Y E A S T

A News Letter for Persons Interested in Yeast

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I. Section Levures, Université Claude Bernard (Lyon). 43 Bd. du 11 Novembre 1918. Villeurbanne 69621, France. Communicated by M. C. Pignal.

Since the last issue of the Yeast News Letter, four doctoral theses were defended in our research team, under the direction of Professor Boidin. Here is a list of the titles and abstracts.

S. Poncet. A study of some genera of ascosporogenous yeasts: A comparison of the results of numerical analysis with those of the Guanine-Cytosine content of DNAs. July 1973. (Mme Kockova-Kratochvilova was a member of the thesis committee).

A technique of numerical analysis has been applied to all of the phenotypical (biochemical and morphological) characters of species of three genera of ascosporogenous yeasts (Pichia, Hansenula, and Kluyveromyces); two species were represented by many strains (H. anomala, 75 strains, and H. subpelliculosa, 15 strains).
The main results are:
1- On the basis of the calculated taxonomic distances between the species, the following were put forward:

2 species clusters in the genus Kluyveromyces
3 species clusters in the genus Pichia
3 species clusters in the genus Hansenula

These clusters have been shown to be very stable, since the addition of new species or new characters did not bring any significant variation in the delimitation of the genera.
2- It is proposed that the nomenclatural type-species of each genus be replaced by the "bary-type" species, which would be the closest to the median of the cluster of points of the studied population.
3- Moreover, these calculations of taxonomic distances between species have shown that in the present classification, the term "species" does not contain equivalent entities in the different genera studied, and sometimes not even inside of one genus; therefore, some species were brought down to the rank of varieties.
4- Certain pools of characters, which constitute those of greatest taxonomic significance in the three studied genera were established. Thus, among the two principal factors, one can find assimilation of α-glucosides, β-glucosides, and certain polyols, which would have a predominantly discriminative function in the separation of the species of these three genera.
5- The groups of species established in these genera have been compared with the results obtained by estimating the GC content of the DNAs. In two out of three genera (Hansenula and Kluyveromyces), a strong correlation between the groupings obtained through numerical analysis and the GC% could be observed; this could not be shown with the genus Pichia, where it was not always possible to show common phenotype species groupings and close GC%; moreover, false phenotypical relationships have been noted between certain species (ex: P. rhodanensis, P. bovis, and P. toletana) since their GC% differed considerably (up to 10% deviation).

For the three other theses, J. P. van der Walt, whom we were happy to welcome in Lyon for a few days, was part of the thesis committee.

J. B. Fiol. Yeast biosystematics: oxidase assays, vitamin requirements, guanine-cytosine content of DNAs. September 1973.

In this work, the author has sought to improve the systematics of the sporogenous genera Kluyveromyces, Pichia, Hansenula, Debaryomyces and Saccharomyces; first, by refining the fundamental tests for assimilation of hydrocarbons, lactose, maltose, cellobiose, trehalose, melibiose and sucrose with assays of intracellular oxidases in the non-assimilative species; secondly, by defining the exact growth factor requirements. These two methods of indirect approach of the genotype have been complemented, in two genera (Kluyveromyces, round-spored Pichia), with the GC content of the DNAs (in collaboration with S. Poncet). Finally, the author has compared the results obtained in these three ways, in order to propose more natural grouping of species than that determined with the traditional systematics.

For example, in the genus Kluyveromyces, a synthesis of these results leads to consider two very distinct groups, separated by the presence or absence of β-glucosidase, nicotinamide requirements, and by the GC contents. In the genus Hansenula, the GC contents, the individual needs in pyridoxine, thiamine, and biotin, the constant presence of α-glucosidase, β-glucosidase, trehalase, and, eventually, α-galactosidase, lead the author to divide this genus into two large entities, each of them further subdivided into several groups that are very different from those in Wickerham's classification. A similar type of splitting of the genus Pichia is considered, which is based on the GC contents, the presence of α-glucosidase and of β-galactosidase, and the requirement for thiamine, pyridoxine, and biotin.

F. Jacob. A contribution to the study of the yeasts of vegetative tanning liquors. September 1973.

The plant-originated tanning liquors are a yeast-rich environment. Several samples, taken in tanneries of different regions of France, were characterized according to a certain number of physical and chemical assays, and 680 strains of yeast were isolated from them. The isolates belonged to 37 species of 8 genera. Among these, 5 new species had to be described. It is possible to distinguish three groups in these microorganisms: one contains the strains isolated from the "brewing heads" concentrated liquors, a second one groups the strains obtained along any part of the process, and a third one contains the species found in the less concentrated liquors.

The examination of the growth of these yeasts in tanning media shows that the hydrolysable tannings are more toxic than the condensed tannins, which partly accounts for the distribution of the yeasts. The non-tanning aromatic fraction does not play a significant role.

The isolated yeasts have a reduced phenoloxidase activity; moreover, it was observed that only the gallotannins were hydrolysed by a few species. The yeasts, by not attacking the active fraction of tanning extracts, and by generally not displaying a proteolytic action of importance, have a useful function in tanning liquors, by competing with undesirable microorganisms, and by helping to lower the pH of the liquors.

M. C. Pignal. A study of some tree-inhabiting coleoptera-associated yeasts. September 1973.

A study of the microflora of over 250 samples of insects belonging to various families of tree-inhabiting coleopterae (buprestids, cerambicids, curculionids, scolytids and platypodids, scarabeids and

palissadids, ...) has enabled us to bring light upon a rich and diversified yeast flora. Samples were taken simultaneously in insect tunnels and in neighboring plant tissues.

The yeasts belong to 31 different species, in 8 genera. A predominance of Hansenula and Pichia (or of their imperfect forms) is obvious.

It has been possible to show a few stable associations:

-in Chalcophora virginiensis (Bupr., North America): Candida obtusa var. oregonensis and C. shehatae;

-in the scolytids and the platypodids of Equatorial Africa: Pichia monospora, P. membranaefaciens and Candida berthetii;

-in Ruguloscolytus rugulosus (Scolyt., Europe): Hansenula holstii, and Candida diddensii;

-in Dorcus and Cetonia (Scarab., Europe): Pichia stipitis.

Among the different microfloral elements surveyed, yeasts are important in number and in relative frequency. Their presence can be advantageous at different levels for the insects.

-Vitamin synthesis and growth factor excretion

-Degradation of sugars and tannins of plant tissues

-Utilization of certain nitrogen sources.

II. Ecole Nationale Superieure Agronomique de Montpellier, Laboratoire de Recherches de la Chaire de Genetique, I.N.R.A., Montpellier, France. Communicated by P. Galzy.

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

MOULIN, G., GALZY, P., FERRON, F. - Le Lait, T L III, n° 525-526, 1973, p. 237-245. Ten strains of yeasts have been isolated during the manufacture of some cheeses. They have been classified in the genera Torulopsis and Saccharomyces. The growth on lactose and composition of the cells have been studied.

VEZINHET, F., PELLECUER, M., GALZY, P. Third Internat. Spec. Symp. on Yeasts. Metabolism and regulation of cellular processes. HELSINKI. Finland 4-8 June 1973. A sporulating a/α strain and a non-sporulating α/α strain were compared during incubation in sporulation medium. We have followed the change in composition of the cells in dry weight, protein, trehalose, glycogen, mannans, glucans, fatty acids and sterols as well as the development of respiratory metabolism.

PELLECUER, M. Contribution à l'étude du caractere sexuel chez Saccharomyces cerevisiae HANSEN. Thèse de Doctorat de Spécialité. 1973.

ROGER, M. Quelques aspects de l'évolution de la cellule de levure au cours de la sproulation. Thèse de Doctorat de Spécialité. 1973.

SAQUET, Hélène. Action de quelques substrats à 2 carbones sur le métabolisme endogène de la levure. Thèse de Doctorat de Spécialité. 1973.

DOUILLET, Aline. Contribution à l'étude comparative des genres Saccharomyces et Kluyveromyces. Thèse de Doctorat de Spécialité. 1973.

ARNAUD, A., GALZY, P. On the role of pH in the sporulation of Saccharomyces cerevisiae HANSEN. Can. J. Microbiol. 19, 1973. Helix pomatia juice has been used to dissolve in situ the cell wall of two yeast strains, a wild strain and a mutant "smooth colony" strain. The mutant strain had lost one or more antigenic sites. One fraction of mannoprotein

was much less prevalent in the mutant strain.

GALZY, P., MOULIN, G., BEZARD, J. Observations sur la composition de lipides de quelques levures (Le Lait - In press). We have studied, the effect of different carbon sources on the fatty acid composition of Kluyveromyces fragilis. The fatty acid compositions of some species of yeast are compared after culture on lactose as sole source of carbon.

GUIRAUD, J. P., BIZEAU, C., ARNAUD, A., SAQUET, Hélène et GALZY, P. Action des composés à 2 carbones sur le métabolisme endogène de la levure. Mycopath. Mycol. Appl. (In press). Ethanol, acetic acid, ethanal, glycolic acid, glycolaldehyde increase oxydation of endogenous reserves of non-growing cells.

III. Departamento de Microbiologia Geral, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil and Department of Dermatology, College of Physicians and Surgeons of Columbia University, New York, New York 10032. Communicated by Luiz R. Travassos.

A paper entitled "Microbiological assay of carnitine" by L. R. Travassos and C. O. Sales was accepted for publication and will appear in a forthcoming issue of Analytical Biochemistry.

Summary: A method for carnitine assay has been devised by the growth response of the yeast Torulopsis bovina. As little as 0.0005 µg/ml of l (-) carnitine could be assayed by this method. Free carnitine was assayed in several biological materials together with the carnitine present as soluble esters. Lipid-bound carnitine was precipitated by trichloroacetic acid and assayed independently. Average recovery of added carnitine in the free-carnitine assays was 95%. In lipid-bound carnitine assays recovery of added carnitine ranged from 76.2 to 95%. Treatment of materials for the microbiological assays included separation in ion-exchange resins and alkaline or acid hydrolyses. Materials containing negligible amounts of carnitine esters could be assayed directly without further treatment.

The carnitine-requiring strain of Torulopsis bovina has been accessioned in the CBS collection under the assigned number CBS 6471 and in the ATCC under no. 26014.

IV. Department of Plant Sciences, University of Western Ontario, London, Canada. Communicated by A. W. Day and J. E. Cummins.

Conjugation in the yeastlike anther smut fungus, Ustilago violacea (a basidiomycete) is being studied as a major morphological event controlled by a single though possibly complex locus with two alleles. We have shown that:-

1. The two alleles are under different cell cycle controls. One allele (a_1) is active during G_1 only, while the other allele (a_2) mates in all phases of the cell cycle (ref. #2).
2. These cell cycle controls of the individual alleles remain operative in heterozygous diploids so that these cells are sexually neutral during G_1 (probably because both alleles are active and neutralize each other) but have a_2 activity during the rest of the cycle. This apparent dominance of allele a_2 represents a new kind of dominance deriving from "temporal allelic interaction" (ref. #3).
3. Experiments using wild type and UV sensitive cells show that both mating types must complete transcription of "sex message" for conjugation to proceed. Transcription of the sex message is interfered with by low doses of UV (ref. #5).

4. Experiments with inhibitors of transcription and translation and radioactive tracer studies of macromolecule synthesis indicate that transcription of the last sex message is completed some 60 minutes before assembly of the conjugation tube, while translation of this message is completed about 30 minutes before assembly (ref. #6).
5. The morphology of conjugation has been analysed under the electron-microscope and 5 stages have been identified (ref. #4).

Our current work is twofold (1) to isolate and characterize the "sex message" and protein products (2) to find mutants with altered cell cycle controls and to use these mutants in the study of cell cycle controls and temporal allelic interactions.

References

1. Day, A. W. 1972. Dominance at the mating-type locus in bipolar heterothallic forms. Nature New Biology 237: 282-283.
2. Cummins, J. E. and Day, A. W. 1973. The cell cycle regulation of mating type alleles in a smut, Ustilago violacea. Nature 245: 259-260.
3. Day, A. W. and Cummins, J. E. Temporal allelic interaction, a new kind of dominance. Nature 245: 260-261.
4. Poon, N. H., Martin, J. and Day, A. W. 1973. Conjugation in Ustilago violacea. I. Morphology. Accepted for publication - Can. J. Microbiol.
5. Day, A. W. and Cummins, J. E. 1973. Transcription and translation of the sex message in Ustilago violacea. I. The effect of UV. Accepted for publication in J. of Cell Science.
6. Cummins, J. E. and Day, A. W. Transcription and translation of the sex message in Ustilago violacea. II. The effects of inhibitors. Submitted to J. of Cell Science. (Oct.).
7. Cummins, J. E. and Day, A. W. Invited contribution to "Cell Cycle Controls". (ed. Padilla, Cameron and Zimmerman) to be published by Academic Press, 1974. Manuscript title "The cell cycle regulation of sexual morphogenesis".

V. Noda Institute for Scientific Research, Noda-Shi. Chiba-Ken, Japan. Communicated by Haruhiko Mori.

The following is the summary of a recent paper: Life Cycle in a Heterothallic Haploid Yeast, Saccharomyces rouxii. Haruhiko Mori. J. Ferment. Technol., 51: 379-392. (1973).

Summary: The life cycle in a heterothallic haploid yeast, Saccharomyces rouxii, was demonstrated using strains marked with auxotrophic genetic traits and was proved to be similar to that of a typical diploid yeast, S. cerevisiae. These strains and the segregants were divided into two groups according to their mating response. The segregation patterns of mating types in the haploid pedigrees from the diploids constructed in this investigation showed approximately equal frequency for the mating types by random spore analysis. Thus, I have designated two allelic genes, a and α, as controlling the two mating types and have used two original strains, NRRL-2547 and NRRL-2548, as standards for the a and α mating types, respectively. Separation of each daughter cell from the zygote formed between two cells of the opposite mating types demonstrated three different fates of zygote, (1) heterocaryosis, (2) diploidization and direct meiosis of zygotic nuclei (sporogenesis)

and (3) diploidization and mitosis of diploid nuclei to produce diploid cells. A relatively long and stable period of heterocaryosis of the zygote was observed: the zygote produced new buds marked with the same genotype as either of its parental haploid clones. The zygote could undergo mitotic reproduction only under anaerobic conditions, while the conjugation and sporulation processes required aerobic conditions. Estimation of ploidy of the large cell clones, constructed by cell fusion between two haploid clones, was made by testing the segregation pattern of genetic markers by random spore isolation and by determination of the 2-deoxyribose content, cell size, and cell weight of each segregant clone. It was indicated that these large cell clones were diploids heterozygous for the mating-type alleles. Moreover, diploid cells homozygous for the mating-type alleles occurred in a certain heterozygous diploid clone, spontaneously. From this culture, it was possible to isolate the a/a or α/α diploid clones by micromanipulation. The genetic mechanism of the occurrence of the diploid homozygous for the mating-type allele is obscure. However, using these diploid clones homozygous for the mating-type allele, triploid and tetraploid clones could be constructed.

VI. Biology Department, Brooklyn College, Brooklyn, New York, 11210. Communicated by Nasim A. Khan.

Progress report on the Isolation and Characterization of Nonfermenters from a Yeast Strain Carrying the SUC 3 Gene. Investigators are R. A. Hackel, N. A. Khan, F. K. Zimmermann and N. R. Eaton.

Fermentation of the sucrose in yeast is under the control of a polymeric gene system. So far six non-allelic genes have been identified (Mortimer and Hawthorne, 1969, "Yeast Genetics," in The Yeasts, Vol. I). Any one of these sucrose (SUC) genes is sufficient to allow a yeast strain to utilize sucrose. However, one of the unsolved questions is whether these SUC genes are independent structural genes for invertases or genes with some regulatory function. In order to resolve this question, we have isolated five sucrose nonfermenters after UV mutagenesis from a strain EK6B carrying the SUC 3 gene which is tightly linked to the MAL 3 gene in Saccharomyces cerevisiae. These five mutants can be classified into the following three categories:

1) Two mutants ferment sucrose slowly but do not ferment raffinose;
2) Two mutants cannot ferment sucrose or raffinose;
3) One mutant cannot ferment these sugars and also cannot ferment maltose.

All of the above mutants were crossed to a segregational sucrose nonfermenter, AZ5A, and diploids obtained were tested for sucrose fermentation. Only one of the five diploids was found to ferment sucrose in eight days at 30 C. Tetrad analysis is in progress to determine whether or not these mutations are allelic to SUC 3.

Invertase activity in these five mutants grown under various culture conditions was found to be absent or negligible, compared to the parental strain.

Since these nonfermenters have virtually no invertase, it is possible that in some cases the mutation has occurred in the structural genes for invertase. In order to identify unambiguously the role of these known SUC genes, an attempt was made to isolate temperature-sensitive revertants from one of the above nonfermenters.

Thirty spontaneous temperature-sensitive revertants were isolated. These mutants have the following properties: 1) at 23 C they ferment sucrose, maltose and glucose; and 2) at 35 C they ferment maltose and glucose, but fail to ferment sucrose. Invertases are being isolated from these temperature-sensitive revertants and their physical and chemical properties will be compared with the invertases from the parental strain. Any differences found could constitute unambiguous evidence for the structural nature of the known SUC 3 gene, if these revertants are allelic to the SUC 3 gene.

VII. Mykologie/Genetik, Technische Hochschule, 61 Darmstadt, Schnittspahnstr., German Federal Republic. Communicated by F. K. Zimmermann.

