YEAST

A Newsletter for Persons Interested in Yeast

Official Publication of the
International Commission on Yeasts and Yeasts-like Microorganisms
of the International Association of Microbiological Societies (IAMS)

June 1979 Volume XXVIII, Number 1

Herman J. Phaff, Editor
University of California, Davis, California 95616

****

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

The strains listed below have been added to the ATCC since October 17, 1978. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

Candida valida ATCC 36897	B. Kirsop, NCYC Surrey, England
Debaryomyces vanriji ATCC 36898	"
Saccharomyces capensis ATCC 36899	"
Saccharomyces cerevisiae ATCC 36900-36901	"
Saccharomyces diastaticus ATCC 36902	"
Hansenula anomala ATCC 39603-36904	"
Hansenula subpelliculosa ATCC 36905	"
Kluyveromyces drosophilarum ATCC 36906	"
Kluyveromyces fragilis ATCC 36907	"
Pichia membranaefaciens ATCC 36908	"
Torulopsis glabrata ATCC 36909	"
Kluyveromyces lactis ATCC 36940-36944, chloramphenicol and oligomycin resistant mutant	A. Brunner U.N.A.M. Inst. de Biologia Mexico
Saccharomyces acidifaciens ATCC 36946 agent of food spoilage	E. Magno Best Food Research Center Union, New Jersey
Saccharomyces bailii ATCC 36947 agent of food spoilage	"
Candida humicola ATCC 36992 hydrolyzes L-aminolactam	T. Fukumura Osaka City University Japan

Trichosporon cutaneum ATCC 36993 hydrolyzes L-aminolactam	T. Fukumura Osaka City University Japan
Rhodotorula rubra ATCC 36994 hydrolytic activity	Y. Yamaguchi Takasago Perfumery Co. Japan
Hansenula anomala ATCC 36995 hydrolytic activity	"
Coccidioides immitis ATCC 38142-38150 human pathogen	M. Huppert Veterans Administration Hospital Long Beach, California
Saccharomyces cerevisiae ATCC 38198 mode of action of tryptophan analgin	P. Niedeberger ETH-Zentrum Switzerland
Saccharomyces cerevisiae ATCC 38202 genetic studies	B. J. Barclay York University Ontario, Canada
Candida sake ATCC 38233 isolated from diseased fish kidney	K. Hatai Sankyo Company Tokyo, Japan
Candida tropicalis ATCC 38234 isolated from diseased fish kidney	"
Candida albicans ATCC 38245-38248 mutant resistant to polyene antibiotics	A. M. Pierce Simon Fraser University B.C., Canada
Candida boidinii ATCC 38256-38257	W. J. Middelhoven Agricult. University The Netherlands
Candida albicans ATCC 38289, human isolate	V. Hopsu-Havu University Turku Finland
Candida guilliermondii ATCC 38290, human isolate	"
Candida parapsilosis ATCC 38291, human isolate	"
Candida tropicalis ATCC 38292, human isolate	"
Candida krusei ATCC 38293, human isolate	"

Candida humicola ATCC 38294, human isolate	V. Hopsu-Havu University Turku Finland
Candida pseudotropicalis ATCC 38296, human isolate	"
Endomycopsis lipolytica ATCC 38295, human isolate	"
Cryoptococcus albidus ATCC 38297, human isolate	"
Cryptococcus uniguttulatus ATCC 38298, human isolate	"
Rhodotorula rubra ATCC 38299, human isolate	"
Trichosporon cutaneum ATCC 38300, human isolate	"
Kluyveromyces lactis ATCC 38322, human isolate	"
Kloeckera apiculata ATCC 38323, human isolate	"
Saccharomyces cerevisiae ATCC 38324, human isolate	"
Debaryomyces hansenii ATCC 38325, human isolate	"
Torulopsis glabrata ATCC 38326, human isolate	"
Schizosaccharomyces pombe ATCC 38364-38440 genetic studies	P. Munz University of Bern Switzerland
Candida albicans ATCC 38483 human pathogen	F. B. Greatorex Public Health Lab. Somerset, U.K.
Saccharomyces rouxii ATCC 38528 xerotolerant	M. Edgley Univ. Wollongong N.S. Wales, Australia
Saccharomyces cerevisiae ATCC 38531, baker's yeast	"
Cryptococcus cereanus ATCC 38532	D. Yarrow CBS The Netherlands

Candida lusitaniae ATCC 38533 isolated from citrus peel juice	D. Yarrow CBS The Netherlands
Saccharomyces cerevisiae ATCC 38451-38463, 38513-38527, 38530 genetic studies	R. B. Wickner NIH Bethesda, Maryland

II. Research Institute of Fermentation, Yamanashi University, Kitashin, 1-13 -1, Kofu, 400, Japan. Communicated by Shoji Goto.

Shoji Goto: "A new yeast species, Candida acutus, isolated from sulfited grape must". J. GEN. APPL. MICROBIOL., 25 (3), 1979 (in press).

A new yeast species, Candida acutus, was isolated from sulfited grape must. This species was characterized by the morphological properties of the formation of septated or short mycelical cells and budding cells from tapering base on lateral of cells in ring or pellicle on YM broth and the biochemical properties of a high GC content (54.7%), positive urease and DNase activities, and the coenzyme Q_9 system.

III. Laboratory for Medical Microbiology R.U., Oostersingel 59, Groningen, The Netherlands. Communicated by N. J. W. Kreger-van Rij.

Dr. Lodder and I have made a formal proposal for the conservation of the genus name Debaryomyces Lodder et Kreger-van Rij (TAXON, 27:306-307, 1978). After the appearance of the proposal, van der Walt and Johannsen (PERSOONIA, 10:146-148, 1978) have described a new name Debaryozyma, replacing Debaryomyces. However, Recommendation 15A of the International Code of Botanical Nomenclature (1972) states that botanists should follow existing nomenclature as far as possible pending the General Committee's recommendation on the proposal. It means that the name Debaryomyces is still in use.

The following is a recent publication from this laboratory:

N. J. W. Kreger-van Rij, A comparative ultrastructural study of the ascospores of some Saccharomyces and Kluyveromyces species. ARCH. MICROBIOL. 121:53-59, 1979.

IV. Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pushchino, Moscow Region 142 292 USSR. Communicated by W. I. Golubev.

Below follows the abstract of a recent paper from our laboratory:

THE SIGNIFICANCE OF THE ABILITY TO ASSIMILATE D-GLUCURONIC ACID, D-GLUCONO-δ-LACTONE AND 5-KETO-D-GLUCONATE IN IDENTIFICATION OF SPECIES OF CANDIDA BERKHOUT

W. I. Golubev and V. M. Blagodatskaya

MIKROBIOLOGIA 47:841-848 (1978)

117 Candida strains representing 76 species and varieties were studied. Most of them readily assimilated glucono-δ-lactone with the exception of species which were imperfect analogues of Pichia species with round ascospores, Saccharomyces species and Kluyveromyces species. The test for assimilation of 5-keto-D-gluconate can be used to differentiate varieties of C. guilliermondii, C. pelliculosa, and the species C. zeylanoides and C. vinaria. The group of glucuronate-positive species belonging to the genus Candida comprises both ascomycetous and basidiomycetous organisms. The former lack urease activity. Among the latter, C. curvata, C. humicola, C. marina, and C. podzolica produce extracellular polysaccharides identical in the monosaccharide composition to polysaccharides of cryptococci. These four species should be included into the genus Cryptococcus.

Orlova, S. A., Gulevskaya, S. A., Golubev, W. I., Fichte, B. A., 1978. Cytological aspects of life cycle of pedogamic yeasts Nadsonia Sydow. In "Experimental study of development of microorganisms", Pushchino, 149-154.

Golubev, W. I., Manukian, A. R., 1979. Capsule formation in saprophytic yeast. MIKROBIOLOGIA, 48, N 2, 314-318.

V. United States Department of Agriculture, Northeastern Region, Beltsville Agricultural Research Center, Beltsville, Maryland 20705. Communicated by L. R. Batra.

Below follows the announcement of a work recently edited by me. It should be on the market by the end of this month. It discusses some aspects of yeasts that serve as ambrosia fungi.

TAXONOMY AND SYSTEMATICS OF THE HEMIASCOMYCETES (HEMIASCOMYCETIDAE)

Lekh R. Batra

Reprinted from: Subramanian, C. V. (editor) 1978. Taxonomy of Fungi. Proc. Int. Symp. Taxonomy of Fungi, Univ. Madras, held in 1973, pp. 187-214, Fig. 1-43 and Pl. I.

ABSTRACT

The comparative morphology, ecology, life cycle, and nutrition of several Hemiascomycetes have been investigated during the past 15 years. Ultrastructure and conidial ontogeny of several seemingly related species have revealed fundamental differences among them. These developments coupled with the discovery of many new forms found in mutualistic symbiosis with insects necessitates a revision of the existing classification of the Hemiascomycetes.

The objectives of this paper are: (1) to present information on the comparative morphology and life history of selected Hemiascomycetes, particularly those described during the past 10 years; (2) to evaluate criteria used to distinguish species, genera, and supergeneric taxa; and (3) to present a classification of the group with a key to the recognized genera.

The Hemiascomycetes are presumed to have their antecedents in the phycomycetous fungi rather than in the red algae. Spermophthora gossypii Ashby & Nowell, a merogamous and diplobiontic species, is considered to be a primitive member of the group. With few exceptions, the remaining species are haplobiontic. To date, the paucity of fungus fossil records has impeded an evaluation of the degree of proximity between seemingly related taxa. In addition, the technology enabling comparisons of DNA nucleotide composition has not been used extensively to determine the degree of genetic homology necessary for proposing a phylogenetic classification. Thus, the definition of hemiascomycete taxa, like most other groups of fungi, is dependent upon a critical evaluation of their developmental, morphological, and ecological characteristics. Conidial ontogeny, gametangial or gametic development and their ensuing fusion, the origin of the ascus and its wall characteristics, and the nature of agents or mechanisms involved in the dissemination of ascospores are used in delineating supergeneric taxa. Generic and infrageneric taxa are distinguished on the basis of characteristics of the thallus, including ultrastructure, ascus, conidium, and ascospore morphology, and nutrition and habitat of the species.

Five orders, including the doubtful Taphrinales, are recognized for the Hemiascomycetes: SPERMOPHTHORALES (monotypic)--merogamous, diplobiontic, the two thalli filamentous; DIPODASCALES (Dipodascaceae, Eremascaceae, and Hemiascosporiaceae)--haplobiontic, thalli haploid or rarely diploid, filamentous or non-filamentous, asexual reproduction by arthroconidia or lacking, sexual reproduction by gametangial copulation; CEPHALOASCALES (monotypic)--haplobiontic but with a characteristic multicellular, uninucleate, diploid, ascus-bearing stalk derived from a conjugant cell, asexual reproduction by blastoconidia, sexual reproduction gametangiogamous; ASCOIDEALES (Ascoideaceae, Nematosporaceae, and Saccharomycetaceae)--haplobiontic or diplobiontic, filamentous or nonfilamentous, asexual reproduction by thin- or thick-walled blastoconidia, sexual reproduction by ascosporic or somatic copulation, gametangia occasionally present; TAPHRINALES (monotypic)--haplobiontic but with a characteristic dicaryotic, parasitic mycelium, asexual reproduction by blastoconidia, sexual reproduction by somatogamous copulation.

