YEAST

A Newsletter for Persons Interested in Yeast

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

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The Editor wishes all readers of the Yeast Newsletter a happy and scientifically rewarding New Year.

I. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852. Communicated by Kathryn A. Schmeding.

The strains listed have been added to the ATCC since April 30, 1977. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

Bullera piricola ATCC 34640 Type Culture	F. Stadelmann Swiss Federal Research Station Liebefield-Bern Switzerland
Candida albicans ATCC 20032, Released patent Citric Acid Production	John B. Routien Charles Pfizer & Co., Inc. Groton, CT
Candida albicans ATCC 36082	J. E. Edwards Jr. Harbor General Hospital Torrance, CA
Candida muscorum ATCC 34886 Type Culture	CBS The Netherlands
Candida oleophila ATCC 20372, ATCC 20373, Released patents Citric Acid Production	John A. Benckiser Rhein, W. Germany
Candida shehatae ATCC 34887 Type Culture	CBS The Netherlands
Candida utilis ATCC 34962, ATCC 34963, ATCC 34964 Antimycin resistance	C. Grimmelikhuijzen Laboratory European de Biologie Moleculaire Heidelberg, W. Germany
Cryptococcus albidus ATCC 34632, ATCC 34633	A. Kockova-Kratochvilova Czechoslovak Collection of Yeasts Czechoslovakia
Cryptococcus albidus var. diffluens ATCC 34634	" "
Cryptococcus gastricus ATCC 34636	" "
Cryptococcus laurentii ATCC 34635	" "
Cryptococcus neoformans ATCC 34631, ATCC 34663, ATCC 34664	" "

Cryptococcus neoformans ATCC 36069, ATCC 36070, ATCC 36071 Filamentous mutants	M. A. Gordon New York State Department of Health Albany, N. Y.
Cryptococcus neoformans ATCC 34868-ATCC 34883	K. J. Kwon-Chung N. I. H. Bethesda, Md.
Filobasidium capsuligenum ATCC 34639	A. Kockova-Kratochvilova Czechoslovak Collection of Yeasts Czechoslovakia
Hansenula anomala ATCC 20029, Released patent Citric Acid Production	John B. Routien Charles Pfizer & Co. Groton, CT
Kluyveromyces blattae ATCC 34711 Type Culture	W. Henninger Deutsche Sammelung von Mikroorganism W. Germany
Kluyveromyces fragilis ATCC 36142	NCYC Surrey, England
Pichia amethionina var. amethionina ATCC 36080 Type Culture	Mary Miranda University of California, Davis Davis, CA
Pichia amethionina var. pachycereana ATCC 36079 Type Culture	" "
Pichia burtonii ATCC 20030, Released patent Citric Acid Production	John B. Routien Charles Pfizer Co., Inc. Groton, CT
Pichia cactophila ATCC 34932, Type culture ATCC 34933-ATCC 34935	Mary Miranda University of California, Davis Davis, CA
Pichia heedii ATCC 34936-ATCC 34941	" "
Pichia pinus ATCC 34972 Assimilation of methanol	R. J. Mehta Smith Kline & French Philadelphia, PA
Saccharomyces bayanus ATCC 36022, ATCC 36023 Wine Yeasts	Ralph Kunkee University of California, Davis Davis, CA

Saccharomyces bisporis var. mellis ATCC 34890 Spoilage of soft drinks	V. Grinsven Naarden International Naarden-Bussum The Netherlands
Saccharomyces cerevisiae ATCC 34893-ATCC 34895 Inositol requiring mutants	Susan A. Henry Albert Einstein College of Medicine Bronx, N. Y.
Saccharomyces cerevisiae ATCC 36011-ATCC 36013 Yeast alcohol dehydrogenase mutant	Christopher Wills University of California, San Diego San Diego, CA
Saccharomyces cerevisiae ATCC 36024-ATCC 36027, ATCC 36029 Wine Yeasts	Ralph E. Kunkee Dept. of Viticulture & Enology University of California, Davis Davis, CA
Saccaromyces fermentati ATCC 36030 Wine Yeast	" "
Saccharomycopsis lipolytica ATCC 34922 Hydrocarbon degradation	F. C. Stormer National Institute of Public Health Norway
Saccharomyces montanus ATCC 34891 Spoilage of soft drink	V. Grinsven Naarden International Naarden-Bussum The Netherlands
Saccharomyces rouxii ATCC 36141 Osmophilic spoilage yeast	A. R. Isaacs Sandy Trout Food Preservation Research Laboratories Queensland, Australia
Saccharomyces sp. ATCC 20488, Released patent Amylase assay	Norman F. Oblon Arlington, VA
Saccharomycopsis capsularis ATCC 20033, Released patent Citric Acid Production	John R. Routien Charles Pfizer Co., Inc. Groton, CT
Sporobolomyces antarcticus ATCC 34888 Type Culture	CBS The Netherlands
Sporobolomyces holsaticus ATCC 34889 Type Culture	CBS The Netherlands

Torulopsis apis ATCC 34638	A. Kockova-Kratochvilova Czechoslovak Collection of Yeasts Czechoslovakia
Torulopsis inconspicua ATCC 34637	" "
Torulopsis sp. ATCC 20031, Released patent Citric Acid Production	John B. Routien Charles Pfizer Co., Inc. Groton, CT

II. Inst. Biochemistry and Physiology of Microorganisms, USSR Acad. Sciences, Pushchino, Moscow Region, 142192, USSR. Communicated by W. I. Golubev.

Candida lactativora (Fell et Phaff) Golubev nov comb. Basionym: Cryptococcus lactativorus Fell et Phaff, Antonie van Leeuwenhoek 33:464 (1967).

The yeast species Cr. lactativorus is atypical of the genus Cryptococcus Kütz. emend. Phaff et Spencer. It was included in the latter because of its ability to utilize i-inositol for growth and inability to form pseudomycelium. This species does not produce starchlike compounds, does not hydrolyze urea and thus differs from the majority of Cryptococcus spp. The % G + C of Cr. lacativorus was found to be lower than in other species of this genus. The mol% G + C of the type strain is 46.3 (buoyant density) and 43.2 (chemical determination) (Phaff et al., 1974, Golubev, Vagabova, 1976). Its extracellular polysaccharides contain glucose, galactose and mannose but no xylose. Besides, in Dalmau plate cultures I observed the formation of primitive pseudomycelium in the type strain of Cr. lactativorus on bacto-yeast morphology agar and meat-peptone agar. For the above mentioned reasons I propose to transfer Cr. lactativorus to the genus Candida Berkhout as C. lactativora nov comb.

Golubev, W. I. and Vagabova, L. M. 1976. Yeast. A Newsletter 24(2).
Phaff, H. J., Miller, M. W., Miranda, M. Heed, W. B. and Starmer, W. T. 1974. Int. J. Syst. Bacteriol. 24:486-490.

The following article has recently been published:

Golubev, W. I., Bab'eva, I.P., Blagodatskaya, V. M. and Reshetova, I. S., 1977. Taxonomic study of yeasts isolated from spring sap of birch (Betula verrucosa Ehrh). Mikrobiologia 46(3).

SUMMARY

The taxonomy of yeast organisms isolated from spring sap of birch was studied, particularly of those whose properties deviated from the standard descriptions of species or even the genus (Nadsonia, Cryptococcus). Additional information is presented for red, carotenoid-containing, fermenting yeast.

III. University of California, Davis, Department of Internal Medicine, U.C.D. Professional Bldg., 4301 X Street, Sacramento, California, 95817. Communicated by Michael G. Rinaldi.

Recently, we have come across some "black" yeast isolates which we found to be of some interest.

