Y E A S T

A News Letter for Persons Interested in Yeast

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Many thanks to those who have contributed to this issue by sending in news items and accounts of research projects.

The Editor extends to the readers of the Yeast News Letter his warmest wishes for a happy and productive new year ahead.

H. J. Phaff

I. University of Bristol, Department of Agriculture and Horticulture, Research Station, Long Ashton, Bristol, BS18 9AF, England. Communicated by R. Davenport.

1. Sporing Metschnikowia isolates have been obtained from several habitats within an English vineyard. This is the first time that this ascomycetous stage has been reported in England. Strains have been tentatively identified as M. pulcherrima and M. reukaufii but further tests are being carried out as these particular yeasts are morphologically somewhat different from other published descriptions.

2. I have completed an M.Sc. (Bristol 1970) thesis 'Epiphytic' yeasts associated with the developing grape vine. It was established that Saccharomyces spp. were absent and in general the vine yeast microflora was very similar to that of the surrounding orchards except for some minor differences including the isolation of Nadsonia spp. and a Schizosaccharomyces sp. Other yeast genera present were Candida, Cryptococcus, Torulopsis, Rhodotorula, Kloeckera, Sporobolomyces, Aureobasidium and Trichosporon.

3. I am continuing my vineyard yeast ecology studies, under the guidance of Dr. F. W. Beech, for a doctoral dissertation to be submitted next year. So far it has been found that the vineyard soil contains few yeasts. Certain species were common to all habitats, Aureobasidium, Trichosporon, Rhodotorula, Cryptococcus, Candida, Torulopsis and a species of Schizosaccharomyces. Yeasts isolated from soil invertebrate vectors and/or leaf litter, or on weeds, included Rhodosporidium spp. (first time in England as far as known), Kloeckera spp., Bullera sp., Debaryomyces sp., Nadsonia sp. and Metschnikowia spp.

I would like to receive and exchange cultures of Metschnikowia spp. from interested workers.

II. Chonnam National University, Department of Biology, Chonnam, Korea. Communicated by M. S. Park.

This paper was presented on November 7, 1970, at the 14th Korean Biological Society, Seoul, Korea.

Studies on the Wild Yeasts of Korea - with Emphasis on Natural Habitats

ABSTRACT

This research is based on the presumption that cultivated yeast originates from wild yeast, and that the former can be isolated from the latter, which is widely scattered in the natural world. Accordingly, research on the geographical and ecological distribution of yeast is extremely useful.

In this study, an attempt is made to investigate and analyse the locality, and to clarify the growth cycle of yeast with reference to the seasons and the ecological niche.

A total of 205 strains of wild yeasts were isolated and gathered during the period of March 1969 - Sept. 1970 from more than 30 areas throughout the country, including Kwangju and Mt. Chiri, and catalogued by season and by area.

These samples were further classified as those yielding yeasts isolated from the nectar of flowers (7), the crops of Drosophila (8), from slime fluxes of oak trees (29), from the gills of mushrooms (2), and from edible fruits and other tree fruits (159).

These wild yeasts (total 205 strains) were classified according to standard morphological and physiological criteria.

S. cerevisiae var. tetrasporus (Beijerink), the ancestor of Saccharomyces cerevisiae as Phaff called it, could be isolated from the epidermis of apples produced in the Taegu area, and also from the slime flux of Quercus variabilis BLUME in the Mt. Chiri area.

It has also been found that Drosophila is inclined to follow the smell of fruit, the slime flux of trees, and mushroom gills in quest of food and a place to inhabit - and are necessarily drawn to yeast that has high capacity for fermentation.

The slime flux of trees and the gills of mushrooms were found to be suitable places for Drosophila to lay eggs. Some yeast from Drosophila crops showed the same pattern as that of the yeast from slime flux.

In particular, it was noted that S. florentinus (Castelli), isolated from the crop of Drosophila rufa and S. cerevisiae (Hansen) isolated from D. nigromaculata have a high value of fermentation on maltose; and both of them, it is believed, can be put to practical industrial use.

Hansenula anomala sp 1, isolated from Ligustrum lbota var. angustifolium BLUM (privet) and from the epidermis of apples also is extremely effective in producing fermentation in maltose. It has the smell of ethyl acetate.

There is a high probability that it can be effectively used in bakeries and confectioneries.

In this research, about 200 strains were morphologically classified to the genus level. For more accurate classification, physiological experiments should be made on the wild yeasts of Korea, the first attempt of its kind, until a clear-cut system of classification of yeasts has been constructed, providing a contribution to purposes of industry.

III. Georgia State University, 33 Gilmer Street, S.E., Atlanta, Georgia 30303. Communicated by D. G. Ahearn.

Recent Publications:

Systematics of yeasts of medical interest. Ahearn, D. G. Proc. Int. Symp. Mycoses. PAHO Sci. Publ. No. 205:64-70. Washington, D. C. (1970).

The utilization of alkylbenzene sulfonates by yeasts. P. G. Standard and D. G. Ahearn. Appl. Microbiol. 20:646-648 (1970).

Incidence and characterization of fungi in eye cosmetics. J. Kuehne and D. G. Ahearn. Dev. Industr. Microbiol. 12:173-177 (1971).

The role of yeasts in the decomposition of oils in marine environments. Dev. Industr. Microbiol. 12:173-177 (1971).

Characterization of yeasts in Barataria Bay. S. P. Meyers, D. G. Ahearn, and P. G. Miles. Louisiana State Univ. Coastal Studies. Bull. 5:111-124 (1971).

A limited number of copies of "Recent Trends in Yeast Research (D. Ahearn, ed.) Spectrum, Monograph Series in the Arts and Sciences, Georgia State University. 206 pp. (1970)" are still available at $4.50 per copy. Please make checks payable to Georgia State University.

Current Research:

Microbiological investigations of eutrophic regions of Lake Sidney Lanier, Georgia; Biodegradation of oil pollutants in fresh and marine waters; Microbial contamination of eye cosmetics; Gas chromatographic identification of yeasts, cell wall composition of Prototheca. Work in these areas is being pursued by Dr. Warren L. Cook, Dr. Donald J. Reinhardt, Mrs. Joan Kuehne, M.S. and Mr. Wayman Turner, M.S.

The microbiology staff at Georgia State University now includes Drs. D. G. Ahearn, W. L. Cook, P. E. Gaffney, J. G. Haller, A. Ibrahim, D. J. Reinhardt, and D. Verma.

IV. Muséum National D-Histoire Naturelle, Laboratoire D'Ethologie, Parc Zoologique, Paris-XII, France. Communicated by H. Saëz.

I am primarily interested at this time in the fungi (including yeasts) in the lungs of primates: factors involved in survival or development of these

microorganisms in the respiratory tract. Below follow some recent publications:

I/ In primates:

-H. Saëz and J. Rinjard - Candidose et oedème aigu du poumon chez un Primate. Le Poumon et le Coeur, 26, 6, 701-709, 1970.

-H. Saëz - Levures de la cavité **buccale** du Babouin, Papio papio (Desm.). Zentralblatt für Veterinärmedizin, B 17, 381-388, 1970.

-H. Saëz - Stomatite myco-infectieuse du Babouin - Papio papio (Desm.). Annales de Médecine Vétérinaire, 5, 309-314, 1969.

-H. Saëz - Flore levuriforme intestinale du Babouin, Papio papio (Desm.). Mycopathologia et Mycologia Applicata, 41, 3-4, 383-395, 1970.

-H. Saëz - Micromycetes hébergés par des Macaques à face rouge, Macaca speciosa F. Cuvier. Expérimentation Animale, 3, 3, 183-198, 1970.

II/ In birds:

-H. Saëz et J. Rinjard - Levures du tube digestif d'oiseaux sauvages captifs, Ansériformes, à régime alimentaire granivore. L'Oiseau et la Revue Française d'Ornithologie, 40, 2, 104-114, 1970.

-H. Saëz - Levures et candidoses du Nandou, Rhea americana, en captivité. Les Cahiers de Médecine Vétérinaire, 39, 4, 199-209, 1970.

-H. Saëz - Champignons isolés du poumon et du tube digestif de quelques Psittacidés. L'Animal de Compagnie, 15, 27-41, 1970.

-H. Saëz - Bilan mycologique de quelques oiseaux ayant souffert de "marée noire". Mykosen, 14, 1, 31-40, 1971.

III/ Various: yeasts and Geotrichum:

-H. Saëz et J. Rinjard - Levures isolées du tube digestif de Mammifères adultes. Résultats en fonction du régime alimentaire. Les Cahiers de Médecine Vétérinaire, 39, 6, 290-304, 1970.

-H. Saëz - L'incidence saisonnière sur les levures isolées chez des animaux sauvages captifs. Bulletin de la Sté. Mycologique de France, 85, 2, 255-271, 1969.

-H. Saëz - Geotrichum loubieri Morenz, un champignon arthrosporé formant également des endospores. Bulletin de la Sté Linnéenne de Lyon, 39, 9, 283-288, 1970.

V. Institute of Medical Microbiology, University of Aarhus, 8000 Aarhus C, Denmark. Communicated by A. Stenderup.

The work here continues on yeast DNA. Our main interests have been on species in the genera Torulopsis and Candida. GC contents, genome sizes, and amounts of repetitive DNA are currently being investigated. Other studies include mitochondrial DNA and immunological research on the pathogenesis of yeast infections. In addition, the yeast work includes a cooperative program with the School of Dentistry, Aarhus (Dr. E. Budtz-Jørgensen) and the Protein Laboratory, University of Copenhagen (Dr. N. H. Axelsen).

"Repetitive DNA in Yeasts" (C. Christiansen, A. Leth Bak, A Stenderup and Gunna Christiansen) was recently published in Nature New Biology 231: 176, 1971. Several papers were presented on the yeast DNA work at European meetings in Czechoslovakia, Paris, Edinburgh and Utrecht.

Several yeast friends were able to visit the institute in recent months either before or after some European meetings. These included Dr. H. J. Phaff (California), Dr. R. Mayorga (Guatemala), Dr. J. F. T. Spencer (Canada) and Dr. David Yarrow (Delft).

In January Dr. Sally Ann Meyer will complete a year's stay at the institute. She has been working with DNA from Candida and Torulopsis species. She tells us that she has enjoyed her year in Denmark very much.

She returns to the U.S. to take a position at the American Type Culture Collection, Rockville, Maryland.

VI. Institut National Agronomique, Chaire de Génétique, 16 Rue Claude-Bernard, Paris-5°, France. Communicated by H. Heslot.

Utilization and interconversions of purine derivatives in the fission yeast Schizosaccharomyces pombe.

Jacques Pourquie and Henri Heslot

In the fission yeast Schizosaccharomyces pombe, growth responses of mutant strains in the de novo purine synthesis pathway, in the purine interconversion system, and of various double mutants, have been studied upon different purine-supplemented media. The results show that exogenous purine utilization as nucleotide source is based exclusively upon pyrophosphorylation of purine bases, and they make it possible to identify most of the enzymic steps acting, in this organism, upon a purine ring to give another purine ring.

(Abstract of a paper to be published shortly in Genet. Res. Camb).

Regulation of purine metabolism in Schizosaccharomyces pombe. Two forms of the Phosphoribosyl-amido-transferase, the first enzyme in the de novo pathway.

Maria Nagy, Uwe Reichert* and Anne-Marie Ribet

Depending on growth phase and cultivation conditions, two forms of the PRPP amidotransferase (EC 2.4.2.14; ad4 locus) are found in the wild type and purine auxotrophs. The two forms, co-purified in the same $(NH_4)_2\ SO_4$, as well as in a Sephadex G-200 fraction (apparent M.W. 320.000) differ in the following properties:

--Thermostability
--Sensitivity to the feedback inhibitors AMP, IMP and GMP
--pH dependence of enzyme activity.

The thermolabile form, more sensitive to feedback inhibitors and pH variations, is present in the wild type at the beginning of the exponential growth in yeast extract medium, whereas the thermostable form is found in stationary cells.

In contrast to these findings, auxotrophs affected in GAR synthetase (EC 6.3.1.3), adenylosuccinate lyase (EC 4.3.2.2) and adenylosuccinate synthetase (EC 6.3.4.4) maintain the labile form till the end of growth under the same conditions. However, when cultivated in the presence of high adenine concentrations, the same mutants form the stable enzyme. Otherwise, addition of adenine at the end of the exponential phase does not change the labile state of the enzyme.

Measurements of pool formation of purine derivatives (NAD, ATP, nucleoside diphosphates, AMP, GMP, adenosine, inosine, hypoxanthine) during the growth of wild type and mutants indicate that the pool pattern of adenosine, one of the purine storage forms, is the only one correlated with the appearance of the two enzymic forms.

At present, our results favor the assumption of two non-convertible modifications of the same protein encoded by one gene. The appearance of one or the other form seems to depend on the intracellular level of adenosine. Such a mechanism could be important for the coordination between de novo and salvage pathways.

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Freien Universität Berlin.

VII. Laboratorio di Mutagenesi e Differenziamento del C.N.R., and Istituto di Genetica Dell'Universita' - viale Matteotti, 1/A, 56100-Pisa, Italy. Communicated by A. Abbondandolo.

The following is a summary of some of the lines of research currently under investigation:

a) Temperature sensitive mutants in Schizosaccharomyces pombe. 71 mutants, able to grow on complete medium at 25C but not at 37C, were isolated in S. pombe. A preliminary characterization of 13 mutants, selected on the basis of a morphological character (cell lengthening at 37C), showed that 7 mutants have a depressed DNA synthesis at the restrictive temperature. Four of them seem to be affected primarily in cell division. These results have been submitted to J. Bacteriology for publication. Present experiments deal with characterization of mutator phenotype exhibited by three of the ts1 mutants.

b) X-rays mutagenesis in Schizosaccharomyces pombe. The relationship between nuclear stage and X-ray-induced mutations is being investigated by using G1 and G2 pure populations. Reversions to adenine independance are induced preferentially in G2. G1 cells do not mutate and do not incorporate H^3-thymidine into DNA when plated on minimal medium after irradiation. However, if incorporation (i.e. DNA synthesis) is stimulated before plating on minimal medium, G1 cells do produce mutations.

Since our last contribution (January 1970) the following papers have been published or presented as communications to meetings:

A. CINCI and C. BAUER: Simplified automatic determination of pH-curves of enzymes: application to acid phosphatases of S. pombe. Technicon Symposium, Roma-EUR, October 1969 (Italian).