GENETICS OF MALTOSE FERMENTATION IN SACCHAROMYCES CEREVISIAE

Recent developments have shown that of the known MAL-genes (maltose-genes) two are regulatory genes: MAL6 (ten Berge et al., Molec. Gen. Genetics 123, 139, 1973) and MAL4 (Khan et al., Molec. Gen. Genet. 124, 365, 1973). Uptake of maltose and sucrose is under the control of a large number of so-called dsf-genes (disaccharide fermentation) which are not identical with any of the other MAL-genes (Zimmermann et al., Molec. Gen. Genet. 123, 29, 1973). In order to explore the function of other MAL-genes I have concentrated on MAL2. Five maltose non-fermenting mutants were isolated from a MAL2 strain without a SUC (sucrose)- gene, and fermenting only maltose but not sucrose. These mutants were crossed to a segregational nonfermenter for maltose and sucrose (mal suc). The resulting diploids did not ferment maltose. This showed that the mutants were not of the dsf-type described previously. Tetrad analysis gave only maltose non-fermenting progeny. Some of the mutants reverted easily, and were identified by that criterion amongst segregants from crosses. The revertants obtained fell into three classes: 1. normally inducible maltose-fermenters; 2. maltose fermenters with constitutive maltase synthesis in the absence of glucose; 3. maltose fermenters with constitutive maltase synthesis resistant to glucose. All constitutives fermented sucrose as predicted for maltase constitutives (Khan et al., Molec. Gen. Genet. 123, 43, 1973) and also alpha-methylglucoside. The latter sugar is fermented only slowly by the original parent strain from which I had isolated the maltose non-fermenting mutants, but not at all by the strains used in crosses. All types of constitutives were crossed to segregational non-fermenters. All segregation patterns showed that maltose fermentation was associated always with fermentation of sucrose and alpha-methylglucoside, and all maltose fermenters were constitutive for maltase synthesis. These properties were also dominant in crosses with MAL2 wild type, mutants isolated from wild type and segregational non-fermenters. These properties were not due to a mutation in a gene allelic or closely linked to MAL4, the classical gene for constitutive maltase synthesis, but allelism could be demonstrated to MAL2. These results suggest that MAL2 is also a regulatory gene involved in induction of maltase synthesis by maltose, and also in glucose repression of maltase synthesis. A remarkable side effect of constitutive maltase synthesis in these mutants was the fermentation of alpha-methylglucoside. Some of the constitutive segregants fermented alpha-methylglucoside in one day, others from the same tetrad took over a week and fermentation was very slow. This suggests that constitutive MAL2 alleles complement the alpha-methylglucoside genes MGL1 and MGL3 for rapid fermentation, and also allow for a very

slow fermentation of this sugar in the absence of one of those genes.

GENETIC TINKER TOYS

I am interested in the stability of chromosome numbers in diploid cells of Saccharomyces cerevisiae. A strain was constructed with chromosome VII marked on both sides of the centromere in the following order: met13 cyh2 (recessive resistance to cycloheximide) trp5 leu1 (tightly linked to the centromere) centromere ade3 (causing a simultaneous requirement for adenine and histidine) and on chromosome XV ade2-40. This haploid is white (ade3 prevents the accumulation of the pigment precursor usually formed in ade2 strains), it requires adenine, histidine, leucine, tryptophan, methionine, and it is resistant to cycloheximide. It was crossed to strain 2.2^{c}-24A with his4 and ade2-40. The resulting diploid was red, required adenine and was sensitive to cycloheximide. Non-disjunction should give a white diploid resistant to cycloheximide, requiring histidine, leucine, tryptophan and methionine. Such a clone can also be obtained by two cross overs, one between ade3 and the centromere, the other between the centromere and the tightly centromere linked marker leu1 on the other side of the centromere. Non-disjunction is indicated when the incidence of leucine requiring types is higher among the white cycloheximide resistant colonies than among the red cycloheximide resistant ones. Preliminary experiments have shown that among 2×10^{6} untreated cells of this diploid, called D6, none of the cycloheximide resistant colonies expressed all the recessive markers in chromosome VII. The same result was obtained by analysing cells treated with nitrous acid. This showed that non-disjunction must be very rare in mitotic yeast cells, and moreover, expression of recessive markers in diploids due to aneuploidy is very rare too and not interferring with studies of mitotic crossing over. This strain is available for distribution.

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

Genetic Analysis of Non-complementing Fatty Acid Synthetase Mutants in Saccharomyces cerevisiae. Patric Tauro, Ulrich Holzner, Helga Castorph, Frank Hill and Eckhart Schweizer.

Of 43 non-complementing alleles of the Saccharomyces cerevisiae fatty acid synthetase locus fas 1 studied, 27 are reverted by external suppressors whereas 16 are susceptible only to interagenic suppression. Of the externally suppressible mutants 12 are reverted by both, amber and ochre suppressors, another 12 only by amber suppressors, and 3 are not suppressed by either of them. According to their response to the acridine mustard, ICR-191, one of the mutants not suppressible by external suppressors appears to be a frameshift mutation whereas the others are suggested to be missense nutations. By X-ray induced mitotic recombination a genetic fine structure map of the complementing as well as of the non-complementing fas 1-alleles was constructed. It was found that the alleles of the non-pleiotropic complementation groups II, Va, Vb and Vc map at distinct and characteristic regions with fas 1, each of them covering 9.8, 13.5, 5.3, and 1.6 X-ray map units, respectively. On the other hand, the non-complementing fas 1-alleles are distributed randomly over the entire locus, no matter whether they were nonsense or missense mutations. These non-

complementing alleles are spread over a DNA region of 72 X-ray map units length and all the complementing alleles studied map within this region. From these results it is concluded that fas 1 encodes only one, functionally complex, poly-peptide chain.

A saturated Fatty Acid Mutant of Saccharomyces cerevisiae with an intact Fatty Acid Synthetase. Karl Heinz Meyer and Eckhart Schweizer.

A Saccharomyces cerevisiae conditional mutant, LK 181, is described which grows at 37°C only when supplemented with a saturated fatty acid of 12-14 carbon atoms chain length. At 22°C, however, no fatty acid supplementation is required for growth. The fatty acid concentration required for optimal growth at 37°C is about 4 times lower for LK 181 than for fatty acid synthetase deficient mutants. In contrast to all fatty acid synthetase mutants so far examined, mutant LK 181 cannot grow with palmitic acid. The addition of palmitic, palmitoleic or oleic acid to the culture medium prevents LK 181 growth of temperatures between 22 and 37°C. In vivo as well as in vitro, cellular de novo fatty acid biosynthesis from acetate is unimpaired, in the mutant. It is suggested that endogeneously synthesized fatty acids, due to their chain lengths of 16 and more carbon atoms, cannot supplement the mutant LK 181. It is concluded that the exogeneously supplied fatty acids act as allosteric effectors for a mutationally altered cellular protein to restore its biological function at elevated temperatures, rather than as a substitute for endogeneously synthesized long chain fatty acids.

IX. Donner Laboratory, University of California, Berkeley, California, 94720. Communicated by R. Mortimer.

A. Yeast Genetics Stock Center:

In approximately four months the Yeast Genetics Stock Center expects to publish a revised and expanded culture list. Those who have previously requested a stock list or yeast strains will receive the new list when it is available. In order to get on our mailing list or to request yeast strains write to: Dr. John Bassel, Donner Laboratory, University of California, Berkeley, California 94720.

B. The following articles have appeared recently:

R. K. Mortimer and D. C. Hawthorne (1973). Genetic mapping in Saccharomyces cerevisiae. IV. Mapping of temperature sensitive genes and use of disomic strains in localizing genes. Genetics 74: 33-54.

L. H. Hartwell, R. K. Mortimer, J. Culotti, and M. Culotti (1973). Genetic control of the cell division cycle in yeast. V. Genetic analysis of cdc mutants. Genetics 74: 267-286.

G. E. Jones and R. K. Mortimer (1973). Biochemical properties of yeast L-Asparaginase. Biochemical Genetics 9: 131-146.

J. Bassel and R. Mortimer (1973). Genetic analysis of mating type and alkane utilization in Saccharomycopsis lipolytica. J. Bacteriol. 114: 894-896.

K.S.Y. Ho and R. K. Mortimer (1973). Induction of dominant lethality by X-rays in a radiosensitive strain of yeast. Mutation Res. 20: 45-51.

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

Below follow two abstracts based on work done in our laboratory. The articles appeared in the June 1973 issue of Genetics (74: No. 2).

ALLELIC COMPLEMENTATION IN THE FIRST GENE FOR HISTIDINE BIOSYNTHESIS IN SACCHAROMYCES CEREVISIAE. I. CHARACTERISTICS OF MUTANTS AND GENETIC MAPPING OF ALLELES by Christopher T. Korch and Richard Snow.

A number of his1 mutants were tested for suppressibility, for reversion by EMS, ICR-170, and nitrous acid, for their allelic complementation response, and for their temperature sensitivity and osmotic remediability. None of 52 mutants tested was suppressible by a known ochre suppressor. This is a very surprising result compared with other studies of suppressibility in yeast and suggests that another function essential to the cell is associated with the his1 gene product, the polarity effect of a nonsense mutation destroying the activity of the his1 enzyme and this second function.

Sixty-four his1 alleles were ordered by allelic mapping methods utilizing gamma rays, X-rays, and MMS. The three maps agree well in placement of alleles and have been oriented on chromosome V of yeast with respect to the centromere. The 18 noncomplementing alleles are localized in the distal half of the gene, whereas the complementing alleles are distributed more or less evenly. Mutations which revert to feedback resistance map in the proximal end. Also at this end are mutations having a very high X-ray or MMS induced homoallelic reversion rate. This suggests that a number of missense mutations can occur in this region which result in innocuous amino acid substitutions in the enzyme. One X-ray map unit is estimated to correspond to about 131 base pairs or 43 amino acids, in agreement with results for the cytochrome-c protein obtained by Parker and Sherman (1969).

ALLELIC COMPLEMENTATION IN THE FIRST GENE FOR HISTIDINE BIOSYNTHESIS IN SACCHAROMYCES CEREVISIAE. II. COMPLEMENTATION MAPPING OF MUTANTS AND A SUBUNIT MODEL OF THE ENZYME by Christopher T. Korch.

Sixty-two alleles of the histidine-1 (his1) gene were tested for complementation. The 44 complementing mutants fell into 31 complementation groups which were used to construct a complex complementation map with 18 complementation units. Cluster analysis of the complementation map by either visual inspection or the computer method of Gillie and Peto (1969) shows two very definite clusters.

The molecular weight estimate of the his1 enzyme, phosphoribosyl adenosine triphosphate: pyrophosphate phosphoribosyltransferase, is $1.8 \cdot 10^5$ by sucrose density gradient analysis and $2.4 \cdot 10^5$ by Sephadex gel chromatography. Correlating the length of the his1 gene to the molecular weight of the enzyme indicates that this enzyme is composed of 6 subunits, as is the analogous enzyme in Salmonella typhimurium.

A model of the subunit and tertiary and quaternary structure of the enzyme has been developed from consideration of the genetic and complementation data, the distribution of the various mutant types within the gene, and the biochemical properties of the enzyme encoded by the his1 gene.

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

The following papers summarize the work being done on yeast.

Published:

1) Diffusion of Acridine Molecules to a Genetic Site in Living Cells. Takashi Ito, Biochim. Biophys. Acta 329 190-196, 1973.

Summary: A method which constitutes the measurement of induced photosensitized reaction at a specified site in living cells by illumination of short duration was developed to study a dynamic aspect of the interaction between small molecules and a specific cellular site. By using this method it was demonstrated that the diffusion of acridine orange molecules, starting outside of the cell, to a particular site of nuclear DNA could be measured in yeast cells. The temperature dependence of the rate constant suggests that the viscosity of cytoplasm is not a major barrier in this process.

2) Some Differences Between 470 nm and 510 nm in the Acridine-Orange-Sensitized Photodynamic Actions on Yeast Cells. Takashi Ito, Mutation Res. 20 201-206, 1973.

Summary: The two wavelengths that correspond to the absorption of two complexes formed in the interactions of acridine orange (AO) with nucleic acids were different in a few aspects of photodynamic action on yeast, Saccharomyces cerevisiae. (1) The dose-survival curve at 470 nm is not the same in shape as that at 510 nm. (2) The efficiency for the induction of gene conversion at 510 nm is higher than that obtained at 470 nm on the basis of both dose and survival.

In Press:

Induction of Genetic Change by Drying in Yeast. K. Hieda and T. Ito. Will be included in the Proceedings of International Symposium on Freeze-Drying held in Sapporo, Japan, October, 1973.

Summary: The genetic effects of freeze- and vacuum-drying were investigated with several strains of diploid and haploid cells of Saccharomyces cerevisiae. The results indicated that genetic changes were induced by drying in 3 diploid strains heterozygous for the ade1 or ade2 locus, but not in a diploid strain heteroallelic for the leu1 nor in a haploid strain. Genetic change at the ade locus appeared as whole- and fractional-colony types. Genetic tests suggested that the fractional colonies were not induced through gene conversion and haploidization. The length of drying time, the suspending media during freeze-drying, the pre-drying culture media, and the reconstituting media before plating were studied as modifying factors in the induction of genetic change. Particularly, the effect of the suspending media indicates that the induction mechanism of genetic change in yeast by freeze-drying may be different from that in aerosoled bacteria.

XII. Arbeitsgruppe Mikrobengenetik, Fachbereich Biologie, J. W. Goethe Universität, 6 Frankfurt/Main, Siesmayerstr. 70, Germany. Communicated by M. Brendel.

We have built up a yeast lab and are currently working on problems of radiation sensitivity and DNA metabolism in S. cerevisiae. The following summaries of papers accepted for publication or just published may give a more detailed scope of our activities.

Published: Interactions among Genes Controlling Sensitivity to Radiation and Alkylation in Yeast. Martin Brendel and Robert H.

Haynes, Molec. Gen. Genetics 125: 197-216 (1973).

In the simple eucaryote Saccharomyces cerevisiae there are at least three phenotypically distinct classes of mutants sensitive to inactivation by radiations and alkylating agents: class I mutants are sensitive to ultraviolet light and nitrogen mustard (HN2); class II mutants are sensitive to X-rays and methylmethane sulphonate (MMS); and class III mutants are sensitive to all four of these agents. We have constructed doubly mutant strains of types (I,I), (I,II), (I,III), and (II,III) and have measured their sensitivity to UV, X-rays, HN2 and MMS in order to characterize the interactions of the various mutant gene pairs. Class (I,III) double mutants proved to be supersensitive to UV and HN2 and class (II,III) double mutants proved to be supersensitive to X-rays and MMS. All other double mutants showed little or no enhancement of sensitivity over their most sensitive single mutant parents. Mutants of class I are known to be defective in excision repair and our results are consistent with the idea that there exist at least two additional pathways for dark repair in yeast, one capable of repairing X-ray and MMS damage to DNA, and another, possibly analogous to post-replication repair in bacteria, that competes with the other two for damaged regions in DNA.

In Press: Exogenous Thymidine-5'-Monophosphate as a Precursor for DNA Synthesis in Yeast. Martin Brendel and Robert H. Haynes. (Accepted for publication in Molec. Gen. Genetics, 1973).

Strain MB1015-5C of Saccharomyces cerevisiae can utilize exogenous thymidine 5'-monophosphate (5'-dTMP) for its DNA synthesis. Studies with either P^{32} or 2-C^{14} labelled 5'-dTMP reveal first that some of the precursor molecules are taken up intact in DNA synthesis and secondly that 3'-digests of highly purified P^{32} DNA yield up to 94% of all P^{32} as 5'-dTMP P^{32}. Under the conditions used in these experiments more than 90% of the exogenously supplied 5'-dTMP is broken down into orthophosphate and thymidine by an acid phosphatase. Only the orthophosphate is utilized by the yeast cells, mainly for RNA synthesis, and thymidine is not taken up. Suppression of the phosphatase activity is possible by addition of inorganic phosphate to the medium; under these conditions breakdown of 5'-dTMP is suppressed but uptake and incorporation of the molecules into the DNA of strain MB1015-5C is still not very effective.

To be published: Specific DNA-Labelling by Exogenous Thymidine-5'- Monophosphate in Saccharomyces cerevisiae. Wolfgang W. Fäth and Martin Brendel. (Submitted for publication in Molec. Gen. Genetics).

Strain 211-1aM of Saccharomyces cerevisiae was found to specifically incorporate into its nuclear and mitochondrial DNA exogenous 5'-dTMP when it is incubated in a synthetic medium N rich in inorganic phosphate. 3'-digestions of DNA of cells labelled by ^{32}P-5'-dTMP revealed that the 5'-dTMP molecules pass through cell wall and membranes to the sites of DNA synthesis without being broken down. It is possible in medium N to generate DNA which derives its thymine-contents almost totally from external sources. However, rather high concentrations of 5'-dTMP (approx. 100 µg/ml) must be offered to achieve this.

XIII. Albert Einstein College of Medicine of Yeshiva University, Department of Biochemistry, 1300 Morris Park Avenue, Bronx, N.Y. 10461. Communicated by Claire D. Goldthwaite.

Below follows the summary of a manuscript by Claire D. Goldthwaite and Dennis R. Cryer we are about to submit for publication.

Summary: Genetic crosses were made using strains of Saccharomyces cerevisiae which carried cytoplasmically inherited markers conferring resistance to erythromycin, oligomycin and chloramphenicol. The frequency of transmission of these mitochondrial loci to diploid progeny was found to be influenced by the physiological state of the haploid parents, and was not affected by the cis or trans configuration of the three resistance markers. No recombinational polarity was seen in any of the crosses.

Growth of a haploid parental strain to stationary phase in a yeast extract peptone medium containing glycerol as a carbon source resulted in a high level of transmission of mitochondrial markers when crossed with a strain grown to stationary phase in the same medium but with glucose as carbon source.