* * *

Reprints of this article are available to readers of the Yeast Newsletter upon request.

VI. United States Department of Agriculture, Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604. Communicated by C. P. Kurtzman.

The following are abstracts of two brief articles to appear in MYCOLOGIA, 1979:

Kurtzman, C. P., and M. J. Smiley. Taxonomy of Pichia carsonii and its synonyms P. vini and P. vini var. melibiosi: Comparison by DNA reassociation.

Ascospores of extant strains of P. vini and P. vini var. melibiosi were examined by scanning electron microscopy, but the spores from both varieties were smooth to slightly wrinkled and indistinguishable from

one another. Single-spore isolates from four-spored asci produced sporogenous colonies, which indicated all strains to be homothallic. Attempts to clarify relatedness between the varieties through mating tests using auxotrophic mutants were inconclusive because of low mating frequency and a high rate of reversion. Consequently, we examined relatedness between the varieties and one apparent synonym by DNA reassociation experiments. DNA relatedness between type strains was as follows: P. vini + P. vini var. melibiosi = 93%; P. vini + P. carsonii = 99%; P. vini var. melibiosi + P. carsonii = 98%. These high relatedness values showed all strains to belong to the same species. Zimmermann failed to provide a Latin diagnosis when he described P. vini, and the valid name of the species now must be Pichia carsonii Phaff & Knapp.

Kurtzman, C. P., C. J. Johnson, and M. J. Smiley. Determination of conspecificity of Candida utilis and Hansenula jadinii through DNA reassociation.

Single-ascospore isolations suggested Hansenula jadinii and H. petersonii to be homothallic and, therefore, not easily compared by standard genetic tests. The relationship between these two species and Candida utilis was examined by comparing their DNA base sequence complementarity. The following DNA relatedness values were obtained: C. utilis + H. jadinii = 85%; C. utilis + H. petersonii = 4%; H. jadinii + H. petersonii = 5%. These data confirm the suspected conspecificity of C. utilis and H. jadinii and demonstrate H. petersonii to be a separate species.

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

The following papers have been published or are in press:

1. W. T. Starmer, H. J. Phaff, M. Miranda, M. W. Miller, and J. S. F. Barker (1979). Pichia opuntiae, a new heterothallic species of yeast found in decaying cladodes of Opuntia inermis and in necrotic tissue of cereoid cacti. INT. J. SYST. BACTERIOL. 29:159-167. (An abstract is given in the December 1978 issue of the Yeast Newsletter, XXVII, Number 2).

2. H. J. Phaff and M. A. Amerine (1979). Wine. Chapter 5 in MICROBIAL TECHNOLOGY, 2nd Edition, Vol. II, 131-153. Academic Press, New York (in press).

3. C. W. Price and H. J. Phaff (1979). Debaryomyces polymorphus and D. pseudopolymorphus, new taxonomic combinations. MYCOLOGIA 71, 444-445 (1979).

4. Donald L. Holzschu, Heather L. Presley, Mary Miranda, and Herman J. Phaff.

IDENTIFICATION OF CANDIDA LUSITANIAE AS AN OPPORTUNISTIC YEAST IN MAN

JOUR. CLIN. MICROBIOL. (accepted for publication, Aug. 1979)

ABSTRACT

Four yeast strains, causally associated with infection in a patient with acute myelogenous leukemia, were identified by standard methods currently used in yeast taxonomy as representatives of Candida lusitaniae van Uden et do Carmo-Sousa. Because this species has not been recognized previously as an opportunistic yeast in humans, molecular taxonomic methods were applied to confirm its identity. The nuclear deoxyribonucleic acid (DNA) base composition of two clinical isolates was shown to be 45.1 mol% guanine + cytosine (G+C) as compared to 44.7 mol% G+C for the type strain of this species. DNA/DNA reassociation experiments revealed more than 95% complementarity between the DNAs from the clinical isolates and that of the type strain of Candida lusitaniae, thus confirming their classification by conventional taxonomy. A key is provided to differentiate C. lusitaniae from two phenotypically similar Candida species.

VIII. University of East Anglia, School of Biological Sciences, Norwich NR4 7TJ, England. Communicated by James A. Barnett.

The following paper has been published:

Sims, A. P., and Barnett, J. A. (1978). The requirement of oxygen for the utilization of maltose, cellobiose, and D-galactose by certain anaerobically fermenting yeasts (Kluyver effect). JOURNAL OF GENERAL MICROBIOLOGY 106:277-288.

The following book is in the press:

Barnett, J. A., Payne, R. W., and Yarrow, D. (1979). A Guide to Identifying and Classifying Yeasts. Cambridge University Press. (About 350 pages)

This reference work is designed for those who need to identify yeasts or want special kinds of information about them. It is intended particularly for those who are not specialist yeast taxonomists.

There are 18 identification keys, made from information derived from the records of the Centraalbureau voor Schimmelcultures. These keys are as follows: for all yeasts, for yeasts which form ascospores, for yeasts using methanol or hydrocarbons, for clinical yeasts, for yeasts associated with foods, wine, or brewing. Some keys include microscopical and others only physiological characteristics. There is a simple account of how to identify yeasts.

One chapter is for checking the identity of any yeast with minimum trouble. For every yeast species, there are short lists of tests, distinguishing that species from the others.

There is an up-to-date list of all yeast species and a table of their physiological and microscopical characteristics. For each species, this table details what is known of its ability to utilize 34 organic compounds and ferment 11 sugars, the requirements for growth factors,

and the response to cycloheximide. It is the most comprehensive table of yeast characteristics ever published and should help people in industry or the laboratory select the right yeast with appropriate chemical activities.

The following items will help cope with the many recent changes in yeast names: a chapter explaining the principles of yeast classification, a table of generic characteristics, brief descriptions or explanations for 189 recently accepted species (with full references), a cross-referenced index of generic and specific names. There are 273 references.

Full use has been made of the latest methods of constructing keys and tables by computer, and these methods are described. In order to avoid errors, most of the book has been printed by computer-controlled typesetting.

IX. The University of Sydney, Sydney, N.S.W. 2006, Australia.
Communicated by A. M. Bersten.

Below follows the summary of a recently completed dissertation on the morphogenesis of Trigonopsis variabilis by Nicolle H. Packer in my laboratory. Two papers on the work in the dissertation have already been published in the JOURNAL OF GENERAL MICROBIOLOGY and a third and final one is in preparation.

SUMMARY

The morphogenesis of the triangular and ellipsoidal forms of the yeast Trigonopsis variabilis and the role that lipids may play in determining the shape of the cell was investigated.

No apparent ultrastructural differences were observed between the two types of cell when viewed by electron microscopy. Both cell shapes developed from a small spherical bud, and the determination of morphology depended on the external environment of the newly formed bud and not on the shape of the parent cell.

Growth of the cells in batch culture at 25°C with glucose as carbon source and methionine as nitrogen source produced triangular cells. The maximum number (82%) of triangular cells occurred in the mid-exponential phase of growth and was decreased by changing the carbon source, growth temperature, or aeration of the medium. An increase in redox potential of the medium coincided with the formation of the maximum number of triangular cells.

Continuous cultivation of the organism, in medium containing methionine as nitrogen source and with glucose as the limiting nutrient, differentiated between the effects of growth temperature and growth rate on morphology and enabled cultures consisting entirely of either triangular or ellipsoidal cells to be obtained without changing the nitrogen source. A growth rate of 0.075 h^{-1} at 25°C, with a concentration of 230 µM oxygen in the medium, produced 100% triangular cells. Changing any of these growth conditions resulted in the formation of ellipsoidal cells.

Growth in batch culture in medium containing ammonium sulphate as nitrogen source and glucose as carbon source produced ellipsoidal cells. Addition of the detergent Tween 80 (polyoxyethylene sorbitan mono-oleate) at a concentration of 1% (v/v) induced the formation of triangular cells (98%). The presence of the sorbitan-oleate moiety of the detergent was essential for the induction, since other Tween detergents had no effect on morphology and adding Span 80 (sorbitan mono-oleate) also produced triangular cells (67%). The phospholipids of the triangular cells grown with Tween 80 were enriched in $C_{18:1}$ fatty acid. Increasing the growth temperature, changing the carbon source, or adding short-chain (C_1 to C_3) alcohols to Tween 80-supplemented medium inhibited triangular cell formation. These growth conditions offered an alternative to the use of methionine as nitrogen source for obtaining completely triangular or ellipsoidal cell populations.

It has been suggested that methionine exerts its effect on the morphology of the cell by providing an excess of methyl groups for the increased synthesis of phosphatidyl choline in triangular cells. This postulate was supported by the data that only ellipsoidal cells were formed when homocysteine was used as nitrogen source for growth, and that the intracellular concentration of methionine increased at the time of maximum triangulation in medium containing methionine as nitrogen source. The methyl group of methionine was used in the synthesis of phosphatidyl choline, as shown by studies using (^{14}C-methyl) methionine, but the amount of phosphatidyl choline in the triangular cells was the same as that in the ellipsoidal cells grown to the same phase of growth in medium containing ammonium sulphate as nitrogen source.

An increased proportion of the methyl group of methionine which was incorporated into the cellular lipids was used for the synthesis of sterols during triangular cell formation in medium containing methionine as nitrogen source. The major, if not the only, sterol in Trig. variabilis was identified as ergosterol. No esterified sterols were detected. Triangular cells grown with methionine as nitrogen source contained more ergosterol than ellipsoidal cells grown with ammonium sulphate as nitrogen source. A role for sterols in determining the shape of the cell was further indicated by the prevention of the formation of triangular cells in both methionine and Tween 80-supplemented medium by the antibiotic homoazasterol, an inhibitor of methionine:Δ^{24}-sterol methyltransferase.

An attempt was made to discover whether lipids influence the determination of the shape of the cell wall by modifying the function of the underlying plasma membrane. The composition of the membrane lipids in the cell and the activity of some membrane-bound enzymes responsible for the synthesis of cell wall polysaccharides were compared in triangular and ellipsoidal cells obtained by growth in the presence of different morphological inducers. The ergosterol content, phospholipid fatty acid composition, and the activity of mannan synthetase varied widely between cells which were grown in the different cultural conditions. The extent of these metabolic changes in triangular cells obtained by growth with methionine as nitrogen source differed from that in triangular cells obtained by supplementing medium containing ammonium sulphate as nitrogen source with Tween 80. Similarly, the ellipsoidal cells obtained by growth in the various media exhibited different lipid profiles. Thus, contrary to the existing hypothesis, there appeared to be no correlation between the lipid composition and the shape of the cell.

X. Georgia State University, Department of Biology, Atlanta, Georgia 30303. Communicated by Donald G. Ahearn.

The following is a recently published paper:

Holzschu, D. L., F. W. Chandler, L. Ajello, and D. G. Ahearn. 1979. Evaluation of industrial yeasts for pathogenicity. SABOURAUDIA. 17:71-78.

ABSTRACT

Eleven yeasts representative of species of industrial interest were compared with Candida albicans for their potential pathogenicity for untreated and cortisone-treated mice. Only C. tropicalis produced a progressive infection similar to that produced by C. albicans. Candida lipolytica, Torulopsis species, and Hansenula polymorpha were not recovered from mice 6 days after inoculation. Kluyveromyces fragilis, C. pseudotropicalis, C. utilis, C. guilliermondii, and C. maltosa were recovered from mice but did not produce evidence of infection.