Dr. G. S. de Hoog of the CBS, Baarn, The Netherlands, recently described the "black" yeast genus Phaeococcus (Studies of Mycology, #15, CBS, 1977). The genus is characterized as follows: colonies restricted, glistening, black, with sharp margin. Budding cells thick-walled, dark olivaceous brown, globose to broadly ellipsoidal, giving rise to similarly shaped secondary conidia from 1-3(-5) loci. Hyphae absent; sometimes chains of globose cells are formed.

de Hoog points out that, physiologically, Cryptococcus ater (Castell. ex W. B. Cooke) Phaff and Fell can be regarded as a good species of Cryptococcus Kützing emend. Phaff and Spencer, whereas the species with dark budding cells are significantly different in assimilation of melibiose, glycerol, citric acid and inositol. For these species only the invalid generic name, Nigrococcus Novák and Zsolt, is available. Hence, Dr. de Hoog created the new genus Phaeococcus.

de Hoog recognizes three species within the genus: Phaeococcus exophialae (mature colonies smooth or slimy), Ph. nigricans (mature colonies heavily wrinkled; cells variable: globose to ellipsoidal, subhyaline to dark brown), and Ph. catenatus (cells all similar: globose, mid brown, rarely septate).

Dr. Michael McGinnis (University of North Carolina Memorial Hospital) and I have recently come across four strains of Phaeococcus (3 from clinical material; one from pine litter) which, when compared with the type strains of the three species in the genus, indicated they were different species. Work is underway at present to characterize and describe these organisms.

The medical mycological significance of these isolates remains unknown; however, de Hoog suggests that the relationship of Phaeococcus with the dematiaceous hyphomycete genus Exophiala is apparent from the overall similarity in carbohydrate constitution. Also, Ph. nigricans is morphologically similar to budding states of Exophiala species. de Hoog, therefore, considers Ph. exophialae to be a complex with accompanying conidial states of Exophiala jeanselmei (Langer.) McGinnis and Pahyre var. heteromorpha (Nannf.)de Hoog, E. dermatitidis (Kano) de Hoog = Wangiella dermatitidis (Kano) McGinnis, and Rhinocladiella spinifera (Nielsen and Conant) de Hoog = Exophiala spinifera (Nielsen and Conant) McGinnis.

All of these moulds and their varying morphological forms have been implicated as etiologic agents in human cases of subcutaneous mycoses, i.e. mycetoma, subcutaneous nodules and cysts, collectively referred to as "phaeohyphomycoses" (Ajello,

1974). It is, therefore, of some interest to determine the status of our new isolates of Phaeococcus as regards taxonomy, nomenclature, and role in human disease.

IV. Consejo Superior de Investigaciones Cientificas, Instituto Jaime Ferran de Microbiologia, Joaquin Costa 32, Madrid 6, Spain. Communicated by C. Ramirez.

The following paper: "Torulopsis navarrensis sp. nov., a new species of yeast isolated from an acid washed brown soil in the province of Navarra, Spain", by C. Ramírez and I. Moriyón, has been accepted for publication in MYCOPATHOLOGIA. The main features of this organism are the capacity to ferment glucose, galactose, raffinose, trehalose (latent and weak), and to assimilate glucose, galactose, sucrose, maltose, cellobiose (latent), trehalose, raffinose, melezitose, ribitol, D-mannitol, D-glucitol, α-methyl D-glucoside, salicin, succinic acid, D-xylose, and citric acid.

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

1. The following papers previously listed as in press have now been published:

H. J. Phaff (1977). Enzymatic yeast cell wall degradation (a review). Adv. in Chemistry Series #160:241-82. American Chemical Society.

T. G. Villa, M. A. Lachance and H. J. Phaff (1977). On the structure of the β-(1→3)-glucan component of the cell wall of baker's yeast. FEMS Letters 1:317-19.

Other recently published papers:

Melvin T. Meyer and H. J. Phaff (1977). Survey for α-(1→3)-glucanases among yeasts. J. Bacteriol. 131:702-06.

M. A. Lachance, T. G. Villa, and H. J. Phaff (1977). Purification and partial characterization of an exo-β-glucanase from the yeast Kluyveromyces aestuarii. Canad. J. Biochem. 55:1001-1006.

2. The following papers are in press:

H. J. Phaff, W. T. Starmer, Mary Miranda, and M. W. Miller (1978). Pichia heedii, a new species of yeast indigenous to necrotic cacti in the North American Sonoran Desert. Int. J. Syst. Bact. 28(1978-April issue).

Abstract

A novel member of the yeast genus Pichia has been recovered 67 times during the period 1971-76. We have named this new species Pichia heedii in honor of Professor William B. Heed. Most strains were isolated from the soft-rot of Lophocereus schottii and from

Drosophila pachea, which utilizes L. schottii as a host plant. All strains were found in the Sonoran Desert. The species has four-spored asci and is heterothallic. P. heedii has metabolic capabilities similar to those of P. membranaefaciens, but the base composition of its nuclear DNA is 10-12 mol% lower than that of P. membranaefaciens. P. heedii may be differentiated from other cactus-specific Pichia species by its ability to assimilate D-xylose and from P. membranaefaciens by its cactus habitat, small cell size, and relatively high maximum temperature of growth. The type strain of P. heedii is UCD-FST 76-356 (= ATCC 34936 = CBS 6930).

PICHIA CACTOPHILA, A NEW SPECIES OF YEAST FOUND IN DECAYING TISSUE OF CACTI

W. T. Starmer, H. J. Phaff, Mary Miranda and M. W. Miller (1978).

Int. J. Syst. Bacteriol. April issue.

ABSTRACT

A novel representative of the yeast genus Pichia has been recovered 190 times during the period 1971-76. We regard this organism as belonging to a new species, Pichia cactophila. Strains were found in the necrotic tissue of 16 species of cactus and in the crops of 3 species of Drosophila which utilize the cacti as host plants. Isolates were obtained from widely separate geographic localities (throughout Mexico, Southwestern USA, and Hawaii). The new species forms predominantly two-spored asci. Both homothallic and heterothallic strains have been observed.

The metabolic capability of P. cactophila, like that of P. membranaefaciens, is limited to oxidative utilization of only a few compounds. P. cactophila can be differentiated from P. membranaefaciens by its strong growth on D-glucosamine and by the lower guanine plus cytosine content (36.3-37.5 mol%) of its nuclear deoxyribonucleic acid. The type strain of P. cactophila is UCD-FST 76-243A (= ATCC 34932 = CBS 6926).

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3. Below follows the abstract of the doctoral dissertation of M. A. Lachance, University of California, Davis (1977). A study of yeast exo-β-glucan hydrolases as phylogenetic markers in yeast.

Summary

Yeast exo-β-glucanases (EC 3.2.1.58) were studied with the intent of unravelling their phylogenetic significance. The principal properties studied were their molecular weights, as determined by gel exclusion chromatography on polyacrylamide (Bio-Gel P), and their primary sequence differences, as revealed by the microcomplement fixation technique.

A general molecular weight survey of this enzyme in ascomycetous yeasts showed a range from 24 to 59 kilodaltons in the yeast species

studied, and somewhat higher values for exo-β-glucanases from basidiomycetous yeasts, which were observed to synthesize considerably lower amounts of this enzyme. The molecular weight distribution in ascomycetous yeasts was centered over 40 kd, which was the approximate value found for the exo-β-glucanases from Debaryomyces, Hansenula, Kluyveromyces, Pichia, Saccharomyces Group III, Schwanniomyces, and from species of several other genera. The species of Kluyveromyces were investigated in depth, and most exo-β-glucanases extracted from these yeasts had a molecular weight of ca. 41 kd. The species of Saccharomyces sensu stricto (Group I) surveyed in this work synthesized exo-β-glucanases with molecular weights of ca. 50 kd. The intrinsic value of molecular weight determinations as they apply to the phylogenetic study of yeast exo-β-glucanases was discussed, along with some factors affecting the reliability of these determinations. A computer algorithm was designed to calculate molecular weights from raw data obtained during chromatography of standard proteins and partially purified proteins of unknown molecular weights, using combined log linear and log parabolic regression fits of the elution curves.