When cells were grown under the same conditions as those used in the genetic crosses they were found to contain more mitochondrial DNA relative to nuclear DNA when glycerol was used as a carbon source than when glucose was used. Two criteria were used to determine the amount of mitochondrial DNA present: i) incorporation of radioactive precursors into the different DNA species; and ii) measurement of the mass of DNA from amounts of ultraviolet-absorbing material at the appropriate buoyant densities in isopycnic CsCl gradients.

It is proposed that the two to three fold difference in the ratio of mitochondrial DNA to nuclear DNA in stationary phase cells grown in the presence of glycerol or glucose reflects an increased number of mitochondrial genomes in depressed mitochondria. This difference, by a "genome dosage" effect, could account for the variations in the genetic parameter of the frequency of transmission, i.e., strains grown with glycerol as a carbon source contain more mitochondrial genomes than glucose-grown strains and thus will contribute more mitochondrial markers to the zygote.

XIV. Centre De Génétique Moléculaire, 91190 Gif-Sur-Yvette, France. Communicated by P. Slonimski.

Below follows a list of recent publications from my laboratory. Requests for reprints should be sent to me at the above address.

PAJOT, P. - Affinity of protocheme for the apoprotein of cytochrome b_2. Biochem. Biophys. Res. Commu., 45, 887-892, 1971.

VERDIERE, J., LEDERER, F. - Présence de la εN-trimethyllysine dans 1'iso-1 et 1'iso-2 cytochromes c synthétisés par des souches de levure à déficience respiratoire (ρ^-). FEBS Letters, 19, 72-74, 1971.

BAUDRAS, A., SPYRIDAKIS, A. - Etude de la L(+)-lactate: cytochrome c oxydoréductase (cytochrome b_2) de la levure Hansenula anomala. Biochimie, 53, 943-955, 1971.

LABEYRIE, F., BAUDRAS, A. - Differences in quaternary structure and constitutive chains between two homologous forms of cytochrome b_2 (L-lactate: cytochrome c oxydoreductase). Eur. J. Biochem., 25, 33-40, 1972.

JACQ, C., LEDERER, F. - Sur les deux formes moléculaires du cytochrome b_2 de Saccharomyces cerevisiae. Eur. J. Biochem., 25, 41-48, 1972.

CASEY, J., COHEN, M., RABINOWITZ, M., FUKUHARA, H., GETZ, G. S. - Hybridization of mitochondrial transfer RNA's with mitochondrial and nuclear DNA of Grande (wild type) yeast. J. Mol. Biol., 63, 431-440, 1972.

RENOT-FAURES, M. - Etude du DNA mitochondrial et de ses produits de transcription chez les mutants à déficience respiratoire de Saccharomyces cerevisiae. Thèse de Doctorat d'Etat ès-Sciences Naturelles, soutenue le 28 février 1972. Université Paris VI. 110 p.

MEVEL-NINIO, M. - Subunit structure of L-lactate dehydrogenase (cytochrome b_2) of Saccharomyces cerevisiae. Ultracentrifugation studies. Eur. J. Biochem., 25, 254-261, 1972.

HENAUT, A., LUZZATI, M. - Contrôle de l'aptitude à recombiner pendant la phase végétative chez Saccharomyces cerevisiae. Mol. Gen. Genetics, 116, 26-34, 1972.

LEDERER, F., SIMON, A. M., VERDIERE, J. - Saccharomyces cerevisiae iso-cytochromes c: revision of the amino acid sequence between the cysteine residues. Biochem. Biophys. Res. Commun., 47, 55-58, 1972.

BAUDRAS, A., CAPEILLERE-BLANDIN, C., IWATSUBO, M., LABEYRIE, F. - Formation of a stable complex between cytochrome b_2 and cytochrome c and study of its role in the overall electron transfer by rapid kinetics. "Structure and Function of oxidation reduction enzymes" (Å. Åkeson, Å. Ehrenberg, Eds.) Permagon Press - Oxford and New York, 273-290, 1972.

GRIVELL, L. A., NETTER, P., SLONIMSKI, P. P. - Mutant ribosomes of yeast mitochondria. Abstract. Abstr. N° 623. Commun. Meet. Fed. Eur. Biochem. Soc. 8, Amsterdam (20-25 August, 1972).

LEDERER, F., JACQ, C. - Some properties of Baker's yeast physiological cytochrome b2 (L-lactate cytochrome c oxidoreductase). Abstract. Abstr. N° 588. Commun. Meet. Fed. Eur. Biochem. Soc. 8, Amsterdam (20-25 August, 1972).

GROUDINSKY, O. - Liaison hème-protéine du cytochrome b2 de la levure. Renseignements apportés par l'étude des propriétés physicochimiques du noyau cytochromique b_2. Thèse de Doctorat d'Etat ès-Sciences Naturelles, soutenue le 26 October 1972, Université Paris VI. 73 p.

FOUCHER, M., VERDIERE, J., LEDERER, F., SLONIMSKI, P. P. - On the presence of a non-trimethylated iso-1 cytochrome c in a wild-type strain of Saccharomyces cerevisiae. Eur. J. Biochem., 31, 139-143, 1972.

LEDERER, F. - Candida krusei cytochrome c: a correction to the sequence. Glutamine-16, an invariant residue in mitochondrial cytochrome c ? Eur. J. Biochem., 31, 144-147, 1972.

CLAISSEE, M. L., COSSON, J. J. - Comparison des protéines mitochondriales de souches "grandes" et de souches "petites" de Saccharomyces cerevisiae (Abstract). Réunion de la Société de Chimie Biologique, Symposium sur les Mitochondries "Membranes mitochondriales - Perméabilité et biogénèse", Grenoble 7-9 Février 1973.

PAJOT, P., CLAISSE, M. L. - "Phosphorylation oxydative du lactate par la levure en présence d'antymicine A (Abstract) Réunion de la Société de Chimie Biologique, Symposium sur les Mitochondries "Membranes mitochondriales - Perméabilité et biogénèse", Grenoble, 7-9 Février 1973.

SOMLO, M., AVNER, P., DUJON, B., KRUPA, M. Distinct submitochondrial particles carrying ARPase activity and their mutual relations during mitochondrial biogenesis (Abstract). Réunion de la Société de Chimie Biologique, Symposium sur les Mitochondries "Membranes mitochondriales - Perméabilitié et biogénèse", Grenoble, 7-9 Février 1973.

HENAUT, A. - La recombinaison mitotique spontanée chez Saccharomyces cerevisiae. Thèse de Doctorat d'Etat ès-Sciences Naturelles. soutenue le 28 février 1973. Université Paris-Sud. 178 p.

GUIARD, B., GROUDINSKY, B., LEDERER, F. - Yeast L-lactate dehydrogenase (cytochrome b_2) chemical characterization of the heme binding core. Eur. J. Biochem., 34, 241-247, 1973.

NASLIN, L., SPYRIDAKIS, A., LABEYRIE, F. - A study of several hypersensitive to proteases in a complex flavohemoenzyme, yeast cytochrome b_2: modification of their reactivity with ligand-induced conformational transitions. Eur. J. Biochem., 34, 268-283, 1973.

FAYE, G., FUKUHARA, H., GRANDCHAMP, C., LAZOWSKA, J., MICHEL, F., CASEY, J., GETZ, G. S., LOCKER, J., RABINOWITZ, M., BOLOTIN-FUKUHARA, M., COEN, D., DEUTSCH, J., DUJON, B., NETTER, P., SLONIMSKI, P. P. Mitochondrial nucleic acids in the petite colonie mutants: deletions and repetitions of genes. Biochimie, 55, 779-792, 1973.

MICHAELIS, G., PETROCHILO, E., SLONIMSKI, P. P. - Mitochondrial Genetics III. Recombined molecules of mitochondrial DNA obtained from crosses between cytoplasmic petite mutants of S. cerevisiae: physical and genetic characterization. Mol. Gen. Genetics, 123, 51-65, 1973.

AVNER, Ph., COEN, D., DUJON, B., SLONIMSKI, P. P. - Mitochondrial Genetics IV - Allelism and mapping studies of oligomycin resistant mutants in S. cerevisiae. Mol. Gen. Genetics, 125, 9-52, 1973.

WOLF, K., DUJON, B., SLONIMSKI, P. P. - Mitochondrial Genetics V - Multifactorial mitochondrial crosses involving a mutation conferring Paromomycin-Resistance in Saccharomyces cerevisiae. Mol. Gen. Genetics, 125, 53-90, 1973.

RISLER, J. L., GROUNDINSKY, O. - Magnetic circular dichroism studies of cytochrome c and cytochrome b_2. Eur. J. Biochem., 35, 201-205, 1973.

GRIVELL, L. A., NETTER, P., BORST, P., SLONIMSKI, P. P. - Mitochondrial antibiotic resistance in yeast: ribosomal mutants resistant to chloramphenicol, erythromycin and spiramycin. Biochim. Biophys. Acta, 312, 358-367, 1973.

PAJOT, P., CLAISSE, M. - Antimycin-A-resistant oxydative phosphorylation of lactale in yeast. Abstract. 9th Internat. Congress of Biochemistry, Stockholm 1-7 July 1973. Abstract. 41-6, p. 239.

CLAISSE, M. L., HAWTHORNE, D. C. - Studies on strains of Saccharomyces cerevisiae with a growth deficiency on glycerol medium: strains with amber suppressors derived from tyrosyl transfer RNA's. Proceedings of the Third Internat. Specialized Symposium on Yeast. Metabolism and Regulation of Cellular Processes Otaniemi/ Helsinki, Finland. 4-8 June 1973. Abstracts. Part I. Section 9, pp. 144-145. (Suomalainen H., Waller Ch. Eds).

RISLER, J. L. - Etude de quelques propriétés structurales de la L-lactate deshydrogenase (cytochrome b_2) de la levure: structure quaternaire et relations spatiales entre l'hème et la flavine prosthétiques. Thèse de Doctorat d'Etat ès-Sciences Physiques, soutenue le 7 mars 1973. Université Paris-Sud. 84 p.

LEDERER, F. - On the mechanism of lactate oxidation by baker's yeast L-lactate dehydrogenase (cytochrome b_2). Abstract 9th Internat. Congress of Biochemistry. Stockholm, July 1-7, 1973. Abstract 2d 46, p. 59.

GUIARD, B., LEDERER, F. - Structure of the heme binding site of baker's yeast L-lactate dehydrogenase (cytochrome b_2). Abstract. 9th Internat. Congress of Biochemistry, Stockholm, July 1-7, 1973. Abstract 2n 7, p. 92.

XV. Polish Academy of Sciences, Institute of Biochemistry and Biophysics, 36 Rakowiecka Str., Warszawa 12, Poland. Communicated by W. Gajewski.

In the Department of Genetics of the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences the following researches on yeast are now in progress:

1. Mutagenesis in yeast. We have shown that in yeast neither hydroxylamine nor methoxyamine can be considered as capable of inducing specifically GC → AT transitions. Presently we are studying the induction by manganese of mitochondrial respiratory deficient and antibiotic resistant mutations. The evidence so far available does not contradict with the assumption that manganese induces both types of mutations through its interaction with mitochondrial DNA polymerase(s).

2. Another line of investigations is concerned with the physiology of conjugation process in yeasts. A method of synchronous mass production of zygotes was worked out and we hope to use it to investigate mitochondrial transmission and segregation in young zygotes and in early stages of their budding.

3. We started a study on the interactions between the nuclear and mitochondrial genes in mitochondrial biogenesis. It was demonstrated that the activity of mitochondria does influence the resistance of cytosol ribosomes to cycloheximide and we intend to extend this kind of

approach to different problems of mutual interactions between nuclear and mitochondrial genes in yeast.

Publications:

A. Putrament and H. Baranowska: Induction of intragenic mitotic recombination in yeast by hydroxylamine and its mutagenic specificity. Molec. Gen. Genetics 111, 89-95, 1971.

T. Bilinski: Amino Acid Incorporation into Cell Fractions of Yeast Strains Sensitive to Antibiotics and Cytoplasmically Resistant. Bulletin de l'Academie Polonaise des Sciences. Serie des sciences biologiques Cl. II. Vol. XIX, No. 6, 1971.

A. Putrament, H. Baranowska, T. Bilinski and W. Prazmo: On the specificity of caffeine effects. Molec. Gen. Genetics 118, 373-379, 1972.

A. Putrament, H. Baranowska and J. Pachecka: Mutagenic action of hydroxylamine and methoxyamine on yeast. Molec. Gen. Genetics 122, 61-80, 1973.

A. Putrament, H. Baranowska and W. Prazmo: Induction by manganese of mitochondrial antibiotic resistance mutations in yeast. Molec. Gen. Genetics 126, 357-366, 1973.

T. Bilinski and W. Jachymczyk: The influence of mitochondria on ribosomes. Cycloheximide resistance of ribosomes from petite mutants of Saccharomyces cerevisiae. Biochemical and Biophysical Research Communications. Vol. 52, No. 2, 1973.

T. Bilinski, J. Litwinska, J. Zuk and W. Gajewski: Sunchronization of zygote production in Saccharomyces cerevisiae. J. Gen. Microbiol. 79, 1973.

T. Bilinski, Z. Kotylak and W. Jachymczyk: The dependence of Cytosole Protein Biosynthesis resistance to Cycloheximide in Yeast on changes in Mitochondrial activity. Molec. Gen. Genetics (in press).

T. Bilinski, W. Jachymczyk, J. Litwinska, J. Zuk and W. Gajewski: Mutual inhibition of DNA synthesis in a- and α-cells of Saccharomyces cerevisiae during conjugation. (Submitted to J. Gen. Microbiol.).

Bilinski, T. and Litwinska, J.: The various binding affinities to Dowex 1 resin of some strains of Saccharomyces cerevisiae. (Submitted to Bull. Acad. Polon. Sci.).

Rytka, J.: Positive Selection of General Amino Acid Permease Mutants in Saccharomyces cerevisiae (will be submitted to M.G.G.).

XVI. Department of Biophysics, All-India Institute of Medical Sciences, Ansari Nagar, New Delhi, India. Communicated by V. K. Jain.

I have now joined the department of biophysics, All India Institute of Medical Sciences, New Delhi as an assistant professor. Previously I was at the department of radiation biophysics, Society for Radiation and Environmental Research, D. G. Frankfurt/M, Germany.

Given below is a summary of one of our papers due to appear in Biophysics.

Influence of Energy Metabolism on the Repair of X-Ray Damage in living cells. III. Effect of 2-Deoxy-D-Glucose on the Liquid-Holding Reactivation in Yeast. By V. K. Jain, W. Pohit and S. C. Purohit.

Summary: Effects of the glucose antimetabolite, 2-deoxy-D-glucose (2DG) on liquid-holding reactivation (LHR) in X-irradiated yeast were studied. It has been observed that LHR in respiratory-deficient mutants is completely inhibited at a molar concentration ratio 2DG: glucose equal to 1, whereas for the wild type this ratio is 10. Significance of these results for the radiochemotherapy of tumours is discussed.

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

Dr. Calleja has been awarded a Canadian International Development Agency (CIDA) Research Associateship. This will allow him to work in my lab for three months each year for three years. His present address is University of the Philippines, Natural Sciences Research Center, Diliman, Quezon City, The Philippines.

The following is an abstract of a paper accepted for publication by Experimental Cell Research.

KILLING OF YEAST CELLS AND INDUCTION OF THE CYTOPLASMIC PETITE MUTATION BY PARTIAL CELL IRRADIATION WITH AN ULTRAVIOLET MICROBEAM by Byron F. Johnson and D. H. Williamson, National Institute for Medical Research, Mill Hill, London, NW 7, U.K. P. P. Dendy and J.M.R. Hatfield, Department of Radiotherapeutics, University of Cambridge, Tennis Court Road, Cambridge, U.K.

Summary: Individual cells of Saccharomyces cerevisiae (NCYC 239) were irradiated with a UV-microbeam to characterize the target for induction of the cytoplasmic petite mutation. The organisms were taken from synchronous cultures at a stage prior to migration of the nucleus into the bud, thus permitting irradiation of either the nucleated parental portion of the cell or the anucleate (but mitochondria-containing) bud. Whole cell irradiation was carried out for comparison. The LD_{50} was 0.061 ergs/cell for whole cell irradiation, about 0.047 ergs/cell for nuclear irradiation and about twice that value for bud irradiation. The spontaneous petite frequency was 4/7108 (0.056%). The overall induced frequency was 9/540 among nucleus-irradiated cells (significantly enhanced) and 2/832 among bud-irradiated cells (insignificantly enhanced). Thus induction of the mutation did not require direct irradiation of all the mitochondria in the cell and the target was apparently associated with the nucleus.

XVIII. Department of Biology, York University, 4700 Keele Street, Downsview 463, Ontario, Canada. Communicated by J. G. Little.

The following is a summary of a paper presented at the International Genetics Congress in August, 1973.

Little, J. G. and R. H. Haynes. DNA-Specific labelling yeast mutants:

Yeast cells lack thymidine kinase and therefore do not incorporate exogenous thymine or thymidine. The consequent absence of a DNA-specific labelling procedure has hindered studies of DNA metabolism in yeast. To permit DNA-specific labelling in Saccharomyces cerevisiae we have devised techniques for the isolation of mutant strains which efficiently incorporate exogenous thymidine monophosphate (dTMP). Wild-type cells do not take up dTMP. A mutant sensitive to aminopterin was first isolated. When plated at high cell densities ($\sim 5 \times 10^7$ cells/plate) on media containing aminopterin (100 µg/ml) and dTMP (100 µg/ml), outgrowths were obtained. These derivatives, when cultivated in aminopterin media, have an absolute requirement for dTMP, and some of these strains show an additional adenine dependency. The amount of dTMP required for growth is strain dependent. Some require 5 µg/ml; with others, no growth is observed at dTMP concentrations below 50 µg/ml. Thymine or thymidine do not substitute for dTMP in relieving growth inhibition

by aminopterin. In the absence of aminopterin, these mutants do not exhibit a nutritional requirement for dTMP. Nevertheless, the strains thus selected incorporate dTMP in the absence of aminopterin and incorporation occurs throughout the growth cycle. When yeast DNA was labelled in this manner with radioactive dTMP, isopycnic gradient analysis revealed that incorporation had occurred into both nuclear and mitochondrial DNA. An unusually high proportion (92%) of the dTMP-permeable mutants obtained by these selection procedures were found to be petites. These mutants can be exploited in the analysis of DNA replication and repair and such studies are in progress.