XI. The University of Connecticut, Marine Sciences Institute, Marine Research Laboratory, P.O. Box 278, Noank, Connecticut 06340. Communicated by John D. Buck.

Below follow the references of two recent articles from our laboratory:

Buck, J. D. 1978. Comparison of in situ and in vitro survival of Candida albicans in sea water. MICROBIOL. ECOL. 4:291-302.

Matson, E. A., S. G. Hornor, and J. D. Buck. 1979. Effect of upgrading a municipal waste water effluent on pollution indicator and other microorganisms in river water. ENVIRONM. SCI. TECHNOL. 13:460-465.

Our current yeast work is in two general areas, and I would appreciate greatly receiving any appropriate reprints or comments.

(1) Occurrence of yeasts, particularly human-associated species, with marine mammals. Candidiasis is being more frequently reported in these animals; and some basic studies are required on the ecology and survival of yeasts in aquariums, etc.

(2) Relationships between pathogenic yeasts and accepted bacterial indicators in sewage treatment processes and receiving waters.

Thank you for your cooperation.

XII. Biology Department, UCLA, Los Angeles, California 90024. Communicated by T. W. James.

Dominic Montisano and T. W. James. Mitochondrial Morphology in Yeast with and without Mitochondrial DNA. JOURNAL OF ULTRASTRUCTURE RESEARCH (in press, 1979).

We have examined the morphology of yeast mitochondria in Schizosaccharomyces pombe and a petite (rho^0) and wild type strain of Saccharomyces cerevisiae. Our major objective was to determine the effect of the

complete loss of mitochondrial DNA on the morphology of mitochondria in the strains of S. cerevisiae. In thin sections of wild type S. cerevisiae which contained a full complement of mitochondrial DNA, cristae were readily discernible; while in the petite mutant strain completely lacking mtDNA, normal cristae were totally absent despite the appearance of a well-preserved inner and outer mitochondrial membrane.

S. pombe was used as a test strain for developing a fixation procedure that would yield well-defined cristae. Several different fixation techniques were employed yielding a variety of results. Optimum results were obtained when protoplasts were fixed in glutaraldehyde alone for a period of less than one hour. In addition to allowing better fixation, the plastic infiltration of protoplasts became optimal and made thin sectioning feasible. The cells appeared to be well fixed displaying clearly defined organelles including nuclei, Golgi apparatus, and endoplasmic reticulum. The structure of the inner and outer mitochondrial membranes could be discerned as well as cristae. The use of this fixation procedure on S. cerevisiae yielded similar results except a Golgi apparatus was lacking. A careful comparison of the mitochondrial structure in S. pombe and the two strains of S. cerevisiae lead us to postulate the cristae membranes to be different from the inner and outer membranes.

B. Hyman and T. W. James. Visualization and Analysis of Mitochondrial DNA in Wickerhamia fluorescens.

Staining of the sporogenous yeast Wickerhamia fluorescens (Robinow, 1975) with the DNA specific fluorescent probe 4'6 Diamidino-2-phenylindole (DAPI) often reveals loop-like structures in the cytoplasm of some cells. We attribute these structures to the staining of mitochondrial DNA (mtDNA), which appear to be congruent with the mitochondria observed by Robinow (Robinow, 1975). Such loops are also observed in the ρ^+ state of Saccharomyces cerevisiae. In an effort to understand the nature of these structures, we have begun a physical characterization of the mtDNA from Wickerhamia.

The buoyant density of this mtDNA is 1.686, indicative of a rather high A+T content (73%). This base composition is also characteristic of Saccharomyces mtDNA. Restriction enzyme analysis yields a unit genome size with a molecular weight of approximately 35×10^6 (17 μm). Using the GC site specific enzymes, Hha I or Hae III, we find that this mitochondrial genome lacks the GC rich site clusters so prominent in Saccharomyces mtDNA.

Due to our inability to generate cytoplasmic petites by treatment with ethidium bromide, chloramphenicol, Hoechst H33258, or acriflavin, we tentatively categorize Wickerhamia as a petite negative yeast and propose that the small genome size of this mtDNA is characteristic of petite negative species. Yet, the high percent A+T content of this mtDNA is similar to S. cerevisiae, a petite positive yeast. This mtDNA might represent an interesting addition to the wide variations of mitochondrial genomes now being obtained with yeasts.

[1]Robinow, W. F. (1975). In: Methods in Cell Biology, XI, pp 1-22 (D. M. Prescott, ed.).

XIII. University of California, Bodega Marine Laboratory, Bodega Bay, California 94923. Communicated by Eric A. Johnson.

The following paper has been accepted for publication in the JOURNAL OF GENERAL MICROBIOLOGY:

ASTAXANTHIN FORMATION BY THE YEAST PHAFFIA RHODOZYMA

E. A. Johnson and M. J. Lewis

ABSTRACT

The production of carotenoid pigments by the yeast Phaffia rhodozyma depended upon the culture conditions. Astaxanthin, which is the primary carotenoid (xanthophyll) in this yeast, was produced mainly during the exponential phase of growth. The concentration of carotenes in P. rhodozyma remained relatively constant (about 5 μg g^{-1}) throughout growth in a 1.5% (w/v) glucose medium, but the xanthophyll concentration increased from 90 to 406 μg g^{-1} during the fermentation. Active xanthophyll synthesis occurred during the period of accelerating growth and after exhaustion of glucose from the growth medium. In media containing more than 1.5% glucose, however, yeast and carotenoid yields were considerably reduced. The pH of the medium affected yeast yields and carotenoid production; the optimum pH was 5.0. At pH 3.5, the carotene β-zeacarotene was found to accumulate in P. rhodozyma. β-Carotene was the primary carotene in the yeast under all other conditions tested. The optimum temperature for yeast growth and pigment formation was 20 to 22°C, and the best carbon source was cellobiose. Oxygen was an important substrate for optimum yields of yeast and astaxanthin; under microaerophilic growth conditions, astaxanthin production was drastically decreased and P. rhodozyma accumulated β-carotene and the monoketone echinenone.

XIV. Department of Food and Nutrition, Faculty of Science of Living, Osaka City University, Sugimoto-cho, Sumiyoshi-ku, Osaka 558. Communicated by Akira Misaki.

Below follows the abstract of a recent paper from our laboratory:

"Comparative Structural Studies on Acidic Heteropolysaccharides Isolated from "Shirokikurage", Fruit Body of Tremella fuciformis Berk, and the Growing Culture of Its Yeast-like Cells[1]"

Mariko Kakuta, Yoshiaki Sone, Tomiyo Umeda, and Akira Misaki[2]

AGRIC. BIOL. CHEM. 43 (in press) 1979

Acidic heteropolysaccharides, D-glucurono-D-xylo-D-mannans were isolated from the water and alkaline extracts of the fruit body of Tremella fuciformis Berk. The similar polysaccharides were isolated

---

[1]A part of this work was presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, 1974.

[2]To whom the request for reprints should be addressed.

from the growing culture of the haploid cells of two strains (T-19 and T-7) of T. fuciformis, when they were cultured in sucrose or glucose-yeast extract medium. The extracellular polysaccharides contain D-glucuronic acid, D-xylose, and D-mannose [molar ratios, 1.3:1.0:3.5 (T-7) and 0.8:1.0:2.1 (T-19)] and, in addition, small proportions of L-fucose and O-acetyl groups. Methylation and Smith degradation studies indicated that both fruit body and extracellular polysaccharides are built up of α-(1→3)-linked D-mannan back-bone chain to which β-linked D-glucuronic acid and single or short chains of β-(1→2)-linked D-xylose residues are attached at the C-2 positions. L-fucose residues in the extracellular polysaccharides may form the single branches. The structural features of these polysaccharides are discussed in comparison with similar polysaccharides from other fungi.

XV. Centro de Investigaciones en Fisiologia Celular, Universidad Nacional Autónoma de México, Apartado Postal 70-600, México 20, D.F. Communicated by Antonio Peña.

Below follow abstracts of three recent papers from this laboratory:

Antonio Peña. Effect of Ethidium Bromide on Ca^{2+} Uptake by Yeast. J. MEMBRANE BIOL. 42, 199-213 (1978).

SUMMARY

Ethidium bromide and other cationic dyes have been found to inhibit monovalent cation uptake. This dye also produces in a K^+-free medium an efflux of K^+ which could be of the electrogenic type.

The study of the effects of the same cationic dyes on Ca^{2+} uptake showed a large stimulation of the uptake rate of the divalent cation of more than tenfold.

The analysis of the effects of one of the cationic dyes on Ca^{2+} uptake indicated that the efflux of K^+ is of the electrogenic type and can drive the uptake of the divalent cation.

Kinetic data on Ca^{2+} uptake indicate that, both under "normal" or under stimulated conditions, the divalent cation is taken up by the same transport system.

The addition of ethidium bromide, besides, can stimulate the uptake of Mn^{2+} and ^{14}C-glycine and could be a good weapon to magnify and study some of the characteristics of ion transport systems in yeast.

* * *

Antonio Peña, Miguel A. Mora, and Nancy Carrasco. Uptake and Effects of Several Cationic Dyes on Yeast. J. MEMBRANE BIOL. 47, (1979), in press.

SUMMARY

Several cationic dyes were found to behave as inhibitors of K^+ uptake in yeast. When added at high concentrations or in a K^+-free medium, dyes can also produce an efflux of K^+. The dyes are taken up by

the cells in a process that, in different degrees, for several cations requires glucose and is inhibited to a higher degree by K^+ than by Na^+.

The inhibition of cation uptake is of the competitive type with ethidium bromide and close to this type with other dyes. CA^{2+} inhibits the uptake and effects of dyes and in some cases also seems to change the inhibition kinetics on Rb^+ uptake closer to a pure competitive type.

According to preliminary experiments, the efflux of K^+ seems to be of the electrogenic type, and not due to the disruption of the cells. The data indicate that, independently of the existence of other types of interaction (which do exist), dyes seem to interact with the system for monovalent cation uptake of yeast in different degrees of specificity and energy requirement. This interaction can be followed by fluorescence or metachromatic changes or reduction of the dyes as observed in the dual wavelength spectrophotometer and can be inhibited specifically by K^+, but not by Na^+.

* * *

A. Peña, S. M. Clemente, M. Borbolla, N. Carrasco, and S. Uribe. Multiple Interactions of Ethidium Bromide with Yeast Cells (submitted for publication).

SUMMARY

Experiments were carried out to determine the effects of different energy states of the yeast cell on the uptake of ethidium bromide (EB). By varying the substrate, oxygenation, and by the use of uncouplers of respiratory inhibitors, it is possible to have energization or not, of the whole cell, but also to deenergize specifically the mitochondria. The energy state of the whole cell can be determined by several means.

With this system, three kinds of interactions of EB with the cell can be detected. The first one is a binding to the cell that does not seem to require energy. A second interaction is represented by the uptake of the dye into the cell, which does require energy, and is accompanied by an increase of the fluorescence of EB. The third interaction that can be monitored seems to be the uptake or binding of the dye by the mitochondria of the yeast cell; it requires specifically the energization of this organelle and manifests itself as a quenching of the fluorescence.