The exo-β-glucanases from Saccharomyces cerevisiae, Kluyveromyces fragilis, K. aestuarii, and K. phaseolosporus were purified to antigenic homogeneity and the enzyme from K. aestuarii was partially characterized. The latter was found to be a glycoprotein (ca. 24% carbohydrate) with a molecular weight of 43 kd, and no essential thiol group or cation in its active site. Its pH-activity curve was found not to differ significantly from those reported in the literature for exo-β-glucanases from other yeasts, suggesting the involvement of carboxyl and imidozolium groups in the catalytic sites of these enzymes. The substrate specificity and kinetic properties of K. aestuarii's exo-β-glucanase were studied, along with a restricted number of properties of the enzymes extracted from K. phaseolosporus and K. polysporus.

A survey of the immunological relatedness of exo-β-glucanases from various yeast species was performed, using Saccharomyces cerevisiae, K. fragilis, K. aestuarii, and K. phaseolosporus as reference species. The enzyme from S. cerevisiae was found to be a poor immunogen, but the exo-β-glucanases from the three species of Kluyveromyces produced monospecific antisera with microcomplement fixation titers from 9000 to 17000.

High degrees of immunological cross-reactivity were found between the exo-β-glucanase from S. cerevisiae and those from other species of Saccharomyces Group I, but no measurable reactions occurred when the anti-S. cerevisiae glucanase serum was titrated with extracts from yeasts belonging to Saccharomyces Groups II, and III, and from species of several other genera.

The serum prepared against the exo-β-glucanase from K. fragilis reacted strongly with cytoplasmic extracts from K. bulgaricus, K. cicerisporus, K. marxianus, and K. wikenii, revealing their close phylogenetic relatedness. High immunological distances were found between K. aestuarii's glucanase and all other species tested. K.

phaseolosporus was found closely related by this technique to K. delphensis, K. drosophilarum, K. lactis, and K. vanudenii.

The potential impact of molecular studies on yeast systematics was discussed, and it was proposed that macromolecular sequence comparisons be given exclusionary value. It was suggested that the concept of sibling species be introduced in the taxonomy of yeasts, and that more importance be given to population genetics and ecological factors in the delimitation of yeast species.

The nature of yeast exo-β-glucanases was discussed in the light of their known properties, and of their universality in yeasts. A mechanism was proposed for their catalytic action on β-glucans, with evidence that they act as non-inverting glucan glucohydrolases (as shown for the enzyme extracted from K. aestuarii). It was suggested that one of the in vivo functions of yeast exo-β-glucanase may be the synthesis of β-glucan in addition to its terminal hydrolysis. Circumstantial evidence in support of this was reported from the literature, and an experimental study of the effect of water activity upon the activity of K. aestuarii's exo-β-glucanase was discussed in this context. Transglycosylation by this enzyme, however, could not be demonstrated.

4. Dr. Tomas G. Villa from the University of Salamanaca, Spain, has spent one year as a postdoctoral visitor in the laboratory of Professor H. J. Phaff. He will continue his residency at Davis during the academic year 1977-78. His studies are concerned with the enzymology of the yeast cell envelope.

VI. Department of Soil Biology, Moscow State University, Moscow, USSR. Communicated by I. P. Babjeva.

At this laboratory we have been studying for about 15 years the biology of soil-inhabiting yeasts of the genus Lipomyces. At present our collection consists of more than 200 strains of all five known species. Recently we began to study in detail the process of ascospore formation in L. tetrasporus and L. starkeyi in connection with the taxonomic problems of the lipomycetes. Below follows a summary of this work.

Ascospore formation in Lipomyces tetrasporus

I. P. Babjeva, S. E. Gorin, M. M. Voostin

SUMMARY

Twenty-five strains of L. tetrasporus isolated from various soils in different parts of the distribution area of this species were studied by microscopic examination of ascus development. The following medium was selected for abundant sporulation (per 100 ml): meat-peptone broth - 10 ml; succinic acid - 0.2 g; KH_2PO_4-0.1 g; $MgSO_4 \cdot 7H_2O$ - 0.05 g; yeast autolysate - 0.4 ml; agar - 2.0 g. Other media were also used. As a result we may distinguish three main types of ascus formation in L. tetrasporus.

1. The pear-shaped ascus is developed from an individual cell, which forms a protuberance that copulates with the so called

"active bud" on the mother cell.

2. The ascus is formed after copulation of two active buds on the same cell or on different cells. The zygote resulting from conjugation of these buds produces a sac-like ascus on a broad base or on two "podes". Active buds involved in copulation are always separated from the mother cells by septa.

The process of ascus formation according to types 1 or 2 closely resembles primitive gametangiogamy as it takes place in Dipodascopsis, Dipodascus, Endomyces, and Eremascus spp.

3. The ascus with ascospores is formed from an enlarged bud separated from the mother cell by a septum without any evidence of copulation. In such a way both a pear-shaped and a sac-like ascus on a broad base may be formed. The latter is formed when the growing ascus curves and touches the mother cell wall for the second time. The mother cells of such asci often lose their contents and look empty.

The proportion of asci of the different types varied in a single strain depending on the medium used.

It has been supposed (Henninger, Emeis, Arch. Mikrobiol., 1974, 101:365) that ascospore formation in multispored species is not connected with meiosis, and that ascospores and vegetative cells have the same ploidy and are nonhaploid. As far as L. tetrasporus is concerned, we think that this species is a homothallic, haploid yeast. This conclusion is based on the following observations. All isolated single-spore and single-cell clones were able to form ascospores. When zygotes of various ages were transfered to a medium supporting vegetative reproduction only the 12-hour zygotes started to bud and gave rise to vegetative cells but those 24 hours old formed ascospores. Hence L. tetrasporus may be supposed to be a haploid yeast. The final confirmation of ploidy may be obtained after carrying out additional cytological and genetical investigations.

VII. National Research Council, Ottawa, Canada KIA OR6. Communicated by G. B. Calleja.

Below is an abstract of a subsection in a forthcoming volume of the multi-volume CRC Handbook of Nutrition and Food Series, edited by Dr. Miloslav Rechcigl, Jr.

The effects of nutritional factors on microbial aggregation

G. B. Calleja and B. F. Johnson

A widespread phenomenon in the microbial world, cell aggregation, is defined as the gathering together of cells to form fairly stable, contiguous, multicellular associations under physiological conditions. In addition to well-known phenomena such as flocculation of brewer's yeast, aggregation of cellular slime molds, and agglutination of gametes in unicellular algae, our list of aggregation systems includes mating-aggregate formation during bacterial conjugation, bacterial star formation, agglutination associated with competence

to bacterial transformation, dental plaque formation, aggregation in myxobacteria, sex-directed flocculation in fission yeast, and sexual agglutination in Hansenula, Saccharomyces, and other yeasts. It also includes mating reactions in protozoa and the formation of strands, rhizomorphs, sclerotia, synnemata, and coremia in the filamentous fungi. The stimulatory, inhibitory, and indifferent responses of the various aggregation systems to nutritional factors and conditions are summarized in tabular form from over 400 references.

VIII. Université de Dijon, E.N.S.B.A.N.A., Laboratoire de Botanique appliquée section Levures, 21000 Dijon, France. Communicated by J. -M. Belin.

About seven years ago I began work on the biology and physiology of yeasts from vineyards and cellars of Burgundy. Now I am identifying the yeasts isolated from the vineyards and I prepared a new simple key to identify all the yeasts.