(Supported by the N.R.C., Canada)

XIX. Indiana University, Department of Microbiology, Jordan Hall 438, Bloomington, Indiana 47401. Communicated by Marjorie Crandall.

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

In a search for sex hormones in the sexually agglutinative yeast Hansenula wingei, culture filtrates of mating types 5 and 21 and the 5x21 diploid hybrid were tested for their effect on inducing a sexual response in the nonagglutinative, nonmating diploid. A substance was found which induces synthesis of 5-factor (the agglutination factor from strain 5) in the diploid. This 5 inducer (5I) is present in maximal concentrations in exponential phase and appears to be destroyed in stationary phase. 5I must be a normal cellular constituent since it is present in culture filtrates of 5, 21 and 5x21 as well as in culture filtrates of mating types a and α of Saccharomyces cerevisiae. However, 5I is specific in its effect since it induces only 5-factor not 21-factor synthesis. 5I is amphipathic since it is extractable by lipid solvents.

Although 5I is present in diploid cultures the diploid remains nonagglutinative i.e., 5-factor is not induced because diploid cells are responsive to 5I only in stationary phase and 5I is present only in exponential phase. Furthermore, the pH of a diploid culture is normally too acid to allow induction. It has been discovered recently (Crandall and Caulton; ECR 1973 in press) that vanadium + molybdenum also induce 5-factor in the diploid. It appears that when diploid cells are grown in the presence of V + Mo they become responsive to 5I at the time 5I is still present in the culture (late exponential). Then by late stationary phase the V + Mo grown diploid cells become highly agglutinative with 21 cells, in some experiments almost as strongly as haploid strains 5 with 21.

The following papers have been published recently or will appear soon:

1. Crandall, M. (1973). Comparison of Hansenula wingei, a petite-negative, obligately aerobic yeast to the petite-positive yeast Saccharomyces cerevisiae. J. Gen. Microbiol. 75: 363-375.
2. Crandall, M. (1973). A respiratory-deficient mutant in the obligately aerobic yeast Hansenula wingei. J. Gen. Microbiol. 75: 377-381.
3. Crandall, M. and R. H. Richter. (1973). Genetics of resistance to ethidium bromide in the petite-negative yeast Hansenula wingei. Mol. General Genetics. 125: 279-293.

4. Crandall, M. and C. F. Robinow. (1973). The chromosome number of the yeast Hansenula wingei observed in mitosis and meiosis. Can. J. Genet. Cytol. (To appear in the December issue).
5. Crandall, M. and J. H. Caulton. (1973). Induction of glycoprotein mating factors in diploid yeast of Hansenula wingei by vanadium salts or chelating agents. Exper. Cell Res; In press.
6. Crandall, M., L. M. Lawrence and R. M. Saunders. (1974). Molecular complementarity of yeast glycoprotein mating factors. P.N.A.S. (To appear in the January issue).

XX. Biological Institute of the Carlsberg Foundation, DK-2200 Copenhagen, Nlb, Tagensvej, Denmark. Communicated by Erik Zeuthen.

Below follows a summary of a paper to be submitted for publication in Comptes Rendus des Traveaux du Laboratoire Carlsberg during the spring of 1974:

Membranous structures in the fission yeast Schizosaccharomyces pombe. By: Birte Kramhøft[1], E. Zeuthen[1], Helene Ravn[2], and A. Birch-Andersen.

Summary: The cortical zone of Schizosaccharomyces cells has been studied by electron microscopy of thin sections. It was found that this zone contains hitherto undescribed membranous structures most often composed of triple-layered membranes. Each triple-layered membrane has more or less the dimensions of two unit membranes, i.e, 14-20 nm. The membranes often develop into very complicated structures at presumably active sites in the cell surface.

[1] Biological Institute of the Carlsberg Foundation, DK-2200 Copenhagen N, Denmark.
[2] Department of Biophysics, Statens Seruminstitut, DK-2300 Copenhagen S, Denmark.

XXI. Department of Biology, Faculty of Medicine, Purkyne University, Brno, Czechoslovakia. Communicated by Marie Kopecka.

The following papers have been published recently:

1. Necas, O., Svoboda, A.: Effect of urea on the plasma membrane particles in yeast cells and protoplasts. Protoplasma 77, 453-466, 1973.
2. Kopecka, M., Svoboda, A., Brichta, J.: Effect of osmotic stabilizers and glycerol on yeast cell envelopes. Z. Allg. Mikrobiol. 13, 481-487, 1973.
3. Necas, O., Svoboda, A.: Granules of the cytoplasmic membrane in yeast cells and protoplasts. Folia microbiol. 18, 158, 1973.
4. Kopecka, M.: Reaggregation of a fibrillary component of the cell wall of yeast protoplasts. Folia microbiol. 18, 158-159, 1973.
5. Havelkova, M.: Controlled production of sphaeroplasts in the yeast Nadsonia elongata. Arch. Mikrobiol. 90, 77-78, 1973.

The following papers are in press:

5. Kreger, D. R., Kopecka, M.: On the nature of the fibrillar nets formed by protoplasts of Saccharomyces cerevisiae in liquid media. Proceedings III. Int. Symp. on Yeast Protoplasts, Acad. Press, London.

Summary: The protoplast nets of Saccharomyces cerevisiae have been found to contain, in contrast to native walls, free chains of β-1,3-linked D-glucose residues aggregated to form crystalline structures. These structures form part of the long microfibrils constituting the nets. The crystalline packing of the chains is slightly different from that of hydroglucan and corresponds to that of paramylum.

The nets contain chitin which is formed de novo by protoplasts and, in contrast to the chitin in the native walls, is crystalline. The chitin to glucan ratio of the nets is normally higher than in native walls.

The structure of the nets is disturbed by dilute alkali, which dissolves about 40%, the residual part consisting of more or less microfibrillor clusters and chitin. The X-ray diagram of the clusters is indicative of the presence in them of β-1,3-glucan chains, but their true nature has not yet been established.

The compositional and structural difference between the nets and the glucan-chitin moiety of normal walls have tentatively been explained by differential removal of 1,6 and 1,3 linking enzyme(s) and removal of a chitin complexing factor from their natural locus of action by the liquid medium.

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6. Necas, O., Svoboda, A.: The influence of proteases, phospholipases and polysaccharide-degrading enzymes on plasma membrane particles and on the synthesis of the fibrillar component in yeast protoplasts. Folia microbiol., in press.

Summary: Plasma membrane particles demonstrable by the freeze-etching technique, according to some authors, play a certain role in the synthesis of cell walls. On the model of yeast protoplasts, which can regenerate a new cell wall, we studied the morphology of the plasma membrane particles and the synthesis of the fibrillar component of the wall after treatment of protoplasts with different enzymes and lysolecithin. We used proteases (trypsin, papain, Pronase), polysaccharide-splitting enzymes (complex of snail enzymes, mannosidase), phospholipases (A, C, D), and lipase. When treated living protoplasts with these agents we could not demonstrate any morphological changes in the structure of the particles or their distribution in the plasma membrane. The fibrillar component was formed even in the presence of the proteases and phospholipases. If the plasma membrane particles represent enzymic systems synthetising the fibrillar component of the wall they cannot be exposed on the surface of the plasma membrane or they are protected in some way from corresponding enzymes.

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7. Kopecka, M. Gabriel, M., Necas, O.: A method of isolating anucleated yeast protoplasts unable to synthesize the glucan fibrillar component of the cell wall. J. Gen. Microbiol., in press.

Summary: A mixture of nucleated and anucleated protoplasts was produced when log-phase cells of Saccharomyces cerevisiae were treated with snail enzymes. The mixture was separated by centrifugation and anucleated protoplasts were studied by means of light and electron microscopy. Anucleated protoplasts did not synthesize glucan fibrils even though they seemed to contain all other basic structures in their cytoplasm, and the structure of the plasma membrane was unchanged. This was in sharp contrast

to ordinary nucleated protoplasts which synthesized glucan fibrils even after inhibition of protein synthesis by cycloheximide. The reason for this behaviour of anucleated protoplasts is not clear. Such anucleated yeast protoplasts represent the first clear example of uniform anucleated fungi produced by a reproducible method.

The following is a summary of the Ph.D. Dissertation of Dr. Miroslav Gabriel. The research was done under the guidance of Professor O. Necas.

REGENERATION OF PROTOPLASTS OF LOWER PLANT CELLS.
J. E. Purkyne, University, Brno, 1973.

The formation of protoplasts, their growth and cell wall regeneration were studied in lower heterotrophic plants (the mold Rhizopus nigricans; the yeast Trichosporon pullulans) and lower autotrophic plants (the green alga Uronema gigas; the blue-green alga Cylindrospermum sp.) using light microscopy, time-lapse cinematography, electron microscopy, chemical methods and effect of enzymes.

Cells of Rhizopus nigricans, Trichosporon pullulans and Uronema gigas treated with snail enzymes yielded a high percentage of protoplasts; a lower yield of protoplasts was obtained from Cylindrospermum sp. after combined treatment of snail enzymes and lysozyme.

The regeneration of the protoplasts was studied in these organisms with the exception of Cylindrospermum, in which conditions favourable to cell wall regeneration were not found.

It appeared that the protoplasts grew if they were not able to form a complete cell wall or if the regeneration of the wall was partly inhibited by enzymes. When an incomplete cell wall, usually consisting of polysaccharide fibrillar nets, is synthesized, this incomplete wall is not able to control cytodieresis. The growing organisms are of irregular shapes and unable to multiply.

In contrast to bacterial and budding yeast protoplasts, the organisms studied did not require special physical culture conditions for complete cell wall regeneration. Only in the case of the green alga was it necessary to regulate the relation of the cytoplasmic turgor to the rigidity of the synthesized cell wall by adjusting nutrient and osmotic conditions during the course of regeneration.

Cell wall regeneration commences by synthesis of the fibrillar wall component, regardless of whether it consists of chitin (Rh. nigricans), of glucan (Tr. pullulans) or of cellulose (U. gigas). Gradually, this fibrillar net is masked by the amorphous matrix till the completion of the cell wall. The new cell wall has all of the basic features of the original cell wall, though some differences were found both in morphological and physico-chemical properties. The shape of the regenerated wall is determined primarily by shape of the protoplast. Cultivation of mould protoplasts under semiaerobic conditions or with partial inhibition of oxydative phosphorylation showed that the regenerating protoplast distributes the available energy preferably to the regeneration of the new cell wall.

The formation of a rigid cell wall on the surface of a regenerating protoplast is a necessary step before cytokinesis is triggered. The protoplast which has regenerated a cell wall is capable of reversion into a cell (a mycelium) which corresponds

in its main features to the cells of the original culture. Revertants give rise to normal cell cultures.

XXII. Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Czechoslovakia. Communicated by V. Farkas.

The studies carried out in our laboratories are concerned mainly with the processes involved in the biosynthesis of the yeast cell wall. Two groups of problems currently being studied: a) the degrading processes in the cell wall, and b) the synthesis of the cell wall constituents, especially of mannan, and the mechanism of their incorporation into the growing walls.

The approaches used for the study of degrading processes are: 1) the use of antimetabolites interferring in the synthesis of the cell wall, such as 2-deoxy-D-glucose (2DG) and 2-deoxy-2-fluoro-D-glucose. 2) The location and characterization of yeast glucanases and their possible role in the cell wall extension.

The synthetic processes involved in the cell wall formation are being studied mainly by using high-resolution autoradiography.

The results of our studies can be summarized as follows: Addition of 2FG into a growing culture of S. cerevisiae results in extensive lysis of the cell population due to the dissolution of the cell walls by autohydrolytic enzymes. A comparison of the 2FG effects on metabolic activity of protoplasts, simultaneous secretion of mannan-proteins and formation of glucan fibrils on their surface demonstrated that the cell wall glucan synthesis is the most 2FG-sensitive process in S. cerevisiae. Lysis of the cell walls appears to be a consequence of the disturbed balance between synthetic and degrading processes involved in the cell wall growth.

DEAE-cellulose fractionation of the proteins secreted by yeast protoplasts into the growth medium showed the presence of at least three β-glucanase fractions, one of them being an endo-β-1,3-glucanase, the second an enzyme splitting both β(1,3) and β(1,6) linkages in laminarin and pustulan in an endwise fashion. The third fraction obtained appeared to be a mixture of two enzymes and has, so far, not been investigated in detail.

Autoradiography experiments show that the overall rate of the cell wall synthesis in buds of S. cerevisiae is not constant throughout the cell cycle but that it is proportional to the size of the bud. The incorporation of radioactive material into the walls of mother cells was always negligible when compared with the radioactivity incorporated into the growing buds. The cell walls of Schizosaccharomyces pombe were labelled almost exclusively at the growing tips. The incorporation is of tritium-labelled carbohydrates into non-extensile regions of the yeast was found to be only about 5% of the total wall labelling.

The results of pulse-chase experiments with selective labelling of mannan with D-mannose-^{3}H show that this polysaccharide is being synthesized in the cytoplasm from where it is transported and incorporated into the cell wall. Additional experiments with fractioned cell membranes strongly support the evidence that the plasmalemma is not involved in the biosynthesis of yeast mannan.

Another series of autoradiography experiments showed that the mode of incorporation of mannan into the growing cell walls depends largely on the size of the growing bud. In the first phase, when the bud is small and its shape is nearly spherical, the mannan is incorporated uniformly over the bud surface. As the growth proceeds, a gradual polarization of cell wall growth is observed. In the maturation phase

of bud development the polarization of growth apparently ceases and more or less uniform distribution of new mannan over the entire bud surface is observed.

The following papers were recently published or are to be published soon:

1. P. Biely, J. Kovarik and S. Bauer: Lysis of Saccharomyces cerevisiae with 2-deoxy-2-fluoro-D-glucose, an inhibitor of the cell wall glucan synthesis. J. Bacteriol. 115, 1108-1120 (1973).
2. V. Farkas, P. Biely and S. Bauer: Extracellular β-glucanases of the yeast Saccharomyces cerevisiae. Biochim. Biophys. Acta 321, 246-255 (1973).
3. P. Biely, J. Kovarik and S. Bauer: Cell wall formation in yeast: An electron-microscopic autoradiographic study. Arch. Microbiol. (in the press).
4. V. Farkas, J. Kovarik, A. Kosinova and S. Bauer: An autoradiographic study of mannan incorporation into the growing cell walls of Saccharomyces cerevisiae. (Should appear in January 1974 issue of the Journal of Bacteriology).
5. A. Kosinova, V. Farkas, R. Machala and S. Bauer: Site of mannan synthesis in yeast. (Manuscript in preparation).

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

Dr. Graham H. Fleet has completed his Ph.D. Dissertation entitled: "Beta-Glucan hydrolases of Schizosaccharomyces versatilis." The work was done under the guidance of Professor H. J. Phaff. A condensed summary follows:

1. The four yeast species Schizosaccharomyces pombe, S. versatilis, S. octosporus, and S. malidevorans were screened for the presence of β-(1 → 3)-, β-(1 → 6)-, and α-(1 → 3)-glucanases. β-(1 → 3)- and β-(1 → 6)-glucanase activities were found in cell extracts of all four species. Washed cell wall preparations exhibited β-(1 → 3)-glucanase activity. Conclusive evidence for the occurrence of an α-(1 → 3)-glucanase in any of the species was not obtained. The highest levels of β-glucanases were found in S. versatilis. This yeast in contrast to several other species excreted an exo-β-glucanase into the culture medium.

2. The β-(1 → 3)-glucanases in the cell-free extracts and in the culture fluid of S. versatilis were exo-glucanases. The enzyme from the cell-free extract was purified 420-fold, after which it was electrophoretically homogeneous. On the basis of migration in an SDS polyacrylamide gel the molecular weight of this enzyme was estimated to be 43,000. The properties of the intra-and extracellular β-(1 → 3)-glucanases were identical. The purified enzymes hydrolyzed both laminarin and pustulan in an endwise fashion although the latter at a much reduced rate. The exo-β-glucanase followed Michaelis-Menten kinetics and the V_{max} values for laminarin and pustulan hydrolyses were 345 and 53 μmoles of glucose released/min/mg of protein, respectively. The corresponding K_m values were 6.25 mg/ml for laminarin and 166.6 mg/ml for pustulan. The pH optimum of hydrolysis for either substrate was 5.0.

3. The ratios of the activities toward laminarin and pustulan remained constant during enzyme purification; the two activities were inactivated at the same rate upon heating of the purified enzyme at three different temperatures; the inhibitor glucono-delta-lactone gave the same K_i values when tested with either substrate (inhibition by glucono-delta-lactone was non-competitive); both enzyme activities migrated together during polyacrylamide gel electrophoresis. These observations suggest that this single enzyme can hydrolyze both laminarin and pustulan.

4. Purified exo-β-glucanase does not cause significant hydrolysis of isolated cell walls of S. pombe or of S. cerevisiae.

5. Isolated washed cell walls of S. versatilis underwent autolysis at pH 5.0, during which the wall-associated β-(1 → 3)-glucanase activity became partially solubilized. Fractionation of the solubilized wall glucanase by DEAE-cellulose chromatography yielded two enzymes. At pH 5.0 an exo-β-(1 → 3)-glucanase was firmly bound to the column while an endo-β-(1 → 3)-glucanase did not adsorb to the DEAE-cellulose under the conditions used. Further purification and study of the exo-β-(1 → 3)-glucanase showed its properties to be identical to the exo-β-glucanases purified from the cell-free extracts and from the culture of S. versatilis.