A. CINCI: Automatic determination of pH-curves of enzymes. A new technique for obtaining pH gradients applied to characterization of acid phosphatases in cell suspensions of S. pombe. Technicon Symposium, Roma-EUR, October 1969 (Italian).

C. BAUER, A. CINCI, and G. DIBENEDETTO: Biochemical analysis of acid phosphatases deficient mutants in S. pombe. Boll. Soc. Ital. Biol. Sperim. 46, n° 20bis (1970) (Italian).

G. DIBENEDETTO and C. LEPORINI: Purification of aspecific acid phosphatases in S. pombe. Boll. Soc. Ital. Biol. Sperim. 46, n° 20bis (1970) (Italian).

G. DIBENEDETTO and C. LEPORINI: Acid phosphatase of S. pombe: an enzyme repressed by phosphate. 7th Meeting of the Federation of European Biochemical Societies (1971).

C. BAUER, G. BRONZETTI, A. CINCI and G. DIBENEDETTO: Preliminary results of genetic and biochemical studies on non specific acid phosphatases of S. pombe. Atti Ass. Genet. Ital. 16, 23-25 (1971).

G. CORTI, R. GUGLIELMINETTI, M. NOZZOLINI and S. SIMI: UVS-2, 44:a mutant of S. pombe UV-sensitive and conditional lethal. Atti Ass. Genet. Ital. 15, 157-8 (1970).

R. GUGLIELMINETTI: Caffeine resistance and UV-sensitivity in S. pombe. Atti Ass. Genet. Ital. 16, 16-18 (1971).

M. SCHÜPBACH: The isolation and genetic classification of UV-sensitive mutants of S. pombe. Mutation Res. 11, 361-71 (1971).

N. LOPRIENO and M. SCHÜPBACH: Effect of caffeine on mutation and recombination. EMS Newsletter, n° 3, June, 1970.

N. LOPRIENO and M. SCHÜPBACH: On the effect of caffeine on mutation and recombination in S. pombe. Molec. Gen. Genetics 110, 348-54 (1971).

N. LOPRIENO: UV radiation-sensitivity and mutator activity in yeast S. pombe. Proc. IVth Intern. Congress of Radiation Research, Evian,

June 28-July 4, 1970 (in press).
N. LOPRIENO: Analyses of gene mutators in S. pombe. Atti Ass. Genet. Ital. 16, 19-22 (1971).
N. LOPRIENO: The danger of environmental chemical mutagens for man. Tecnico Scientifica Ed., Pisa, 1971 (Italian).
N. LOPRIENO: Specificity of chemical mutagens at the DNA level. Scientia 65, 411-7 (1971).
N. LOPRIENO, R. BARALE and S. BONATTI: Further data on gene mutators in S. pombe. Atti Ass. Genet. Ital. 17 (1972) (in press).
S. BONATTI, M. SIMILI and A. ABBONDANDOLO: Isolation of temperature sensitive mutants in S. pombe. J. Bacteriol. (in press).
S. BONATTI, M. SIMILI and A. ABBONDANDOLO: Isolation and characterization of temperature sensitive mutants in S. pombe. Atti Ass. Genet. Ital. 16, 26-8 (1971).
S. BONATTI and A. ABBONDANDOLO: The influence of nuclear stage on mosaicism induced by nitrous acid in S. pombe. Atti Ass. Genet. Ital. 15, 145-6 (1970).
A. ABBONDANDOLO and S. SIMI: Mosaicism and lethal sectoring in G1 cells of S. pombe. Mutation Res. 12, 143-50 (1971).
A. ABBONDANDOLO: Induction of mutations by X-rays during defined nuclear stages in S. pombe. Atti Ass. Genet. Ital. 17 (1972) (in press).

VIII. Institute of General Genetics, University of Oslo, P. O. Box 1031, Blindern, Norway. Communicated by Dr. Øistein Strømnaes.

THE INDUCTION OF MITOTIC RECOMBINATION BY AMINOPTERIN IN A HETEROZYGOUS DIPLOID AT THE ade2 LOCUS OF SACCHAROMYCES CEREVISIAE.

B. A. Siddiqi

Aminopterin (4-aminopteroylglutamic acid) (Apt) is an inhibitor of DNA synthesis. The results of this work demonstrate conclusively that aminopterin at the concentrations and conditions used is neither a haploidising agent, nor a mutagen. Nor, surprisingly, does it decrease the viability of the spores treated with it.

When diploid cells heterozygous at the ade2 were treated with aminopterin, it inhibits cell division and increases sectoring frequency at a concentration as low as 25 µg/ml for six hours. The increase in sectoring frequency may arise as a result of either somatic crossing-over followed by segregation or by gene conversion. The only real test to distinguish between these two phenomena in this system is tetrad analysis of asci which are formed from both the red and white parts of the same colony. The cells of ten 1/2 white/1/2 red sectored colonies were analysed in this way and the reciprocal genotypes which were recovered showed that all could be the result of somatic crossing-over and segregation. However, since particular cultures were selected (1/2 red 1/2 white) results of further work involving the tetrad analysis of cells from other types of colonies would be needed before it can be shown conclusively whether gene conversion was also taking place in the formation of red cells following Apt treatment. That gene conversion may play a part is suggested by the results of testing the additional requirements of samples of red cells taken from all types of colonies. The red cells so tested show a high proportion (127 out of 197) which have no requirement for either of the linked genes ser-1/+, his-8/+. Since the ade-2/+ gene is nearer the centromere than either the ser/+ or the his/+ gene it is difficult to conceive that such a high proportion of the reds could have resulted from somatic recombination which would have to involve a double cross-over - one between the ade-2 locus and the centromere

and the other between the ade-2 locus (unknown distance to the centromere) and the ser-1 locus (mapped meiotically at 26.55 units from the ade-2 locus). Further, in view of Fogel and Hurst's (1964) results who showed a coincidence relation between gene conversion and mitotic recombination in Saccharomyces, it would be surprising if further work did not reveal that many of the red cells arising as a result of Apt treatment were the results of gene conversion.

IX. Instituto Politecnico Nacional, Departamento: Genetica y Biologia Celular, Apartado Postal 14-740, Mexico 14, D. F. Communicated by John R. Johnston*.

(Received May 24 but too late for inclusion in the Spring issue)

More extensive analysis of 33 resistant mutants, isolated from nystatin, pimaricin and amphotericin B, respectively, and displaying various cross-resistance patterns, has shown that 19 carry alleles of nys1. Another 12 are not allelic with nys1, nys2 or nys3 (Ahmed and Woods, 1967) and allelism tests by tetrad analysis define 4 more loci for polyene resistance. Mutations at 3 of these loci are recessive and at one locus, dominant. It is proposed that these loci be designated nys4, nys5, nys6 and NYS7. Previous unexpected results of allelism tests, showing 2:2 segregations, (Yeast News Letter, January, 1971) have been shown to be due to the strong selective advantage, by both growth and mating, of sensitive revertants of some mutants.

*Present address: University of Strathclyde, Department of Applied Microbiology, Royal College, George Street, Glasgow C1, Scotland.

X. Department of Biology, York University, 4700 Keele Street, Downsview 463, Ontario, Canada. Communicated by J. C. Game.

Below follows a summary of part of my D.Phil. thesis, submitted at the University of Oxford (Britain), in August 1971. The work was carried out in the Department of Botany, Oxford, in the laboratory of Dr. B. S. Cox.

Radiation-sensitive (= rad) mutants of Saccharomyces cerevisiae were studied, with the aim of elucidating dark repair processes in this species. Allelism tests were done between mutants isolated by different workers, allowing a nomenclature to be worked out (see Game and Cox, Mutation Res., 12, 328-331, 1971). This also allows phenotypes conferred by different alleles of the same loci, described by different workers, to be correlated.

Haploid strains mutant at two or more rad loci simultaneously were constructed by tetrad dissection to determine the interactions that occur between various loci. In theory, one can predict that two mutations which each block the same pathway of repair of damaged DNA will have an epistatic interaction. That is, no further increase in sensitivity will be observed in the double mutant with respect to one or other of the single mutants, because a second block will not be expressed if the pathway concerned is already non-functional. However, if two mutations act on independent repair pathways, an enhancement of sensitivity is to be expected in the double mutant.

Work with double mutants was primarily aimed at determining the interactions between pairwise combinations of mutants of seven loci. These were: rad 1, rad 2, rad 3, rad 4, and rad 6; and two mutations that are X-ray sensitive but confer only a very moderate degree of uv-sensitivity, namely X_1^s (Nakai and Matsumoto, Mutation Res., 4, 129-136, 1966) and UVS-5 (Cox and Parry, Mutation Res. 6, 37-55, 1968). The principal findings were as follows:

No increase in sensitivity is found in double mutants involving the loci rad 1, rad 2, rad 3, and rad 4, with one exception. The exception is the

double mutant rad 1-5 rad 4-4, which differs from the double mutant rad 1-18 rad 4-3 in having a sensitivity greater than that of either single mutant. This allele-specific interaction can be interpreted as resulting from leakiness of the two mutations involved, such that each alone only partially blocks the pathway upon which both mutations act. Data from triple mutants involving three of these four loci, and from the quadruple mutant strain rad 1-18 rad 2-1 rad 3-2 rad 4-3 support double mutant data, i.e. no increase in sensitivity is found. It is inferred that all these loci block the same repair pathway, and biological properties of the single mutants suggest that this may be the equivalent of the "excision-repair" pathway in E. coli.

In contrast, some very striking increases in sensitivity occur when various double and multiple mutants involving rad 1, rad 2, rad 3 or rad 4 in combination with rad 6, X_1^s and uvs5 are constructed. Interactions are found that are synergistic; that is, the double mutant has a sensitivity much greater than that predicted by a simple addition of sensitivities in terms of log. per cent killing of the single mutants. One interpretation of synergistic interactions is that they arise when two pathways acting on the same type of uv-damage are blocked. The availability of a second pathway would reduce the expression of each mutation alone, in this case; but the mutations would become fully expressed when both pathways are blocked.

The particular pattern of synergistic interactions that occurs suggests that the seven loci mentioned above mediate three types of repair of one type of uv-damage. A strain has been constructed that is mutant at all seven loci. It has a sensitivity substantially greater than that arising from any of the interactions of the single loci rad 6, X_1^s and uvs5 in combination with one or more of the loci rad 1, rad 2, rad 3 and rad 4. This "super-synergiom" supports the hypothesis that there exist at least three repair pathways. In addition, strains mutant at several loci simultaneously have proved very useful in unmasking interactions between mutations that are not fully expressed in a wild-type genetic background.

XI. Centre National de la Recherche Scientifique, Laboratoire D'Enzymologie, 91 Gif-Sur-Yvette, France. Communicated by H. de Robichon-Szulmajster.

NONSENSE MUTATION IN THE REGULATORY GENE ETH2 INVOLVED IN METHIONINE BIOSYNTHESIS IN SACCHAROMYCES CEREVISIAE

M. Masselot and H. de Robichon-Szulmajster

SUMMARY (manuscript submitted to Genetics)

Ethionine resistant mutants, mapping at the locus eth2 - the product of which is involved in pleiotropic regulation of methionine biosynthesis - have been isolated in a strain carrying five ochre nonsense mutations. Selection for nonsense suppressors in such a strain led to characterization of several allele-specific but gene non-specific suppressors which are active on the recessive heteroallele eth2-2 (resulting in partial recovery of sensitivity toward ethionine) as well as on the five other suppressible alleles. Two of these suppressors are unlinked to the eth2 gene and either dominant or semi-dominant. It is concluded that the mutation eth2-2 resulted in a nonsense codon. Enzyme studies indicate that this mutation results in a complete absence of an active product of gene eth2, in contrast with the effect of a former mutation eth2-1 which was interpreted as leading to a modified product of this gene (Cherest et al., 1971). This conclusion is based on the absence of repressibility of methionine group I enzymes and the observation that in a heteroallelic diploid, eth2-1 expression is not masked by eth2-2. The nonsense suppressors studied lead to at least partial recovery of repressibility of methionine group I

enzymes. All these results support the idea that the product of gene ETH2 is an apo-repressor protein.

XII. Institut für Biochemie, Universität Würzburg, 87 Würzburg, Röntgenring 11, Germany. Communicated by Eckhart Schweizer.

rDNA Replication in a Synchronized Culture of Sacch. cerevisiae. Gerlinde M. Gimmler and Eckhart Schweizer.
From a synchronized culture of Saccharomyces cerevisiae cells were harvested at different intervals during S-phase. The purified DNA of each sample was challenged with ribosomal RNA by specific DNA/rRNA hybridization. From the constant rDNA/DNA ratio observed it is concluded that in yeast, the replication of the rRNA cistrons is not restricted to a distinct interval but proceeds at a constant rate throughout the entire S-period.
(Biochem. Biophys. Res. Comm., in the press)

The Use of Disomics in Mapping Ribosomal RNA Genes in Yeast. Gerlinde M. Gimmler and Eckhart Schweizer.
The isolation of 4 different disomic Sacch. cerevisiae strains is described. Two of them harbor an extra copy of chromosome III, two others one of the chromosomes V and IX, respectively. In these strains, as well as in 3 additional ones, disomy is established for one of the chromosomes III, V, VI, VII, VIII, and IX, respectively, by means of their specific UV resistance, sporulation ability, mating behaviour, UV induced mitotic recombination and meiotic marker segregation characteristics. The purified nuclear DNA of these disomics as well as that of a haploid Sacch. cerevisiae strain was challenged with 32-P-ribosomal RNA by specific DNA/rRNA hybridization. In all cases there was a constant proportion of 2.2 $\pm$ 0.1 percent of the nuclear DNA homologous to ribosomal RNA. From this result it is concluded that, in Sacch. cerevisiae, the chromosomes III, V, VI, VII, VIII and IX are either free of rRNA cistrons, or alternatively, that the 140 rRNA cistrons of yeast are randomly scattered over the entire genome.
(Manuscript in preparation and to be submitted to the European Journal of Biochemistry. Contents based on a doctoral dissertation completed last summer.)

XIII. Edinboro State College, Department of Biology, Edinboro, Pennsylvania 16412. Communicated by Frank T. Bayliss.

Abstract Ph.D. dissertation by F. T. Bayliss of work done at the University of California, Davis.