The following papers dealing with this work have appeared:

1. J. -M. Belin, R. Bessis, L. Mouillet, P. Henry. Influence de deux fongicides sur la croissance et le métabolisme de deux espèces de levures. Conn. Vigne Vin 2:199-216. 1971.

2. J. -M. Belin, P. Henry. Contribution à l'étude écologique des levures dans le vinoble. Répartition des levures à la surface du pédicelle et de la baie de raisin. C. R. Acad. Sc. 274:2318-2320. 1972.

3. J. -M. Belin. Recherches sur la répartition des levures à la surface de la grappe de raisin. Vitis 11:135-145. 1972.

4. J. -M. Belin, P. Henry. Répartition des levures à la surface de la tige de vigne. C. R. Acad. Sc. 277:1885-1887. 1973.

5. J. -M. Belin. Morphologie inframicroscopique de Vitis vinifera L., son incidence sur la répartition des levures. Conn. Vigne Vin, in press.

Summary

Studies with the S.E.M. report the ultramicroscopic morphology and the aspect of waxes of the various parts of the vine: stem (trunk, rod, ripening branches, herbaceous branches), young and adult leaves (blade, petiole), grapes (grape-stalk, pedicel, berries). This work allowed us to determine the propitious zones for yeast accumulation: stomata and lenticels on the various parts of the grape; along the veins and the edges of the leaves; on the "lenticels" and breaks of the bark of the old stem parts.

6. J. -M. Belin. Contribution à l'étude des levures des chais. Taxinomie. Répartition des levures. Submitted for publication.

The following are papers from other main areas of research on yeasts.

7. J. -M. Belin. A study of budding of Saccharomyces uvarum Beijerinck with the scanning electron microscope. Antonie van Leeuwenhoek 38:341-349. 1972.

8. J. -M. Belin, P. Henry. Organisation structurale et répartition des lipides extracellulaires dans les colonies du genre Rhodotorula. Rev. IBANA. 21:11-17. 1974.

9. C. Bizeau, J. -M. Belin, P. Galzy. Action of "colonie lisse" mutation on cell morphology of Saccharomyces cerevisiae. Can. J. Genet. Cytol. 17:395-399. 1975.

IX. Central Research Laboratories Mitsubishi Chemical Industries Limited, 1,000 Kamoshida-cho, Midori-ku, Yokohama-shi, Kanagawa-ken, Japan. Communicated by Norio Gunge.

The following is a summary of our work which was recently done to obtain genetic evidence for protoplast fusion in yeast.

Protoplasts of Saccharomyces cerevisiae were prepared from two haploid strains both of mating type a, which carried different nuclear (adel, ural, his4, leu2 and thr4) and mitochondrial (ρ, ω, C^R, E^R and O^R) markers, and were fused with the aid of 30% polyethylene glycol 4000. Cells of fused products (prototrophs) displayed mating phenotype a and were crossed to mating type α/α diploids having auxotrophic markers, e.g. trp1. On sporulation of these crosses, as a rule, there were three tetrad types for the mating types; (I) 4 non-mater (n), (II) 2a:2α and (III) a:α:2n. The relative frequencies of these three tetrad types were close to ones theoretically predicted from tetraploids of mating type a/a/α/α, showing that the fusion products were diploids of mating type a/a. Auxotrophic markers involved in the crosses, which were located on four different chromosomes, were also segregated to yield the tetrad distribution expected from the parentages. It was concluded thus that the protoplast fusion proceeded to karyogamy to produce a stable diploid. A study of mitochondrial recombination demonstrated that the fusion products accepted the mitochondrial genome (the polar gene ω as well as the drug resistance genes) from one protoplast parent.

Details of the study will be published in Jap. J. of Genetics.

X. Department of Microbiology, Attila Jozsef University, H6701, Szeged, P. O. Box 428, Hungary. Communicated by L. Ferenczy.

The following publications on protoplast fusion have recently appeared:

1. Transfer of mitochondria by protoplast fusion in Saccharomyces cerevisiae. L. Ferenczy and Anna Maraz. Nature 268:524-525 (1977).

2. High-frequency fusion of fungal protoplasts. L. Ferenczy, F. Kevei, and M. Szegedi. Experientia 31:1028-30 (1975).

3. Factors affecting high-frequency fungal protoplast fusion. L. Ferenczy, F. Kevei, M. Szegedi, A. Franko, and I. Rojik. Experientia 32:1156-58 (1976).

4. Some characteristics of intra- and interspecific protoplast fusion products of Aspergillus nidulans and Aspergillus fumigatus. L. Ferency. In Cell genetics in higher plants (D. Dudits and P. Maliga eds.), 171-182 (1976).

5. Interspecific protoplast fusion and complementation in Aspergilli. L. Ferenczy, M. Szegedi, and F. Kevei. Experientia 33:184-85 (1977).

XI. Department of Applied Microbiology, University of Strathclyde, Glasgow, G11XW, Scotland. Communicated by J. R. Johnston.

Genes in Saccharomyces conferring flocculation in late fermentation

SUMMARY

The following defined genes conferring flocculation of dispersed cells at a late stage of fermentation of brewers wort (or 10% malt extract medium) have been reported: Flo 1, Flo 2 (both dominant), flo 3 (recessive) (2nd Int. Symp. Genetics of Ind. Microorganisms, Sheffield, England, 1974 pp 339-355; J. Inst. Brewing, 82, 158, 1976) and Flo 4 (Steward and Russell, Can. J. Microbiol. 23, 441, 1977). The latter publication reports the centromere-linkage of Flo 4 and also its location on chromosome I at 37 cM from ade 1 but on the opposite chromosome arm. It was this latter information which led Stewart and Russell to conclude that Flo 4 was a different gene to Flo 1 and Flo 2. These workers have also, however, obtained data indicating that Flo 4 and Flo 2 are in fact allelic (Stewart, personal communication).

Recently, H. P. Reader has obtained the following data from crosses of strains carrying genes Flo 1 and Flo 2 (hitherto reported as separate but linked genes, mapping 8 cM apart): 53 tetrads all gave a 4:0 ratio for flocculent:non-flocculent. We conclude from this that Flo 1 and Flo 2 are not separate genes but are in fact allelic, the gene to be designated Flo 1. This gene, Flo 1 (and the formerly designated gene Flo 2) shows linkage with ade 1 (PD : NPD : T = 22 : 1 : 52) and a map distance between them of approximately 39 cM. It is therefore evident that the dominant genes reported as Flo 1, Flo 2 and Flo 4 are allelic and that only the recessive gene, flo 3, defines a different locus from Flo 1.

XII. Department of Genetics, University of California, Berkeley, California 94720. Communicated by John Cummings.

Hybridization of Wine Yeasts

by John Cummings

Abstract

Seven commercial yeast strains were obtained from the Department of Enology and Viticulture, U. C. Davis. Of the seven, two (519, Sherry and 530, Tokay) failed to sporulate and three (505, Champagne 513, Distillers and 585, Adelaide) sporulated at low frequency and produced few viable ascospores. The Burgundy (51) and the Montrachet (522) strains sporulated readily and produced an abundance of viable ascospores. These two wine yeasts were spore-mated to laboratory strains carrying marker genes on thirteen chromosomes and to a strain of S. uvarum (C126) isolated from soured figs. The Burgundy, Montrachet and S. uvarum strains are homothallic diploids (genotype: a/α HO/HO HMα/HMα HMa/HMa), chromosomally homologous to heterothallic, laboratory strains of S. cerevisiae. The intervarietal hybrid between the two wine yeasts, the hybrids between the two wine yeasts and the laboratory strain, X2180-1B, and the interspecific hybrids with the strain of S. uvarum, sporulate readily and produce viable ascospores. Identification of these genetically homologous strains should prove useful in a genetic analysis of economically important characteristics.