The results are consistent with the hypothesis that the selectivity of EB for mitochondrial DNA can be partially explained by the ability of this organelle to concentrate the dye.

XVI. Department of Biochemistry, Yeast Study Group, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India. Communicated by J. Jayaraman.

1) BIOGENESIS OF MITOCHONDRIA IN SYNCHRONOUS CULTURES.

T. Somasundaram and J. Jayaraman.

Studies with synchronously growing populations of Saccharomyces cerevisiae have given the following results:

a) Mitochondrial protein synthesis is restricted to late S and early G_2 phases of the cell cycle.

b) The cytoplasmically-made components of cyt oxidase and ATPase are made earlier than their mitochondrial counterparts during the cell cycle. They accumulate in the cytoplasm till the mid-S-phase and only then transported to mitochondria.

c) Evidence is found that the cytoplasmic components of cytochrome oxidase are made as a large molecular weight precursor protein and transported to mitochondria where presumably the processing takes place.

2) ROLE OF CYCLIC AMP IN MITOCHONDRIOGENESIS.

C. Rajamanickam, T. Somasundaram, K. Chandrasekaran, and J. Jayaraman.

In continuation of our earlier work (FEBS letters), we have confirmed that mitochondrial protein synthesis is stimulated by cAMP. Addition of cAMP also prevents the release of mitochondrial components from the membrane by glucose. It has also been found that cAMP reduces glucose uptake by yeast cells by more than 50%. Thus, it is hypothesized that cAMP could counteract the repressive effects of glucose by (a) reducing the cellular concentration of glucose or its catabolites and (b) by stimulating mitochondrial protein synthesis.

XVII. Laboratory of Microbial Biochemistry, Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan. Communicated by Kenji Soda.

The following is the summary of our paper on lysine production by lysine analog-resistant mutants of yeast published recently:

Lysine Production by S-(β-Aminoethyl)-L-cysteine Resistant Mutants of Candida pelliculosa

Eiichi Takenouchi, Tatsuo Yamamoto, and Kenji Soda, The Laboratory of Microbial Biochemistry, Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan.

Hidehiko Tanaka, The Laboratory of Biochemistry, Kyoto College of Pharmacy, Kyoto 607, Japan.

Donka K. Nikolova, Department of Microbiology, Higher Institute of Food Industry, Plovdiv, Bulgaria.

S-(β-Aminoethyl)-L-cysteine (SAEC), a sulfur analog of L-lysine, significantly inhibited the growth of wild-type strains of Candida species. The growth inhibition of C. pelliculosa depended on SAEC concentrations, but L-lysine and L-α-aminoadipate restored growth effectively. SAEC-resistant mutants were induced from the wild-type strain of C. pelliculosa by ultraviolet irradiation and N-methyl-N'-nitro-N-nitrosoguanidine treatment. Almost all resistant mutants excreted some

L-lysine into the medium. Lysine excretion increased after repeated mutations. The mutant strain SR-V-1263 extracellularly produced about 2 mg/ml of L-lysine after shaking culture for 96 hr. The effect of various factors on lysine accumulation was investigated with strain SR-V-1263. The concentration of extracellular lysine reached a maximum (3.2 mg/ml) in medium containing 2% polypeptone under the experimental conditions.

XVIII. University of Oregon, Institute of Molecular Biology and Department of Biology, Eugene, Oregon 97403. Communicated by Ira Herskowitz.

The following are abstracts of four papers in press or submitted for publication:

1. Jeffrey N. Strathern, Lindley C. Blair, and Ira Herskowitz

Healing of mat Mutations and Control of Mating Type Interconversion by the Mating Type Locus in Saccharomyces cerevisiae

PROC. NAT. ACAD. SCI. (in press)

ABSTRACT

Homothallic yeasts switch cell types (mating types a and α) at high frequency by changing the alleles of the mating type locus, MATa and MATα. We have proposed in the cassette model that yeast cells contain silent MATa and MATα blocs ("cassettes"), copies of which can be substituted at the mating type locus for the resident information. The existence of silent cassettes was originally proposed to explain efficient switching of a defective MATα locus (matα) to a functional MATα locus. We report here that this "healing" of mat mutations is a general property of the mating type interconversion system and is not specific to the class of matα mutations studied earlier: a defective MATa (matal) switches readily to MATa, and various matα loci switch readily to MATα. These observations satisfy the prediction of the cassette model that all mutations within MATa and MATα be healed. These studies also identify the MAT functions which control the switching process: the same functions which promote sporulation and prevent mating in a/α cells also inhibit the switching system in a/α cells. Finally, we present additional characterization of a natural variant of MATα, MATα-inc (Takano et al., 1973), which is insensitive to switching. Our observation that MATα-inc acts in cis suggests that it may be altered in a site concerned with excision of MATα-inc or its replacement by another cassette.

2. Jasper Rine, Jeffrey N. Strathern, James B. Hicks and Ira Herskowitz

A Suppressor of Mating Type Locus Mutations in Saccharomyces cerevisiae: Evidence for and Identification of Cryptic Mating Type Loci

GENETICS (submitted for publication)

ABSTRACT

A mutation has been identified which suppresses the mating and sporulation defects of all mutations in the mating type loci of S. cerevisiae. This suppressor, sir1-1, restores mating ability to matα1 and matα2 mutants and restores sporulation ability to matα2 and matA1 mutants. MATa sir1-1 strains exhibit a polar budding pattern and have reduced sensitivity to α-factor, both properties of a/α diploids. Furthermore, sir1-1 allows MATa/MATa, matα1/matα1, and MATα/MATα strains to sporulate efficiently. All actions of sir1-1 are recessive to SIR1. The ability of sir1-1 to supply all functions necessary for mating and sporulation and its effects in a cells are explained by proposing that sir1-1 allows expression of mating type loci which are ordinarily not expressed. The ability of sir1-1 to suppress the matα1-5 mutation is dependent on the HMa gene, previously identified as required for switching of mating types from a to α. Thus, as predicted by the cassette model, HMa is functionally equivalent to MATα since it supplies functions of MATα. We propose that sir1-1 is defective in a function, Sir ("silent information regulator"), whose role may be to regulate expression of HMa and HMα.

3. Jeffrey N. Strathern and Ira Herskowitz

Asymmetry and Directionality in Production of New Cell Types During Clonal Growth: the Switching Pattern of Homothallic Yeast

CELL (submitted for publication)

SUMMARY

Homothallic Saccharomyces yeasts efficiently interconvert between two cell types, the mating type a and α. These interconversions have been proposed to occur by genetic rearrangement ("cassette" insertion) at the locus controlling cell type, the mating type locus. The pattern of switching from one cell type to the other during growth of a clone of homothallic cells has been followed by direct microscopic observation, and the results summarized as "rules" of switching. (1) When a cell divides, it produces either two cells with the same mating type as the original cell or two cells that have switched to the other mating type. This observation suggests that the mating type locus is changed early in the cell cycle, in late G1 or during S. (2) The ability to produce cells that have switched mating type is restricted to cells that have previously divided ("experienced cells"). Spores and buds ("inexperienced cells") rarely, if ever, give rise to cells with changed mating type. A homothallic yeast cell thus exhibits asymmetric segregation of the potential for mating type interconversion--at each cell division, the mother but not the daughter is capable of switching cell types in its next division. Homothallic cells also exhibit directionality in switching: experienced cells switch to the opposite cell type in more than 50% of cell divisions. These results show that the process of mating type interconversion is itself controlled during growth of a clone of homothallic cells. By analogy and extension of these results, we propose that multiple cell types can be produced in a specific pattern during development of a higher eukaryote according to a model involving sequential cassette insertion.

4. Ira Herskowitz, Lindley Blair, Douglass Forbes
James Hicks, Yona Kassir, Peter Kushner
Jasper Rine, George Sprague, Jr.
and Jeffrey Strathern

Control of Cell Type in the Yeast *Saccharomyces cerevisiae* and a Hypothesis for Development in Higher Eukaryotes

To appear in THE MOLECULAR GENETICS OF DEVELOPMENT
W. Loomis and T. Leighton, editors; Academic Press

TABLE OF CONTENTS

A. Inversion of the G Segment in Mu

B. Phase Variation in Salmonella

C. Immunoglobulin Synthesis

D. Transposable Drug Resistance Elements

E. Controlling Elements in Maize

F. Analogies with Mating Type Inverconversion in S. cerevisiae

XI. A Hypothesis for Development in Higher Eukaryotes

A. Production of Multiple Cell Types

B. Production of Specific Cell Lineages

5. John McCullough and Ira Herskowitz (1979). Mating pheromones of Saccharomyces kluyveri: pheromone interactions between S. kluyveri and S. cerevisiae. J. BACTERIOL. 138:146-154.

XIX. Laboratoire de Génétique, Institut National Agronomique - 16, Rue Claude Bernard, 75231 Paris Cédex 05, France. Communicated by H. Heslot.

The following papers have been published recently:

1. B. TRETON, M. T. LE DALL, H. HESLOT (1978). Excretion of citric and isocitric acids by the yeast Saccharomycopsis lipolytica. EUR. J. APPL. MICROBIOL. BIOTECHNOL. 6, 67-77.

2. B. TRETON, H. HESLOT (1978). Etude de quelques propriétés de l'aconitase de la levure Saccharomycopsis lipolytica. AGR. BIOL. CHEM. (Tokyo) 42, 1201-1206.

3. J. M. BECKERICH, H. HESLOT (1978). Physiology of lysine permeases in Saccharomycopsis lipolytica. J. BACTERIOL. 133, 492-498.

4. J. RIVIERE, H. HESLOT (1978). Production de protéines microbiennes á partir de mouture de blé. ANN. TECHNOL. AGRIC. 27, 585-607.

The following papers have been accepted for publication:

5. C. GAILLARDIN, H. HESLOT (1979). Evidence for mutations in the structural gene for homocitrate synthase in Saccharomycopsis lipolytica. MOLEC. GEN. GENET.

ABSTRACT

Eight strains devoid of homocitrate synthase (HS) activity were found among lysine-less mutants of Sm. lipolytica. Genetic analysis of these strains showed that they were all affected at the same locus LYS 1. Three lines of evidence suggest that this locus defines a structural gene for HS. (i) The mutations show various

degrees of intragenic complementation; in some cases the hybrid enzyme formed in vivo displays modified properties in vitro. (ii) Reversion of some of these mutations results in a modified enzyme (desensitized). (iii) A feedback mutant of HS was directly isolated from the wild type strain and shown to carry a single mutation at or near LYS 1.

We also present here the first attempts at genetic fine mapping in Sm. lipolytica.

6. C. GAILLARDIN, L. POIRIER, A. M. RIBET, H. HESLOT (1979). General and lysine specific control of saccharopine dehydrogenase levels in the yeast Saccharomycopsis lipolytica. BIOCHIMIE.

ABSTRACT

Lysine supplementation of the growth medium of a wild type strain of Sm. lipolytica specifically results in saccharopine dehydrogenase (SDH) repression. On the other hand, starvation of the strain for histidine triggers a general derepression of various histidine, leucine, arginine, and lysine biosynthetic enzymes, including SDH. These two types of control, specific and general, act independently on SDH expression, since mutants which fail to respond to the specific control still are sensitive to the general one. These mutants were first selected as unable to catabolize lysine, suggesting that a link may exist between SDH specific regulation and activity of the catabolic pathway.

