontaneous mutants of brewing bottom yeasts with the partial respiration deficiency.

Yeasts, models in science and technics

Recently the Proceedings of the First Specialized International Symposium on Yeasts, Smolenice, June 1-4, 1971 appeared, edited by A. Kocková-Kratochvílová and E. Minárik with the Editorial Board consisting of F. Böttcher, J. Šandula and D. Yarrow. Publishing House of the Slovak Academy of Sciences, Bratislava, 1972, 670 pp.

3. Fourth International Symposium on Yeasts, Vienna, Austria, July 8-12, 1974.

This meeting will be the next general Symposium following the last general symposium in Delft - the Hague (1969). Those wishing to participate should request further information from the Secretariat, "Fourth International Symposium on Yeasts" c/o Hochschule für Bodenkultur, Institut für Lebensmitteltechnologie, Gregor Mendel Strasse 33, A-1180 Vienna, Austria.

4. The Third International Symposium on Yeast Protoplasts held in Salamanca, Spain, October 2-5, 1972 was extremely well organized by Professor Julio Villanueva and a dedicated staff of coworkers. The numerous scientific contributions will be published by Academic Press and this volume is expected to be published in early 1973.

5. The Fifth Yeast Genetics Conference, Japan.

The fifth meeting of the Yeast Genetics Conference-Japan was held on December 9 and 10, 1972, with 45 participants, at the Metropolitan Labor and Welfare Hall, Tokyo. Following major areas were discussed: mutation and radiation effects, sexuality and its genetic control, extrachromosomal inheritance and respiratory deficient mutants, gene action and regulation, genetic fine structure, metabolism and cell structure.

The morning session on December 9 was chaired by Y. Oshima (Osaka) and T. Ito (Tokyo). Kobayashi and Ito (Tokyo) reported the photodynamic studies in various growing stages of synchronous yeast culture, and Ito discussed the holding speed of acridine dye molecules unto nuclear DNA in vivo. T. Yamasaki (Kofu) demonstrated the life cycle of Saccharomycodes ludwigii and reported mutant isolation from this species and genetic recombination. Doi, Yanagishima (Osaka) and Takahashi (Suita) reported mendelian segregation of a UV-induced large cell mutant characterized by about eight times the cell volume at stationary phase. Hieda (Tokyo) reported the increase of auxotroph recovery by the drying of heterozygote on ade1 or ade2 and discussed the structural change of the DNA molecule. Takahashi (Suita) explained various chromosomal aberrations induced by some ρ-mutagens and linkage studies with the use of the assumed monosomics induced in the experiments.

N. Gunge (Yokohama) and H. Tamaki (Kyoto) were chairmen of the afternoon session on the first day. Sando (Tsuruoka) presented the physiological studies on the ascospore germination. Tsuboi (Osaka) discussed the effects of cyclic-AMP, theophylline and caffeine on yeast sporulation in connection with the repression of sporulation by glucose. Shimoda, Hagiya, Kitano and Yanagishima (Osaka) reported the characteristics of cell agglutination factor, sexual hormones and β-1,3-glucanase activity on the cell wall in relation to the mating reaction between a- and α-cells. H. Mori (Noda) explained a stable diplophase in Saccharomyces rouxii.

C. Shimoda (Osaka), H. Kasahara (Tokyo) and M. Tsuboi (Osaka) chaired the morning session on the second day. Gunge (Yokohama) reported the elimination of cytoplasmic drug resistance factors by ρ-induction with the use of acriflavine and discussed the duplication and recombination mechanisms of mitochondrial-DNA. Suda (Nara) and Uchida (Kobe) explained similar results on the cytoplasmic drug resistance factors and presented a presumable arrangement of mitochondrial genes. Nagai (Nara) demonstrated the induction of ρ-mutants and unstable strains by the use of acid dyes. Yuwamoto (Shizuoka) reported the effects of ρ-mutagens on S. cerevisiae and Candida utilis. Oshima and Toh-e (Osaka) reported the regulation mechanisms of acid phosphatase formation in Saccharomyces. Tamaki (Kyoto) explained the pseudoallelic relation of three amylase genes (STA1, STA4, and STA5) in S. diastaticus based on genetic and enzymatic studies. Oshima reported new complementary relations between various maltose mutants and postulated the regulation mechanism of the maltose fermentation in Saccharomyces.

The afternoon session on December 10 was chaired by H. Ohnishi (Noda) and Y. Yamamoto (Takasaki). Nishimura (Nara) and Ueda (Osaka) demonstrated the acetic acid metabolism in Sake yeast. T. Yamamoto (Osaka) reported the abnormal cell behavior of strontium resistant yeast in Sr-free medium. Kitamura and Y. Yamamoto (Takasaki) explained the new cell wall enzyme isolated from the culture medium of Arthrobactor luteus. Iguti (Mito) reported spheroplast production of Saccharomyces by the use of snail digestive juice. Osumi (Tokyo), Teranishi and Fukui (Kyoto) discussed the relation of the structure of microbody and catalase activity in the hydrocarbon-utilizing yeast based on electron microscopic studies. Iwata et al. (Tokyo) reviewed the toxic substances produced by strains of Candida albicans and other toxigenic yeasts and fungi.

The following resolutions were proposed in the business meeting.