XIII. Institut für Mikrobiologie und Biochemie, Lehrstuhl für Biochemie der Universität Erlangen-Nürnberg, Egerlandstrasse 7, 8520 Erlangen, W. -Germany. Communicated by E. Schweizer.

CONTROLLED MITOCHONDRIAL INACTIVATION OF TEMPERATURE-SENSITIVE SACCHAROMYCES CEREVISIAE NUCLEAR PETITE MUTANTS.

E. Schweizer, W. Demmer, U. Holzner and H. W. Tahedl
(submitted for publication)

Temperature-sensitive nuclear petite mutants genetically attributable to 106 different complementation groups have been studied biochemically by using either whole cells or isolated mitochondria obtained in temperature shift-up experiments (22°C → 36°) with continuous chemostat cultures.

The following results were obtained:

1. In 20 of the 106 complementation groups studied respiratory competence is retained at 36°C, although cells were unable to grow on lactate as a carbon source. Depending on the complementation group studied, P/O-ratios were either normal or reduced by a factor of 40 - 70 percent. ADP-dependent respiratory control was normal in some groups and absent in others with either both or only one of the two substrates used, succinate and α-ketoglutarate. Only a single strain (ts-1416) exhibited the characteristics expected of a partially uncoupled mutant.

2. Of 12 complementation groups studied, 2 are deficient in mitochondrial protein synthesis. Probably as a consequence of this defect, the ρ+ character is lost in these mutants after 2-3 generations growth at 36°C.

3. Two groups specifically deficient in cytochrome oxidase and one other with a specific defect in the NADH-cytochrome c oxidoreductase were characterized. Upon grown at 36°C all other respiratory-deficient nuclear petites lose cytochrome oxidase activity, but at the same time - or after a slight delay - they also lose NADH/cytochrome c oxidoreductase and ATPase or NADH/cytochrome c oxidoreductase, ATPase and ρ+ character, or NADH/cytochrome c oxidoreductase alone. One strain, possibly a cytochrome c mutant, retains all three activities although respiration is blocked at 36°C.

4. A sizeable portion of the petite-ts mutants studied undergoes a $\rho^{+} \rightarrow \rho^{-}$ transition after 2-4 generations growth at 36°C. Careful kinetic studies indicate that in most cases this transition is the consequence of, and not the reason for, the loss of respiratory competence in these strains. In 2 mutants studied, the loss of ρ+ character could be prevented by performing the shift-up experiment in lactate instead of glucose-containing medium.

XIV. Department of Biology, York University, 4700 Keele Street, Downsview, Ontario M3J 1P3, Canada. Communicated by Robert H. Haynes.

I have been invited by Dr. S. V. Shestakov of Moscow State University to speak on the Genetic Control of DNA Repair in Yeast at the XIV International Congress of Genetics which will be held in Moscow, August 21-30, 1978. The abstract of this lecture is given below.

GENETIC CONTROL OF DNA REPAIR IN YEAST

Thirty-two distinct genetic loci (rad) are known to confer radiation sensitivity in Saccharomyces cerevisiae; 22 further complementation groups, 17 of which show cross-sensitivities to radiation, have been identified among mutants selected for their sensitivity to methyl methanesulfonate. On statistical grounds, it is likely that additional loci will be found in each category. No one knows how many further loci could affect sensitivity to other mutagens. Some of these loci have highly pleiotropic phenotypes and it is unlikely that all of them control the repair of radiation or chemically induced lesions in DNA. Measurements of the sensitivity of various double rad mutants have revealed the existence of three epistatic groups of loci which can be associated with three biochemically distinct pathways for DNA repair. The gene which is thought to control the first step in each pathway has proven to be a mutator: the rad3 pathway contains at least six epistatic loci and controls the error-free excision of UV-induced pyrimidine dimers; the rad51 pathway may control the repair of X-ray strand breaks; the rad6

pathway is highly pleiotropic and controls some mode of mutagenic (error-prone) repair. It is likely that a second mutagenic pathway also exists in yeast. Wild-type rad6 and 9 genes are required for mutagenesis by all agents so far tested, including nitrosoguanidine and ethyl methanesulfonate. At least seven genes are required for normal UV-mutagenesis but some of these lower the reversion frequencies of only certain alleles in the cycl locus. Heteroduplex repair is considered to be essential for pure mutant clone formation after UV; it is related to, but not identical with excision repair in that loci of the rad3 pathway differentially affect the ratio of sectors to pure clones in the ade2 forward mutation system. The kinetics of UV-induced mutation in yeasts are linear for low UV doses in both excision-deficient and excision-proficient strains. At higher doses reversion frequencies rise as the square and ultimately higher powers of dose, whereas in the ade2 forward system the curves rise linearly to a maximum and then decline. Thus, there is a constitutive level of mutagenic repair activity in yeast, although mathematical analysis of the reversion data indicates that an inducible component also exists. The character of the mutation induction kinetics are influenced by the assay conditions employed, and the magnitude of the inducible component of mutagenic repair can be modified by post-irradiation incubation under non-nutrient conditions. Clearly the genetic control of DNA repair in yeast is more complex than that found in E. coli, and the bacterial models for repair and mutagenesis cannot be extended in any straightforward way to this simple eucaryote.

XV. Zentralinstitut für Mikrobiologie und Experimentelle Therapie der Akademie der Wissenschaften der DDR, 69 Jena, Beuthenbergstr. 11, German Democratic Republic. Communicated by H. Weber.

In our laboratory of yeast genetics we recently began a genetic study of the alkane-utilizing yeast Saccharomycopsis (formerly: Candida) lipolytica. Below follow abstracts of papers relating to substructural features of sporulation and multiple forms of alcoholdehydrogenase of this yeast which have been submitted for publication to the "Zeitschrift für Allgemeine Mikrobiologie."

ABSTRACT

Light and electron microscopic studies of sporulating diploids from crosses between different strains of the yeast Saccharomycopsis lipolytica confirmed reports about striking irregularities in spore number per ascus. Using serial sections it could be shown by means of electron microscopy that ascospores derived from asci with different spore number possess never more than one nucleus. In one-, two- and three-spored asci additionally to the nucleus enclosed in mature spores there occur unenclosed "naked" nuclei. In 2 - 4-spored asci four products of meiosis in the form of enclosed and free nuclei could be demonstrated which indicate a normal meiotic division in this yeast. Meiosis and spore formation seem to represent parallel and coordinated development processes. It is assumed that a defect in spore formation gives rise to the spore irregularities. It is concluded that sporulation in Sm.lipolytica in general is not different from that observed in Saccharomyces cerevisiae and Hansenula wingei.

H. Weber. Substructural studies on sporulation of Saccharomycopsis lipolytica. Z. Allg. Mikrobiol. (in press).

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ABSTRACT

In the "oxidative" yeast Saccharomycopsis lipolytica one NAD^+-dependent alcohol dehydrogenase (ADH I) and three $NADP^+$-dependent alcohol dehydrogenases (ADH II, III, IV) were detected by polyacrylamide gel electrophoresis. These four alcohol dehydrogenases react better with octanol than with ethanol. The ADH I is not or only minimally subject to glucose repression. Besides the ADH I band no additional inducible NAD^+-dependent ADH band is detectable gel electrophoretically during growth in ethanol or paraffin-containing medium. The synthesis of ADH II, ADH III and ADH IV is dependent on the carbon source of the medium. In paraffin-containing medium the ADH II is detectable during logarithmic growth phase and the ADH III during stationary phase. The ADH IV is only demonstrable during growth in medium with glucose or ethanol as carbon source. The tolerance of Sm. lipolytica to allyl alcohol was tested. Resistant mutants of our strain can only be isolated in concentrations of about 1 M allyl alcohol in the medium.