7. J. M. BECKERICH, H. HESLOT (1979). Genetic control of lysine permeases in Saccharomycopsis lipolytica. ARCH. MICROBIOL.

ABSTRACT

In order to obtain strains impaired in the active transport of L-lysine, we made use of three toxic compounds: L-canavanine, an arginine analog, L-4-5-transdehydrolysine and L-5-aminoethylcysteine, L-lysine analogs which are competitive inhibitors of the high affinity lysine transport system. After UV mutagenesis, mutants resistant to different mixtures of these compounds, taken either all three or two by two, were isolated. The resistance pattern and the transport capacities of the mutants were studied. In all the mutants, both the high affinity and low affinity lysine permeases were impaired. In recombination tests, the mutants mapped into four loci.

8. C. GERBAUD[1], P. FOURNIER[2], H. BLANC[2, 3], M. AIGLE[4], H. HESLOT[2], and M. GUERINEAU[1]. Transformation of yeast with chimeric plasmids carrying part or entire 2 µm plasmid. GENE, 1979, 5, (in press).

---

(1) Institut Gustave Roussy, Villejuif
(2) Laboratoire de Génétique, Institut National Agronomique, Paris
(3) Centre Génétique Moléculaire, Gif S/ Yvette
(4) Institut de Biologie Moléculaire et cellulaire, Strasbourg

ABSTRACT

Hinnen et al. (P.N.A.S. 1978, 75, 1929-1933) have shown that yeast can be transformed at a frequency of 10^{-7} with a yeast gene carried on a bacterial vector. This frequency was increased 1000 times by using the 2 μm yeast plasmid (Beggs, NATURE, 1978, 275, 104-109). We have developed a similar system with another yeast gene (URA 3 instead of LEU 2) and constructed several chimeric plasmids carrying part or all of the 2 μm plasmid. The 2 μm plasmid cloned in pCR1 was partially digested by Hind III and ligated to the URA 3 gene extracted from a gene bank (this DNA fragment of 1.1 Kb has been shown to bear the URA 3 sequence by Bach et al., P.N.A.S., 1979, 76. (1), 386-390). As pCR1 has one Hind III target in the kanamycin resistance gene, and the 2 μm plasmid has 3, we got two kinds of hybrid plasmids. In the first, the URA 3 sequence was inserted in pCR1, the 2 μm remaining entire; in the second, the URA 3 sequence is in the 2 μm. We chose one plasmid (G.18) of the first type and one (G.9) of the second, where a Hind III fragment of the 2 μm has been deleted and replaced by the 1.1. Kb URA 3 fragment. This G.9 plasmid can be easily separated in two parts with Eco R1 digestion: a yeast one carrying URA 3 and a bacterial one. The two plasmids G.9 and G.18 are able to transform and to replicate in an E. coli pyr F strain as well as a S. cerevisiae URA 3^- strain. (G.18 conferring the kan^S phenotype to E. coli and G.9 the kan^R one.) The transformation frequency (of yeasts) is 10^{-5} to 10^{-4}. Hybridization experiments carried out on the total DNA of transformants show the presence of the URA 3 sequence on the bands corresponding to the 2 μm plasmid but don't show any integration in the chromosome. Genetic studies reveal a mitotic segregation in haploids and in diploids and a non-mendelian behaviour at meiosis; this arguing for the maintenance of URA 3 on a cytoplasmic genetic element. Plasmids recovered in E. coli from yeast transformants have also been studied: it is clear that recombination events took place in yeast between the hybrid plasmid and the endogenous 2 μm of the recipient strain. Finally, the specific activity of the enzyme encoded by URA 3 (OMP-decarboxylase) is increased about 25 times in the transformants.

XX. Institut für Mikrobiologie, Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, 6000 Frankfurt/Main, Federal Republic of Germany. Communicated by M. Brendel.

We have moved into new laboratories located on the Medical Campus of Frankfurt University. Below follow abstracts of papers recently published or submitted.

A. Ruhland, R. Fleer, and M. Brendel. Studying genetic activity of chemicals in yeast: DNA alterations and mutations induced by alkylating anti-cancer agents. MUTATION RES. 58, 241-250 (1978).

The simple eucaryotic organism baker's yeast parallely allows demonstration of primary DNA lesions and measuring of mutagenicity and lethality after treatment with alkylating chemicals. Several anti-cancer

drugs were shown to form cross-linked DNA molecules and to be genetically active. Mutagenicity and lethality of these drugs vary substantially and are dependent on the function of some processes of DNA dark repair.

* * *

A. Ruhland and M. Brendel. Mutagenesis by cytostatic alkylating agents in yeast strains of differing repair capacities. GENETICS 91, May issue (1979).

Reversion of two nuclear ochre nonsense alleles and cell inactivation induced by mono-, bi-, and tri-functional alkylating agents and by UV has been investigated in haploid, stationary-phase cells of yeast strains with differing capacities for dark repair. The ability to survive alkylation damage is correlated with UV repair capacity, a UV-resistant and -mutable strain (RAD REV) being least and a UV-sensitive and -nonmutable strain (rad1 rev3) most sensitive. Mutagenicity of alkylating agents is highest in the former and is abolished in the latter strain. Deficiency in excision repair (rad1 rad2) or in the RAD18 function does not lead to enhanced mutability. Mutagenesis by the various agents is characterized by a common pattern of induction of locus-specific revertants and of suppressor mutants. Induction kinetics are mostly linear, but UV-induced reversion in the RAD REV strain follows higher than linear (probably "quadratic") kinetics. The alkylating agent cyclophosphamide, usually considered inactive without metabolic conversion, reduces colony-forming ability and induces revertants in a manner similar but not identical to the other chemicals tested. These findings are taken to support the concept of mutagenesis by mis-repair after alkylation which, albeit sharing common features with the mechanism of UV-induced reversion, can be distinguished therefrom.

* * *

M. Kircher, R. Fleer, A. Ruhland, and M. Brendel. Biological and chemical effects of mustard gas in yeast. MUTATION RES., submitted.

Mustard gas induces inactiviation and mutation in yeast. Both effects are dose proportional, indicating single-hit events. Induction of both effects is influenced by the cell's capacity for DNA dark repair, whereby the probability of reversion is highest in repair proficient cells. Binding of mustard gas to cells and probably to DNA is independent of DNA repair systems. The number of interstrand cross-links as determined by assaying for renaturability of alkali-denatured DNA increases in a dose proportional manner. At 37% survival an excision-deficient strain contains 55 interstrand cross-links. Chromatographical analysis yields several alkylation products of DNA. Their relative frequencies resemble the values reported for E. coli and bacteriophage T7.

* * *

R. Fleer and M. Brendel. Formation and fate of cross-links induced by polyfunctional anti-cancer drugs in yeast. MOLEC. GEN. GENET., submitted.

A method to detect low levels in interstrand cross-links in DNA of Saccharomyces cerevisiae is described. Isopycnic ultracentrifugation of alkali-treated, unpurified Eaton press homogenates allows the detection

of less than one cross-link per yeast chromosome. Efficient separation of single- and double-stranded DNA requires low cell density and addition of glycerol during homogenization. Using a yeast strain defective in excision repair, a dose dependent formation of interstrand cross-links after treatment of cells with biological doses of nitrogen mustard, Triaziquone and Chlorambueil could be demonstrated. The most powerful of these alkylating agents is Triaziquone: half of the DNA molecules are shown to be cross-linked after a 12 minute exposure to 9×10^{-9} g/ml of the drug. The cross-linking reaction continues after excessive alkylating agent is removed. After having reached a maximum, the fraction of renaturable DNA decreases upon further incubation. The speed of the "after reaction" depends on temperature: 48 hours after the end of treatment, renaturability of DNA has almost completely disappeared when cells are kept at 36°C.

XXI. Lehrstuhl für Genetik, Technische Universität Braunschweig, Postfach 3329, D-3300 Braunschweig, Federal Republic of Germany. Communicated by Herbert Gutz.

1. Dr. Chikashi Shimoda from Osaka City University, Japan, spent eight months in our laboratory. The purpose of his visit was to study techniques for mapping ts mutants of Schizosaccharomyces pombe which are defective in ascospore germination.

2. In our work with Schizosaccharomyces pombe, we encountered difficulties with some dehydrated culture media produced by Difco Laboratories. Malt extract agar is the standard sporulation medium for S. pombe. Previously, Bacto-malt extract was very suitable for this purpose[1]. However, all batches of Bacto-malt extract which we bought in the last few years did not support sporulation of S. pombe. Now we are using "Malzextrakt für die Mikrobiologie" produced by E. Merck, Darmstadt, which (at a concentration of 3%, 2.5% agar added) yields an excellent sporulation medium.

   We found a similar situation with Bacto-yeast extract several years ago. On yeast extract dextrose agar plates (YEA), prepared with batches of this product bought prior to 1967, copulation and sporulation of S. pombe occurred. With all purchases of bacto-yeast extract after 1967, these results were no longer achieved. We found that this deficiency can be overcome by adding vitamins to the YEA (vitamins and their concentrations as in S. pombe minimal medium, reference 1).

   [1]H. Gutz et al.: Schizosaccharomyces pombe, pp. 395-446, In: HANDBOOK OF GENETICS, Vol. 1, edited by R. C. King. Plenum Press, New York and London 1974.

XXII. Institut für Mikrobiologie, Technische Hochschule Darmstadt, Schnittspahnstr. 10, D-6100 Darmstadt, Federal Republic of Germany. Communicated by F. K. Zimmermann.

F. K. Zimmermann: Selection of Wine Yeasts. The 1978 season was a cellar master's night mare. The musts came in very cold; they tended to foam, and the usual procedure of spontaneous fermentation was often a complete failure. There are suspicions that excessive

pesticide use lead to fermentation problems. However, use of selected yeast strains inoculated from actively fermenting tanks overcame these difficulties; and fermentation went either to completion or at least down to tolerable residual sugar levels. Reisolation of yeast clones from fermentation tanks in different wineries yielded clones that are vigorous fermenters, aromatically neutral, cold tolerant and did not form H_2S when tested on a variety of musts.

* * *

K. D. Entian has completed his thesis: Genetics of carbohydrate metabolism in Saccharomyces cerevisiae: Isolation and characterization of genes involved in carbon catabolite repression and carbon catabolite inactivation. He is now working at the Physiologisch-chemisches Institute, University of Tübingen, West Germany. In addition to previously published data (Entian et al.: MOLEC. GEN. GENET. 156, 99, 1977; Entian: MOLEC. GEN. GENET. 158, 201, 1977), he determined all the glycolytic enzymes and glycolytic intermediates in three types of mutants resistant to 2-deoxyglucose on raffinose media (Zimmermann and Scheel: MOLEC. GEN. GENET. 154, 75, 1977). The mutants were all recessive and called hex1 (reduced hexose phosphorylation), hex2 (increased hexose phosphorylation), and cat80 (normal hexose phosphorylation). As previously described, hex1-mutants showed reduced growth on glucose and lacked catabolite inactivation of gluconeogenic and glyoxylate shunt enzymes. Mutants of the hex2-type were normal in all respects; like, growth rates, fermentation rates, glycolytic intermediates on glucose medium, and catabolite inactivation. Only repression of invertase, alpha-glucosidases, malate-, succinate-, and NADH-dehydrogenase was completely or partly abolished. cat80-Mutants were only nonrepressible for invertase, alphaglucosidases, and malate dehydrogenase; growth on and fermentation of glucose was normal, glycolytic enzymes and metabolites at wild type level and catabolite inactivation active. This does not support the concept that a mere increase in glycolytic intermediates in cells transferred into a repressing glucose medium triggers catabolite repression. All hex2-mutants are strongly inhibited on maltose media; there was an accumulation of up to 300 µmoles/g dry weight of glucose. Maltose resistant hex2-suppressor mutants could be located in genes CAT1 and CAT3, the recessive mutants of which prevented derepression of gluconeogenic and glyoxylate shunt enzymes.