STUDIES IN ANTIBIOTIC-SENSITIVE AND COLD-SENSITIVE MUTATIONS IN SACCHAROMYCES CEREVISIAE RESULTING IN ALTERED RIBOSOMES

Abstract

Wild type Saccharomyces cerevisiae is highly resistant to streptomycin and neomycin. By screening a number of histidine auxotrophs one was found which could grow without histidine if high concentrations of streptomycin were added. Selection for derivatives of this strain which could be suppressed by much lower concentrations of streptomycin yielded streptomycin-sensitive mutants. Other mutants sensitive to neomycin were selected from wild type (S288C) by nystatin counterselection. The mutations are nuclear (segregate 2:2) and in the case of one of them (AA-1) no centromere linkage, or linkage with some 40 widely distributed genes located on the genetic map, could be demonstrated. Linkage of one of these mutations (strl) with an unmapped cycloheximide resistance gene, cyh-x (ac^r_x) was found, establishing a new linkage group fragment.

A mutant, AA-89, was found to be streptomycin-, neomycin-, and cold-sensitive and also to have an altered ribosome profile when grown at 15C. The ribosomes from this mutant are unable to incorporate ^{14}C-phenylalanine into protein in a polyU-directed in vitro system when neomycin or streptomycin are added. Studies of in vitro protein synthesis with combinations of subunits isolated from mutant AA-89 and S288C (wild type) in the presence and absence of streptomycin or neomycin show that the drug-sensitivity is a property of the 40S ribosomal subunit of the mutant (AA-89). Streptomycin, labelled with tritium, was found to be preferentially bound to the mutant 40S subunit, in vitro, and not to the mutant 60S or wild type 60S or 40S subunits.

Publications:

"Selection of Ribosomal Mutants by Antibiotic Suppression in Yeast", Bayliss, Frank T. and Vinopal, Robert T. Science 1971 (in press).

"A Mutation in Saccharomyces cerevisiae Conferring Antibiotic - Sensitivity and Affecting Ribosome Formation and Function", Bayliss, Frank T. and J. L. Ingraham. To be submitted to the Journal of Bacteriology.

XIV. Department of Biology, Brooklyn College of The City University of New York, Brooklyn, New York 11210. Communicated by Norman R. Eaton.

GENETICS OF MALTASE FORMATION IN YEAST

N. R. Eaton, N. A. Khan and F. K. Zimmermann

Strains of the yeast Saccharomyces carrying different non-allelic maltose genes produce a basal level of maltase in glucose, and this level is increased up to several hundred fold in presence of an inducer, such as maltose. Recently Khan and Eaton (Molec. Gen. Genetics, 112:317-322, 1971) have described a strain (1403-7A) carrying the MAL_4 gene which produces enzyme 15-500 fold higher in the absence of inducer than does any other non-allelic maltose gene tested. The factor responsible for high basal level of maltase in absence of inducer is genetically dominant and closely linked to or allelic to the structural gene.

In order to establish whether the high basal level or constitutive synthesis of maltase in strain (1403-7A) is due to mutation in some regulatory gene or is the property of the MAL_4 locus itself, we have obtained mutants from this strain after U.V. treatment of the following phenotypes.

(a) No fermentation; no enzyme

(b) Slow or no fermentation; high uninduced levels of enzyme. Three of these ferment glucose slowly or not at all.

All mutants were crossed to non-fermenter strains, and the diploids obtained were tested for maltose fermentation. All diploids fermented maltose except those of class (a).

Those mutants of class (b) which are not blocked in gluocse fermentation may be permease mutants or regulatory mutants.

Further biochemical and genetic studies on these mutants are in progress.

Induction of maltase synthesis as a complex process: Yeast strains carrying any one of the genes MAL1, MAL2, MAL3 and MAL6 produce high levels of maltase only when maltose is present, and when glucose is absent at the same time. Induction of enzyme synthesis can be observed only after a lag period of about 90-120 min. This lag period is independent of the previous growth history of the cultures used i.e. growth on glucose or glycerol medium. When cells are induced for a period long enough to allow maltase activity to appear, removal of inducer results in a complete stop of further synthesis within about 15 min. A strain carrying the gene MAL2 (# Z3-2B) was induced for 150 min in 2% maltose YEP, and cells were resuspended in 2%

glucose YEP. At various intervals after this change of media, cells were returned to 2% maltose YEP. An interruption for only 30 min allowed for an immediate resumption of maltase synthesis. Longer periods of interruption prevented this immediate resumption of synthesis leading to an increasing lag period which, after 2-2.5 hr of interruption, was the same as that observed with cells not previously exposed to maltose. The question was asked, whether higher levels of maltase in the cells would allow for an immediate reinitiation of further synthesis. Therefore, a culture was induced for 4 hrs. These cells were then incubated in 2% glucose YEP or in plain YEP for 3 hrs. Maltase activity per volume cell suspension remained constant during the period of incubation without maltose. Resumption of maltase synthesis was observed only after the usual lag of 2 hrs in the cultures exposed either to glucose or to plain YEP. This showed that a high level of maltase activity in the cells does not alleviate the long lag period. There appears to be an additional system that has to be induced before induction of maltase synthesis can be initiated. This additional system seems to decay relatively fast. Once it is established or still functional as in the experiment with a 30 min interruption, maltase induction is very fast. These results resemble those described previously by Halvorson and collaborators and Axelrod and co-workers on the labile permeation system.

Effect of sucrose on maltase induction: Induction of maltase synthesis in yeast strains carrying an inducible MAL - gene requires not only the presence of maltose but also the simultaneous absence of glucose, i.e. synthesis of maltase is under a dual control by the presence and absence of inducer and by catabolite repression. Sucrose is split in certain yeast strains to yield fructose and glucose. Sucrase activity can be genetically associated with the genes MAL1, MAL2, MAL3 and MAL4, but other strains can carry sucrase genes not associated with MAL-genes. The question to be asked was whether sucrose interferes with maltase synthesis directly or else indirectly after being cleaved. A strain carrying a MAL1 gene without a sucrose gene and a strain with a MAL2 gene which allows for very slow sucrose fermentation could be induced to form maltase in the presence of sucrose. A strain carrying this same MAL1 gene and a SUC2 gene as well as a strain carrying MAL3 and the associated SUC3 gene could not be induced in the presence of sucrose. This shows that sucrose does not interfere with maltase induction as such but only when it is cleaved at a fast rate.

XV. Indiana University, Department of Microbiology, Bloomington, Indiana 47401. Communicated by Marjorie Crandall.

Last year I was an exchange postdoctoral fellow of the NSF and the French CNRS studying under the sponsorship of Professor Piotr Slonimski, Centre de Génétique Moléculaire, C.N.R.S., 91-Gif-sur-Yvette, France. Below follow the summaries of research projects recently completed.

In a search for mitochondrial mutants in the yeast, Hansenula wingei, chloramphenicol (C^R) and erythromycin (E^R) resistant mutants were isolated in haploid strains. Diploid hybrids of these mutants did not show mitotic segregation indicating that these mutants were not mitochondrial. All the hybrids were 100% resistant to both chloramphenicol and erythromycin, but cells able to grow on medium containing both antibiotics resulted from only one cross. A difficulty with these experiments was that the wild type parental strains were partially resistant to these mitochondrial inhibitors even at the high pH (7.7) employed. For this reason, the diploid hybrids had to be plated directly on selective medium because the wild type would grow on chloramphenicol and erythromycin media following replica plating from non-selective medium. To test the idea that selection was occurring

in the cross where 100% of the cells were C^RE^R, colonies from C medium were resuspended in saline and plated on E medium and vice-versa. Again all cells were found to be C^RE^R. Thus, it was concluded that all the different C^R and E^R mutants were nuclear and other types of potentially mitochondrial mutants were sought.

Hansenula wingei is an obligate aerobe and, therefore, the loss of the respiratory chain of enzymes as occurs in the petite mutation would be expected to be lethal. To test this hypothesis, spontaneous and induced petite mutants were sought. Untreated cells were plated on differential medium (0.1% glucose + 2.0% glycerol) and then replica plated to glucose plates and glycerol plates. All colonies were able to grow on both carbon sources. Following UV mutagenesis to 4% survival, a population of cells containing 1% auxotrophs was diluted and plated on differential medium followed by replica plating as above. Only one glycerol-negative mutant was obtained and this was not a petite because (a) it had a normal component of cytochromes, (b) it reverted to glycerol-positive (frequency of 10^{-7}) and (c) it could respire ethanol. This mutant was specifically blocked in glycerol respiration (33% of the rate of ethanol respiration). Finally, ethidium bromide (EB) was used as a mitochondrial mutagen to induce the petite mutation in H. wingei. Cells were plated on either glucose + EB or glycerol + EB media. The wild type is sensitive on both carbon sources to 20 µg/ml (= 50 µM) EB. At 2 µg/ml, the inhibition of growth was only partial. To isolate EB resistant mutants, cells were treated with UV as above and plated at high cell densities on EB media. EB^R mutants on glucose were present at a frequency of 10^{-4} and EB^R glycerol mutants at 10^{-6} after mutagenesis. Both frequencies were increased 100-fold by UV mutagenesis. The EB^Rglucose mutants were sensitive to EB on glycerol but the EB^Rglycerol mutants were cross resistant to EB on glucose. There were stable and unstable mutants in both classes. One EB^Rglucose mutant reverted to sensitivity concurrently with becoming ethanol-negative. Several EB^Rglycerol isolates when cultured on non-selective medium lost their EB resistance on glycerol but retained EB resistance on glucose. These data indicate that the EB^Rglucose mutation is different from the EB^Rglycerol mutation and in order for a cell to grow on glycerol it must have both resistance markers. None of these EB^R mutants were petites since they were all glycerol-positive. Thus, it can be concluded from this stringent search for petites in Hansenula wingei that this type of mitochondrial mutation does not occur in this yeast.

It was next of interest to determine if the glycerol-negative (gly^-) mutant and an EB^R mutant were nuclear or mitochondrial. The gly^- mutant would not sporulate even though the parent did, and it did not segregate during mitosis of the heterozygous diploid (gly^+/gly^-). Only one gly^- diploid was isolated from all the experiments making the frequency of this event around 10^{-4} to 10^{-5}, typical for nuclear mitotic recombinational events. One EB^Rglucose mutant segregated at meiosis 13:16 in a random ascosporic analysis as did other markers (lys^-, Actidione resistance). Yet this same EB^Rglucose mutant when mated to a sensitive strain gave a population of diploids containing about 1% resistant diploid cells on aliquot plating or replica plating. The presence of the gly^- gene in the hybrid increased the percentage of EB^R segregants. The presence of the gly^- gene also decreased the cycloheximide resistance phenotype of the diploid heterozygous for A^R/A^S on 20 µg cycloheximide/ml. These effects of the gly^- gene in a hybrid which is phenotypically glycerol-positive are not understood, but this gly^- mutant may be a semi-dominant suppressor since it affects: sporulation, diploid mitotic segregation, diploid antibiotic resistance expression, haploid growth rates on glucose, as well as the specific decrease in glycerol respiration.

The results obtained with the EB^Rglucose gene showing segregation both during meiosis and mitosis do not allow a conclusion to be drawn as to whether this gene is nuclear or mitochondrial. However, the evidence strongly suggests that this EB^Rglucose gene is nuclear because (a) a roughly 2:2 segregation at meiosis was obtained, (b) the zygotes are EB^S, and if the gene were mitochondrial, the zygotes would be expected to be resistant and lose this resistance with growth, (c) there is no change in the proportion of EB^S + EB^{R++++} segregants as a function of the number of generations of growth after conjugation, and (d) the only thing which is unusual is that the segregational frequencies are high (10^{-1} to 10^{-2}) which is the same order of magnitude for mitochondrial segregation and 3 orders of magnitude higher than usually obtained for mitotic segregation. For example, the gly^- marker gave only 1 gly^- diploid out of about 10^4 gly^+/gly^- diploid colonies scored. However, this same unusually high frequency of segregation was obtained for another gene (A^R) in this cross which is considered nuclear so that the high mitotic segregation frequency is probably more a function of the strain than of the nature of the gene. Since the segregation occurred under non-selective conditions and since the segregants bred true, the high frequency of diploid mitotic segregation cannot be due to effects of these inhibitors on the cells.

In order to determine whether a marker resides in the nuclear or the mitochondrial genome, it is more definite to do a tetrad analysis rather than a random ascoporic analysis. For example, the 13:16 segregation obtained (see above) could be from a 2:2 segregation in all tetrads or could be the result of the average of mixed tetrads (0:4, 1:3, 2:2, 3:1, and 4:0) due to segregation of several nuclear genes or to sporulation of diploids not pure for one mitochondrial gene. I attempted tetrad dissections but could not separate the sexually agglutinative ascospores even after digestion with snail enzyme plus cysteine or sonication. I would be grateful to workers in this field for suggestions as to methods to separate spores like these which are strongly attracted to each other.

Future experiments will be performed to study the lethal reaction caused by ethidium bromide and to study the biochemical basis for ethidium bromide resistance mutation. Presently I have a USPHS research grant to study the complementary glycoproteins involved in the mating reaction in Hansenula wingei. I am now also studying derepression of the synthesis of these two haploid agglutination factors in the nonagglutinative diploid.

XVI. Department of Biochemistry, Komensky University, Bratislava, Czechoslovakia. Communicated by L. Kováč.

For 6 years, our group has been engaged in a systematic study on the organization and function of yeast mitochondria with twofold aims: To use yeast as a model organism for research on oxidative phosphorylation and genesis of mitochondria, and to clarify specific problems of energetics of the yeast cell. Under the pressure of external circumstances, the group has recently undergone reorganization and this prompts us to present an account of the work hitherto accomplished. Some work was done in collaboration with foreign laboratories, and the contribution of Drs. Galeotti, Groot, Hess, Lachowicz, Racker, Schatz and Slonimski is gratefully acknowledged.

A simple and univeral method for isolation of yeast mitochondria was elaborated[1,2]. Properties of mitochondria from wild-type yeast were described in detail[1,3,4,5]. Promitochondria from anaerobically grown cells were also prepared by this method and shown to have preserved translocation systems for ions and adenine nucleotides[5] and energy-transfer reactions[6].