The endo-β-(1 → 3)-glucanase in the eluate from the DEAE cellulose was purified further to electrophoretic homogeneity by a combination of CM-cellulose and Sephadex G-100 chromatography. On the basis of migration in SDS polyacrylamide gels, this endo-β-(1 → 3)-glucanase was estimated to have a molecular weight of approximately 97,000.

6. The endo-β-(1 → 3)-glucanase was specific for the β-(1 → 3)-glucosidic linkage but it did not hydrolyze laminaribiose. Laminaritriose was readily cleaved by the enzyme. Laminarin hydrolysis appeared to follow sigmoidal rather than Michaelis-Menten kinetics. The V_{max} for laminarin hydrolysis was 5 μmoles of glucose equivalents released/min/mg of protein. The substrate concentration which yielded half this value was 0.33 mg/ml of laminarin. Laminarin hydrolysis was optimal over the pH range 5.0 to 6.0

7. The purified endo-β-(1 → 3)-glucanase from S. versatilis caused extensive but incomplete hydrolysis of isolated S. pombe and S. cerevisiae walls. S. pombe walls still retained the shape of the cell after enzymic digestion, although they appeared much thinner. Treatment of such walls with a α-(1 → 3)-glucanase resulted in a loss of cellular integrity.

8. Saccharomyces cerevisiae, Saccharomyces rosei, Hansenula anomala, Pichia pastoris, Kluyveromyces fragilis, and Candida utilis also exhibited various levels of endo-β-(1 → 3)-glucanase activity in their cell walls.

9. Endo-β-(1 → 3)-glucanase and endo-β-(1 → 6)-glucanase were extensively purified from the culture fluid of Bacillus circulans WL-12 grown on Baker's yeast cell walls. The two enzyme activities were well separated suring Sephadex G-100 chromatography. The endo-β-(1 → 3)-glucanase was further purified by DEAE-cellulose and hydroxyapatite chromatography. The bacterial β-(1 → 6)-glucanase could be purified further by DEAE- and CM-cellulose chromatography. The purified endo-β-(1 → 3)-glucanase was specific for the β-(1 → 3)-glucosidic linkage but it did not hydrolyze laminaribiose and in contrast to the yeast enzyme it cleaved laminaritriose very weakly. The kinetics of laminarin hydrolysis were complicated but appeared

on the whole to follow Michaelis-Menten theory. The pH optimum for this reaction was 5.0. Glucono-delta-lactone was inhibitory to enzyme activity. The bacterial endo-β-(1 → 3)-glucanase caused only a small degree of hydrolysis of isolated S. pombe walls. The endo-β-(1 → 6)-glucanase caused negligible release of reducing sugars from S. pombe walls but when total carbohydrate was measured a small amount of solubilization was apparent.

S. cerevisiae cell walls underwent negligible hydrolysis by the purified bacterial endo-β-(1 → 3)-glucanase and only a very limited hydrolysis was caused by the endo-β-(1 → 6)-glucanase. Acting together the two enzymes caused a greater degree of hydrolysis but this was still very limited in comparison to that obtained with the yeast endo-β-(1 → 3)-glucanase.

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1. Part of this work will appear in Proceedings of the 3rd Internat. Symp. on Yeast Protoplasts (J. R. Villanueva et al. eds.) Academic Press, London, Dec. 1973.
2. Glucanases in Schizosaccharomyces: Isolation and properties of the cell wall-associated β-(1 → 3)-glucanases. G. H. Fleet and H. J. Phaff. J. Biol., Chem. Febr. 1974.

Dr. Fleet is currently doing postdoctoral work in the laboratory of Professor D. J. Manners, Dept. of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh EH1-1HX, Scotland. He expects to remain there until September 1975.

Dr. F. M. Rombouts from the Agricultural University, Wageningen, Holland has joined our group for a year. He is currently working on the enzyme from the culture liquid of Bacillus circulans responsible for the lysis of the cell wall glucan of Saccharomyces cerevisiae.

Dr. Leda C. Mendonca has returned to Rio de Janeiro after a two-year stay in our laboratory. She has studied the base composition and has done DNA-DNA hybridization experiments among various psychrophobic yeasts associated with warm blooded animals. The work is now being prepared for publication and fuller details will appear in the Spring issue of the Yeast News Letter.

XXIV. Research Laboratories State Alcohol Monopoly, Alko, Box 350, SF-00101 Helsinki 10, Finland. Communicated by H. Suomalainen and Chr. Waller.

A list of our work published or accepted for publication since June 1973 follows below.

FORMATION OF FATTY ACIDS C_4-C_{10} DURING WILD YEAST FERMENTATION by V. Arkima.

Abstract of paper presented at the 14th International Congress of the European Brewery Convention, Salzburg 1973.

Formation of fatty acids C_4-C_{10} in the fermentation of beer wort inoculated with wild yeasts, Saccharomyces cerevisiae var. ellipsoideus, S. pastorianus, S. diastaticus and Brettanomyces anomala was compared with corresponding results from bottom yeast (S. carlsbergensis) fermentation. Fermentation was performed at 8 C and 20 C on a laboratory scale. Fatty acids in the fermentation solution were determined by gas chromatography.

Quantitative sequence of acids after wild yeast fermentation was as follows: isobutyric acid, caprylic acid, caproic acid, isovaleric acid, valeric acid, capric acid and butyric acid. Isobutyric acid and isovaleric acid were formed more by wild yeasts than in beer fermentation. The amount of caproic and caprylic acid was lower in wild yeast fermentation. A small increase in total acids was found when the temperature was raised from 8 C to 12 C.

THE EFFECTS OF NUCLEOTIDES ON THE α-GLUCOSIDASE FORMATION IN BAKER'S YEAST PROTOPLASTS by S. Haarasilta and E. Oura.

Submitted for publication in Acta Chem. Scand. Some of the results were published in Proc. 3rd. Int. Spec. Symp. Yeasts, Otaniemi, Finland 1973. Part I, Abstract, pp. 126-127.

Effects of certain nucleotides and Mg^{2+} and Mn^{2+} ions on the formation of α-glucosidase in baker's yeast protoplasts were investigated. Mg^{2+} and Mn^{2+} ions had a repressive effect on the formation of α-glucosidase, which effect, as well as the repressive effect of glucose, could be reversed partially by cAMP. A cAMP concentration of about 0.3 mM gave the maximum de-repressive effect. ATP had a de-repressive effect, but butyryl derivatives of cAMP were repressive.

SOME ASPECTS OF THE GROWTH PROCESS OF BAKER'S YEAST by E. Oura.

Proc. 3rd Int. Spec. Symp. Yeasts, Otaniemi, Finland 1973. Part I, Abstracts, p. 25, Part II, pp. 215-230.

The substrate used for growth of baker's yeast decisively affects the activity of various enzymes in the cell and the nature of the whole metabolism. Some enzymatic determinations concerning growth on various substrates - glucose, glycerol, pyruvic acid and ethanol - are reported.

For a given substrate, the rate of growth and enzymatic properties depends on the culture method. A fermentation metabolism allows a higher rate of growth than an oxidative one, and yeast cultured on excess glucose, even under aerated conditions, follows a fermentation mechanism to a significant extent. On the other hand yeast shows a clear tendency to switch to an aerobic metabolism in the presence of low glucose levels. The potential capacity of yeast for aerobic metabolism is greatest in conditions where it is prevented by the limited supply of oxygen. Under these conditions yeast uses an oxidative metabolism as much as possible, and satisfies its remaining energy requirements by fermentation. By certain assumptions it is possible to estimate the total energy used for the growth process.

THE EFFECT OF STORAGE ON THE NUCLEIC ACID COMPOSITION OF BAKER'S YEAST by E. Parkkinen, E. Oura and H. Suomalainen.

Submitted for publication in J. Inst. Brew. Some of the results were published in Proc. 3rd Int. Spec. Symp. Yeasts, Otaniemi, Finland 1973. Part I, Abstracts, pp. 62-63.

General changes are described in the nucleic acid content of yeasts stored for 20 days under extreme conditions (35 C). The total amount of nucleic acid increased in the first phase of 6 to 8 days storage. In the second phase, during which the yeast started to autolyse, the nucleic acid content diminished, first slowly and then more quickly. The quantity of acid soluble nucleotides increased with storage. The amount of dead yeast cells is low during the first period, but starts to increase quickly during the second period.

Changes in the rRNA, 5S RNA, tRNA, mRNA and DNA, fractionated on MAK columns, were investigated over a 20 days' storage period.

ON THE ENZYMES AND LIPID COMPOSITION OF CELL ENVELOPE FRACTIONS FROM SACCHAROMYCES CEREVISIAE by T. Nurminen and H. Suomalainen.

Proc. 3rd Int. Spec. Symp. Yeasts, Otaniemi, Finland 1973. Part I, Abstracts, pp. 36-37. Part II, pp. 169-189.

Lipid and enzymatic composition of cell envelopes, cell walls and plasma membranes of aerobic baker's yeast (Saccharomyces cerevisiae) have been studied. Saccharase and various acid phosphatases were mainly located outside the plasma membrane. The Mg^{2+} -dependent ATPase with an optimum activity at pH 6.8, the adenyl cyclase preferentially activated by Mn^{2+} and with a pH optimum of 5.7, the lipase and the bulk of phospholipase apparent in the cell envelopes were intrinsic characteristics of the plasma membrane.

Phosphoglycerides comprised 30% of the envelope lipids and were exclusively constituents of the plasma membrane. Neutral lipids amounted to 50% of the envelope lipids. The wall lipids consisted of neutral lipids to the extent of 75%, principally of triacylglycerols and lower acylglycerols. The plasma membrane also contained a considerable amount of triacylglycerols. The envelope sterols were mainly components of the plasma membrane. The main sterol was ergosterol. The major envelope fatty acids were $C_{16:1}$ and $C_{18:1}$ acids.

The applicability of zonal centrifugation for the large-scale isolation of plasma membranes from anaerobic and aerobic Sacch. cerevisiae was also studied. A clean separation from soluble and slowly sedimentable cell components was achieved. A further purification of plasma membranes was achieved by centrifugation in Urografin gradients.

ON THE STRUCTURE OF THE PLASMA MEMBRANE IN ANAEROBIC AND AEROBIC YEAST by H. Suomalainen and T. Nurminen.

Int Congr. Biochem., 9th, Stockholm 1973, Abstract book, p. 281.

Lipid and enzymatic analysis of whole cell envelopes of aerobic Saccharomyces cerevisiae and anaerobic Sacch. carlsbergensis, and of the cell walls and plasma membranes isolated from the envelopes have been made. Aerobic envelopes contained more total lipid (5.1% dry wt.) than anaerobic envelopes (3.1%) but a smaller fraction of this was neutral lipid (50%, compared with 65%). The cell walls from aerobic and anaerobic yeast contained only 1.6 and 1.4% total lipid, of which 75% and 80% were neutral lipids, including some sterols. The plasma membrane of both the yeasts contained a considerable amount of triacylglycerols. The main sterol in aerobic yeast was ergosterol comprising two-thirds of the total sterol and being mostly in a free form. Zymosterol and three other minor sterols were esterified. The aerobic membrane contained less lower acyglycerols, more sterols, more $C_{16:1}$, $C_{18:1}$ and other unsaturated fatty acids, and less of other fatty acids, than the anaerobic membrane. The effect of anaerobiosis on the composition of the membranes obtained by zonal centrifugation from Sacch. cerevisiae was also studied.

The following publications have appeared since the last communication. The abstracts of the reports have been given in Yeast News Letter 21:1, 17, 1972 and 21:2, 80, 82, 1973.

Suomalainen, H. and Nurminen, T., Isolation and properties of the plasma membrane of yeast cell. Proc. 4th Int. Ferment. Symp., Kyoto 1972, Ferment. Technol. Today, pp. 825-831.

Suomalainen, H. and Nykanen, L. Formation of aroma compounds in alcoholic beverages. Wallerstein Lab. Commun. 35:118, 185-202, 1972.

Suomalainen, H., Nurminen, T. and Oura, E., Aspects of cytology and metabolism of yeast, in Progress in Industrial Microbiology, vol. 12, ed. by D.J.D. Hockenhull, Churchill Livingstone, Edinburgh and London 1973, pp. 111-167.

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

1. The accumulation of large quantities of uric acid crystals (ρ = 1.89) in the vacuole of Candida utilis (A. H. Roush (1961), Nature 190, 449) has been used to test the intracellular stability toward mechanical stress. Both cells and spheroplasts with an intravacuolar load of crystals survived centrifugation in high gravitational fields as judged by plating, ultraviolet micrography, and spectrophotometry (K. D. Nakamura and F. Schlenk, J. Bacteriol. 116, December issue, 1973).

2. The isolation of vacuoles from Candida utilis has been studied. Experimental details included, a special culture medium for the cells, osmotic lysis of the spheroplasts, and isolation of the vacuoles by differential centrifugation. The vacuolar fraction appeared uniform as judged by ultraviolet micrography and showed retentiveness for solutes [K. D. Nakamura, 1974. Preparative Biochem. (in press)].

XXVI. School of Biological Technology, University of New South Wales, Kensington, N.S.W. 2033. Australia. Communicated by P. A. D. Rickard.

The following are summaries of two recent publications: -

1. QUANTITATIVE STUDIES OF THE DEVELOPMENT OF S. CEREVISIAE MITOCHONDRIA by M. Ferdouse, Pamela A. D. Rickard, F. J. Moss, and H. W. Blanch. Biotechnology and Bioengineering, (1972), 14, 1007.

The quantitative changes in mitochondria and cytochromes during transition of Saccharomyces cerevisiae from one steady state to another, while growing in continuous culture under controlled environmental conditions, were followed.

No mitochondria, or mitochondria-like structures, were detectable in electron micrographs of permanganate-fixed anaerobic cells. Microaerobiosis (3 μM dissolved oxygen) was sufficient to visualize mitochondrial profiles and induce cytochromes. Cells grown in excess glucose contained a low concentration of cytochromes and their sections had a reduced number of mitochondrial profiles compared with cells grown in limiting glucose.

In the presence of ergosterol and Tween 80 mitochondriogenesis, whether induced by aerobiosis or glucose limitation, involved enhanced definition of cristal and outer mitochondrial membranes and increased number of profiles. Where membrane formation was limited, by the

absence of ergosterol and Tween 80 from the medium, adaptation from anaerobiosis to aerobiosis involved cytochrome induction and profile visualization, but limited profile proliferation; the adapted cells consequently contained fewer, but more cytochrome-enriched, mitochondria than cells adapted in the presence of ergosterol and Tween 80.

Increase in dissolved ozygen from 3 μM to 52 μM further enhanced membrane definition and increased the size, but not the number, of mitochondrial profiles.

Evidence, obtained by measurement of cytochrome concentration per unit mitochondrial volume and per unit cristal area, support the concept that mitochondriogenesis and cytochrome synthesis are not synchronized processes and that cytochromes are added to or depleted from the mitochondrial cristae in response to culture conditions.

2. THE RESPONSE OF THE ADENOSINE PHOSPHATE POOL LEVEL TO CHANGES IN THE CATABOLIC PATTERN OF S. CEREVISIAE by M. D. Akbar, Pamela A. D. Rickard and F. J. Moss. Biotechnology and Bioengineering (in press).

Changes in the catabolic pattern of Saccharomyces cerevisiae, growing in continuous culture, were effected by altering the glucose feed rate or the dissolved oxygen concentration. The cytochrome concentrations and the adenosine phosphate pool level of the yeast in a series of steady states and during three transitions were measured and compared with the glucose uptake rate (Q_G), the respiration rate (Q_{O_2}) and the rate of ethanolic fermentation (Q_E).

Respiration was decreased at high glucose feed rates only if oxygen was low but cytochromes were glucose repressible at both high and low oxygen concentrations. In the main, Q_E and the levels of ATP, ADP and AMP were decreased and cytochrome concentrations were elevated at low Q_G values. No consistent relationship between any of the adenosine phosphate parameters and Q_{O_2} was discernable.

Evidence is presented for the concept that the Q_G directly controls the adenosine phosphate pool level and that a relationship between the concentration of adenosine phosphate anhydride bonds and the adenosine phosphate level is constantly maintained.

XXVII. Department of Comparative Biochemistry, Janssen Pharmaceutica, Research Laboratories, B-2340 Beerse, Belgium. Communicated by H. Van den Bossche.

The following paper will appear in a forthcoming issue of Biochemical Pharmacology.

Van den Bossche, H. Biochemical effects of miconazole on fungi. 1. Effects on the uptake and/or utilization of purines, pyrimidines, nucleosides, amino acids and glucose by Candida albicans.

Abstract: The antifungal and antibacterial drug miconazole (1-[2,4-dichloro-β-(2,4-dichlorobenzyloxy) phenethyl] imidazole nitrate) has been shown to inhibit, at concentrations lower than those affecting growth, the utilization of adenine, guanine and hypoxanthine by C. albicans in suspension culture. The observed decrease in the incorporation of purines into nucleic acids seems to be the consequence of an inhibitory effect on their uptake into the cells. When the purines were replaced by nucleosides, miconazole induced an increased uptake and incorporation of the radioactivity derived from the nucleosides into macromolecules. The data obtained suggest that the drug-induced increase of nucleoside incorporation into nucleic acids is secondary to enhanced nucleoside transport. Miconazole also slightly affected the uptake of orotic acid. Glucose, glycine and leucine transports were not affected by micona-

zole whereas in some way the drug affected glutamine uptake.