1. The foundation of Yeast Information Service Center in Japan

The center will make an effort for information service for Japanese yeast investigators, and it will act in laison to the International Commission of Yeasts and Yeast-like Microorganisms, IAMS. The business of the center will be governed by the delegates from the scientific and technological research groups on yeasts, and the chairman will be elected in the near future.

2. Election of the members of the organizing committee of Yeast Genetics Conference-Japan

The term of the members has expired, and N. Yanagishima (Chairman), N. Gunge, T. Hirano, Y. Oshima, C. Shimoda, T. Takahashi and K. Wakabayashi were elected as the new members.

3. Next meeting

The next meeting will be held at Osaka University in 1973 after the XIIIth International Congress of Genetics in Berkeley, California.

Communicated by Toshiaki Takahashi

XXXIII. Brief News Items

A. Employment

1. Dr. Robert Roth would appreciate hearing about available post-doctoral positions. A mature female Ph.D. would like to obtain such a position starting either in the summer or fall of 1973. If necessary she would be willing to do some teaching in Microbiology or Biology as part of her responsibilities; she is an able and experienced undergraduate instructor. She would prefer to work in yeast biochemistry or physiology, but also has some experience in yeast genetics. Her thesis is titled "Mechanisms of purine nucleoside utilization in Saccharomyces cerevisiae." For further information contact:

   Dr. Robert Roth
   Biology Department
   Illinois Institute of Technology
   Chicago, Illinois 60616

2. Job opportunity for Marine microbiologist (M.S. degree preferred) to head laboratory work for isolation of marine microorganisms and their evaluation for antibiotic production. Excellent lab facilities on Florida Keys. Association with a major pharmaceutical firm. Salary commensurate with qualifications. Please send complete resume giving personal, academic, and job history (if any), salary requirements and recent photo.

   Bio-Marine Research, Inc.
   Box 324
   Marathon, Florida 33050

B. Change of address (see also below)

1. Dr. Jorgen Friis
   From: Bringstrup, DK-4100
   Ringsted, Denmark

   To: Institute of Molecular Biology
   Odense University. Niels
   Bohr Alle. DK5000 Odense,
   Denmark

2. The new address of Dr. F. K. Zimmermann (after April 1, 1973): Fachbereich Biologie (10), Technische Hochschule Darmstadt, 61 Darmstadt, Schnittspahnstr. 3, G.F.R.

3. Dr. Norman H. Giles
   From: Dept. of Biology
   Kline Biology Tower
   Yale University
   New Haven, Conn. 06520

   To: Dept. of Zoology
   University of Georgia
   Athens, Ga. 30602
   (Callaway Professor of
   Genetics)

4. Dr. D. W. R. Mackenzie has assumed the Directorship of the Mycological Reference Laboratory, Public Health Laboratory Service, London School of Hygiene and Tropical Medicine, Keppel Street and Gower Str., London WC1E7HT, England.

C. Research news

1. Dr. N. J. W. Kreger-van Rij writes: Below follow two recent publications:

   R. T. Moore and N. J. W. Kreger-van Rij, Ultrastructure of Filobasidium Olive, Can. J. Microbiol., 18, 1949-1951, 1972.

   N. J. W. Kreger-van Rij and M. Veenhuis, Electron microscopy of

some special cell contacts in yeasts, J. Bacteriol., in press. January 1973.

I am moving to a new laboratory on January 1, 1973. The new address is:

Department of Medical Microbiology
University of Groningen
Oostersingel 59
Groningen, The Netherlands

2. Dr. Helen R. Buckley, whose new address is: Harvard School of Public Health, Department of Microbiology, 665 Huntington Avenue, Boston, Mass. 02115, U.S.A., writes: Mr. Wilfred Lee and I are developing a synthetic medium for the growth of the yeast and mycelial phases of Candida albicans. The purpose is to study the antigens produced during the different phases of growth.

3. Department of Microbiology, Botanical Institute, Carl Skottsbergs gata 22, University of Göteborg, S-413 19 Göteborg, Sweden. Communicated by Birgitta Norkrans.

Recent publications: Determination of 2-oximinopropanoic acid in microorganisms. Andersson, I., B. Norkrans and G. Odham. Anal. Biochemistry 47:620-628, 1972.

Oximinoacids in the inorganic nitrogen metabolism. Andersson, I., B. Norkrans and G. Odham. Experientia, 1972 (in press).

Current research: Halotolerant yeasts. Oximes in hydroxylamine (HA) tolerant yeasts - Endomycopsis lipolytica - and "normal", not HA-tolerant yeasts as e.g. Cryptococcus albidus, and the possible role of oximes in inorganic nitrogen metabolism.

4. The following paper was recently published:

Synthesis of 3-Deoxy-3-fluoro-D-glucose 1- and 6-Phosphates and their Interaction with Phosphoglucomutase and UDPG-Pyrophosphorylase. By J. A. Wright, N. F. Taylor, R. V. Brunt and R. W. Brownsey (Biochemistry Group, School of Biological Sciences, University of Bath, Bath BA2 7AY). Journal of the Chemical Society Chemical Communications, 691-692, 1972.

5. The volume and year of the last issue were inadvertantly omitted for the last issue. This should be Vol. XXI, No. 1, June 1972.