1. W. Künkel und G. Barth. Alkoholdehydrogenase (ADH) in Hefezellen II. NAD^+- und $NADP^+$-abhängige ADH in Saccharomycopsis (Candida) lipolytica. Z. Allg. Mikrobiol. (in press).

2. G. Barth und W. Künkel. Alkoholdehydrogenase (ADH) in Hefezellen III. ADH-aktivität in Saccharomycopsis (Candida) lipolytica in Abhängigkeit von Substrat und Wachstumsphase. Isolation allylalkoholresistenter Mutanten. Z. Allg. Mikrobiol. (in press).

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

Below follow several items for inclusion in the next issue of the Yeast Newsletter.

1. Abstract of the Ph.D. dissertation in Genetics submitted by Dr. Frederick Knauert to the Graduate Division, University of California, Davis, California.

The Isolation and Characterization of Nitrous Acid Sensitive (nas) Mutants of the Yeast Saccharomyces cerevisiae.

Putative cross-link repair mutants of the yeast Saccharomyces cerevisiae were selected on the basis of unusual sensitivity to nitrous acid. Thirty-nine nas mutants were isolated by this procedure and were found to fall into at least 23 complementation groups. Three mutants were not placed in complementation groups, two because they were non-maters and one because it was dominant. The sensitivity of the nas mutants to the cross-linking agents nitrogen mustard and psoralen plus 360 nm light and to UV and X-ray

irradiation was tested to separate strains that were uniquely sensitive to cross-linking treatment from those that were sensitive to radiation. None of the mutants were found to be uniquely sensitive to the cross-linking treatments. These preliminary tests revealed that many of the nas mutations were pleiotropic affecting the ability to mate, spore survival, chromosome segregation, cell viability during "liquid holding" and stationary phase morphology. Further tests revealed that 19 of the mutants required inositol, 3 required pantothenate, 6 required para-amino benzoic acid and one had a double inositol-para-amino benzoic acid requirement. Because of their precursor relationship to phospholipids the phenotypic anomalies expressed by the inositol and pantothenate requiring strains are explained in terms of membrane alterations caused by a precursor insufficiency, due either to a defect in de novo vitamin synthesis or to a defect in a regulatory element that coordinates cell growth with cell division. The basis for the para-amino benzoic acid requirement is unclear. The requirement is not alleviated by folic acid supplementation making a deficiency in a folic acid precursor an unlikely explanation for the pleiotropic defects manifested by these strains. Alternate explanations for this requirement are considered.

Two of the UV sensitive nas strains, nas10 and nas14, were found to be allelic with previously isolated rad loci, rad18 and rad12. Other studies have implicated rad18 as a mutant in the branched portion of the error prone repair pathway in yeast. Explanations for the UV sensitivity of nas10 in terms of a membrane deficiency and in terms of a defect in a regulatory element of the cell division cycle are presented.

2. Abstract of the M.S. thesis in Food Science submitted by Kevin Scott, Department of Viticulture and Enology, to the Graduate Division, University of California, Davis, California.

Effects of Genetic Manipulation of Wine Yeast Characteristics

Kevin E. Scott

Genetically altered yeasts were used for wine fermentation. Mutants of the Montrachet yeast strain, which were auxotrophic for specific amino acids-including arginine, cysteine, methionine or cysteine, and leucine-were generally poor fermentors. Most strains left excessive residual sugar in the finished wine and some showed a decreased efficiency of conversion of sugar to ethanol. The yield of n-propanol was reduced in the wines fermented by two strains requiring either methionine or cysteine. Another wine which was found to contain a low concentration of n-propanol was probably fermented by wild yeasts. Only slight changes in the contents of other higher alcohols were detected. No conclusions could be drawn concerning minor differences in wine flavor and aroma, except for the presence of sulfide or mercaptan-like odors. A coincidence of sulfur amino acid requirement, inability to adapt to high levels of sulfur dioxide, and the formation of hydrogen sulfide was observed for the two strains which required either methionine or cysteine. A technique for rapid detection of low

hydrogen sulfide-producing strains of yeast was developed. This technique involved the plating of yeast cells on nutrient agar, spraying the mature colonies with particulate sulfur, and overlaying the plates with agar containing lead acetate. Poor fermentative character was observed not only in the auxotrophic strains, but also in strains derived from the four isolated spores of a single ascus of the parental "wild-type" yeast. In both cases this may have been due to homozygosity of clones derived from ascospores.

3. Abstract of a paper presented at the Molecular Biology of Yeast meeting, Cold Spring Harbor Laboratory, New York, August 18-21, 1977.

Genetic Evidence for Complementation between Non-homologous Proteins in Saccharomyces cerevisiae

Richard Snow

Cells with the allele his1-1 cannot grow without histidine at 30° but can grow without it at 15° if they carry either the allele THR4 or a suppressor, SUP(his1-1). SUP(his1-1) is not as efficient as THR4 as judged by poorer growth on histidineless medium at 15° of SUP(his1-1)/sup(his1-1); thr4/thr4 diploids compared to sup(his1-1)/ sup(his1-1); THR4/thr4 diploids. SUP(his1-1) segregates independently of his1-1 and thr4 and is not centromere-linked.

Revertants of three types have been obtained from a his1-1 sup(his1-1) thr4 strain. One class, selected for growth without histidine at 30°, will also grow without it at 15°. Three of these are revertants to HIS1. A second class was selected for growth on histidineless medium at 15°. Growth at 30° without histidine was not restored. One of these is a revertant to SUP(his1-1). A third class was selected for growth on threonineless medium. One of these is a revertant to THR4.

his1-30, a complementing EMS-induced allele, responds to THR4 similarly to his1-1. his1-68, a complementing HNO_2-induced allele, will also respond to THR4 at 15° if the medium is 1M in KCl.

Cells which are his1-1 sup(his1-1) thr4 thr1 will not grow at 15° without histidine. Since THR1 catalyses the step immediately before THR4, this observation supports the idea that the effect is not due to an excessive accumulation of the substrate of THR4, O-phosphohomoserine.

The best explanation of the THR4 results invokes physical interaction between the his1-1 and the THR4 proteins such that the conformation of the mutant his1-1 protein is sufficiently corrected to restore some activity at 15°. It is not likely that low temperature activity of his1-1 is inhibited by the simultaneous presence of thr4 and SUP(his1-1) proteins because if this were true THR4/thr4; SUP(his1-1)/sup(his1-1) diploids would not be expected to grow without histidine at 15°, whereas they do. Also in this case, his1-1 sup(his1-1) thr4 thr1 strains would be expected to grow

without histidine at 15°, whereas they do not.

The non-homologous protein complementation explanation can also be applied to the SUP(his1-1) observations, but it is also possible that this allele is a missense suppressor which at low temperature causes translation of a small amount of wild-type HIS1 enzyme.

4. Kevin Scott has developed an extremely quick and easy way to make glass needles for micromanipulators using inverted agar slabs. A 1/8 inch glass rod is drawn out to a very thin filament in a small, very hot flame. Sections of the filament are selected according to the desired diameter and short segments about 1/4 inch long are cut with sharp scissors. A support rod of 1/8 inch glass is cut to a length suitable for the micromanipulator, and the working end is made slightly blunt. This end is dipped into a small drop of cement and touched to a short piece of the filament, so it is attached at right angles to the support rod. After the cement has hardened and the needle is placed in the micromanipulator, the tip of the filament can be snipped off with the scissors until a suitable length is obtained. A tip which is too long is excessively flexible. A needle of this type has been in regular use for several months in my lab. About a dozen were made in a half-hour or so.