Studies on the distribution of miconazole and/or its metabolites in the Candida cell indicate that in log-phase cells most of the radioactivity was found in the fraction containing cell walls and plasmalemma. In stationary-phase cells the highest radioactivity was found in the fraction which seemed to contain the microsomes.

Although more information will be needed, the data presented seem to indicate that at low concentrations (10^{-9} - 10^{-8} M), miconazole acts primarily on the yeast cell membranes (cell wall and plasmalemma) resulting in a selective and irreversible inhibition of the uptake of precursors of RNA and DNA (purines) and mucopolysaccharide (glutamine). Higher doses and longer incubation periods also seem to alter the activities of microsomal and/or mitochondrial membranes.

XXVIII. Mikrobiologisches Institut, Swiss Federal Institute of Technology, Weinbergstrasse 38, 8006 Zürich, Switzerland. Communicated by A. Fiechter.

1. Recent Publications:

U-Flury: Isoenzyme der Malat-Dehydrogenase in Schizosaccharomyces pombe. Thesis No. 5129.

K. Weibel: Zur Energetik von Saccharomyces cerevisiae Thesis No. 5155.

A. Einsele and A. Fiechter: Die kontinuierliche Kultur von Candida tropicalis mit Hexadecan in vollständig gefüllten Reaktoren. Kurzreferate 3. Symp. Techn. Microbiol., Berlin, 1973.

J. R. Mor: Automatic Assay of Microbial Growth. Paper B 77, Symp. on "Rapid Methods and Automation in Microbiology", Stockholm, 1973.

2. In Press

H. W. Blanch and Fiechter: Dispersion and coalescences phenomena in the hydrocarbon fermentation. (Accepted for publication in Biotechnol. Bioeng.).

S. Divjak and J. R. Mor: On the activity of Carbon Dioxide Fixation in Growing Yeasts. (Accepted for publication in Arch. Mikrobiol.).

XXIX. Instituto de Enzimologia del C.S.I.C. Facultad de Medicina de la Universidad Autonoma, Madrid-34, Spain. Communicated by C. Gancedo.

A preliminary account of our work on hexose kinases from Rhodotorula glutinis has been presented at the 3rd International Specialized Symposium on Yeasts, June 1973, Otaniemi, Finland. The work has now been completed and will soon be submitted for publication.

An hexokinase (EC 2.7.1.1) and a glucomannokinase (EC 2.7.1.2) from the red yeast Rhodotorula glutins are described. Both enzymes have been separated and their properties studied. The glucomannokinase does not phosphorylate fructose and is not inhibited by it up to 50 mM. Otherwise both enzymes share many properties, the K_m for glucose is 0.1 mM in both cases and the K_m values for ATP are 0.5 mM and 0.6 mM respectively for hexokinase and glucomannokinase. Both enzymes are constitutive, show competitive inhibition by N-acetylglucosamine, have weak affinity for glucosamine and exhibit a broad pH optimum. The molecular weights determined by gel

filtration are 110.000 for glucomannokinase and 96.000 for hexokinase. The maximal activity of both hexose kinases roughly accounts for glucose utilization by Rhodotorula glutinis and it is concluded that phosphorylation is the first intracellular step in glucose metabolism by this yeast.

Recent publications

"Concentrations of intermediary metabolites in yeast" J. M. Gancedo and C. Gancedo. Biochimie 55, 205-211 (1973).

The concentrations of some intermediary metabolites in yeast in different metabolic situations have been tabulated from data available in the literature. A critical examination of the extraction procedures and their influence on the values obtained is performed.

"Contribution of the pentose-phosphate pathway to glucose metabolism in Saccharomyces cerevisiae: a critical analysis on the use of labelled glucose" J. M. Gancedo and R. Lagunas. Plant Science Letter, 1 193-200 (1973).

The validity of the procedure of Katz and Wood for the determination of the contribution of the pentose-phosphate pathway (PPP) to glucose metabolism in yeast is established, and it is shown that a number of methods are liable to yield erroneous results. In yeast growing on glucose with NH_4^+ as nitrogen source, the pentose-phosphate pathway was found to account for 2.5% of the total metabolism of glucose, while in yeast growing on an enriched medium where NH_4^+ had been replaced by Difco yeast extract the contribution was lowered to 0.9%. These results confirm that a major role for this pathway is to supply NADPH for biosynthetic reactions.

"Reduced pyridine-nucleotides balance in Glucose-growing Saccharomyces cerevisiae". R. Lagunas and J. M. Gancedo, Eur. J. Biochem., 37, 90-94 (1973).

The quantitative importance of transhydrogenation from NADH to NADP in Saccharomyces cerevisiae growing on glucose has been investigated. To this aim, the contribution of the different metabolic pathways to glucose metabolism has been measured and a balance between the requirements and the production of reduced pyridine nucleotides has been established. The results suggest that the oxidative hexose monophosphate pathway accounts for all, or almost all of the cellular requirements for NADPH and that the reoxidation of NADH is effected in part by the respiratory chain, but mainly by enzymatic reactions coupled to the reduction of organic compounds like acetaldehyde or dihydroxyacetone-phosphate. A significant transhydrogenation between pyridine nucleotides is therefore not likely to occur in S. cerevisiae growing on glucose.

"Assay of yeast enzymes in situ. A potential tool in regulation studies". R. Serrano, J. M. Gancedo and C. Gancedo. Eur. J. Biochem., 34, 479-482 (1973).

A permeabilization method which allows the assay of several intracellular enzymes within the boundaries of the yeast cell wall is described. The kinetic properties of hexokinase and pyruvate kinase examined in the permeabilized cells, including the allosteric activation of the latter by fructose disphosphate, are essentially the same as in cell-free extracts.

XXX. Moscow State University, Moscow, USSR. Communicated by I. P. Babyeva.

The following is the abstract of a paper presented at the Fifteenth Plenary Meeting of COSPAR, Madrid, Spain, May 10-24, 1972. The full paper appears in Life Sciences and Space Research XI, pp. 55-61, Akademie Verlag, Berlin, 1973.

ON THE MECHANISM OF ADAPTATIONS OF MICRO-ORGANISMS TO CONDITIONS OF EXTREME LOW HUMIDITY by S. I. Aksenov, I. P. Babyeva and V. I. Golubev.

Low humidity is considered one of the main obstacles for life on Mars. Mechanisms of adaptation to extreme low humidity are investigated in asporogenic yeasts. Cryptococcus albidus var. diffluens inhabits the high mountain deserts of the Pamirs and the Tien Shan. The spin-echo nuclear magnetic resonance method shows that different ways of drying do not extract all liquid water from the cells. Residual humidity of the Cryptococcus cells may reach 30%. The polysaccharide capsule of Cryptococcus delays the drying process noticeably and also collects moisture at low relative humidity. The considerable quantity of conserved liquid water allows Cryptococcus to adapt to the great periodic oscillations of relative humidity resulting from the great diurnal temperature changes in the high mountain deserts.

Data obtained indicate the presence of a humidity regulating mechanism on the cellular level in lower plants.

XXXI. University of Bristol. Cider and Fruit Juices Section. England. Communicated by R. R. Davenport.

1. Next March I shall be in Sao Paulo at the Institute de Botanica to give a seminar on Mycology and Taxonomy of fungi in fruit juices. I would welcome the opportunity to make arrangements to meet any persons interested in yeasts and yeast-like organisms, particularly in the fields of taxonomy and microecology.

2. Abstract. This abstract is of an environmental study which was made of an English vineyard. In this project the vineyard was considered as an ecosystem in which precise strategy was used for monitoring and sampling procedures at the times indicated by a biological calendar based on the development states of the grape vine. This ecological approach was complemented by a series of well defined laboratory programmes and field observations in order to give the maximum information about the organisms and their habitats.

Yeasts and yeast-like organisms were identified using specially developed techniques and identification keys designed to handle large numbers of isolates with a wide variety of characteristics. These micro-organisms belong to nine morphological groups further sub-divided into 21 genera which included 80 species and six varieties: new species were discovered within the genera Endomycopsis (2), Sporobolomyces (1), Sporidiobolus (3), Rhodosporidium (1), Bullera (2), Leucosporidium (2), Lipomyces (1), Candida (4), Pichia (1), Nadsonia (2) and Schizosaccharomyces (1).

Different pleomorphic forms were observed in the genera Schizosaccharomyces, Sporobolomyces, Bullera and Kloeckera. Endospores were found in strains of Aureobasidium pullulans and also in several Trichosporon

ssp. that have not previously been reported. Hat-shaped ascospores were seen in a new yeast, previously identified as Endomycopsis sp.A. Three black yeasts have been investigated and tentatively named as Trichosporonoides spp. but other features of these isolates may require the establishment of a new genus after further investigation. Sporing isolates of Metschnikowia pulcherrima and M. reukaufii were also observed but had some morphological features hitherto not reported. Saccharomyces spp. (fermenting yeasts) were rarely found. More importantly the following potential spoilage organisms of industrial significance were isolated: Saccharomyces aceti, Sacch. baillii, Sacch. cerevisiae, Sacch. montanus, Sacch. rouxii, Schizosaccharomyces sp.A., Schizosaccharomyces spp., Torulopsis lactis condensii, T. magnoliae, T. candida/Debaryomyces hansenii, T. castellii, Candida valida, C. vini, C. zeylanoides, Pichia farinosa, Hansenula anomala var. anomala, Hanseniaspora valbyensis/Kloeckera apiculata, Hanseniaspora uvarum, Kloeckera africana, Kl. corticus, Kl. javanica var. lafarii, Nadsonia sp.X.

DAVENPORT, R. R. (1973). Vineyard Yeasts - an environmental study. In Sampling - Microbiological Monitoring of environments. Eds. R. G. Board, D. W. Lovelock. The Society for Applied Bacteriology Technical Series No. 7.

DAVENPORT, R. R. An environmental study of an English vineyard with particular reference to yeasts and yeast-like organisms. Ph.D. Thesis, University of Bristol: to be submitted shortly.

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

Since the last publishing of the Yeast News Letter, a M.Sc. dissertation has been submitted and approved in Dr. Blackwood's research group in yeast microbiology.

M. A. Lachance*. Production of single-cell-protein from waste pasta products by Endomycopsis fibuligera.

The production of Single-Cell-Protein from pasta wastes was studied with a strain of Endomycopsis fibuligera (Lindner) Dekker [syn. Saccharomycopsis fibuligera (Lindner) Klöcker] Mac-Y-4373 isolated from pasta wastes, and with mutants of this strain. The growth process was found to occur in three phases, and the factors affecting them were examined.

The first phase, amylase production by the yeast, occurs best at a pH of 5.5, a temperature of 32 C, in a medium containing 0.5% starch, plus 0.5 to 0.8% $(NH_4)_2SO_4$ and 0.5% KH_2PO_4. The second phase, degradation of starch by the extracellular enzymes, is highest at pH 4.8, in a temperature range of 35 to 45 C; increasing amounts of certain salts and carbohydrates, especially maltose, inhibit the amylolytic activity. The third phase, growth of the yeast on hydrolysed starch, has pH and temperature optima identical to those of the first phase. Growth is less affected by the salt concentration and responds to carbohydrate levels, with the equivalent of 2% maltose giving high yields. The overall process is stimulated by the presence of various salts and growth factors. Pasta was examined as a nutrient source and was found to supply sufficient nutrients for this yeast.

A three-phased batch fermentation process was designed, that ensures more than 90% conversion of pasta starch, in approximately 22 hours, into a biomass that contains as much as 40% protein, with

a cell yield of up to 50% of the starch consumed.

*Present address: Dept. of Food Science and Technology, University of California, Davis, California 95616.

XXXIII. Korean Institute of Science and Technology (KIST), Dept. of Biology, Chonnam National University, Chonnam, Korea. Communicated by Myung Sam Park.

STUDIES ON THE WILD YEASTS IN KOREA (4) - ON THE PRODUCTION OF FERMENTED FEEDS.

Abstract. Fermented feeds using rice, barley, wheat and defatted rice brans as the raw materials were prepared with three species of wild yeasts, which were selected among 35 strains of yeasts isolated. The results were as follows:

1. The two yeasts were identified as H. anomala var. anomala (No. 225), Candida utilis (No. 400). Also Irpex-cellulase (consors) (No. 403-A) was used.

2. The optimum pH, and sugar concentration for the yeasts in liquid culture were pH 5.0 and 10° Balling each. The optimum temperature was 30 C for No. 225 and No. 403-A, 27 C for No. 400. No. 225 and No. 403-A grow at higher temperature than 37 C and 40 C each.

3. The No. 225 yeast had a large vegetative cell and strong sugar fermentability. The No. 225 and 403-A culture could assimilate cellobiose, xylose, KNO_2 and KNO_3. These properties were suitable for bran fermentation.

4. The No. 403-A microorganism was a yeast-like microbe and showed cellulase activity which might help in the propagation of other yeasts on the brans.

5. The analytical data of fermented feed are in the following order of useful value: rice-wheat-barley bran 4:4:2, rice-wheat bran 5:5, rice-barley 5:5, rice-defatted rice bran 5:5.

6. The fermented feeds were prepared by mixing brans, 0.3% ammonium sulfate and 5% (w/w) inoculum of yeast suspension 4% glucose solution. Water content 70-80%, fermentation temperature 25-30 C and fermentation time 2-3 days.

7. The rice-wheat bran 5:5 and rice-barley bran 5:5 fermented feeds showed 11.17-11.45% protein increase, and the rice-barley-wheat bran 4:4:2 and rice-defatted bran 5:5 showed 3.75-6.03% protein increase.

The fermented feeds prepared in this experiment by the author might work as a nutritive feed using microbial cell body, enzymes produced by microbes and other cell constituents.

XXXIV. Institute of Technology of Fermentation and Microbiology, Technical University of Lódź, Poland. Communicated by J. Jakubowska.

Third International Specialized Symposium on Yeasts. Helsinki, Finland. Abstracts, section 9, p. 142-143.

THE EFFECT OF INDOLE ACETIC ACID (IAA) ON GROWTH AND ACTIVITY OF BAKER'S YEAST GROWTH IN CONTINUOUS CULTIVATION by J. Jakubowska and M. Wlodarczyk.

In our previous experiments (1) the stimulatory effect of auxins on rate of growth and oxydative consumption of sugar by baker's yeast, especially in early logarithmic phase of growth, was noticed. The optimal dose of IAA not exceeding 10 µg/ml in the culture medium did not influence any physiological changes in yeast population. The research reported herein was focused on the possibility of obtaining

a high yield of active biomass of baker's yeast growing in steady state conditions in presence of optimal concentration of IAA.

A pure culture of baker's yeast strain Saccharomyces cerevisiae Hansen Ja-64 of our collection was cultivated in yeast extract solution with 2% glucose. The concentration of IAA was 1,5 or 10 µg/ml in the growing medium. Continuous cultivation was carried out in one-stage laboratory fermentor. The steady state conditions (pH 6.0 and 30 C) were maintained with D=0.20 hr^{-1} and doubling time 3,5 hrs.

In presence of IAA (1.5 or 10 µg/ml) in the medium during 100 hrs of continuous flow culture, the biomass level was higher by 20 and 60% over the control. Simultaneously the rate of sugar utilization was accelerated. The activity of cells was shown to be markedly higher than those grown without IAA, namely: (1) two-fold amound of D-xylose was accumulated in the cells, (2) the increase in dehydrogenase activity by 150 to 250%, (3) the oxydative decarboxylation in sucrose and maltose attained 6 and 3 times higher level, respectively. The carbon dioxyde output always predominated over the oxygen uptake.

These results confirmed our earlier statements (Acta Microbiol. Pol. Ser. B. 1970,2(19):75-81.) for yeast grown in batch culture on the same medium supplied with IAA, and led us to the conclusion that betaindoliloacetic acid in optimal dose can function as a useful activator in baker's yeast propagation. Stimulatory effect of IAA can be responsible for transport of sugar into the yeast cell, and for oxydative intracellular metabolism.

INDUCTION OF MUTATION OF BAKER'S YEASTS BY N-METHYL-L-NITRO-N-NITROSOGUANIDINE (MNNG) by J. S. Szopa and A. Borodziej.

The influence of MNNG on the diploid Saccharomyces cerevisiae Ja-64 strain was tested. After 120 min of incubation of the cells (10^8 cells/ml) with 50 µg/ml of MNNG the viability of cells was rather high (about 10%). By the use of tetrazolium-overlay technique and testing growth of MNNG-treated population on different cultivation media: Go + molasses, G- + maltose, Go + raffinose and Go + melibiose, we have isolated many strains differentiated in their fermentation ability. The most promising strain marked N_4 produced about 15% higher biomass than initial Ja-64 strain.

Zesz. Nauk. P. L. Chemia Spozywcza 1973, (in press).

THE EFFECT OF BETA-INDOLILOACETIC ACID ON SCHIZOSACCHAROMYCES ACIDODEVORATUS NO. 2 by Danuta Kusewicz.

Beta-indoliloacetic acid (IAA) had little effect on growth of Sch. acidodevoratus Nr. 2. However, the cells grown with IAA showed higher activity in the reduction test with tetrazolium blue (BT) than those grown without IAA.

In the experiments carried out by Warburg's method IAA markedly increased endogenous oxygen uptake. A similar effect was observed in the presence of malate, citrate, tartrate and glucose. Resting cells examined with BT confirmed the above results.

Zeszyty Naukowe Politechniki Lodzkiej, Chemia Spozywcza (in press).

ORGANIC ACID FORMATION BY RESPIRATION-DEFICIENT MUTANTS OF BAKER'S YEAST Ja-64 by H. Oberman, J. S. Szopa and J. Pikala.

The respiration-deficient mutants of baker's yeast Saccharomyces cerevisiae produced citro-malic acid in fermentation media. Irrespective of the respiratory deficiency of the tested yeasts, lactic and α-oxoglutaric acids were also detected in such media.