* * *

M. Grossmann: Genetics of Invertase Formation. A new gene SUC7 has been identified. It is present in a silent form in all strains studied so far. Active mutant alleles can be induced by UV-irradiation and then selected as raffinose fermenters in the case of suc0-strains (no invertase made) or as inuline utilizers in e.g., SUC4-strains. They are dominant in crosses with suc0-strains and form an invertase with an electrophoretic mobility similar to that of the fast forms found in strains carrying one of the genes SUC2, SUC3, SUC4, or SUC5. Strains with SUC1 form a slow invertase. Strains with SUC1 and one of the other SUC-genes form three invertases: the fast,

the slow, and a third with intermediate mobility. The SUC7-allele isolated from an inuline utilizer in the heterozygous condition with SUC1 gave the expected three bands. In contrast to this, the SUC7-alleles isolated from de novo raffinose fermenters in a suc0-strain in combination with SUC1 showed only the SUC1-band of invertase. This looks like a suppression of the formation of the SUC7-invertase in the presence of SUC1. This difference between alleles of the same gene in respect to hypostatic behavior towards SUC1 is striking but cannot be explained at present.

* * *

Inuline cannot be utilized sufficiently well to support growth of our raffinose fermenting strains. Therefore, it is possible to select mutants utilizing inuline efficiently. This way, it was hoped to isolate mutants with regulatory effects increasing the formation of invertase. In fact, a dominant mutant was found which formed on a raffinose medium 18-20 units of invertase, whereas the parent SUC4-strain had only 4-6. The inuline-utilizing mutant was a double mutant with an additional active SUC7-allele and another mutation leading to the excretion of large amounts of invertase already during log-phase.

* * *

M. Ciriacy: Isolation and identification of cis- and trans- acting genetic elements regulating the synthesis of glucose-repressible alcohol dehydrogenase (ADHII) in Saccharomyces cerevisiae.

In continuation of previous work concerning regulation of ADHII-synthesis (MOLEC. GEN. GENET. 145, 327-333, 1976), additional regulatory mutants with glucose-resistant, ADHII-formation have been found. Most of them could be attributed to semi-dominant mutations in gene ADR1 which was previously suggested as a positive regulatory gene. A recessive mutant allele of gene CCR1 (cf MOLEC. GEN. GENET. 154, 213-220, 1977) was epistatic over the function of ADR1; i.e., ADHII-synthesis was almost completely repressed by glucose in ccr1 $ADR1^c$ double mutants. Another allele of ADR1 could be isolated which was independent of the function of gene CCR1. These results suggested that the expression of the ADHII-structural gene (ADR2) is controlled by a combined action of at least two regulatory genes. Furthermore, four cis-dominant mutations at a site near or inside the ADHII structural gene could be identified (controlling site mutations, ADR3). They differ mainly with regard to derepressed ADHII-levels. In some of these mutants, association of the ADHII-subunits to the catalytically active tetramer is obviously affected. Therefore, we are currently investigating whether the primary structure of the ADHII-polypeptide is altered in these controlling site mutants.

XXIII. Université de Bordeaux II, Institut d'Oenologie, 351. Cours de la Libération, 33405 Talence, France. Communicated by P. Ribéreau-Gayon.

The following papers have been published:

I) The various functions of steroids on yeast metabolism in grape must during fermentation; the notion of survival factor. F. LARUE, S. LAFON-LAFOURCADE, P. RIBÉREAU-GAYON. ANN. MICROBIOL. (Inst. Pasteur) 1979, 130 A, 231-243.

ABSTRACT

The physiological effects of steroids supplied to yeasts in grape must is complicated. These substances may function as inhibitors, as growth factors or survival factors. The steroids have a "survival factor" action when they act on aerobically cultivated yeasts, fermenting under anaerobic conditions, in grape must of high sugar concentration. In this case, the substances have no effect on the cellular multiplication, but they maintain viability and fermentation activity in "resting cells". Consequently, a greater quantity of sugar is consumed; and the content of secondary products is modified by the end of the fermentation.

Certain growth conditions during fermentation affect the "survival factor" action of steroids: high temperature, low pH, vitamin deficiency, low amount of inoculum, insufficient aeration, excessive sugar concentration.

* * *

II) Relationship between the sterol content of yeast cells and their fermentative activity in grape must. The role of steroid. Francoise LARUE, Suzanne LAFON-LAFOURCADE and Pascal RIBÉREAU-GAYON. C. R. Acad. Sci. Paris 287, série D, p. 1445-1448.

ABSTRACT

In grape musts of high sugar concentration, the metabolic activity of the resting cells, which are responsible for the last stages of fermentation, may be determined by the cellular sterol content. It is possible to alleviate the sterol deficiency of the yeast cells by the addition of certain steroids to the must. According to the conditions of fermentation, steroids act as: a) inhibitor, b) growth factors, c) survival factors.

The following papers have been submitted for publication:

I) Fermentation metabolism of Saccharomyces cerevisiae in musts infected by Botrytis cinerea. Formation of acetic acid. S. LAFON-LAFOURCADE, D. DUBOURDIEU, V. LUCMARET, F. LARUE, P. RIBÉREAU-GAYON. C. R. Acad. Sci. Paris.

II) Origine de l'acidité volatile des vins. S. LAFON-LAFOURCADE. Congrès International--Microbiologie et Industrie Alimentaire. Paris, Octobre 1979.

III) Quelques observations sur les problèmes microbiologiques de la vinification en blanc. S. LAFON-LAFOURCADE et P. RIBÉREAU-GAYON. Connaissance de la Vigne et du Vin (in preparation).

XXIV. Institut Für Mikrobiologie Und Weinforschung, Johannes Gutenberg-Universität, 6500 Mainz, Federal Republic of Germany. Communicated by F. Radler.

Below follow abstracts of work conducted in our laboratory:

Tittel, D., and Radler, F.: Über die Bildung von 2, 3-Butanediol bei Saccharomyces cerevisiae durch Acetoin-Reduktase, Mschr. Brauerei 1979 (in press).

SUMMARY

On the formation of 2, 3-butanediol by acetoin reductase in Saccharomyces cerevisiae.

Five strains of Saccharomyces cerevisiae when grown in a synthetic medium or in grape must produced amounts of 2, 3-butanediol varying between 850 to 1800 mg/l as determined by gas liquid chromatography. High values were observed in the synthetic medium. Of the butanediol formed, 32 to 49% belonged to the meso form, the remainder being optically active. Valine, which is known to inhibit the formation of diacetyl, had little influence on the formation of 2, 3-butanediol. Therefore, it is assumed that the diol is formed by the acetoin forming system and not via α-acetolactate.

Commercial yeast alcohol dehydrogenase neither oxidizes 2, 3-butanediol nor reduces acetoin, whereas diacetyl is reduced. Cell free extracts from Saccharomyces cerevisiae were able to reduce acetoin in the presence of NADH, but the reverse reaction, the dehydrogenation of 2, 3-butanediol, was not observed. An acetoin reductase which required NADH as coenzyme was partially purified from Saccharomyces cerevisiae. The following steps led to a 10fold purification of the enzyme: removal of nucleic acids by protamine sulfate; gel filtration; and precipitation with ammonium sulfate. The acetoin reductase was partially separated from the alcohol-dehydrogenase by gel filtration with Sepharose 6B. Acetoin reductase was completely inactivated within 20 min at a temperature of 45°C but was unaffected at 35°C. The optimum pH value for the reduction of acetoin was 6.2. The molecular weight of the acetoin reductase as determined by gel chromatography was 95 000. Using a partially purified preparation of acetoin reductase, the following K_m values were found: 2.8 mM for acetoin, 0.02 mM for NADH.

E. Heerde and F. Radler: Metabolism of the Anaerobic Formation of Succinic Acid by Saccharomyces cerevisiae, ARCH. MICROBIOL. 117, 269-276 (1978).

ABSTRACT

1. Succinic acid is formed in amounts of 0.2-1.7 g/l by fermenting yeasts of the genus Saccharomyces during the exponential growth phase. No differences were observed between the various species, respiratory deficient mutants, and wild type strains.

2. At low glucose concentrations the formation of succinic acid depended on the amount of sugar fermented. However, the nitrogen source was found to be of greater importance than the carbon source.

3. Of all nitrogen sources, glutamate yielded the highest amounts of succinic acid. Glutamate led to an oxidative and aspartate to a reductive formation of succinic acid.

4. A reductive formation of succinic acid by the citric acid cycle enzymes was observed with malate. This was partially inhibited by malonate. No evidence was obtained that the glyoxylate cycle is involved in succinic acid formation by yeasts.

5. Anaerobically grown cells of Saccharomyces cerevisiae contained α-ketoglutarate dehydrogenase. Its activity was found in the 175 000 x g sediment after fractionated centrifugation. The specific activity increased 6-fold after growth on glutamate as compared with cells grown on ammonium sulfate.

6. The specific activities of malate dehydrogenase, fumarase, succinate dehydrogenase, succinyl-coenzyme A synthetase, α-ketoglutarate dehydrogenase, and glutamate dehydrogenase (nicotinamide adenine dinucleotide dependent) were determined in yeast cells grown on glutamate or ammonium sulfate. Similar results were obtained with a wild type strain and a respiratory deficient mutant. The latter did not contain succinate dehydrogenase.

7. In fermenting yeasts succinic acid is mainly formed from glutamate by oxidation.

XXV. Research Laboratory, Arthur Guinness Son & Co. (Park Royal) Ltd., Park Royal Brewery, London NW10 7RR, England. Communicated by J. R. M. Hammond.

The following is an abstract of a paper recently published in the Journal of the Institute of Brewing:

The Immunofluorescent staining Technique for the detection of wild yeasts--practical problems. J. R. M. Hammond and M. Jones, J. INST. BREW., 85, 26-30, (1979).

ABSTRACT

An apparent heavy wild yeast infection in pitching yeast has been detected using the immunofluorescent detection method. This infection would not be detected by conventional liquid forcing or plating techniques. The yeasts responsible were isolated and identified as Saccharomyces cerevisiae. The yeasts were very similar to the pitching yeast but varied in a number of respects associated with the cell wall such as flocculation character and giant colony morphology. The results suggest that the immunofluorescent-positive (IP) yeasts are variants of the culture yeast. As a result of this work, it is felt that although immunofluorescence is of value for the rapid detection of infection, it must always be used in association with more conventional microbiological techniques.

XXVI. The Finnish State Alcohol Monopoly, Alko,, Box 350, SF-00101 Helsinki 10, Finland. Communicated by Heikki Suomalainen.