The use of yeast mutants as a new tool for examination of oxidative phosphorylation was proposed[7,8]. It was found that mitochondria from cytoplasmic

respiration-deficient mutants exhibit normal osmotic and permeability properties[5] and normal phospholipid composition[9], catalyze hydrolysis of ATP[10], but the ATPase is oligomycin-insensitive[10] and energy of ATP hydrolysis cannot be channelled into the charge separation system[4].

A mutant deficient in oxidative phosphorylation was described[7,11] and the lesion was localized at the step of adenine nucleotide translocation across the mitochondrial membrane[12].

It was demonstrated that both cytoplasmic and nuclear mutants simultaneously deficient in cytochromes a and b are not able to carry out mitochondrial protein synthesis[13,14,15]. Complex deficiency in mitochondrial respiration and energy transfer systems of these mutants appears to be a secondary consequence of this lesion in mitochondrial protein synthesizing machinery. Nuclear single-gene mutants deficient either in cytochrome a or in cytochrome b were shown to have preserved mitochondrial protein synthesis[15] and to display oxidative phosphorylation with suitable electron acceptors[16].

In studies with intact yeast cells, a procedure for determination of efficiency of energy transformation in growing cells was developed[17], loose coupling between respiration and phosphorylation in non-growing cells was indicated[12,18,19], a mechanism of action of uncouplers under anaerobic conditions was formulated[20], relationship between respiratory activity and lipid composition of yeast cells was analyzed[21], and interaction of uncouplers and antimycin A with cytochrome b under anaerobic conditions was described[22].

Graduate and undergraduate students have greatly contributed to the results obtained. In fact, this research orientation has proven to be particularly suitable to serve pedagogical purposes, allowing to teach students modern biochemistry by means of relatively simple methods and inexpensive materials and on the most exciting problems of cellular energetics and of protein synthesis.

Two members of the group are no longer employed at Komensky University. Dr. Kužela is now at the Institute of oncology, Slovak Academy of Sciences, Bratislava and Dr. Kováč is at the Psychiatric hospital, Pezinok. It is hoped, however, that all the original participants will be able to continue their collaboration in a joint effort along the previously set line of research.

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9. L. Kováč, K. Poláková, P. Šmigáň and Š. Kužela, Antonie van Leeuwenhoek, 35: G 11, 1969.
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11. L. Kováč and E. Hrušovská, Biochim. Biophys. Acta, 153:43, 1968.
12. J. Kolarov, L. Kováč and J. Šubík, manuscript submitted.
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XVII. Department of Biology, University of Ottawa, Ottawa, Ontario KIN 6N5 Canada. Communicated by Roderick A. McDonald.

Below follow reports on projects in progress in the laboratory of J. Gordin Kaplan.

R. A. McDonald has just completed his doctoral dissertation; the abstract of his thesis, "Enzymological and regulatory aspects of isoleucine, valine and leucine biosynthesis in Schizosaccharomyces pombe", follows below.

Studies were undertaken on the synthesis and regulation of the branched-chain amino acid biosynthetic enzymes in the fission yeast, Schizosaccharomyces pombe. Enzymological studies have shown that this yeast possesses all of the enzymes known to be involved in the biosynthesis of isoleucine and valine. Threonine deaminase (TD), the first enzyme of the isoleucine pathway, is strongly inhibited by L-isoleucine, and this inhibition is relieved by L-valine. Threonine saturation kinetics at pH 9 (the optimum for activity) are Michaelian; the substrate curves become increasingly sigmoidal as the pH is lowered to 7, demonstrating homotropic interactions of L-threonine. At these lower pH values, valine activates TD and normalizes the substrate curve. Isoleucine increases the homotropic effects of threonine. TD could not be desensitized by the usual chemical means, but at pH 10, the enzyme is insensitive to isoleucine while still retaining catalytic activity. A model which accounts for these and other findings is presented. Acetohydroxy acid synthetase (AHAS), the first enzyme in valine biosynthesis, has a pH optimum of 6.5, in contrast to the pH optimum of 7.4-8.0 found in other systems. It is sensitive to feedback inhibition by L-valine, which effector shows homotropic cooperative effects. Substrate saturation of AHAS from glycerol-grown cells, or partially purified from glucose-grown cells, is Michaelian, while that of the crude extract from the latter is strongly sigmoidal. This effect was shown to be due to competition for pyruvate between AHAS and pyruvate decarboxylase, which is present in large amounts in cells fermenting glucose. The first enzyme of the leucine pathway, isopropylmalate synthetase, was shown to be sensitive to L-leucine. Of all the ilva enzymes, only AHAS and isomeroreductase appear to be subject to multivalent repression.

Tony Seah is continuing his work on catalases in Saccharomyces cerevisiae. In collaboration with A. Rashid Bhatti, he is conducting immunological studies on purified catalases from yeast.

In an attempt to study the genetics and regulation of yeast (Saccharomyces cerevisiae) catalase, we have succeeded for the first time in purifying this enzyme. Initially, commercial dry bakers' yeast was used. Three isoenzymes were found to have different properties: optimum pH for activities, molecular weight (high, medium and low), specific activities and absorption spectra. Preparations from a subcloned colony derived from a

single cell of the commercial yeast contain all three enzymes, indicating that all three catalases are present within a single yeast cell. Wild type yeast strains FL-90 and FL-100 used in this laboratory, were found to lack the high molecular weight enzyme. Preliminary studies using antibody prepared from the high molecular weight catalase showed that the two other catalases do not cross-react. Some mutants have been isolated and genetic studies in collaboration with Dr. Fred Sherman are in progress.

Summary of a paper by Michèle Duphil-Denis and François Lacroute, soon to be published in Molecular & General Genetics:

Studies of complementation patterns of ura2 mutants of the pyrimidine pathway in Saccharomyces cerevisiae suggest the existence of at least two different structural genes in the ura2 locus: one coding for carbamyl phosphate synthetase, ura2C, and the other coding for aspartic transcarbamylase, ura2A. An interallelic type of complementation takes place among some ura2A mutants, but was not noted among the ura2C class. Preliminary results of the mapping of suppressible mutants indicate that the complex is transcribed into a single messenger RNA, with the direction of transcription proceeding from the CPSase region to the ATCase region.

The following publications have appeared:

Satyanarayana, T. and J. G. Kaplan. Regulation of the purine pathway in yeast: activity and feedback inhibition of phosphoriboribosyl-pyrophosphate amidotransferase. Arch. Biochem. Biophys. 142, 40-47, 1971.

Lue, P. F. and J. G. Kaplan. Aggregation states of a regulatory enzyme complex catalyzing the early steps of pyrimidine biosynthesis in bakers' yeast. Can. J. Biochem. 49, 403-411, 1971.

Lue, P. F. and J. G. Kaplan. Metabolic compartmentation at the molecular level: the function of a multienzyme aggregate in the pyrimidine pathway of yeast. Biochim. Biophys. Acta 220, 365-372, 1970.

XVIII. Ecole Nationale Superieure Agronomique de Montpellier, Chaire de Génétique, Montpellier, France. Communicated by P. Galzy.

Three theses: (Thèses de spécialité de biochimie) have been completed in this laboratory.

JEANBART F. - Contribution à l'étude physiologique du mode d'action des gènes de la série "pl" chez Saccharomyces cerevisiae HANSEN. The mutation of the gene PL to pl causes a diminution of the cell volume. The action law of the concentration on the respiratory velocity of some substrates is modified by the mutation. The mutation of genes pl_2, pl_3, pl_4, pl_8 and pl_{11} modifies the respiration of pyruvic acid. The mutation of genes pl_3, pl_4, pl_8 and pl_{11} modifies the respiration of acetic acid. The mutation of genes pl_1, pl_5 and pl_7 modifies the respiration of L-lactic acid. The mutation of gene pl_3 modifies the respiration of D-lactic acid.

ARNAUD A. - Contribution à l'étude du métabolisme respiratoire au cours de la respiration.

GUIRAUD J. P. - Métabolisme de quelques substrats à deux carbones chez Saccharomyces cerevisiae HANSEN.

The following publications will be published shortly.

GUIRAUD J. P., GALZY P., ALBERT J. - Remarques sur le métabolisme de l'acide glycolique chez Saccharomyces cerevisiae HANSEN. (Annales Institut Pasteur). Glycollic acid does not allow the growth of yeast and does not seem to be metabolized. According to its concentration however, it may inhibit or activate the respiration of cell reserves.

This result can be compared to those obtained previously with ethanol and acetic acid.

GUIRAUD J. P., VEZINHET F., GALZY P., ALBERT J. - Influence de la nutrition préalable des cellules de levure sur leur aptitude à métaboliser certains substrats. (Archiv. für Mikrobiologie). The medium in which yeasts are grown seems to modify their ability to metabolize ethanol and acetic acid. In certain cases, the two substrates activate the oxidation of reserves. The phenomenon is observed with yeasts grown on synthetic medium with glucose (2%) or ethanol (1%). It is never observed with yeasts grown on the same medium with 0.5% glucose. It cannot be explained either by an elevated cellular level or carbohydrate reserves, by a nutritional imbalance, or by a difference in respiratory activity.

GALZY P., ARNAUD A., PLAN C. - Analyse factorielle chez les champignons. (Rev. Ferm. Ind. Alim.). Bruxelles.

BIZEAU C., BIZEAU E. - Extraction et étude de quelques composés glyco-protéiques de la paroi de Saccharomyces cerevisiae HANSEN. (C. R. Soc. Biol.). Two kinds of glucan - proteins and one of mannan, glucan, and protein have been extracted from the cell wall of Saccharomyces cerevisiae. One of these fractions is perhaps in low concentration in the mutant or is broken down more quickly in alkali.

XIX. Laboratoire de Chimie Bactérienne 4 - C.N.R.S. 31, chemin J. Aiguier, Marseille (9e) France. Communicated by E. Azoulay.

PARTICIPATION DU CYTOCHROME P_{450} DANS L'OXYDATION DES ALCANES CHEZ CANDIDA TROPICALIS.

M. Gallo, J. C. Bertrand et E. Azoulay
FEBS Letters (in press)

Summary

When grown on tetradecane as the carbon source Candida tropicalis strain 101 possesses a hydroxylase system which is active towards hydrocarbons and fatty acids. Cell-free, particulate enzyme preparations of the organism catalyze the oxidation of ^{14}C-labelled decane, decanoate, and dodecanoate to the corresponding fatty acid, and w-hydroxy fatty acids with specific activities of 2.4 to 3.5 units. In this system NADPH is more active than NADH, and may be replaced by NAD with equal efficiency. When cell-free extracts are passed through sucrose layers of discontinuous concentration, a cytochrome P_{450} and NADPH-cytochrome C reductase activity were localized in the concentration range between 15 and 21%. This system of a microsomal nature, which is specifically induced by alkanes, can be considered to be involved in the primary oxidation of aliphatic hydrocarbons. The presence of alcohol and aldehyde deshydrogenase activities associated with this sub-cellular fraction suggests that there exists in this yeast a microsomal metabolic pathway responsible for the transformation of alkanes to the corresponding fatty acids.

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

Earlier studies in this Laboratory on the influences of sulfur amino acid supplement on the digestibility of cell walls by Helix pomatia enzymes have been continued by Dr. K. A. Killick. Her results are summarized in a paper, Modification of the Composition and Structure of the Yeast Cell Wall by Culture in the Presence of Sulfur Amino Acids. K. A. Killick, J. Bacteriol. 106, 931-7 (1971).

Continued research on the effect of massive X-irradiation of yeast cells is reported in a paper by G. Svihla, F. Schlenk, and J. L. Dainko, Growth and Metabolism of Yeast Cells after Lethal X-Irradiation. International. J. Radiation Biol. 20, (in press) (1971).

The yeasts Candida utilis and Saccharomyces cerevisiae were X-irradiated (2×10^5R, 0.1% and 1.0% survivors) and incubated for 24 to 48 hours in culture medium. A three- to five-fold increase in cell weight occurred, but the process of budding was abnormal. In S. cerevisiae, multiple and lateral budding was observed while C. utilis produced one greatly elongated, sometimes bifurcated, bud which was much larger than the parent cell. In C. utilis, irregular structure of the bud wall was indicated by fluorescence staining with Primulin. The buds were deficient in respiration and DNA synthesis, but nitrogen assimilation, RNA, and ATP synthesis were found to resemble that of the parent cell. Ultraviolet micrography revealed that the junction between parent cell and bud was not closed. The buds contained vacuoles which were capable of storing newly synthesized material.

XXI. University of Aarhus, Royal Dental College, Denmark. Communicated by Sven Darling.

The following was reported at the annual meeting of the Scandinavian Society for Electron Microscopy in Gothenburg, June 10-12, 1971.

Structure and chemical composition of prospheroplast envelopes of Saccharomyces cerevisiae and Hansenula anomala. SVEN DARLING, JØRGEN THEILADE, and AKSEL BIRCH-ANDERSEN, Departments of Biochemistry and Electron Microscopy, Royal Dental College, DK-8000 Aarhus C, Denmark, and Department of Biophysics, Statens Seruminstitut, DK-2300 Copenhagen S, Denmark. Under certain conditions of growth some yeasts have elongated cells. Digestion of their cell walls with snail enzyme can produce osmotic sensitive cells which - when osmotically protected - still retain this elongated shape. These cells, the prospheroplasts, represent an intermediate stage between intact cells and spheroplasts to which they are converted after prolonged enzyme treatment (1). In order to study the structure of the prospheroplast envelope, cells of Saccharomyces cerevisiae and Hansenula anomala were digested with snail enzyme under conditions yielding prospheroplasts. These were lysed in distilled water and subsequently centrifuged. The sediment obtained was treated in 8 M urea for 24 hours and centrifuged. The resulting sediment consisted of prospheroplast envelopes. Such envelope material was embedded and sectioned for electron microscopy and thin hollow structures still retaining the elongated form of the original cells were seen. Sections stained with uranyl magnesium acetate and lead citrate showed the envelopes as structures of very low electron density, while phosphotungstic acid staining made them more electron dense. Shadowed preparations of prospheroplast envelopes revealed structures resembling ghosts. They were similar to the original cells in form and size but seemed to be very thin membranes. Varying numbers of anular structures (bud scars) were found on them. Chemical analysis of the envelopes may indicate that a polyglucosephosphate was a major constituent.