XVII. Department of Microbiology, Shizuoka College of Pharmacy, 2-2-1 Shizuoka, Japan. Communicated by Tamotsu Morita.

Below follow summaries of our recent work done in the Laboratory of Molecular Biology, University of Alabama in Birmingham, U.S.A. (Chief: Dr. K. Lemone Yielding).

1. (Published) Tamotsu Morita and K. Lemone Yielding: Induction of respiratory deficient mutants in Saccharomyces cerevisiae by mono- and diazido analogs of ethidium. Mutation Research 56, 21-30, 1977.

SUMMARY

Mono- and diazido analogs of ethidium when photolyzed with yeast cells were highly effective in inducing respiratory deficient (RD) mutants. The monoazide was more mutagenic, though slightly less photosensitive, and under the concentrations and conditions used, both required photolysis to be significantly mutagenic.

Ethidium bromide (EB) competed with either its mono- or diazide analog for RD induction when applied before, but not after, the photolysis step. This suggested that the initial mutagenic binding sites for the azides were identical with those of EB.

There was no self-rescue or recovery in azide mutagenesis in contrast to EB. Furthermore, recovery from azide mutagenesis could not be provoked by EB. This confirmed a simple competition between binding of EB and its azide

analogs to account for the prevention of EB of the azide induced mutations.

2. (Submitted to Mutation Research) Tamotsu Morita and K. Lemone Yielding: Photolytic binding of the monoazido analog of ethidium to yeast mitochondrial DNA-competition by ethidium.

SUMMARY

The [^{14}C] labeled monoazido analog of ethidium, 3-amino-8-azido-5-ethyl-6-phenylphenanthridium chloride, when mixed with yeast cells and photolyzed, produced covalent adducts with both nuclear and mitochondrial DNA via the light-generated nitrene. The binding efficiency was about twelve times higher in mitochondrial than nuclear DNA. Moreover, the parent ethidium bromide at a fivefold excess was an effective competitor for the binding of the monoazido analog with mitochondrial DNA, but not with nuclear DNA.

XVIII. Tata Institute of Fundamental Research, Bombay 400 005, India. Communicated by P. K. Maitra.

The following is a list of recent publications.

P. K. Maitra and Z. Lobo (1977). Pyruvate kinase mutants of Saccharomyces cerevisiae: Biochemical and genetic characterization. Mol. Gen. Genetics 152:193-200.

Z. Lobo and P. K. Maitra (1977). Genetics of yeast hexokinase. Genetics 86:727-744.

Z. Lobo and P. K. Maitra (1977). Physiological role of glucose-phosphorylating enzymes in Saccharomyces cerevisiae. Arch. Biochem. Biophys. 182:639-645.

P. K. Maitra and Z. Lobo (1977). Yeast pyruvate kinase: A mutant from catalytically insensitive to fructose 1,6-bisphosphate. Eur. J. Biochem. 78:353-360.

P. K. Maitra and Z. Lobo (1977 - in press). Molecular properties of yeast glucokinase. Mol. Cell. Biochem.

P. K. Maitra and Z. Lobo (1977 - in press). Genetic studies with a phosphoglucose isomerase mutant of Saccharomyces cerevisiae. Mol. Gen. Genetics.

P. Sinha and P. K. Maitra (1977 - in press). Mutants of Saccharomyces cerevisiae having structurally altered pyruvate kinase. Mol. Gen. Genetics.

Z. Lobo and P. K. Maitra (1977 - in press). Resistance of 2-deoxyglucose in yeast: A direct selection of mutants lacking glucose-phosphorylating enzymes. Mol. Gen. Genetics.

P. K. Maitra and Z. Lobo (1978 - in press). Reversal of glycolysis in yeast. Arch. Biochem. Biophys.

XIX. Biological Laboratory, University of Kent at Canterbury, Kent CT2 7NJ, England. Communicated by Steve Oliver.

1. I have recently published the following papers on yeast:
Oliver, S. G. (1977). On the mutability of the yeast mitochondrial genome. J. Theoret. Biol. 67:195-201.

Oliver, S. G., McCready, S. J., Holm, C., Sutherland, P. A., McLaughlin, C. S. and Cox, B. S. (1977). Biochemical and physiological studies of the yeast virus-like particle. J. Bact. 130:1303-1309.

Oliver, S. G. and McLaughlin, C. S. (1977). Regulation of RNA synthesis in yeast I - Starvation experiments. Molec. gen. Genet. 154:145-153.

Oliver, S. G. and Williamson, D. H. (1977). Mutants of yeast specifically resistant to petite induction by fluorinated pyrimidines. Biochem. Genet. 15:775-783.

2. In addition, two other papers have been accepted for publication. The work involved formed part of Rick Ludwig's thesis entitled "The effects of an amino acid shift-up on growth, macromolecular synthesis and phosphate metabolism in Saccharomyces cerevisiae." Rick's M.S. degree was awarded by the University of California at Irvine this Summer.

Ludwig, J. R., II, Oliver, S. G. and McLaughlin, C. S. The regulation of RNA synthesis in yeast II - Amino acid shift-up experiments. (Molec. gen. Genet., in press).

ABSTRACT

A study has been made of the effects of a casamino acids shift-up on a prototrophic strain of yeast growing under conditions of ammonium repression. The shift-up produced an increase in growth rate some 120 mins. after the addition of amino acids to the medium. This growth rate increase was slightly preceded by an increase in the rate of accumulation of DNA. In contrast, the accumulation of protein increased immediately and that of RNA 15-20 mins. after the shift. RNA was initially accumulated at a rate faster than that required to sustain the new steady state. This was shown to be due to an increase in the rate of synthesis of the rRNA species derived from the 35s precursor. The rate of synthesis of 5s rRNA and of tRNA increased much later and to a lesser extent than that of the 35s-derived species. The implications of these results for general theories of the regulation of RNA synthesis are discussed.

Ludwig, J. R., II, Oliver, S. G. and McLaughlin, C. S. The effect of amino acids on growth and phosphate metabolism in a prototrophic yeast strain. (Biochem. Biophys. Res. Commun., in press).

ABSTRACT

The addition of casamino acids to a log. phase culture of a prototrophic yeast strain under conditions in which their catabolism is repressed caused a stimulation in growth rate. The neutral amino acids and arginine were the principal contributors to this stimulation effect. An early response of the cells to the addition of amino acids was the accumulation of low molecular weight polyphosphates. This accumulation was shown to correlate to the basicity of a given amino acid rather than to its effect on growth rate. A role for polyphosphates in intracellular buffering is therefore suggested.

XX. Rutgers, The State University of New Jersey, Waksman Institute of Microbiology, P. O. Box 759, Piscataway, New Jersey 08854. Communicated by Vivian L. MacKay.

The following items are abstracts of papers recently published or accepted for publication from our laboratory in collaboration with other laboratories.

1. Betz, R., V. L. MacKay and W. Duntze (1977). a-Factor from Saccharomyces cerevisiae: Partial characterization of a mating hormone produced by cells of mating type a. J. Bacteriol. 132: in press.

Conjugation between haploid cells of Saccharomyces cerevisiae is mediated through the action of diffusible mating hormones, two of which have been designated as a-factor and α-factor. Partially purified fractions exhibiting a-factor activity have been obtained from culture filtrates of a cells by ultra-filtration, ion-exchange chromatography, and gel filtration. The a-factor preparations specifically caused both G1 arrest and morphological alterations in cells of α-mating type, whereas a cells, a/α diploids, and nonmating α mutants were not affected. The a-factor activity was found in the culture filtrates of all a strains tested, but not in filtrates of α or a/α cell cultures. The hormone is sensitive to various proteases, showing that it is associated with a peptide or protein. Gel filtration studies suggest an apparent molecular weight > 600,000 daltons; however, this result may be due to aggregation with carbohydrate present in the preparations. Although the biological activities of a-factor are analogous to those described previously for α-factor, the chemical properties of these two hormones appear to be quite different.