Below follow the summaries of our work published since the last issue of the Yeast Newsletter:

Heikki Suomalainen and Matti Lehtonen. YEAST AS A PRODUCER OF AROMA COMPOUNDS. Proceedings of the First European Congress on Biotechnology, Interlaken 1978, Part 3, Dechema Monogr. 82(1978):1693-1703, 207-220.

Alcoholic beverages consist of a multitude of different chemical compounds. The fermentation is one of the most important stages for the production of the principal aroma compounds. Thus, the yeast strain and fermentation conditions have a great influence on the production of the aroma compounds.

The role of yeast in the formation of aroma compounds has been shown by a nitrogen-free sugar fermentation. The yeast produces the same aroma compounds in this fermentation as in normal fermentations. Yeast's central position in the formation of aroma compounds has also been shown by the aroma composition of a genuine Spanish sherry and a Finnish berry wine. The numerically largest group of aroma compounds formed during fermentation consists of fatty acid esters. The amount of esters formed is greatly dependent on the yeast used.

Fatty acids compose another large group of the aroma compounds synthesized during fermentation. The acid contents of whisky and cognac are very similar; but rum contains an acid, 2-ethyl-3-methylbutyric acid, that has so far escaped identification in any other beverages.

Fusel alcohols make up the quantitatively largest group of compounds formed during fermentation. The formation of fusel alcohols is greatly affected by yeast strains and the fermentation conditions such as temperature and yeast nutrients.

Except fermentation, there are other sources of aroma compounds. One of the most important stages is the maturation of alcoholic beverages. During maturation, new aroma compounds can be formed and existing ones can be consumed in chemical reactions.

Flavored beverages form a group of their own in the alcoholic beverages. A wide range of spice distillates and extracts are used in their preparation, including those of juniper, caraway, orange peel, coriander, aniseed, fennel, and dill.

* * *

Heikki Suomalainen and Matti Lehtonen. THE PRODUCTION OF AROMA COMPOUNDS BY YEAST. Paper presented in the Guinness Brewing School, May 25, 1978, London. JOURNAL OF THE INSTITUTE OF BREWING (in press).

The yeast plasma membrane regulates the movement of compounds into the yeast cell and of yeast metabolites from the cell into the medium. The rate of penetration of organic acids into the yeast cell depends on

their lipophilic nature and on their molecular size and degree of branching. During fermentation, yeast synthesizes a vast number of aroma compounds. The numerically and quantitatively largest groups of aroma compounds include fusel alcohols, fatty acids, and fatty acid esters. The yeast used and the fermentation conditions can influence the formation of aroma compounds. The yeast also has a profound effect on the formation of other aroma compounds, such as sulphur compounds and phenols. In addition to fermentation, the maturing of a beverage can also influence the aroma. During the maturation, lactones, phenols, and other compounds are extracted from the oak casks in which the beverage is aged. The presence of the so-called "whisky lactone", β-methyl-γ-octalactone, is characteristic of a beverage that has been matured in oak.

* * *

Slobodan Grba, Erkki Oura, and Heikki Suomalainen. FORMATION OF TREHALOSE AND GLYCOGEN IN GROWING BAKER'S YEAST. Finnish Chemical Letters (in press).

In baker's yeast cultivated with incremental feeding, trehalose and glycogen are not formed in parallel during the growth. Glycogen is formed during the exponential phase of growth and ceases to be formed at the end of this phase, whereupon the total amount of glycogen decreases slowly. In contrast, a rapid degradation of trehalose takes place at the start of the growth; and the concentration is at a minimum during the early logarithmic phase. Trehalose is resynthesized in the last stage of cultivation, during the late exponential growth phase. On the other hand if the cultivation is performed at a somewhat elevated temperature, 37°C, yeast cells synthesize trehalose even during the exponential phase.

Whereas the synthesis of glycogen is regulated by glucose-6-phosphate and adenosine nucleotides, the results obtained indicate that trehalose synthesis is controlled in a different way since a high cellular ATP-level does not inhibit it.

* * *

John Londesborough and Kaija Varimo. THE TEMPERATURE DEPENDENCE OF ADENYLATE CYCLASE FROM BAKER'S YEAST. THE BIOCHEMICAL JOURNAL (in press).

The heat of activation of membrane-bound adenylate cyclase changes from 40 kj/mol above 21°C to 62 kj/mol below 21°C. This change is probably too small to cause a marked temperature dependence of the intracellular concentration of cyclic AMP in yeast and can be explained without assuming that a temperature dependent change in the physical state of the membrane lipids affects the activity of the enzyme.

* * *

John Londesborough. THE HIGH Km CYCLIC NUCLEOTIDE PHOSPHODIESTERASE OF BAKER'S YEAST IS A ZINC METALLO-ENZYME. BIOCHEMICAL SOCIETY TRANSACTIONS 6:1218-1220 (1978).

The soluble high Km cyclic nucleotide phosphodiesterase of baker's yeast, which has no requirement for added bivalent metal ions, contains about 1.6 g atoms of tightly bound zinc per mole (65000 g) of enzyme. Reversible inhibitions by 1, 10-phenanthroline, 8-hydroxyquinoline, CN^- (but not N_3^-) and many thiols, and, especially, the irreversible time-concentration- and temperature dependent inactivation by 8-hydroxyquinoline suggest that this zinc is essential for the enzyme's activity.

* * *

The following publication has appeared since the last communication. The abstract of the report has been given in Yeast Newsletter 26 (1977):2, 27.

Erkki Oura and Heikki Suomalainen. BIOTIN AND THE METABOLISM OF BAKER'S YEAST. JOURNAL OF THE INSTITUTE OF BREWING 84 (1978), 283-287.

XXVII. Research Institute for Viticulture and Enology, 886 15 Bratislava, Matúškova 25, Czechoslovakia. Communicated by E. Minárik.

This is the summary of a paper accepted for publication in Wein-Wissenschaft (Wiesbaden, GFR):

Production of wines with low alcohol content.

The production of table wines with an alcohol content of 8-10 Vol.% is much more delicate than that of wines with "normal" alcohol content (10-12 Vol.%). This is connected with undesirable microbial, above all yeast activity, and/or by increased oxidation processes in table wines. Wine instability is caused by Saccharomycodes ludwigii and by aerobic asporogenous and sporogenous yeast species of the genera Candida, Pichia, and Hansenula. Wines with 10 and less Vol.% alcohol are usually not affected by these yeasts when favorable fermentation conditions and appropriate handling is guaranteed. By increased concentration of free SO_2 coupled with little oxygen access to young wines, film-yeast activity as well as premature oxydation processes may be prevented.

At the International Microbiology and Food Industry Congress organized by APRIA from October 7 to 12, 1979, in Paris, the following paper will be presented:

Pesticides and their influence on must fermentation. A short summary will be given in the next issue of the Yeast Newsletter.

XXVIII. Department of Biochemistry, The Royal Dental College, DK 8000 Arhus C., Denmark. Communicated by Henry Bleeg.

FACILE, LARGE SCALE YEAST DISINTEGRATION

By H. S. Bleeg and F. Christensen

The following is a brief description of a novel approach to yeast cell disintegration which has been used in our department for more than 6 months in a reproducible and satisfactory manner. After a long period of technical and economical problems connected with the production of homogenates from

kg's of baker's yeast, we have turned to a direct rasping of deep-frozen (-20°C) cubes (50 g) of yeast. Taken directly from the freezer, the blocks are immediately rasped on a Braun Multipress MP 50, which is normally used for domestic manufacture of juice from fresh fruit. The grating disc rotates with a constant speed of 3000 r.p.m. As a result of the cell disruption, the homogenate becomes sufficiently fluid to run off the centrifugal drain continuously. The yield of disrupted cells is around 65-70% as judged from cell counts and protein estimations. One kilogram of yeast may be processed in 30 minutes.

The enzyme of concern to us, L-galactono-γ-lactone oxidase, (membrane-bound, probably mitochondrial) retains its specific activity in these homogenates as compared with established procedures like shaking with glass beads or grinding with sea sand. The cells may be suspended in an equal volume of a convenient buffer and frozen in ice cube moulds. When the cell density is lowered to 10%, the yield of disrupted cells is very low.

The method may probably be improved for the purpose of even larger scale processing of cells by increased rotating velocity or another design of the grating disc.

XXIX. Louisiana State University, Dept. of Food Science, Baton Rouge, Louisiana 70803. Communicated by S. P. Meyers.

Below follow some news items from our laboratory:

1. Bioprotein from Banana Wastes is being published in DEVELOPMENTS IN INDUSTRIAL MICROBIOLOGY Vol. 20, 1979 (In Press). This is a portion of work developed by Dr. Chung for her doctoral dissertation here at LSU. Dr. Chung is now at Uncle Ben's Food, 13001 Westerheimer, Houston, Texas 77077.

ABSTRACT

Studies of the ascosporogenous yeast Pichia spartinae in batch culture have included analyses of growth and carbohydrate utilization on banana solubles from waste skin and pulp. Banana broth is a complete medium for yeast development requiring only a metabolizable nitrogen source such as $(NH_4)_2SO_4$. Based on available sugar, total yeast cell yields as great as 58% have been obtained. Protein values of 47% are possible with yeast extract as the sole nitrogen source, with yields of over 6 g/liter. P. spartinae develops readily at pH levels below 3.0, permitting incorporation of low technology production techniques. Amino acid analyses of banana waste-grown yeast show an essential amino acid pattern comparable to FAO reference protein and equivalent to that of torula yeast. Protein levels of waste whole bananas can be increased from 9 to 27% via yeast conversion. Further optimization of yields with accelerated aeration and pH control is indicated.

2. Purification and characterization of alpha-galactosidase from Pichia guilliermondii is a part of a Master's thesis by Mr. Frank Church, who is now studying for his doctorate degree in the Dept. of Food Science at North Carolina State University, Raleigh, North Carolina. Portions of the work are being submitted for publication in MYCOLOGIA and in DEVELOPMENTS IN INDUSTRIAL MICROBIOLOGY.

XXX. Meetings.

1. The next general Symposium on Yeasts and Yeast-like Organisms will be held in London, (Ontario) Canada, from July 20-26, 1980.

Please contact Dr. G. G. Stewart, Chairman of the organizing committee, for further details and application forms. His address is:

Labatt Breweries of Canada, Ltd.
150 Simcoe Str.
London, Ontario, Canada N6A 4M3

2. Dr. J. M. Wiame and Dr. A. Goffeau hope to be able to organize the 10th International Conference of Yeast Genetics and Molecular Biology in Louvain-la Neuve, Belgium, from September 8-12, 1980.

The coordinator is Dr. H. L. Roman, USA; the secretary is Dr. R. C. von Borstel, Canada. The finance committee which is in charge of raising funds for the travel expenses of each participating country includes: Drs. E. A. Bevan (U.K.), B. Carter (Ireland), S. Fogel (USA), R. H. Haynes (Canada), F. Kaudewitz (Germany), L. Kovac (Czechoslovakia), U. Leupold (Switzerland), G. E. Magni (Italy), R. J. Planta (Netherlands), A. Putrament (Poland), G. Simchen (Israel), P. P. Slonimski (France), Ø. Stømnaes (Norway), T. Takahasi (Japan), J. M. Wiame (Belgium).