Zesz. Nauk. P. L. Chemia Spozywcza, 1973 (in press).

CHARACTERISTIC OF SOME CRYOPHILIC WINE YEASTS by Danuta Kusewicz.

A comparison was made of ten strains of cryophilic wine yeasts Saccharomyces cerevisiae var. ellipsoideus: Fendant W-14, W-23, W-24; Johannisberg W-17; W-21/F,W-21-35; W-22/F,W-22-42; Fiedozja I 19-28-115, S. oviformis-Leningradzka 21-22-17, with the strain of mesophilic "Tokay 13". The following features, in the temperature range from 4C to 35 C were tested: rate of growth, utilization of carbohydrates (glucose, sucrose, galactose, maltose, raffinose, melibiose, mannose, lactose, trehalose, inulin, sorbitol, mannitol), sporulation, utilization of ammonium sulphate or asparagine, glucose metabolism (by Warburg method), uptake of sorbose and fermentation yield using apple must.

At lower temperature levels some correlations between the respiratory activity with glucose and the rate of sorbose uptake and fermentation activity were noted. The strains W-22-42 and W-21-35 were proved to be the most active. The strains were selected for the further work.

Roczniki Chemii i Technologii Zywnosci (in press).

XXXV. Labatt Breweries of Canada Limited 150 Simcoe Street, London, Ontario, N6A 4M3, Canada. Communicated by Graham G. Stewart.

The following papers have recently been, or will shortly be published from this laboratory:

1. Russell, I., Garrison, I. F. and Stewart, G. G.: Studies on the Formation of Spheroplasts from Stationary Phase Cells on Sacch. cerevisiae. J. Inst. Brewing 79, 48 (1973).

The rate of spheroplast formation in stationary phase cells of Saccharomyces cerevisiae has been found to be increased as a result of pre-treatment of the cells with protease. The protease has been found selectively to remove the cell wall's mannan-phosphate-protein layer and thus greatly facilitate the action of snail gut enzymes or other such enzymes, in their hydrolytic activity upon the glucan of the cell wall.

2. Wellman, A. M. and Stewart, G. G.: Storage of Brewing Yeasts by Liquid Nitrogen Refrigeration. Appl. Microbiology 26, 577 (1973).

Many yeast strains are difficult to maintain in culture in a stable state, and long-term preservation by lyophilization, which has proved useful for other fungi has given poor results with brewing yeasts. As an alternative to continuous subculture, which maximizes strain variability, various methods of cryogenic storage were investigated. Yeast strains were frozen with or without cryoprotectants (such as glycerol or inositol) and stored at -196 C. Recovery after warming was estimated from plate counts, and survivors were screened to detect changes in the frequency of morphological types, respiratory-deficient mutants, and glycerol-sensitive mutants. Strains varied in their sensitivity to freezing, and

survival was modified by the growth medium, the freezing munstrua, and the freezing conditions. Suspension of cells in 10% (vol/vol) glycerol, cooled at 1 C/min, warmed rapidly and plated on malt-yeast extract-glucose-peptone agar produced the highest percentage of viable colonies with a minimal change in metabolic characteristics. In two of the strains tested, no significant increase in mutation rate was detected under any of the treatments; the strains were maintained in a stable state and were metabolically comparable to unfrozen strains. In one strain of Saccharomyces uvarum after some freezing treatments, the percentage of respiratory-deficient mutants increased markedly, the fermentation rate declined, and a loss of flocculation occurred. The freezing parameters which increased the level of respiratory-deficient cells should be avoided in maintaining this strain. Maintenance of cultures of brewing yeasts by cryogenic storage has several advantages over other preservation techniques: the method is simple and reproducible, the cultures have remained stable over a 3-year test period, and the viability is high.

3. Stewart, G. G.: Some Observations on Maltose and Glucose Metabolism in Sacch. cerevisiae. Amer. Soc. of Brewing Chemists. Ann. Conv. 1973 - in the press.

The uptake rates of glucose and maltose in a number of yeast strains after growth in glucose or maltose have been studied. In all but one of the strains examined maltose uptake did not occur unless the yeast was precultured in maltose (i.e., maltose uptake is an induced system). One strain, however, possessed a constitutive maltose uptake system - even after growth in glucose. This particular strain retained its ability to take up and metabolize maltose.

The uptake and metabolic fate of glucose, fructose and maltose has been compared in strains with constitutive and inductive systems for maltose metabolism. In the strains exhibiting induction glucose caused high rates of aerobic fermentation and low rates of respiration and polysaccharide accumulation whereas with maltose, and to some extent fructose, the converse was true. The constitutive strains, however, showed relatively high levels of aerobic fermentation even during maltose metabolism. The maltose uptake system in an induced strain has been found to be energy dependent and that protein synthesis is required to establish and maintain it.

The implications of these results are discussed in the light of current knowledge and theories on yeast carbohydrate metabolism. Their impact on wort fermentation and beer composition is mentioned briefly.

4. Stewart, G. G., Russell, I. and Garrison, I. F.: Further Studies on Flocculation and Co-flocculation in Brewer's Yeast Strains. Amer. Soc. of Brewing Chemists. Ann. Conv. 1973 - in the press.

The factor that induces co-flocculation in Sacch. cerevisiae strains LAB A/69 and LAB B/69 has been found to be situated on the periphery of the cell wall. Certain amino acids (e.g., aspartic and glutamic acids) when present in excess in the medium, will induce co-flocculation. This ability is not restricted to the L-isomers because the non-metabolizable D-isomers of aspartic and glutamic acids are also capable of inducing co-flocculation. This, therefore, indicates in situ incorporation of the "inducer" material onto the cell wall.

A peptide fraction, high in acidic amino acid residues, has been isolated from wort and this fraction has been found to induce intense co-flocculation (as intense as wort itself). It is proposed that cells in flocs are held together by "carboxyl - Ca^{++}ion complexes", the carboxyl groups being contained in either acidic amino acids or in the acidic amino acid residues of certain peptides (See also Stewart et al. ASBC Conv., 1972, 117-131).

5. Stewart, G.G.: Some Thoughts on the Microbiological Aspects of Brewing and Other Industries Utilizing Yeast - A Review. Advances in Applied Microbiology 17 - in the press.

Modern developments in brewing, and similar yeast-utilizing industries are reviewed. Fermentation technology in both batch and continuous processes is discussed after a brief description of older, more traditional and well-tried procedures. Modern developments are contrasted with their ancestors and by this means it is hoped that the reader is given as complete a picture as possible on the "state of the art" in this area.

XXXVI. Allied Breweries (Production) Limited, The Brewery, Station Street, Burton-on-Trent DE14, 1BZ, England. Communicated by Miss D. A. Lovett.

The following is a summary of the Ph.D. thesis of R. J. Anderson, a member of the staff of the Process Research Department of Allied Breweries. The dissertation was submitted to the University of Birmingham. The work was carried out under the guidance of Professor J. S. Hough.

THE PRODUCTION OF SULPHUR VOLATILES BY BREWERY MICROROGANISMS

The present work has clarified the role of brewing yeasts and spoilage bacteria as potential sources of the volatile sulphur compounds which can significantly affect the flavour of beer.

The hydrogen sulphide sometimes formed during the commercial brewing process arises mainly from yeast activity. Work elsewhere indicates that most of this is usually lost during beer processing and the present studies suggest that its reoccurrence thereafter may be due to infection by the bacterium Zymomonas anaerobia. Radiochemical experiments indicate that hydrogen sulphide produced by yeast and Zymomonas anaerobia is derived mainly from sulphate. Sulphate and any methionine present are assimilated by these organisms but the origin of a large proportion of their cell sulphur is unknown.

The production and assimilation of sulphur compounds by wort bacteria was also studied. A volatile causing 'sulphury' smells in beer was not identified but experiments suggest it may not contain sulphur.

XXXVII. Research Institute for Viticulture and Enology, 886 15 Bratislava, Matuskova 21, Czechoslovakia. Communicated by E. Minarik.

The following manuscripts have been recently accepted for publication:

1. E. Minarik and A. Navara: Sulphite and sulphide formation by wine yeasts. Influence of sulphur containing amino acids (in German) - Mitteilungen Klosterneuburg, Austria 1973.

It could be confirmed that methionine and/or cysteine considerably decrease or prevent the formation of sulphite during the alcoholic fermentation of a synthetic medium with asparagine by so-called SO_2-forming and normal yeast strains. The simultaneous presence of both amino acids in the medium prior to fermentation lead to an especially strong inhibition effect. No correlation could be found between sulphite and sulphide formation under the given fermentation conditions.

2. E. Minarik and A. Navara: The influence of methionine and cysteine on the sulphate uptake and sulphite formation by some species of Saccharomyces (in German). Biologia (Bratislava), No. 12, 1973.

Methionine and cysteine decrease sulphate uptake and sulphite formation in so-called SO_2-forming and normal yeasts under controlled

conditions in a synthetic medium containing asparagine as a sole source of nitrogen. It is assumed that both amino acids participate in the regulation of sulphate activation in the enzymatic sulphate reduction by yeasts of the genus Saccharomyces and thus also in sulphite and sulphide formation, respectively.

XXXVIII. Manuscripts on Yeast Taxonomy.

A recent policy decision by the Editorial Board of the International Journal of Systematic Bacteriology has resulted in the possibility of publishing papers dealing with subjects on the systematics and taxonomy of yeasts and yeast-like organisms in this Journal. The IJSB is the official organ of the International Committee on Systematic Bacteriology and it is published for the International Association of Microbiological Societies (IAMS) by the American Society for Microbiology.

Manuscripts must be written in English and should be submitted in duplicate (original plus one copy) to the Managing Editor, Mr. Robert A. Day, ASM Publications Office, 1913 I Street N.W., Washington, D.C. 20006, U.S.A.

Dr. H. J. Phaff has joined the Editorial Board. Manuscripts submitted for publication will be evaluated by at least two experts in the field as well as by the Editor. Detailed instruction to authors will appear in the January 1974 issue of the IJSB.

Since the name of the Journal does not reflect the subject matter of yeasts, the Editorial Board will discuss at its next meeting in Chicago in May 1974, the possibility of a name change for the Journal.

Authors are invited to submit manuscripts on Yeasts and Yeast-like organisms at any time.

XXXIX. New Books.

1. From Universal Foods Corporation, Milwaukee, Wis. 53201. "Yeast Technology" by G. Reed and H. J. Peppler has been published by AVI Publishind Co., Westport, Conn. (1973). The book contains general chapters on yeast classification, biology, biochemistry, and the microbiological aspects of yeast technology, and applied chapters on baker's yeast production, use of yeast in baking, wine yeast, brewer's yeast, distiller's yeast, sake, feed and food yeasts and yeast derived products. Price $25.

2. Yeast, Mould and Plant Protoplasts. Proceedings of the Third International Symposium on Yeast Protoplasts, held at Salamanca, Spain, in October, 1972. Edited by J. R. Villanueva, I. Garcia-Acha, S. Gascon and F. Uruburu. Departamento de Microbiologia, Facultad de Ciencias Universidad de Salamanca, Salamanca, Spain. December 1973 xxiv + 380 pp., £7.00 0.12.722160.3 Academic Press.

This work constitutes a record of the proceedings of the Third International Symposium on Yeast Protoplasts, held at Salamanca, Spain in October, 1972. The aim of this volume is to bring together papers by leading authorities on yeast, mould and plant protoplasts, in a single volume, and to stress their unity. A special emphasis is placed upon new findings in protoplast science, and the book contains much original research on protoplast formation, morphogenesis, and physiology.

The book falls into two parts, the first dealing with yeast cell wall lytic enzymes, fungal structure and morphogenesis, as well as with different aspects of the physiology and biochemistry of protoplasts.

The second part, which is subdivided into two sections, covers in the first section formation, regeneration, and metabolism of mould protoplasts, and in the second, isolation, fusion and development of plant protoplasts. This latter part is especially valuable in presenting the latest results in the developing research field of mould and higher plant protoplasts, and includes background reviews.

This book makes an important contribution to the study of yeast, fungal and plant protoplasts, and will be of considerable value to botanists, plant and cell physiologists, biochemists, and microbiologists.

Contents: Yeast cell wall lytic enzymes, Structure and morphogenesis, Physiology and biochemistry of yeast protoplasts, Protoplasts of moulds, Protoplasts of plants, Author index, Subject Index.

3. Early in 1974, the North-Holland Publishing Company should bring out a small book entitled: "A New Key to The Yeasts: a Key for Identifying Yeasts Based on Physiological Tests only" by J. A. Barnett and R. J. Pankhurst. The book will be about 160 pages long and the publishers have predicted that it will cost D.fl. 25 [about $ 10 or £4].

This book provides a new computer-made key for identifying 434 yeast species. Unlike any previous key, it is based solely on the results of physiological tests, largely those of growth and fermentation. Features such as microscopical appearance and the ability to produce ascospores are needed only for confirmation of identify and not in the key itself.

The 434 species are those recognized by the Centraalbureau voor Schimmelcultures at the end of November 1972. Three hundred and forty one are detailed in Lodder's The Yeasts (1970). Most of the further 93 species were described too recently for that work. A New Key to The Yeasts gives a brief description and full references for each of these additional species.

A novel feature of the book is a table of the responses of all 434 species, arranged alphabetically, to each of 60 physiological tests. This table makes it practicable (i) to find rapidly the nutritional characteristics of any species, and (ii) to select species with desired combinations of characters.

Some guidance is offered to those who want to use alternatives to the New Key. The authors discuss (i) keys with fewer tests, (ii) partitioned keys, (iii) similarity techniques, (iv) polyclaves and (v) the identification of yeasts from a restricted group.

One large table provides a guide to the yeast genera up to the time of going to press. For each genus, there is a brief account of the chief morphological and physiological characteristics, sexual reproduction (if known) and references.

XL. National and International Meetings.

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

Organizing Committee
Prof. Dr. T. O. Wiken - Honorary Chairman
Prof. Dr. H. Klaushofer - Chairman
Prof. Dr. R. Brunner - Vice Chairman
Doz. Dr. U. Sleytr - Secretary
Prof. Dr. W. Petzelbauer - Treasurer
Prof. Dr. W. Oberzill

All correspondence related to the Symposium should be addressed to:
The Secretariat
"Fourth International Symposium on Yeasts"
c/o Hochschule fur Bodenkultur
Gregor-Mendel-Strasse 33
A-1180 Vienna, Austria

Enrollment fee 1700. - A. Shillings (appr. $88.-) if paid before March 1, 1974.

Second Circular

The Fourth International Symposium on Yeasts is being organized at the request of the International Commission on Yeasts and Yeast-like Microorganisms of IAMS (earlier the Council for International Cooperation in Yeast Science) by a Committee under the leadership of Prof. Dr. H. Klaushofer, Vienna, under the auspices of the Austrian Society of Microbiology, Hygiene and Preventive Medicine, the Austrian Association of Food and Fermentation Technologists and the Experimental Station for Fermentation Industry, Vienna.

Place and Date: The Symposiun will be held in Vienna, Austria, 8-12 July 1974. The official opening will be on July 8th, 1974 at 9:00 a.m., followed by a plenary session of papers by three invited lecturers during the morning. Depending on the final number of participants, the site of informal gathering, official opening and plenary sessions will be announced in the third circular. In the afternoon of July 8th and the whole day on the 9th, 11th and 12th, two or possibly three series of parallel paper sessions will be held at the University of Agriculture, Vienna.

Section Papers: Speakers presenting a paper will be allotted a maximum of 20 minutes, including time for discussion. If less time is required, the organizing committee should be notified accordingly, when the abstract is submitted.

Abstract forms and language to be used: The form for submission of abstracts of communications (Form A), the abstract reproduction form (Form A1) and a model abstract (Form A2) are enclosed. Participants wishing to present a paper should return Form A before December 31, 1973 and the abstract text (Reproduction Form A1) must reach the Secretariat before February 28th, 1974.

The abstract must be written in English. No arrangement will be made for simultaneous translation. Papers should deal with work which has not yet been published in recognized journals.

Only one paper will be accepted for presentation by each participant. Members may present their communication in English, German or French, but speakers are requested to submit 50 copies of an unabridged English translation when registering at the desk if they do not intend to read their paper in English. As a large number of papers are expected, it might be impossible to have all the contributions presented from the platform. In that case the abstracts will be studied by a Programme Committee. The author will be notified before April 30th, 1974 if his paper has not been accepted for presentation.

Panel discussions: Parallel to the short paper sessions the possibility for panel discussions in small groups will be provided. If you are interested in a special topic, please fill in Form B and return it as soon as possible, but not later than February 1st, 1974.

Travel information for participants from North America: Eurotours Inc., 151 West 51st Street, Suite 200, New York, New York 10019 (Tel. 212-581-9060) is representing Primus Travel Organization in Vienna, which in turn has been appointed Official Agent for the above Symposium.

Although every participant, naturally, can make his own travel arrangements, we plan to offer reduced air transportation, and, if so

required, land arrangements and post congress tours to the participants from the United States.

This, however, is only possible if a substantial number (minimum of 15) will travel together on the same transatlantic flight i.e., from one U.S. gateway to one destination in Europe. Traveling within the United States to this gateway would then be possible also on a reduced basis.

The Editor of the Yeast News Letter has made available the addresses of Yeast News Letter subscribers to this Travel Agency and these subscribers will be contacted as to details of the travel arrangements at reduced fares. Mr. Paul Pflamitzer of Eurotours is handling the arrangements for the Yeast Symposium.