1. DARLING, S., et al., J. Bacteriol. 98, 797 (1969).

XXII. National Research Council of Canada, Biochemistry Laboratory, Ottawa 7, Canada. Communicated by Byron F. Johnson.

Below follow the abstracts of two recent papers:

Barras, D. R. 1971. A β-glucan endo-hydrolase from Schizosaccharomyces pombe and its role in cell wall growth. Antonie van Leeuwenhoek 38 (in press).

A β-glucan hydrolase showing a marked specificity for β-1,3-glucosidic linkages was found to be closely associated with the cell wall of the fission yeast Schizosaccharomyces pombe. Intact log phase cells showed

40% of the enzyme activity measured in the cell homogenate. Cellular enzyme activity reached a maximum during the logarithmic growth phase and decreased sharply in the stationary phase. The possible role of the enzyme in cell wall extension is discussed.

Calleja, G. B., and B. F. Johnson. 1971. Flocculation in a fission yeast: an initial step in the conjugation process. Can. J. Microbiol. 17:1175-77.

Schizosaccharomyces pombe NCYC 132, a haploid strain not previously observed to conjugate and sporulate, formed flocs during stationary phase when grown aerobically in malt extract broth. Flocculation was followed by sexual conjugation and sporulation. Cultural conditions which prevented the induction of flocculation, such as anaerobiosis or growth in minimal salts medium, also prevented conjugation and sporulation.

The current address of Dr. Calleja is: Dr. G. B. Calleja, Department of Botany, University of the Philippines at Los Banos, College, Laguna, The Philippines.

The current address of Dr. Barras is: Dr. David R. Barras, Chemistry Department, Swinburne College of Technology, Post Office Box 218, Victoria 3122, Australia.

XXIII. Hiroshima University, Department of Fermentation Technology, Faculty of Engineering, Sendamachi 3, Hiroshima, Japan 730. Communicated by Noboru Kawakami.

Below follow abstracts of two recent publications:

DIGESTION OF LIVING YEAST CELLS WITH PHYSARUM POLYCEPHALUM

Noboru Kawakami

[Bot. Mag. Tokyo 84:35-40 (February 25, 1971)]

Abstract

On a mixed culture of Physarum polycephalum and Candida utilis, yeast cells ingested in the living plasmodium are digested, and remnants of the cell wall are found in the plasmodium in addition to cells in an almost intact form with intracellular highly dense substances. When growing yeast cells are treated with an extract from plasmodia the cell wall and intracellular substances are also disintegrated. The digestion, however, did not advance to produce spheroplasts or protoplasts under the condition employed in this experiment.

SPHEROPLAST FORMATION IN THE YEAST CANDIDA UTILIS BY TREATMENT WITH CRUDE ENZYME ISOLATED FROM THE SLIME MOLD PHYSARUM POLYCEPHALUM

Noboru Kawakami and Hisako Kawakami

[J. Ferment. Technol. 49(6):479-487, 1971]

Abstract

Conversion to spheroplasts from intact cells of the yeast Candida utilis was achieved by incubation in a medium containing digestive enzymes prepared from the plasmodium of the slime mold Physarum polycephalum grown in a two-membered culture with C. utilis. Young cells of C. utilis, after preincubation in an isotonic tris buffer solution containing β-mercapto-ethanol, ethylenediaminetetraacetic acid, and KCl, were incubated in an isotonic digestive solution containing Physarum enzyme, L-cysteine hydro-chloride, and KCl at 35°C for two hours. The fine structures of yeast cells during the spheroplast formation and of the produced spheroplasts were examined with an electron microscope. The direct formation of the spheroplast through a hole which arises from the dissolution of the plug of

the bud scar was observed. Spheroplasts were also formed through elongated prospheroplasts. In the spheroplast, fibrillar remnants of the disintegrated cell wall adhered to the surface of the plasma membrane and intracellular structures were clearly seen.

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

Sequential use of 2-deoxyglucose and snail gut enzyme for spheroplast preparation in wild strain and respiratory deficient mutants of a "petite negative" yeast Schizosaccharomyces pombe 972h⁻

F. Foury and A. Goffeau

Abstract of manuscript submitted:

The effects of snail gut enzyme and 2-deoxyglucose (2-DG) were studied in Schizosaccharomyces pombe 972h⁻ wild type and in four respiratory deficient mutants (COB5A, COB6, M53, M126), in order to prepare respiring spheroplasts potentially able to yield intact isolated mitochondria.

2-DG inhibits growth of all strains except in COB6. 50% growth inhibition of the wild type requires addition of 100 µg/ml 2-DG when cells are grown in glucose containing medium, and of 1 µg/ml 2-DG in glycerol containing medium. Two to three hr after addition of 100 µg/ml 2-DG to cells in log phase growth in glucose, spheroplasts were obtained. In glycerol containing medium, addition of 0.15% of glucose preceding the addition of 100 µg/ml 2-DG, greatly accelerates spheroplast formation. However, the respiration of the spheroplasts obtained by these long 2-DG treatments is inactivated.

Under appropriate conditions, the wild type grown in glycerol, yields 95% respiring spheroplasts after treatment with 2% snail gut enzyme. However, when grown in glucose the wild type and all mutants except COB6 were much less sensitive to snail gut enzyme.

A short pretreatment of growing cells for 10 to 30 min in the presence of 100-500 µg/ml of 2-DG (according to strain and culture conditions) sensitizes cell walls to hydrolysis by snail gut enzyme without inactivation of respiration. Under optimal conditions of enzyme concentration, pH, time of incubation and cell concentration, the sequential use of 2-DG and snail gut enzyme permits the preparation of respiring spheroplasts from all tested strains under all tested growth conditions.

It is suggested that this short 2-DG pretreatment might prevent synthesis of cell wall galactomannans at the cell surface permitting the snail gut enzyme to reach and to hydrolyse glucans.

XXV. National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada. Communicated by J. F. T. Spencer.

Below follows an abstract of a publication soon to appear in the Canadian Journal of Microbiology.

THE GENETIC CONTROL OF THE TWO TYPES OF MANNAN PRODUCED BY SACCHAROMYCES CEREVISIAE

J.F.T. Spencer, P.A.J. Gorin & G.H. Rank

The two mannans formed by different strains of Saccharomyces cerevisiae give proton magnetic resonance spectra (spectral types A and B) which suggest that they differ principally in the presence of α-(1→3)-linked mannopyranosyl end-units of the side chains of the more complex type (giving spectral type B). The presence or absence of such end units was under the control of a single mendelian gene, since the two types of mannan segregated independently during sporulation. The gene controlling this type of mannan produced was apparently not linked to those controlling the requirements for

adenine, uracil, leucine or histidine. The implications of the findings concerning the mechanism of biosynthesis of yeast mannan are discussed.

XXVI. Karl-Marx-Universität, Klinik für Hautkrankheiten, 701 Leipzig, Liebigstrasse 21, DDR. Communicated by Christina Schönborn.

A case report by H. Pöhler and Ch. Schönborn

We observed a case of heavy and generalized candidiasis, which was cured by administration of antibiotics, corticoiden and immune suppressives. From Sept. 6-Nov. 9, 1971 we had in our clinic a patient due to a heavy generalized Candidiasis integumentalis et intestinalis. The 34 year old patient suffers already since his 18th year of a polyarthritic rheumatism and was therefore continually under treatment. In June 1971 he suffered an acute attack with painful swelling of the large joints. He was given antibiotics (OTC, and chloronitrin), Prednison and immune suppressives (Amuno). Under this therapy, an already long existing case of Tinea pedum became worse and at the same time large areas of pustules and granuloma developed near the integuments. At the same time the general condition of the patient deteriorated. The blood sedimentation reaction was maximally increased (112/150) and the antistreptolysinetiter rose to 1025 ASE. C-reactive protein was ++. There was no evidence for diabetes mellitus. From stool, sputum and oral cavity Candida albicans was repeatedly isolated in large numbers, but not from the skin. An intracutaneous test with polysaccharide-N-complex antigen 1:1000 from C. albicans turned out negative, but the lymphocyte transformation test with candidin 1:1000 was positive. A histologic check of skin excision showed a subepithelial infection coupled with penetration of the infiltrate into the epidermis. Fungal elements could not be demonstrated.

After treatment with only amphotericin B (starting every 3 days with 50,000 int. units and increasing to 500,000 units per injection) as well as with oral and local application of Nystatin, the skin eruptions cleared up completely. Also under this therapy the painful swellings of the joints improved.

Clinical symptoms and progress of the case suggest that we dealt with a generalized Candidiasis intestinalis et integumentalis, which was cured by high doses of antibiotics, coticoiden and immune suppressives.

XXVII. Research Institute of Brewing, Takinogawa, Kita-ku, Tokyo, Japan. Communicated by Yataro Nunokawa.

Recently, non-foaming mutants of sake yeast which do not form a high froth head in sake mash were isolated from commercial strains and one of them is now in practical use. Two papers related to the non-foaming sake yeast have appeared or are in press.

(1) Ouchi, K., and Akiyama, H. Non-foaming mutants of sake yeasts: Selection by cell agglutination and by froth flotation. Agr. Biol. Chem. 35:1024-1032, 1971.

Abstract

The non-foaming mutants, which are different both in their affinity to gas bubbles and in their agglutinability from the parent, were concentrated, by removing the wild type cells with froth by the froth flotation method and by removing them by agglutination caused by lactobacillus cells, using the cell agglutination method. Spontaneous non-foaming mutants of Kyokai No. 7 strain were isolated from the concentrates after 9 successive trials of each selection procedure at the rates of 50% by the former and 80% by the latter. UV-induced mutants were also isolated from the concentrates after 7

successions at the rates of 80% and 100%, respectively, by the former and by the latter. There were two types among the non-foaming mutants with respect to the agglutinability; one was non- or almost non-agglutinable (type 1) and the other was weakly-agglutinable (type 2). It is inferred from the concentration rate determined by the froth flotation method in a model experiment that the mutation would occur spontaneously at a rate of 10^{-8} and be stimulated about 100 fold by UV-irradiation in the Kyokai No. 7 strain. The usefulness of the non-foaming mutants is discussed from a practical point of view for sake production.

(2) Nunokawa, Y., Toba, H., and Ouchi, K. Froth flotation of yeast cells. (I) Correlation between high flotability of cells and their froth head forming ability in sake mash. J. Ferment. Technol. (in press, December, 1971).

Abstract

The froth flotation method was applied to yeast cells to find out a correlation between high flotability and froth forming ability in sake mash. Cationic detergent, alkyl dimethyl benzyl ammonium chloride, and nonionic detergent, sucrose monopalmitate, were found to be effective frothers. Some factors affecting the flotability were examined and a frothing condition was established. Flotability of a yeast strain maintained constantly through-out the growth phase. A high froth-forming strain in sake mash could be distinguished from non-frothing strain by froth flotation; high frothing strain had high flotability (3.0 or more in Separation Index), while non-frothing strain had low flotability (less than 1.0 in Separation Index). Intermediate low frothing strain, however, could not be recognized by the froth flotation. Some correlations were found between electrical charge of the cell surface and the flotability of the cell; most of positively charged cells at pH 3.0 had high flotabilities and the negatively charged ones had low flotabilities. As some exceptions were found, however, hydrophobic properties of cell surface were also considered to be concerned to the froth flotability.

Mrs. C. Kumagai has completed her thesis work on the 'Structure of sake yeast mannan', an outline of which has appeared in Report of the Research Institute of Brewing, No. 143, June, 1971.

Mannan was extracted and purified from a sake yeast, Kyokai No. 7 by Peat's method. Structure of the mannan was investigated variously employing methylation, periodic acid oxidation, Smith's degradation, acetolysis and partial hydrolysis accompanied with thin layer and gas-liquid chromatographies and gel filtration. It was recognized that the mannan of sake yeast had a similar structure as that of baker's yeast; the mannose residues are linked by α-1,6-bonds in the main chain and by α-1,2- and α-1,3-bonds in the side chains. Some differences, however, were found in their branch structures between sake yeast and baker's yeast; manno-pentaose was released from the main chain of sake yeast mannan by acetolysis, while manno-tetraose was the longest branch-chain fragment in baker's yeasts. In the mannan of sake yeast, α-1,3-bonds were found twice as much as α-1,2-bonds and they seemed to exist as end groups of the branches. It was presumed that the manno-tetraose was linked to the main chain by α-1,2-bonds and had 2 successive terminal α-1,3-bonds. It is interesting that the structure of mannan is different in sake yeast and baker's yeast, both of which are classified as Saccharomyces cerevisiae.

Further investigations on the side chain fragments derived from various strains showed that the manno-pentaose was released from all of the yeasts which are used for alcoholic beverage industries, including brewer's yeast. In our laboratory, the finger printing of the side chain oligosaccharides was done by developing each fragment on thin layer of Kieselgel G, instead of by gel filtration on Sephadex G-25 as proposed by Ballou.

XXVIII. Massachusetts Institute of Technology, Department of Nutrition and Food Science, Cambridge, Massachusetts 02139. Communicated by Charles L. Cooney.

"Continuous Cultivation of a Methanol Utilizing Yeast". Presented at the 162nd American Chemical Society Meeting held in Washington, D. C., September, 1971 by Charles L. Cooney, David W. Levine, Daniel I. C. Wang, Department of Nutrition and Food Science, M.I.T., Cambridge, Massachusetts 02139

Abstract

Interest in the production of single cell protein has prompted many investigators to examine a wide variety of potential substrates for conversion to protein. One substrate of considerable interest is methanol. Using a continuous enrichment culture, we have isolated a yeast tentatively identified as a species of Candida which utilizes methanol as its sole carbon and energy source. This yeast, when grown on a mineral salts methanol medium supplemented with biotin and thiamine, has a specific growth rate of 0.17 hr^{-1} (doubling of 4 hours). The optimum pH range and temperature for growth are 4.5 to 6.5 and 37°C respectively. This yeast is capable of growth at temperatures up to 45°C. Cellular yields of 0.4 gram of cells per gram of methanol have been measured in both batch and continuous culture. Cells grown in batch culture contain 50% protein and 4.5 % RNA. Studies are continuing to complete material balances on cells growing in continuous culture and to determine the amino acid profile of the yeast protein.