J. M. Wiame and A. Goffeau
Laboratoire D'Enzymologie
Universite De Louvain
Place Croix Du Sud, 1
1348 Louvain-la-Neuve
Belgium

3. The Society for Industrial Microbiology will hold its 30th Annual Meeting from August 12-17, 1979, at Carnegie Mellon Institue, Pittsburgh, Pennsylvania.

For further information contact:

Mrs. Ann Kulback
Society for Industrial Microbiology
1401 Wilson Boulevard
Arlington, Virginia 22209, USA

4. XIth Annual Conference on Yeasts organized by the Committee of Yeasts of the Czechoslovak Microbiological Society, Smolenice, was held February 6-8, 1979.

A. Kocková-Kratochvilová

1) Technology

Stollárová, V.: Study of yeasts from the surface of various fruits in South Slovakia.

Navara, A., Minárik, E.: The production of dihydrosulphide in red wines by the action of wine yeasts.

Minárik, E., Navara, A.: Trends in the microbiology of wines with a low content of alcohol.

Netrval, J., Vojtišek, V., Souhrada, J.: Some new data about the production of phenylacetylcarbinol by yeasts.

Augustin, J., Hal'ama, D., Vasil'ova, D.: Factors determining the inhibitory effect of hydrolyzates of lignin-cellulose material.

Farkaš, V., Kocková-Kratochvilová, A., Vojtková, A.: Growth of selected yeasts on enzymic hydrolyzates of lignin-cellulose materials.

Páca, J.: Effect of specific growth rate on the growth and physiological characteristics of Candida utilis cells in a multistep continuous cultivation system.

Hroncek, J.: Use of the PERT method in the bioengineering of fermentation technologies.

2) Genetics

Kováčová, V., Vlčková, V.: Genetic analysis of nuclear RD mutants of Saccharomyces cerevisiae prepared by MNM action.

Takácsová, G., Subik, J.: Tranfer and recombination of mitochondrial genes involved in the biogenesis of cytochrome b.

3) Biochemistry

Lieblová, J.: Biochemical difference between daughter and mother cells of Saccharomyces cerevisiae.

Hrmová, M.: Induction of hyphal forms of Candida albicans and its biochemical background.

Drobnica, Ľ., Baláž, Š., Šturdik, E., Gemeiner, P.: Our knowledge about the relationship between the structure of naturally occurring and synthetic compounds and their activity against yeasts.

Antalik, M., Kellová, G., Marko, V., Šturdik, E., Drobnica, Ľ.: The reactivity of carbonylcyanidephenylhydrazone derivatives and their action on yeasts.

Šturdik, E., Kellová, G., Drobnica, Ľ.: Vinylfurans, inhibitors of energy metabolism of yeasts and tumor cells.

Biely, P.: Xylan utilizing enzyme system of the yeast Cryptococcus albidus.

Krátký, Z.: Initial transformations of inducers of the system degrading xylan in cells of Cryptococcus albidus.

Vršanská, M.: Some features of the extracellular endo-β-1, 4-xylanase of Cryptococcus albidus.

Šandula, J., Grones, J.: β-xylosidases of some yeasts.

Peciarová, A., Šikl, D., Masler, L., Šandula, J.: Glycoproteins and polysaccharides of Candida lipolytica grown on n-alkanes and glucose.

4) Panel discussion on Aureobasidium pullulans (de Bary) Arnaud moderated by A. Kocková-Kratochvílová

The main themes:

Kocková-Kratochvílová, A.: The characteristics of the species Aureobasidium pullulans.

Slávíková, E.: The ecology of Aureobasidium pullulans.

Zemek, J.: The production of pullulan and glycogen by Aureobasidium pullulans.

Augustin, J.: Characters of α-amylase from Aureobasidium pullulans.

Černiaková, M.: Enzymatic degradation of lignin-cellulose complex by Aureobasidium pullulans.

Zemek, J., Zámocký, J., Gabert, A., Wand, H., Borris, R.: Features of immobilized glucoamylase.

5) Immunology

Tomšíková, A.: Immunological response of organisms on antigens of yeast-like fungi.

Holan, Z.: Use and perspectives of the Czechoslovak product "Zymosan".

6) Minisymposium about eukaryotic cells moderated by O. Nečas

The main themes:

Nečas, O.: Contemporary trends in investigations of eukaryotic cells.

Streiblová, E.: Microtubules and microfilaments in yeasts.

Sipiczki, M.: Fusion of protoplasts of Schizosaccharomyces pombe and S. octosporus.

Svoboda, A.: Fusion of yeast cells.

Havelková, M.: Golgi apparatus in yeast cells.

XXXI. Books and Journals.

1. MICROBIAL TECHNOLOGY
(Second Edition)

Academic Press, Inc.
111 Fifth Avenue
New York, New York 10003

Co-Editors:

Dr. H. J. Peppler
Universal Foods Corporation
P.O. Box 737
Milwaukee, Wisconsin 53201

Professor D. Perlman
School of Pharmacy
University of Wisconsin
Madison, Wisconsin 53706

Volume I, "Microbial Processes", is scheduled for distribution early in July. Volume II, "Fermentation Technology", will follow in mid-August. The publisher is accepting orders now for delivery in time for the 1979-1980 academic year.

2. The following long review article is due to be published in Spring 1979.

John R. Johnston and Helena Oberman, Yeast Genetics in Industry. Progress in Industrial Microbiology 15.

This reviews the applications of selection, mutation, hybridization, and polyploidy, principally within the period 1970-78, within the industries and areas of brewing, baking, winemaking, distilling, food yeasts, and other products. Professor Oberman is a microbiologist and fermentation technologist at the Technical University of Lodz, Poland.

I shall be on sabbatical leave from July to December this year and working in the laboratory of Dr. Robert Mortimer, University of California, Berkeley. I look forward to contacting other yeast biologists and geneticists in California and hopefully in other parts of North America during this time.

J. R. Johnston
Dept. of Applied Microbiology
Royal College Bld.
204 George Str.
Glasgow G1 1XW, Scotland

3. A new journal invites articles in the area of applied biochemistry from readers of the Yeast Newsletter.

NOTICE TO CONTRIBUTORS

JOURNAL OF APPLIED BIOCHEMISTRY (1979) Academic Press is a new international journal devoted to the publication of papers presenting original results, review articles, and other features in the field of applied biochemistry. Manuscripts are welcome that contain new and significant information of general interest to workers in these fields. Acceptance of an article for publication will require that it demonstrate a new concept or approach to a practical problem, or provide a new insight relevant to an understanding of the system under study. Articles dealing with routine investigations and non-innovative studies will not be accepted for publication.

Original papers and review articles will be considered. Normally, however, review articles will be invited by the Editors; any person wishing

to submit an unsolicited review article for consideration should, in the first instance, send a summary of the proposed article to the Editor-in-Chief who will advise the author regarding its potential suitability. Original papers must contain sufficient detail to enable others to repeat the work.

Dr. J. John Marshall
Editor-in-Chief
JOURNAL OF APPLIED BIOCHEMISTRY
Editorial Office
Howard Hughes Medical Institute
P.O. Box 520605
Miami, Florida 33152, USA

A detailed set of instructions regarding the preparation of manuscripts will be published in the first issue of the journal or may be requested from the Editorial Offices of the journal.

XXXII. Brief News Items.

1. The Editor announces, with regret, the death of Professor V. I. Kudriavzev, Moscow, USSR, this last spring. Professor Kudriavzev had a major influence in the systematics of yeast. A species, Pichia kudriavzevii, was named after him in 1965. A book on the classification of the sporogenous yeasts by him was published in Russian in 1954 and translated into German in 1960.

2. The following paper has been publised:

J. B. Fiol and G. Billon-Grand, Étude de quelques enzymes intracellulaires dans les genres Dekkera et Brettanomyces: Conséquences systématiques. MYCOPATHOLOGIA 64:183-186, 1978.

The following paper is in press:

M. C. Pignal and D. Lachaise, Les levures des Drosophiles de savane d'Afrique intertropicale (savanes de Lamto, Gôte d'Ivoire). Accepted for publication in MYCOPATHOLOGIA.

M. C. Pignal
Section Levures, Bat. 405
Université de Lyons
43 Bd. du 11 Novembre 1918
69621, Villeurbanne, France

3. The following articles were published recently:

Moulin, G. and Galzy, P. Remarks on the metabolism of Kluyveromyces lactis Van der Walt. MYCOPATHOLOGIA Vol. 66, 1-2, 73, 1978.

Moulin, G. and Galzy, P. Amylase activity of Torulopsis ingeniosa Di Menna. FOLIA MICROBIOLOGICA Vol. 23, 423, 1978.

P. Galzy, Ecole Nationale Superieure
Agronomique de Montpellier
Chaire de Genetique
Et de Microbiologie
34060 Montpellier Cedex, France

4. Mr. F.E.M.J. Sand has left NAARDEN INTERNATIONAL, HOLLAND on June 1, 1979. Future correspondence in my field (yeast spoilage of soft drinks) should be sent to the following address: Amaliagaarde 52, 1403 Bussum, the Netherlands.

5. The following papers were presented at the VIth international specialized symposium on yeasts in Montpellier from July 2-8, 1978.

Delpech, I., Bizeau, C., Bonaly, R., Galzy, P. Study of the action of the genes of the "pli" series on the biochemical structure of the cell wall of Saccharomyces cerevisiae.

Reisinger, O., Al Bassam, R., Bizeau, C., Bonaly, R., Galzy, P. Microbiological characteristics of a wild strain and of three mutants of Saccharomyces cerevisiae.

Arthaud, J. F., L'Homme, C., Bizeau, C., Galzy, P. Establishment of a model for the growth of the yeast.

Beluche, I., Guiraud, J. P., Galzy, P. Studies of inulinase activity of Debaryomyces cantarellii Capriotti.

Allamel, C., Guiraud, J. P., Galzy, P. Genetical studies of brewing yeast Saccharomyces carlsbergensis.

Moulin, G., Oteng-Gyang, K., Galzy, P. Remarks on the localization of amylase activity of some yeasts.

Vezinhet, F., Dawes, I. W. Mutations in Saccharomyces cerevisiae leading to altered initiation of sporulation: Physiological aspects.

P. Galzy, Laboratoire de Recherches
de la Chaire de Génétique INRA
Ecole National Supérieure Agronomique
34060 Montpellier Cedex, France

6. A new catalogue is now available of the Yeast Genetics Stock Center. Those interested in obtaining a copy should write to Yeast Genetics Stock Center, Donner Laboratory, University of California, Berkeley, California 94720, USA.

7. A research associateship is available as of September 1, 1979. The program concerns the biochemistry, biophysics, and ultrastructure of yeasts and has been recently funded for a further three years by N.I.H. The position is available for all or part of that time by mutual acceptability. We seek a postdoctoral fellow with a biochemical background and the awareness that yeasts are in many respects ideal eukaryotic cell models. Specific and current lines of work include the mechanism of translocation of glycoproteins across the protoplasmic membrane and the influence of glucose and trehalose analogs on carbohydrate metabolism. The program has the cooperation and active participation of an electron microscopy unit at the V.A. Hospital. Interested parties should call or write:

Dr. Wilf. Arnold, Biochemistry
University of Kansas Medical Center
Kansas City, Kansas 66103
Telephone: 913-588-7056 or 588-7005