Application for Travel Grants to N.S.F. by U.S. Citizens. Dr. William J. Riemer, International Travel Program, National Science Foundation, 1800 GST, Washington, D.C. 20550 has informed your Editor that N.S.F. will consider applications from individual U.S. scientists seeking funds to travel to Vienna in 1974 to participate in the 4th International Symposium on Yeasts. However, it should be understood that very little money is available for international travel awards and the likelihood of supporting more than a few persons is not great. Completed applications must be received by N.S.F. by March 1, 1974.

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2. Third Specialized Symposium on Yeasts "Metabolism and Regulation of Cellular Processes". Commission on Yeasts and Yeast-like Organisms of the International Association of Microbiological Societies (IAMS). Communicated by the Chairman Torsten Wiken.

A. The following resolution was proposed by the IAMS Commission on Yeasts and Yeast-like Microorganisms and adopted by the participants of the Third International Specialized Symposium on Yeasts, held at Otaniemi, Finland, June 4-8, 1973.

1. The participants of the Third International Specialized Symposium on Yeasts at Otaniemi June 4-8, 1973, wish to express the most sincere thanks to their Finnish Hosts for having organized a meeting which was extremely profitable and pleasant from a scientific as well as from a social point of view.

2. In accordance with the resolution adopted in Delft and The Hague in 1969 the next symposium, which is a general one, will be held at Vienna, Austria, in the period of July 8-12, 1974.

3. It is proposed that the next specialized symposium is to be held either in the U.S.S.R., France, England, Yugoslavia or the German Democratic Republic.

B. New Member of the Commission: Dr. Peter Lietz, Forschungsinstitut für die Gärungsindustrie, Enzymologie und Technische Mikrobiologie, Alt-Stralau 62, 1017 Berlin, GDR, was nominated as Member of the Commission.

C. Speech given by Professor Dr. T. O. Wiken, Chairman of the IAMS Commission on Yeasts and Yeast-like Microorganisms, at the farewell party in Vuoranta, the Education Centre of Alko, on behalf of the Participants of the Third International Specialized Symposium on Yeasts, held at Otaniemi, June 4-8, 1973.

Dear Drs. Suomalainen, Christine Waller, Enari, Nurminen and Oura,

On behalf of the IAMS Commission on Yeasts and Yeast-like Micro-

organisms and the Participants of the Third International Specialized Symposium on Yeasts I would like to say a few words to you as Chairman, Secretary and Members, respectively, of the Organizing Committee of this Symposium.

We are all very happy that we were allowed to come to Finland in order to listen to invited lectures and papers dealing with the last findings in the fields of metabolism and regulation of cellular processes in yeasts and in order to ponder on and discuss the numerous problems in the fields mentioned. We all highly appreciate that we were allowed to do this in the fence of the famous Centre of the Research Laboratories of the Finnish State Alcohol Monopoly, ALKO, here at Helsinki.

I am convinced that all participants agree with me when I say that this symposium was characterized by a perfect organization, a high scientific level and the charming kindness and thoughtfulness which are inherent properties of the Finnish People.

We were allowed to study the Rajamaki Factories of ALKO by means of a very instructive film and we had the opportunity to admire marvellous Finnish landscapes and sceneries, still practically untouched by air-, water- and soil-pollution and other blessings of our so-called civilization. We have enjoyed Finnish arts and crafts, food and beverages.

The scientific and social events of this symposium have undoubtedly contributed to create new and to fortify old personal contacts. Hence, the symposium implies an important contribution to international understanding.

For all that we were allowed to enjoy here in Finland, we, Participants of the Third International Specialized Symposium on Yeasts, wish to express the most sincere thanks to the Chairman, Dr. Suomalainen, to the Secretary, Mrs. Waller, who has fulfilled her very difficult task in an excellent and charming way, and to the Members, Drs. Enari, Nurminen and Oura, of the Organizing Committee. As Chairman of the IAMS Commission on Yeasts and Yeast-like Microorganisms I would finally like to ask you, Professor Dr. Suomalainen, to forward our most respectful thanks to the Board of Directors of ALKO.

Thank you.

The Symposium was attended by about 200 persons from 24 countries. After the Finnish delegation the largest was from the USSR (34), then England (18), Holland (12), G.F.R. and Yugoslavia (10 each) etc. The program consisted of a plenary session, with Professor F. Lynen giving the opening lecture followed by 20 invited speakers, and section sessions of 6-10 lectures each. The following areas were covered: Intermediate and energy metabolism; biochemistry of macromolecules; special biosyntheses; technology; biochemistry of subcellular structures; permeability and uptake of compounds; nutrition and growth.

The Proceedings of the Symposium containing all invited lectures appeared in November 1973 as Part II. Printed by Print OY, Helsinki. Copies of this book may be ordered from the Symposium Secretariat, Mrs. Chr. Waller, c/o ALKO, Box 350, SF-00101, Helsinki, 10, Finland.

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3. The Sixth Annual Yeast Genetics Conference-Japan.

The sixth annual Yeast Genetics Conference-Japan was held on October 17 and 18, 1973 at the Suntory Memorial Hall, Department

of Fermentation Technology, Osaka University. Fifty investigators met and the following general areas were discussed: mutation and radiation effect, sexuality and its genetic control, cell structure, metabolism, regulation and genetic fine structure, and extrachromosomal inheritance.

The session on the mutation and radiation effect was chaired by Ito (Tokyo) and Nakai (Chiba). Nakai and Machida (Chiba) reported the genetic effect of ethylmercury chloride and furylfuramide. According to them, ethylmercury chloride showed an X-ray-like effect and furylfuramide showed a UV-like effect. Saeki (Chiba) discussed the liquid holding recovery of radiation damage in radiation-sensitive mutants. Yamasaki (Kofu) isolated amino acid-requiring mutants of Saccharomycodes ludwigii and analysed the hybrids between them genetically. Hieda (Tokyo) described the photoreactivation in UV-irradiated ascospores of diploid yeast. Takahashi (Suita) reported the various chromosomal aberrations induced by mitomycin C in yeast mitosis and the linkage data by the use of mitomycin-induced monosomics and telocentrics. Miyamoto (Wakayama) demonstrated a p-nitrophenol-resistant strain.

Banno (Osaka) and Gunge (Yokohama) were the chairmen of the sexuality session. Mori (Noda) isolated stable polyploid strains of Sacch. rouxii and explained their genetic characteristics. Harashima (Osaka) discussed a new hypothesis on the homothallism-controlling genes. According to his report, gene constitutions, HO hm_a hm_α and HO HM_a HM_α, showed the same homothallic phenotype. Hagiya et al. (Osaka) explained α hormone-like effect of n-octanoic acid. Shimoda and Yanagishima (Osaka) studied nucleic acid metabolism during the mating reaction and suggested that the a-factor, which is secreted by a type cells inhibits DNA synthesis in α type cells. Matsushima et al. (Osaka) attempted the isolation of mutants of the mating reaction. Tsuboi et al. (Osaka) reported the effect of ethidium bromide on the sporulation of Sacch. cerevisiae.

The session of cell structure was chaired by Hirano (Tokyo). Osumi (Tokyo) reported the isolation of microbody from Candida tropicalis cultured in hydrocarbon medium. Doi (Osaka) isolated the cell nucleus from tetraploid baker's yeast. Iguchi (Mito) described the characters of spheroplasts produced by the use of snail enzyme.

Wakabayashi (Tokyo) was the chairman of the metabolism session. Nishigaki et al. (Kyoto) discussed the effect of α-phenethyl alcohol on the respiratory adaptation. Nishimura (Nara) and Ueda (Osaka) reported the effects of vitamins on organic acid metabolism in sake yeast.

The session on regulation and genetic fine structure was chaired by Takahashi (Suita). Oshima (Osaka) discussed the regulatory system for acid phosphatase synthesis. Kobayashi (Osaka) reported the relation between pH and the acid phosphatase formation. Kakimoto (Osaka) explained the fine structure of the structural genes on the acid phosphatase synthesis.

Osumi (Tokyo) was chairman of the session on extrachromosomal inheritance. Suda, Inoue (Nara) and Uchida (Kobe) reported the retention of the drug resistance factors and the suppressiveness in an unstable petite strain. Gunge (Yokohama) explained the suppressiveness of cytoplasmic petite mutants. Morita and Mifuchi (Shizuoka) discussed the aberrant mitochondrial DNA in 4NQO-induced respiratory deficient strains. Nagai (Nara) reported the isolation of petite mutants by the use of 4NQO and some xanthene acid dyes.

Finally, Wakabayashi (Tokyo), Oshima (Osaka) and Nakai (Chiba) described the recent progress in yeast genetics in foreign countries.

The next meeting of the Yeast Genetics Conference-Japan in 1974 will be held at Osaka or Shizuoka after the First International Congress on Intersectional Microbiology of IAMS, Tokyo and the Seventh International Yeast Genetics Conference, London.

Toshiaki Takahashi, Suita Laboratory,
Brewing Science Research Institute
Suita

Chikashi Shimoda, Department of Biology,
Faculty of Science, Osaka City
University, Osaka

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4. After profound preparations a community of interests was founded in the G.D.R. in the beginning of 1973 under the reading of Doz. Dr. Koch (Med. Academy, Erfurt). This community of interests being occupied with yeasts is part of the section microbiology of the Biological Society of the G.D.R. Scientists interested in micro-organisms come here from theoretical institutes and from industry to discuss and to solve problems in biology, biochemistry and taxonomy as well as applied problems, such as those pertaining to medical mycology or to the industrial uses of yeasts. Participants include all those who are interested in the above mentioned problems. Meetings with lectures, round-table discussions and a wide exchange of practical experiences will take place twice a year; also yeast-strains can be exchanged and scientific institutions and other enterprises might be visited. The first meeting on problems of taxonomy took place in Erfurt May 25, 1973. This meeting was of high quality. The large number of members who attended documented the necessity of a well developed exchange of information and experience. The main aims of the community are the dissemination and assimilation of scientific results, distribution of information about yeasts as rapidly as possible, and promoting personal contact between the yeastmycologists.

The second meeting took place in Leipzig September 27, 1973. In our laboratory we continue our work on karyokinesis of yeasts, as well as problems of host-parasite relations in medical mycology.

H. A. Koch
Hautklinik Med. Akad. Erfurt
D.D.R.

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5. Recently a Danish Mycology Club was formed for the advancement of human and veterinary mycology in Denmark. In a few months the rules for the organization will be worked out formally, so that connections with national and international societies can be taken up.

President is: Professor A. Stenderup, MD, Institute of Medical Microbiology, University of Aarhus, 8000 Aarhus C, Denmark, and secretary: Dr. Jessie Bodenhoff, Ph.D., Department of Mycology, The State Serum Institute, Amager Boulevard 80, 2300 Copenhagen S, Denmark.

On November 28 the Mycology Club arranged a symposium on yeast infection with the following program:

F. Neukirch, University Hospital, Copenhagen:
Opening Remarks on the Significance of Yeast Infection.

A. Stenderup, Institute of Medical Microbiology, University of Aarhus: Pathogenic Yeasts.

H. I. Winner, Charing Cross Hospital Medical School, London: Yeast Infections.

H. Kirkpatrick, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland: Cellular Immunity in Candidiasis.

D.W.R. Mackenzie, London School of Hygiene and Tropical Medicine: Diagnosis of Candidiasis.

N. H. Axelsen, The Protein Laboratory, University of Copenhagen: Candida Serology.

E. Drouchet, Institute Pasteur, Paris: Chemotherapy of Yeast Infections.

N. Rosmann, Frederiksborg County Hospital, Hillerod: Clinical Manifestation of Candida Infection in Dermatology and Gynecology.

E. Budtz-Jorgensen, Royal Dental College, Aarhus: Oral Candidiasis.

K. Thestrup-Pedersen, Institute of Medical Microbiology, University of Aarhus: Mucocutaneous Candidiasis.

We hope thereby to stimulate interest in mycology in Denmark.

A. Stenderup
University of Aarhus, Denmark.

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6. Wilfred Arnold, The University of Kansas Medical Center, Rainbow Boulevard at 39th Street, Kansas City, Kansas 66103 writes:

A small symposium on the yeast cell wall was held in Chicago November 14-16.

About 150 members of the Society for Complex Carbohydrates attended this (the first) national meeting. The symposium was enthusiastically received by this group, which consists primarily of glycoprotein chemists.

PROGRAM
THE SOCIETY FOR COMPLEX CARBOHYDRATES
CENTER FOR CONTINUING EDUCATION
UNIVERSITY OF CHICAGO

"YEAST CELL WALL CHEMISTRY AND STRUCTURE"

W. N. Arnold U. of Kansas Medical Ctr.	The Role of Endogeneous β-glucanases in Yeast Cell Wall Modification.
E. Cabib N.I.A.M.D., N.I.H.	Polysaccharides in Morphogenesis; the Primary Septum in Yeast.
H. K. Ankel Med. College of Wisconsin	Cell Envelope Glycoprotein Biosynthesis in Cryptococcus laurentii.
K. O. Lloyd Coll. of Physicians and Surgeons, Columbia University	Structural Organization of a Yeast Cell Peptido-phospho-mannan Complex.

C. E. Ballou
U. of California,
Berkeley

Genetic Control of Yeast Mannan Structure-Evidence for a Polyfunctional Mannosyl Transferase System.

XLI. Brief News Items

1. Obituaries

On the 11th of June, 1973 RNDr. Rudolf Müller died after a long illness and enormous suffering. He was the head of the Institute of Microbiology and Experimental Therapy of the German Academy of Sciences in Jena, German Democratic Republic. Dr. Müller belonged to a small group of scientists, who founded the symposial tradition in the field of yeast research. He participated in the organization of the First Symposium on Yeasts held at Smolenice June 4-6, 1964. The next year he was the chairman of the organizing committee of the First Symposium on Yeast Protoplasts held September 21-24, in Jena, in which prominent scientists from the whole world participated. He was elected as a member of the Executive Committee of the International Council for Yeast Science, created in Bratislava, 1966, and later as the member of the International Yeast commission of IAMS where he represented the German Demokratic Republic. The cytological yeast researchers and our organizing efforts in the world organization have lost a very active and initiative personality and we all are missing a very good friend.

A. Kockova-Kratochvilova
Slovak Academy of Sciences
Bratislava, Czechoslovakia.

Dr. Lloyd K. Riggs died October 28, 1973 at the age of 85. Born in 1888 in Castalia, Iowa, Dr. Riggs' early interest in biochemistry and nutrition was encouraged by his father, a druggist. He received his A.B. and A.M. degrees from Leander Clark College in Iowa and his M.S. and Ph.D. from the University of Chicago. During his subsequent professional career, his contributions to nutrition research were of major significance to both the food industry and society.

In 1926 Dr. Riggs became a professor at Rutgers University and later from 1948-53 he was Director of Nutrition Research of Kraftco Corporation. He was considered one of the country's outstanding authorities in the field of physiological chemistry. He was also interested in nutritional aspects of yeast and a faithful subscriber of the Yeast News Letter for many years.

J. L. Yamins
Gar-Baker Laboratories, Inc.
110 W. 18th Str. New York, New York 10011.

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2. In 1949 I needed a culture of Candida guilliermondii. Two Lyophilized tubes of this yeast were obtained from L. J. Wickerham, then at the Northern Regional Research Laboratory, Peoria, Illinois. One tube was opened and a culture obtained, the duplicate lyophilized tube was placed in my desk. On August 1, 1973 this tube was opened and cultured according to directions. The pellet was suspended in 7 ml of dextrose broth. After suspension, a streak was made on dextrose agar, from which 5 colonies developed. Enrichment in dextrose broth from the original pellet gave good growth from which isolations were made. The cultures isolated gave typical reactions for this yeast.

This culture was lyophilized in blood serum on January 4, 1949 and opened August 1, 1973, thus being viable for a period of twenty-four years and seven months.

Though I am retired, am still doing a little work in the laboratory.

Francis M. Clark
Professor Emeritus
Dept. of Microbiology, University of
Illinois at Urbana - Champaign.

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3. A. Stenderup has now returned from a sabbatical year at the University of the South Pacific, Fiji School of Medicine.

Recent publications from the Institute of Medical Microbiology, University of Aarhus, Denmark:

C. Christiansen, A. Leth Bak, A. Stenderup and Gunna Christiansen: Repetitive DNA in Yeast. Nature New Biology, 231, 176, 1971.

A. Stenderup, Sally A. Meyer, A. L. Bak and C. Christiansen: Taxonomy of Candida and Torulopsis. Proc. IV Int. Ferment. Symp., Ferment. Technol. Today p. 793, 1972.

A. Leth Bak, J. F. Atkins, Sally A. Meyer, C. E. Singer and Bruce N. Ames: Evolution of DNA Base Compositions in Microroganisms. Science, 175, 1391, 1972.

A. Leth Bak, C. Christiansen and Gunna Christiansen: Circular, repetitive DNA in yeast. Biochim. Biophys. Acta 269, 527, 1972.

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4. "Professor Dr. T. O. Wiken, Chairman of the Commission on Yeasts and Yeast-like Microorganisms of IAMS, was on October 30, 1973, awarded an Honorary Doctor's Degree at the University of Bordeaux for his outstanding contributions to the knowledge of the physiology of microorganisms, particularly the metabolism of yeasts".

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5. Dr. A. Kockova-Kratochvilova, Bratislava, Czechoslovakia, was elected as a member of the Executive Board of the World Federation for Culture Collections (WFCC). WFCC was established within the International Association of Microbiological Societies.

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6. Mario Luzzati has moved from the Centre de Genetique moleculaire, Gif sur Yvette to:

Laboratoire de Biologie Générale
Faculté des Sciences, Bât. 400
91405, ORSAY (France)

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7. Dr. Anwar Nasim's new address is:

Atomic Energy of Canada Limited
Chalk River, Ontario
Canada.

The Editor extends his best wishes to all readers of the Yeast News Letter for a prosperous and scientifically rewarding 1974!