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

COMPOSITION OF WHISKY FLAVOUR
H. Suomalainen and L. Nykänen

Process Biochemistry 5 (1970), 13-18

Investigations on the flavour of whisky have shown that the aroma fraction consists of aldehydes, fusel alcohols, esters and fatty acids. The composition of the aroma group in different brands of whisky has been reviewed and the effects of the components upon the flavour of whisky are compared. The kinds of aroma components formed in the fermentation do not seem to depend decisively upon the raw material. To date, investigations concerned with the aroma composition of different alcoholic beverages indicate that the same components always appear in the amounts that are quantitatively largest. Nevertheless, the qualitative similarity of the aroma composition, along with the ability of the yeast to produce the same aroma compounds even in nitrogen-free sugar fermentation, gives the yeast a central role in the formation of different aroma components.

The following publications have appeared since the last communication. Abstracts of the reports have been given in Yeast News Letter in June 1970 and in January 1971.

Heikki Suomalainen and Timo Nurminen, The lipid composition of cell wall and plasma membrane of baker's yeast. Chem. Phys. Lipids 4 (1970), 247-56.

Blanka Ries and Heikki Suomalainen, Interaction of phenylmercuric acetate with some enzymes and intact cells of baker's yeast. Suomen Kemistilehti 43B (1970), 392-395.

T. Nurminen and H. Suomalainen, The lipolytic activities of the isolated cell envelopes fractions of baker's yeast. Biochem. J. 118 (1970), 759-63.

Erkki Oura and Heikki Suomalainen, Contents of cytochromes in yeast. J. Inst. Brew. London 76 (1970), 536-545.

Pentti Ronkainen, Saara Brummer and Heikki Suomalainen, α-Hydroxy ketones, acetoin and hydroxy pentanone, in wines. Am. J. Enol. Viticult. 21 (1970), 136-139.

Pentti Ronkainen, Saara Brummer and Heikki Suomalainen, Diacetyl and formic acid as decomposition products of 2-acetolactic acid. Acta Chem. Scand. 24 (1970), 3404-3406.

Heikki Suomalainen, Yeast and its effect on the flavour of alcoholic beverages. J. Inst. Brew. 77 (1971), 164-177.

XXX. Research Institute for Viticulture and Enology, Bratislava, Czechoslovakia. Communicated by Dr. Erich Minárik.

Recently the paper "Study on the yeast flora of marginal wine regions in Czechoslovakia" (in French) had been published in "Connaissance de la Vigne et du Vin" (Talence), No. 2, pp. 185-197, 1971.
Summary: The ecology of natural wine yeast species occurring in two marginal wine regions of Czechoslovakia and that of Saale-Unstrut (GDR) had been studied. It was found that even under excellent wine growing conditions the association of Kloeckera apiculata - Candida pulcherrima - Saccharomyces cerevisiae var. ellipsoideus was responsible for the spontaneous fermentation of grape must. The first phase of fermentation is induced by the asporogenous Kloeckera apiculata and Candida pulcherrima followed by Saccharomyces cerevisiae var. ellipsoideus responsible for the main fermentation and together with Saccharomyces oviformis for the last phase of fermentation. In contrast, in other wine regions musts of marginal regions are marked by a high proportion of aerobic yeast species in the microflora. Among the film-forming yeasts especially Hansenula anomala influences the alcoholic fermentation. These yeasts, possessing little fermentation activity, show a rather strong volatile ester production. The high proportion of film-forming yeasts in musts and young wines are an indicator of the propensity of these wines towards wine flower and underline the importance of preventive measures during wine treatment. Once again the effect of ecological conditions of the wine region, first of all climatic relations, on the qualitative composition of the yeast flora of grapes and musts, could be established.

Following is a summary of a paper published in English in Acta Universitatis Agriculturae 18, No. 4, pp. 645-648, 1970.
Investigations on the contaminating microflora of bottled wines.
The composition of the yeast flora of bottled wines has been investigated in 5 samples originating from 3 different wineries in Czechoslovakia. Within a period of 11 months after bottling 51 yeast strains belonging to 3 sporogenous and 1 asporogenous genera could be detected. Candida mycoderma with 29.4% and Saccharomyces cerevisiae with 25.6% in the yeast flora were the most frequently occurring yeasts in wines. Other species were markedly less common. During storage the proportion of sporogenous yeasts gradually decreased, whilst that of the asporogenous yeasts increased. The occurrence of Hyalodendron sp. in 2 wine samples 11 months after

bottling is interesting and shows the alcohol tolerant character of this fungus. The results of this investigation have also shown that procedures used for wine stabilization against biological clouding and haze are not yet fully satisfactory.

XXXI. Institute of Technology of Fermentation and of Microbiology, Technical University of Lodž, Gdanska 166, Poland. Communicated by J. Jakubowska.

Most of the data cited below were the result of work done at the former Department of Industrial Microbiology, Techn. Univ. of Lodž. The fission yeasts were studied in connection with the wine industry, their resistance to SO_2 and their strong capability to decompose malic acid. S. cerevisiae was studied in the laboratory and in industry with respect to baker's yeast production. Titles of earlier publications and summaries of recent works are presented below.

1. Nowakowska, A. The inhibition of contaminating microorganisms by yeast autolysates. Acta Microb. Pol. 1957, 6, 303-307.
2. Nowakowska, A. Autolysis of brewer's and baker's yeasts. Acta Microb. Pol. 1957, 6, 293-302.
3. Jakubowska, J., Kusewicz, D. The thiamine content in autolysates of yeasts in wine and beer sediments. Acta Microb. Pol. 1962, 11, 363-372.
4. Kusewicz, D. Vitamin B requirements of Schizosaccharomyces. Acta Microb. Pol. 1965, 14, 155-160.
5. Jakubowska, J., Piatkiewicz, A. Metabolism of D-L-malic acid by Schizosaccharomyces pombe and Schizosaccharomyces acidodevoratus. Acta Microb. Pol. 1965, 14, 67-71.
6. Kusewicz, D., Galamon, T. The influence of some analogues of cocarboxylase on Schizosaccharomyces yeast. Zeszyty Naukowe Politechniki Lodzkiej, Chemia Spozywcza 1966, 10, 35-43 (in Polish).
7. Kusewicz, D., Piatkiewicz, A. The effect of lyophilization on the viability and activity of yeasts. Zeszyty Nauk. Politechniki Lodzkiej, Chemia Spozywcza 1968, 14, 61-71 (in Polish).
8. Jakubowska, J., Piatkiewicz, A., Kusewicz, D. Biochemical properties of Schizosaccharomyces used in wine fermentation. Proc. II Int. Symp. on Yeasts held in Bratislava 1966. Publ. Cad. Ac. Sci. Bratislava 969, 121-126.
9. Kusewicz, D. The influence of analogues and derivatives of thiamine on morphology of Schizosaccharomyces. Zeszyty Naukowe Politechniki Lodzkiej, Chemia Spozywcza 1970, 17, 59-72 (in Polish).
10. Kusewicz, D. Pigment formation by Schizosaccharomyces grown on solid media. Zeszyty Naukowe Politechniki Lodzkiej, Chemia Spozywcza 1970, 17, 51-57 (in Polish).
    Abstract
    Schizosaccharomyces when grown on apple-must agar medium produced a red to orange red pigment. After 5 days of cultivation the colour of the colonies, previously cream, became red. The pigment was insoluble in the medium and in liquid medium it was not observed.
11. Wlodarczyk, M. The influence of 6-furfuryloaminopurine (kinetine) on Saccharomyces cerevisiae JA-64. Zeszyty Naukowe Politechniki Lodzkiej, Chemia Spozywcza 1970, 17, 133-153 (in Polish).
    Abstract
    A stimulatory effect of kinetine in concentration 0.5×10^{-4} and 0.5×10^{-5} M/l on rate of growth in liquid media was observed. This substance enhanced the penetration of d-xylose into the yeast cell, and caused a higher decarboxylation rate in glucose, sucrose and maltose.

12. Jakubowska, J., Wlodarczyk, M. Observations on growth and metabolism of Saccharomyces cerevisiae by beta-indoliloacetic acid (IAA). Acta Microbiologica Polonica, ser. B. 1970, 2/19/274-281 and Antonie van Leeuwenhoek 1969, 35/supplement/G17-18.

Abstract

Two regulators of growth, namely IAA and kinetine were used in concentrations of 1 to 10 µg per ml in a cultivation medium or added to the resting cell suspension. A stimulating effect of IAA on Saccharomyces cerevisiae, namely on their rate of growth, was found. Dynamics of glucose consumption as well as the uptake of D-xylose were increased due to IAA and kinetine, the latter being used for comparison. The gaseous exchange of resting cells investigated by the Warburg technique and the activity of alcohol dehydrogenase, measured by Thunberg technique, were significantly enhanced by IAA. The authors suggest that the stimulating effect of IAA on yeast metabolism is mainly connected with the dynamics of sugar transport into the yeast cell.

13. Jakubowska, J., Piatkiewicz, A. The influence of Sodium Deoxycholate and Dimethylsulphoxide on the permeability of the cell membrane of Schizosaccharomyces. Acta Microbiologica Polonica 1966, 15, 241-248.

Abstract

Resting cells of Schizosaccharomyces acidodevoratus and Sch. pombe treated with dimethylsulfoxide (DMSO) or with sodium deoxycholate metabolize substrates thought not be utilized. The 0.006 M solution of sodium deoxycholate stimulates the activity of several yeast organic acid dehydrogenases. The activity of malic acid dehydrogenase was found to be markedly increased in the presence of Mn^{++} ions.

14. Jakubowska, J., Piatkiewicz, A. The ability of Schizosaccharomyces acidodevoratus to utilize some acids of the Krebs cycle. Acta Microbiol. Polon. 1971, in press.

Abstract

In resting cell suspensions of Schizosaccharomyces acidodevoratus using Warburg techniques the ability to utilize some TCA cycle intermediates was investigated. Fresh intact cells dissimilated oxaloacetate, pyruvate and to a small degree fumarate and cis-aconitate; α-oxoglutarate and isocitrate were not used. In the same dried yeast the rate of decarboxylation in pyruvate and cis-aconitate and the consumption of α-oxoglutarate, not used by fresh cells, was markedly enhanced. Using the Thunberg technique the ability of dehydrogenase in both fresh and dried cells in pyruvate and α-oxoglutarate was measured. This effect was stimulated in presence of NAD. In isocitrate the activity of dehydrogenase was stimulated by NADP.

XXXII. International Yeast Meetings

1. The following resolution, proposed by the IAMS Commission on Yeasts and Yeast-like Micro-organisms, was adopted by the participants of the First Specialized Symposium on Yeasts, held at Smolenice Castle, June 1-4, 1972.

   1. The participants of the First Specialized Symposium on Yeasts at Smolenice Castle, June 1-4, 1972, wish to express the most sincere thanks to their Czechoslovak Hosts for having organized an extremely profitable and pleasant meeting.
   2. The following specialized symposia will be held in accordance with the resolution adopted in Delft and The Hague in 1969:
      a. In Kyoto, Japan, March 19-25, 1972, on Taxonomy and Ecology of Yeasts, on Subcellular Structure and Function of Yeasts, and on Sexuality, Gene Action and Breeding of Yeasts.

b. In Tokyo, August 7-9, 1972, on the various aspects of Medical Yeast Science (see program under 4).

c. In Helsinki in 1973 on Metabolism and Regulation of Cellular Processes in Yeasts.

3. The next General Symposium on Yeasts will be held at Vienna, Austria, in 1974.

4. A proposal made by Dr. A. Kocková-Kratochvílová to start an international co-operation on standardization of test-methods applied in various fields of yeast science should be carried into execution.

2. Organizing Committee of the General Symposium on Yeasts to be held at Vienna (Austria) in 1974.

Chairman: Professor Dr. Hans Klaushofer, Institut für Lebensmitteltechnologie, Hochschule für Bodenkultur, Vienna (Austria).

Vice Chairman: Professor Dr. Richard Brunner, Institut für Biochemische Technologie und Mikrobiologie, Technische Hochschule, Vienna (Austria).

Secretary General: Dr. Uwe Sleytr, Institut für Lebensmitteltechnologie, Hochschule für Bodenkultur, Vienna (Austria).

Treasurer: Director Dr. Wolfgang Petzelbauer, Brauerei Schwechat AG, Vienna (Austria).

Scientific Programme: Dr. A. Kocková-Kratochvílová, Chemický ústav, Slovenska akadémia vied, Bratislava (Czechoslovakia).
Dr. E. Minárik, Výskumný ústav vinohradnícky a vinársky, Bratislava (Czechoslovakia).
Professor Dr. J. Meyrath, Institut für angewandte Mikrobiologie, Hochschule für Bodenkultur, Vienna (Austria).
Professor Dr. U. Leupold, Institut für Allgemeine Mikrobiologie der Universität, Bern (Switzerland).
Professor Dr. Ph. Matile, Institut für Allgemeine Botanik, Eidgenössische Technische Hochschule, Zürich (Switzerland).

3. Second Specialized Symposium on Yeasts and Yeast-like Microorganisms. Supplementing the Second Circular of the Fourth International Fermentation Symposium.

For the convenience of program arrangement, financial business and communication, this Yeast Symposium will be included in the Fermentation Symposium as one of its integral parts.

PROVISIONAL PROGRAM

March	morning	afternoon
19 (Sunday)	Assemble:	Registration
20 (Monday)	IFS Opening Ceremony	
21 (Tuesday)	Yeast Symposium	
22 (Wednesday)	"	"
23 (Thursday)	"	"
24 (Friday)	"	" (General Session)
25 (Saturday)	IFS Closing Ceremony	
	Yeast Symposium Closing Session	
		Disperse

1. Taxonomy and Ecology of Yeasts,
convener: Dr. K. Tubaki (Japan)
presiding: Dr. H. J. Phaff (U.S.A.),
Dr. J. P. van der Walt (South Africa)

2. Subcellular Structure and Function of Yeasts,
   convener: Dr. T. Hirano (Japan)
   presiding: Dr. H. Suomalainen (Finland),
   Dr. C. F. Robinow (Canada)
3. Sexuality, Gene Action and Breeding of Yeasts,
   convener: Dr. N. Yanagishima (Japan)
   presiding: Dr. H. O. Halvorson (U.S.A.)
   Dr. P. P. Slonimski (France)
4. Sessions on General Subjects, to be arranged according to the responses to the circular.

Registration fee will be equivalent to U.S. $50.00. The registration entitles you to all the privilege and benefit allowances throughout both the Fermentation and the Yeasts.
Please contact the following when you have enquiries, proposals or requests any further:

Dr. Y. Oshima, Secretary
Local Organizing Committee for Yeasts
Department of Fermentation Technology
Faculty of Engineering
Osaka University
Suita-shi, Osaka
565 Japan

We certainly wish to have you with us in the best season of the year 1972.

Susumu Nagai, in charge of Yeast
Symposium, Subcommittee of IV IFS

4. Second International Specialized Symposium on Yeasts, Tokyo, Japan, August 7-10, 1972.
Theme: Yeasts and Yeast-like Microorganisms in Medical Science

Program

The following fields concerning yeasts and yeast-like micro-organisms with particular reference to medical science will be covered:
A. Taxonomy; B, Morphology and Ultrastructure; C. Genetics;
D. Physiology, Biophysics and Biochemistry; E. Ecology; F. Epidemiology;
G. Pathogenesis; H. Clinical Aspects; I. Laboratory Diagnosis;
J. Immunology; K. Chemotherapy; L. Medicinal and Industrial Application.

All abstracts should be in the hands of the Organizing Committee not later than April 1, 1972.

Preregistration and general information

Those wishing to attend this Symposium are requested to write the secretary as soon as possible. The next circular will include a preliminary scientific program, suggestions for tours after the symposium, formal registration forms, hotel reservation forms, price quotations, and other useful information.

Language

The official language for the Symposium will be English and all Symposium literature will also be published in English. No simultaneous translation service will be available.

Social events and tourist activities

The Organizing Committee has appointed the Japan Travel Bureau (JTB) as the registered official travel agent to handle necessary arrangements with regard to travel and hotel reservations as well as social events.

Correspondence

All correspondence relating to the Symposium should be addressed to:

Prof. Kazuo Iwata
Chairman, Organizing Committee
Second International Specialized Symposium on Yeasts
c/o Department of Microbiology
Faculty of Medicine, University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

5. The Third Yeast Genetics Conference - Japan

The third Yeast Genetics Conference-Japan was held on August 11 and 12, 1971, at Suntory Hall, Department of Fermentation Technology, Osaka University. Fifty-eight investigators assembled, and six research areas were discussed: mutation and radiation effects, sexuality and its genetic control, cell structure and function, gene action and regulation, RD mutants and cytoplasmic inheritance, and chemical components and taxonomy.

H. Mori (Noda) reported on auxotrophic mutants of Saccharomyces rouxii (Soya sauce yeast) induced with nitrosoguanidine. S. Nagai (Nara) demonstrated the identification of foamless mutants of sake yeast by the use of colored media. T. Takahashi (Suita) isolated auxotrophic mutants from a homothallic strain of S. cerevisiae with heat shock (Tamaki 1961) and Nystatin screening (Snow 1966). S. Doi and N. Yanagishima (Osaka) induced variants having an abnormal DNA content from a haploid strain of S. cerevisiae by treatment with a plant hormone. S. Mori (Osaka) described the genetic effects of Linac radiation on uvs and xrs strains.

Y. Oshima (Suita) and I. Takano (Osaka) have obtained a new type of homothallic yeast from Dr. Santa-Maria, and the genetic difference between the new homothallism and homothallism controlled by HO_{α} HM was discussed. Takano isolated sporulation- and mating-positive segregants from a hybrid between homothallic and heterothallic strains. Yanagishima discussed the mating reaction and reported at least four kinds of sex controlling hormone-like substances in Saccharomyces, while C. Shimoda (Osaka) discussed the fate of macro molecules during conjugation of a and α strains. M. Tsuboi (Osaka), Yanagishima and Takahashi showed the accelerating effect of the D gene on the yeast sporulation. N. Gunge (Yokohama) assumed the existence of a mating inhibitor gene, based on analyses of aberrant segregation for mating type. S. Nakai (Chiba) described the production of a strain disomic for chromosome VII and the effect of radiation on this strain. T. Yamazaki (Kofu) discussed the sexual life cycle of Saccharomycodes ludwigii.

N. Sando (Tsuruoka) dealt with the relation of sporulation and metabolism. M. Osumi (Tokyo) presented information on the cell structure of hydrocarbon-utilizing yeasts. N. Kawakami (Hiroshima) reported the production of spheroplasts by the use of myxomycete enzyme.

Oshima and A. Toe (Suita) described the genetic control of acid phosphatase synthesis. H. Tamaki (Kyoto) presented tetrad data on the amylase genes, STR1, STR2 and STR3. Takahashi assumed the existence of a negative suppressor, based on 1:3 and 0:4 segregations of various genetic markers.

T. Morita and I. Mifuchi (Shizuoka) and K. Wakabayashi (Tokyo) presented genetic and biochemical studies on cytochrome mutants of S. cerevisiae. T. Yamamoto (Osaka) reported on the heavy metal sensitivity of an Sr-resistant strain. Gunge discussed the question

of nuclear genes or cytoplasmic factors associated with oligomycin resistance; location of the factor on mitochondrial DNA was postulated.

T. Nakase (Kawasaki) and K. Komagata (Tokyo) discussed the relation between GC content and taxonomy of yeasts, and Y. Yamada (Shizuoka) reported on the relation between coenzyme Q and taxonomic studies.

The outline of the First International Specialized Symposium on Yeasts, which was held in Czechoslovakia, was introduced by Komagata and Yamada.

Schedules of the Second International Specialized Symposium on Yeasts which will be held at Kyoto, Japan were obtained from the members of the local committee of the symposium. The dates are March 21 through 24, 1972. Three focal topics (ecology and taxonomy, subcellular structures and function, and sexuality, gene action and breeding) and sessions for general subjects will be arranged. The general secretary of the local committee is Dr. Y. Oshima, Department of Fermentation Technology, Osaka University, Yamada-kami, Suita 565, Japan.

A committee composed of Gunge, H. Kasahara (Tokyo), K. Kuraishi (Tokyo), Nagai and Y. Yamamoto (Takasaki) was appointed to establish a yeast information service in Japan. Finally, Dr. S. Nagai was elected the President of Yeast Genetics Conference-Japan.

The next meeting will be held on March 27 (M) and 28 (Tu), 1972 at Rakuyu Hall, Kyoto University, along with yeast geneticists who are among the participants of the Fourth International Fermentation Symposium and the Second International Specialized Symposium on Yeasts.

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6. Report on the Fifth Yeast Genetics Conference, Chalk River, Ontario, Canada, 1970.

The 5th International Conference on Yeast Genetics, sponsored by Atomic Energy of Canada, Ltd., was held at Chalk River, Ontario from September 29 to October 2, 1970. Over one hundred visiting scientists from nine countries were welcomed to the AECL facilities by L. G. Elliot, Director of Research, and A. M. Marko, Director of Biology and Health Physics. The conference dealt with the genetics, cytology, and biochemistry of the yeast cell. The theme of the conference encompassed studies of the mitotic cell cycle, intermediary and macromolecular metabolism including mutagenesis, cytoplasmic inheritance, cell fusion, meiosis, spore formation and genetic recombination.

The Mitotic Cell Cycle

Cytological properties of mitosis in several yeasts were reported by McCully and Robinow. Nuclear division in the mitosis of Schizosaccharomyces pombe and Saccharomyces cerevisiae proceeds without breakdown of the nuclear membrane. Mitosis involves centriolar plaque formation, development of an intranuclear spindle, and migration of a portion of the nuclear material into the daughter bud. In contrast, the nuclear division of Leucosporidium scottii and Sporobolomyces occurs after migration of the maternal nucleus into the daughter bud and is complete when one of the nuclei returns to the mother cell. Culotti and Hartwell reported the isolation of temperature-sensitive mutants of S. cerevisiae which are blocked in functions required for cell division cycle (cdc) events; cdc mutants in 15 complementation groups

controlling the initiation of DNA synthesis, DNA polymerization, bud initiation, nuclear division, and cell separation were described. In studies of enzyme function in cells at different stages of the cell division cycle Carter, Sebastian, and Halvorson found that arginase activity precedes ornithine transaminase activity in populations grown under conditions of both induction and non-induction. They concluded that periodic enzyme synthesis during the cell cycle was not due to periodic induction and repression.

Abbondandalo reported that X-ray induced mutation during the mitotic cell cycle of S. pombe is more efficient in G1 than in G2, whereas, nitrous acid and ultraviolet light-induced mutants are recovered from G1 and G2 cells with equal frequency. Experiments on aging of single vegetative cells is isogenic strains of increasing ploidy and size, (2n, 3n, 4n, 6n), indicate that cell size is positively correlated with the number of buds that can be produced only up to 4n (Müller).

Intermediary Metabolism

Various aspects of the genetic and physiological control of amino acid, purine, pyrimidine and carbohydrate metabolism were discussed. De Robichon-Szulmajster, Cherest and Surdin-Kerjan reported that homoserine O-transacetylase, homocysteine synthetase and ATP sulfurylase are encoded by three unlinked structural genes and respond to coordinate repression by methionine, as well as to a regulatory gene eth 2, and ts 296 of McLaughlin and Hartwell which affects methionyl tRNA synthetase. Kahn and Haynes reported studies of maltase and alpha-methyl-glucosidase production in yeast, which suggest a common element in the regulation of these enzymes. Both dominant and recessive non-repressible mutants for enzymes of the pathway of isoleucine-valine synthesis were described by Magee as well as preliminary mapping of the mutants involved. Bussey reported the isolation of trifluoroleucine-resistant variants which are altered in leucine uptake. Meuris has observed that there are two iso-functional enzymes for the first step of aromatic compound biosynthesis (DAHP synthetase). The enzymes differ in their sensitivities to feedback inhibition by tyrosine and phenylalanine. Mutations of the structural gene for threonine dehydratase (ilv 1) have been characterized by Zimmerman and Theuriot. Genetic and biochemical studies indicate that the enzyme (180,000 M.W.) is a multimer of 4 identical subunits (50,000 M.W.). Fink and Shaffer reported that the his 4 gene product which possesses cyclohydrolase (his 4A), pyrophosphorylase (his 4B), and histidinol dehydrogenase (his 4C) activities is a complex which is dissociable into two protein subunits, and studies of nonsense mutants of the his 4C region indicate that one member of the complex has both 4A and 4B activities while the other possesses 4C activity.

Recent progress in our understanding of the regulation of purine and pyrimidine metabolism comes from studies done both in S. cerevisiae and in S. pombe. The ade 4 locus of S. pombe is the structural gene for PRPP amidotransferase, the first enzyme of purine biosynthesis (Heslot and Nagy). The enzyme is feedback inhibited by IMP and GMP; azaguanine resistant mutants of ade 4 and mutations of the structural gene for S-Amp synthetase display altered PRPP amidotransferase feedback inhibition. Purine-excreting mutations of S. cerevisiae map at six unlinked loci (pur 1-6); pur 6, su-pur (suppressor of purine excretion) and dap (sensitivity to 2-6 diaminopurine) are allelic to adenine 4 and all affect the activity of hypoxanthine: guaninephosphoribosyltransferase (Woods). Kaplan, Lue, Satyanarayana and Messmer presented evidence that the first two

enzymes of the pyrimidine pathway of S. cerevisiae, carbamyl phosphate synthesis, and aspartate transcarbamylase are joined in a multifunctional enzyme aggregate. Both activities are controlled by the ura 2 locus and are stimulated by end products of the purine pathway. In contrast the CPase and OTCase activities of the arginine pathway are not complexed. Denis-Duphil and Lacroute have mapped mutants of the ura 2 locus which codes for the ACTase-CPase complex of the pyrimidine pathway. Three different classes of mutants have been receovered. Group A mutants are deficient in ATCase activity, group B mutants are deficient in both activities of the complex and group C mutants are deficient in CPase activity. Fine structure mapping by meiotic recombination and X-ray induced recombination in mitotic cells reveals that group A mutants are clustered at one end of the ura 2 region while group B and group C mutants are intermingled throughout the remainder of the ura 2 region. While several examples of linkage of genes coding for enzymes in the same pathway of synthesis have been reported, as yet no evidence has been obtained that these cistrons are organized for transcription in the manner of the classical operon of Jacob and Monod.

To obtain genetic defects in oxidative phosphorylation Matton and Parker have isolated mutants which respire but grow poorly on non-fermentable carbon sources; 12 cistrons have been identified to date. Ball has compared the respiratory activity of different yeast strains grown in a variety of carbon sources. Parks reported that the synthesis of ergosterol and cytochrome oxidase are correlated with the development of the respiratory activity and that ergosterol bound to cytochrome oxidase cannot be removed without loss of activity of this enzyme. Woods, Ahmed, Molzahn, and Miller have characterized three nystatin resistant mutants (nys 1, nys 2 and nys 3) with respect to their sterol content and find that nys 2 and nys 3 lack ergosterol and 24 (28) dehydroergosterol while nys 1 contains a new sterol. Birnboim reported the results of a survey of methods for lysis of S. pombe which indicated that deoxyglucose grown cells are more sensitive to glusulase treatment.

Macromolecule Synthesis and Metabolism

Studies of the regulation of protein synthesis, RNA metabolism and DNA synthesis and repair were reviewed by several investigators. McLaughlin reported that both the cytoplasm and the mitochondria possess two distinct methionine tRNAs: in each case one of these tRNAs is formylatable. Ts 296 (Hartwell) is deficient in the cytoplasmic methionine tRNA synthetase activity. RNA metabolism has been examined in ten different complementing temperature-sensitive mutants deficient in RNA synthesis; the synthesis of 17s, 25s, and 5.8s RNA is inhibited in these mutants while synthesis of 4s (tRNA) and messenger RNA proceed. These results suggest a coordinate regulation of the synthesis of 35s RNA derivatives and 5s RNA (Warner and Udem). Ito presented evidence that killing following illumination of acridine-treated yeast (photodynamic action) may reflect damage to RNA containing structures.

DNA metabolism seems to be an order of magnitude more complex in yeast than in E. coli; this derives more from genetic rather than biochemical evidence. The genetic evidence for complexity of DNA metabolism is inferred from the presence of a large number of genes conferring radiation-sensitivity. A minimum of 23 different genes (estimated to be about half of them) affect UV sensitivity. Likewise, many different genes affect X-ray sensitivity (independently from UV

sensitivity). Cox presented evidence that at least five different genes affecting UV sensitivity control exision (resembling her strains in E. coli). Several X-ray sensitive mutants lower UV-induced mitotic recombination. Unrau has demonstrated dimer excision using chromatographic techniques. W. Laskowski, working with his own mutants, also described excision deficiency and post-replication repair processes. Nasim has isolated mutants in Schizosaccharomyces sensitive to both UV and elevated temperature. Lemontt selected mutants defective in UV-induced mutability; twenty mutants isolated were at three loci only, all of which lower mitotic recombination frequencies. The ade 3 locus, which does not confer radiation sensitivity, also affects recombination frequencies (Henaut and Luzzati).

Brendel and Haynes also examined excision deficiency in radiation-sensitive strains, and demonstrated the striking enhancement of sensitivity by double mutants. Kilbey and Brown isolated supersensitive mutants from strains already sensitive to UV; some of the new mutants were radiation-sensitive mutants. To obtain information on the biochemistry of genetic recombination and radiation sensitivity Pinon has initiated a study of yeast nucleases. Nucleases specific for single- and double- stranded DNA and enzymatic activities distinguished by pH, response to ATP and Mg^{++}, were reported.

Resnick and Holliday have investigated the effects of UV on transcription and translation of nitrate reductase in wildtype and radiation sensitive strains of Ustilago maydis. UV inhibits synthesis of the enzyme in both the wildtype and radiation sensitive mutant and alters the heat stability and antigenic properties of the enzyme made after irradiation of the UV sensitive strain.

Mutagen specificity has been studied by both genetic and biochemical techniques. Using genetics tests, Magni has observed that 2-amino purine and meiosis induce a specific response whereas most other mutagens, including nitrosoguanidine, ethylmethane sulfonate, ICR-170, nitrous acid, X-rays, and UV, do not. By amino acid replacement analysis of cytochrome c, Sherman and Stewart showed that ethylmethane sulfonate, diethyl sulfonate, nitrosoguanidine, methylmethane sulfonate, nitrous acid, UV and X-ray radiation are not specific. Both Magni and Sherman presented evidence that X-rays induce a high yield of base deletions. Brusick presented his data showing that ICR-170 induces many mutations revertible by ICR-170 but not by ethylmethane sulfonate or by nitrosoguanidine.

James has observed that diploids homozygous for a single genetic defect conferring lethal sectoring yield a high frequency of revertants following meiosis. Tetrad analysis has shown that these revertants arise from tetrads in which two spores fail to survive and two give rise to ascosporal clones which no longer display lethal sectoring. It was suggested that lethal sectoring is the result of loss or damage of linked redundant genes and that meiotic reversion reflects unequal crossing over at the two strand stage. Loprieno demonstrated that many radiation-sensitive mutants and DNA-replication-defective strains of Schizosaccharomyces are mutators. Von Borstel, Cain and Steinberg reported that some radiation-sensitive mutants of Saccharomyces are addition-deletion mutators and that some cytoplasmic petite mutants are base-substituation mutators. Moustacchi has found nuclear mutants that enhance sensitivity for UV induction of cytoplasmic petites. She also described a new class of mutants specifically UV-sensitive to petite production and suggested a partially specific repair machinery for mitochondrial damage.

Cytoplasmic Inheritance

Coen and Netter reported their data and work of Bolotin, Deutsch, Dujon, Petrochilo and Slonimski on genetics of cytoplasmically inherited drug resistance in Saccharomyces. The transmission of these characters and the properties of spontaneous and ultraviolet light stimulated recombination of C^R (chloramphenicol resistance) and E^R (erythromycin resistance) have been examined. Stable recombinants (i.e. C^S E^S and C^R E^R) have been obtained from matings between mitochondrial mutants of different genotypes (i.e. C^S E^R and C^R E^S). In certain crosses both recombinants are recovered with equal frequency, whereas, in others they are recovered disproportionately. Studies of this phenomenon has led to the conclusion that the frequency of recombination and polarity with respect to recombinant classes recovered is governed by a mitochondrial property (mitochondrial sex). Recombination is more frequent and polarity is found in heterosexual crosses, whereas recombination is less frequent and unaccompanied by polarity in homosexual crosses. Mitochondrial sexuality is transmitted cytoplasmically and is independent of cellular mating type.

Lacroute has obtained both chromosomal and cytoplasmic mutants which permit uptake of ureidosuccinic acid and growth of ura-2 mutants in the presence of ureidosuccinic acid and absence of uracil. The cytoplasmic mutation is transmitted to nearly all diploid progeny in matings and is conserved in ethidium bromide induced strains. Nagai reported growth conditions which enhanced the recovery of spontaneous ρ^- petites. Breeding studies by Rank involving ρ^- petites of varying degrees of suppressivity and the wild type ρ^+ may be interpreted in terms of replicative superiority of highly suppressive ρ^- strains. Johnson has employed a UV microbeam to irradiate portions of individual yeast cells and has observed that petites may be induced by irradiation of buds lacking nuclei as well as by irradiation of the nuclear region of individual cells.

Somers presented genetic data indicating that the killer character in Saccharomyces is under the control of two types of extra chromosomal determinants (k and n); cells bearing k release an unstable protein which kills sensitive cells, and cells bearing n are insensitive to the killer factor, while sensitive cells harbor neither particle. The presence of the dominant allele of the nuclear gene Ps is required for the maintenance and/or expression of k, n, and ρ. Presence of the dominant nuclear gene M is required for the maintenance of k and n but not ρ. Mutants of uracil-3 also tend to lose k particles during growth. Bevan and Barry presented preliminary evidence that k and n bearing cells harbor a double-stranded RNA species not found in sensitive individuals.

The characterization of cytoplasmic mutants which affect the expression of nonsense suppressors in S. cerevisiae was summarized by Cox.

Cell Fusion

The fusion of two cells of opposite mating type is a complex process involving several components. MacKay and Manney reported the isolation of mutants that do not produce a diffusible sex factor. The diffusible sex factor is produced by cells of the α-mating type, affects cell morphology by giving them a "zygote-like" appearance, and blocks division of cells of the a-mating type. Duntze has enriched and partially purified this factor, finding that it is an oligopeptide of a molecular weight of about 1500. Yanagishima and Shimoda further report that cells of mating types a and α also produce mating-type specific hormones of a steroidal nature, as well as the nonsteroidal

factor reported by MacKay and Manney. They report that the steroidal factor induces the swelling of cells that occurs prior to cell fusion.

Meiosis and Ascospore Development

Moens and Rapport described the fine structure of meiosis and ascospore development in Saccharomyces as revealed by electron microscopy. They find that the nuclear membrane remains intact during two meiotic divisions each of which involves centriolar plaque duplication and formation of a microtubule spindle apparatus. M. S. Esposito, R. E. Esposito, Arnaud and Halvorson reported that three temperature-sensitive sporulation deficient mutants (spo 1, spo 2 and spo 3) are blocked in meiosis. They determined by temperature shifts that the functions specified by the spo genes are expressed at different times and for periods of varying length during meiosis and sporulation. Magni reported that sporulating cultures require oxygen for only a short portion of the cycle and that it is possible to block sporulation by exposure of cultures to erythromycin.

Recombination

There are three current problems in the domain of genetic recombination that received attention at the conference: these are marker effects, map expansion, and gene conversion. Differences in gene conversion frequencies, and recombination distances which appear to be due to inherited differences between alleles are termed marker effects. Map expansion is a property of intragenic meiotic maps based upon prototroph frequency, where it is observed that the sum of adjacent short intervals is less than the frequencies measured for the most distant of the alleles. Gene conversion refers to heteroallelic recombination and aberrant segregation in heterozygous diploids.

Gutz introduced the discussion of marker effects in intragenic recombination in S. pombe by describing departures from expected recombination frequencies of heteroallelic diploids involving ade 6-M26. Sherman noted that frame-shift mutations give a marker effect, and Leupold noted that transversions of bases gives a strong marker effect and transitions give a weaker effect. Leupold has performed fine structure mapping of nonsense suppressors in S. pombe; mutations at the site of the anticodon exhibit strong marker effects upon heteroallelic recombination and maps of the nonsense suppressors differ significantly in their lengths as well as in the degree of map expansion which they demonstrate. Holliday pointed out that mismatched bases could yield map expansion; he and Fincham have used mismatched base-pairs as a basis for a theory to account for map expansion.

Hurst, Fogel and Mortimer described their experiments on gene conversion; the closer together two alleles are in a gene, the greater the likelihood they will convert together. Gene conversion displays fidelity; while it leads to aberrant segregation it does not generate new alleles. Approximately 50% of conversions are coincident with reciprocal recombination of flanking markers in meiosis. Crossing over associated with conversion leads to interference while conversion not associated with crossing over has no interference. Roman presented evidence that UV-induced mitotic conversion need not be associated with outside marker recombination. Snow reported a fine structure mapping technique based upon the dosage response of heteroallelic recombination to ethylmethane sulfonate.

In conclusion, we wish to acknowledge the gracious hospitality of our hosts, Atomic Energy of Canada, Ltd. and the University of Ottawa, and would like to thank Allen P. James and J. G. Kaplan,

co-chairman of the conference, for their splendid handling of the arrangements. The physical beauty of the Canadian countryside and the sociability of the informal evening gatherings which followed each day's program contributed to making this meeting scientifically useful and pleasant.

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XXXIII. Brief News Items

1. I would like to announce to all the friends whom I met during my research career that I have retired from research since last April. My present address is: Rua Passos Manuel 64-10, Lisboa 1, Portugal.

   Lidia do Carmo-Sousa

   The Editor extends his best wishes for Dr. do Carmo-Sousa's future work.

2. Miss Wilhelmina Slooff has informed the Editor that she has retired from the Yeast Division of the C.B.S. at Delft, the Netherlands. She sends greetings and a farewell to colleagues and friends and thanks them most cordially for their friendly cooperation. Your Editor extends to Miss Slooff our warmest wishes for a happy and relaxed period ahead.

   Miss Maud Smith, a graduate from Leiden University, has joined the Yeast Division in her place.

3. Yeast Genetics Stock Center, Donner Laboratory, University of California, Berkeley, California 95720.

   We are pleased to announce the establishment of a Yeast Genetics Stock Center supported by the National Science Foundation. We will maintain and make available stocks representing most of the commonly studied genetic loci in Saccharomyces cerevisiae. Additionally, wild type, polyploid, and other special stocks will be available. The stock list, currently under preparation, will be available upon request and will also be published in a future issue of the Yeast News Letter. We invite correspondence concerning the deposition of stocks that you believe should be available through the stock center. We expect investigators will continue to maintain stocks pertaining directly to their own research and will deposit only those stocks that are of general utility.

   S. Fogel, R. Mortimer and J. Bassel

4. The work in the laboratory of H. J. Phaff, University of California at Davis, is continuing. Dr. A. Martini has returned to the University of Perugia, Italy after a two-year stay in our laboratory. Dr. Steven Douglas (Ph.D. from the Biochemistry Department at Davis) and Dr. Leda Christina Mendonça (from the Institute of Microbiology, Rio de Janeiro, Brazil) have joined our group and they are continuing the work on DNA base composition and homology of yeasts. Mr. Graham Fleet, graduate student from Australia, is completing his work on lytic enzymes of Schizosaccharomyces and on the cell wall composition of species of that genus. A more detailed progress report will be communicated in the Spring issue of the Yeast News Letter.

5. Dr. James A. Barnett reports a change of address: School of Biological Sciences, University of East Anglia, Norwich, NOR 88C, England. Home address is the same: 365 Unthank Road, Norwich [Telephone

Norwich (0603) 56673].

6. Pichia besseyi sp. n. by C. P. Kurtzman and L. J. Wickerham. Publication scheduled for Antonie van Leeuwenhoek, Vol. 38, No. 1 (March 1972). Pichia besseyi was isolated from a marsh in Quebec, Canada. The hemispheroidal shape of the ascospores makes this species particularly noteworthy. Aside from spore shape, P. besseyi somewhat resembles P. delftensis and P. fluxuum.

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7. The following two articles have appeared recently:
N. J. W. Kreger-van Rij and M. Veenhuis
Some features of yeasts of the genus Sporidiobolus observed by electron microscopy. Antonie van Leeuwenhoek, 37:253-255, 1971.
N. J. W. Kreger-van Rij and M. Veenhuis
A comparative study of the cell wall structure of basidiomycetous and related yeasts. J. Gen. Microbiol., 68:87-95, 1971.

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8. The article listed below will soon be published.
W. A. Maxwell and Edward Spoerl. 1971. Iodoacetic Acid Induced Changes in Saccharomyces cerevisiae. Cytobiologie Zeitschrift für experimentelle Zellforschung (in press).
Some of this information was presented at the Cell Biology meetings in San Diego, November 20, 1970, and an abstract of this work was printed in the Yeast News Letter, Volume XVIX No. 2, January 1971, p. 54.

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9. The following publications have recently been published:
1. Tewari, R. P. and Macpherson, C. R. (1971). A new dimorphic fungus, Oidiodendron kalrai: morphological and biochemical characteristics. Mycologia, 63:602-611.
2. Tewari, R. P. and Macpherson, C. R. (1971). Suppressive effect of streptomycin on the phagocytic activity of mouse peritoneal macrophages for Histoplasma capsulatum. Mycopath. et Mycol. Appl. 44:231-240.

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10. The following publication has appeared recently: "Oleanoic acid, factor for anaerobic growth of wine yeast". A note by P. Bréchot, J. Chauvet, P. Dupuy, M. Croson and A. Rabatu. Comp. Rend. Acad. Sci. (Paris) 272, 890-93, 1971. Series D. This note concerns itself with oleanoic acid (a derivative of ergosterol). This acid was

extracted from the bloom of grapes and it can act as a growth factor required for anaerobic growth of Sacch. cerevisiae var. ellipsoideus.

11. The following publication has appeared in Mycopathologia et Mycologia applicata: "Rapid Diagnosis of Candida albicans" by Carlos Ramírez, Francisco Lanzuela and Domíngo Rodriguez, Instituto "Jaime Ferrán" de Microbiologia, Joaquin Costa 32, Madrid 6, Spain.
The method depends on the inhibitory action of an antibiotic isolated from B. subtilis to which C. albicans is sensitive but related species are not.