2. Tkacz, J. S. and V. L. MacKay (1978). Sexual conjugation in yeast: Cell surface changes in response to the action of mating hormones. J. Cell Biol., accepted for publication.

In the yeast Saccharomyces cerevisiae, sexual conjugation between haploid cells of opposite mating type results in the formation of a diploid zygote. When treated with fluorescently-labeled concanavalin A (a specific staining reagent for the α-mannan in the cell wall), a zygote stains nonuniformly with the greatest fluorescence occurring at the conjugation bridge between the two haploid parents. In the mating mixture, unconjugated haploid cells often elongate to pear-shaped forms ("shmoos") which likewise

exhibit asymmetric staining with the most intense fluorescence at the growing end. Shmoo formation can be induced in cells of one mating type by the addition of a hormone secreted by cells of the opposite mating type; such shmoos also stain asymmetrically. In nearly all cases, the nonmating mutants that were examined stained uniformly after incubation with the appropriate hormone. These results suggest that, prior to and during conjugation, localized cell surface changes occur in cells of both mating types; the surface alterations facilitate fusion and are apparently mediated by the hormones in a manner that is mating-type specific.

The following is the abstract of a paper presented at the annual meeting of the Genetics Society of America, held in Austin, Texas, August 14-17, 1977.

Lemontt, J. F. and V. L. MacKay (1977). A pleiotropic mutant of yeast expressing the mating-specific "shmoo" morphology during vegetative growth in the absence of exogenous mating hormone. Genetics 86:s38 (Supplement, No. 2, Part 2).

ABSTRACT

The umr7-1 allele, previously identified among mutants selected for resistance to ultraviolet light-induced mutation at CAN1 (Lemontt, Genetics 83: s45, 1976), was found to be responsible for several phenotypic changes relating to sexual processes in heterothallic Saccharomyces cerevisiae. The only apparent effect of umr7-1 on a cells is to cause them to aggregate in enormous clumps ("clumpy"). Although α umr7 meiotic haploid segregants are also clumpy, in addition they do not mate at high frequency with a strains (α-specific sterility) nor do they secrete active α-factor (a mating hormone from α cells). Unlike α-specific sterile mutants previously described, α umr7 cultures contain a vast majority of cells with an abnormal shape. Staining of such cells with fluorescent-labelled concanavalin A revealed an asymmetric surface binding pattern indistinguishable from the appearance of normal a strains induced to form "shmoos" by exposure to α-factor. This pattern is entirely different from the uniform staining exhibited by untreated cells that are a umr7, a UMR7, or α UMR7. Genetic data show that 1) all phenotypic effects are attributable to a single umr7 locus, which maps on chromsome III, loosely linked (approx. 20 cM) and distal to thr4, 2) the shmoo-specific staining pattern is produced only by segregants previously scored as α umr7 in tetrads, and 3) revertants of umr7 with normal phenotype may be isolated. It is suggested that the UMR7 gene product plays an important regulatory role in the expression of mating-type-controlled functions.

An undergraduate thesis was completed by G. P. Livi in our laboratory in May 1977; a manuscript is currently in preparation.

SUMMARY

Studies on the mating-type regulation of methyl methanesulfonate sensitivity in Saccharomyces cerevisiae. A number of cellular processes in the yeast Saccharomyces cerevisiae are re-

gulated by the mating-type locus (mat), including mating specificity, meiosis and spore formation, mitotic recombination, and X-ray survival. This investigation has enlarged the influence of mat to involve the control of methyl methane-sulfonate (MMS) survival. This chemical mutagen is radiomimetic in that it simulates the damage and induces the DNA repair mechanisms of yeast indicative of X-irradiation. Heterozygous mat diploids (a/α) are more refractory to the effects of MMS and X-rays than homozygous a/a and α/α diploids; such a mating-type effect has been removed in certain homozygous α/α diploids which have acquired the ability to sporulate.

XXI. J. E. Purkyně University, Faculty of Medicine, Department of Biology, Brno, Czechoslovakia. Communicated by Marie Kopecká.

Papers recently published, in press or in preparation from this laboratory follow below:

Nečas, O.: The role of self-assembly in cell morphogenesis. Evolutionary Biology, Novák V., Paceltová B. (eds.) Prague, 325-334, 1976.

Nečas, O., Gabriel, M.: The relationship between ultrastructure and survival of spores of Rhizopus nigricans damaged by freezing. I. Viability of spores after repeated freezing and thawing shocks. Folia Microbiol., in press.

Nečas, O., Svoboda, A.: Density of particles in osmotically extended plasma membrane. Proc. XVth Czech. Conf. Elect. Microsc. V. Viklický and J. Ludvík (eds.), Czech. Acad. Sci., Prague, Volume A, 229-230, 1977.

Svoboda, A.: Mating reaction in yeast protoplasts. Arch. Microbiol. 110:313-318, 1976.

Hrazdira, I., Havelková, M.: Dynamics and morphology of spore germination of the mould Rhizopus nigricans affected by ultrasonic treatment. Acta Fac. Med. Univ. Brno, in press.

Kreger, D. R., Kopecká, M.: Assembly of wall polymers during the regeneration of yeast protoplasts. Microbial and Plant Protoplasts, J. F. Peberdy, A. H. Rose, H. J. Rogers, E. C. Cocking (eds.) Acad. Press 237-252, 1976.

Kopecká, M., Gabriel, M.: A new cytological method of staining cell nuclei in native yeasts and moulds by lomofungin. In preparation for Nature.

Kopecká, M., Gabriel, M., Svoboda, A., Trávinřk,P.: Osmotically induced changes in the ultrastructure of plasma membrane invagination in yeast cells. Proc. XV. Conf. Electr. Microsc., V. Viklický and J. Ludvík (eds.), Czech. Acad. Sci., Prague, Part A, 177-178, 1977.

Kopecká, M.: Ultrastructure of yeast nuclei after inhibition of DNA-dependent RNA polymerases by the antibiotic lomofungin. Proc. XV. Conf. Electr. Microsc. V. Viclický and J. Ludvík (eds.) Czech. Acad. Sci., Prague, Part A, 175-176, 1977.

Kreger, D. R., Kopecká, M.: The nature of the nets produced by protoplasts of Schizosaccharomyces pombe during the first stage of wall regeneration in liquid media. J. Gen. Microbiol., in press.

Kopecká, M., Horák, J., Farkaš, V.: The effect of lomofungin, an inhibitor of RNA synthesis, on cell wall regeneration in protoplasts of Saccharomyces cerevisiae. In preparation for J. Gen. Microbiol.

Kopecká, M.: One the mechanism of nuclear staining by lomofungin. In preparation for Folia Microbiol.

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Lectures presented on XIIIth Czechosl. Microbiological Congress, April 1977, Gottwaldov:

Gabriel, M.: Ultrastructure of cells and spheroplasts of the blue-green alga Anacystis nidulans.

Svoboda, A.: Differentiation and morphogenesis of yeasts.

Kopecká, M.: Biogenesis of the fibrillar wall component in the cell cycle.

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Course in electron microscopy:

A course, "Methods of preparation of microorganisms for electron microscopy," was arranged by the Committee of General Microbiology, Czech. Microbiological Society (organized by Dr. M. Kopecká and Dr. J. Caslavaská) in the Department of Biology, Faculty of Medicine, J. E. Purkyně University, Brno, from September 5-9, 1977. The course consisted of a series of lectures, demonstrations and practical training. Shadowing, negative staining, freeze-etching and ultrathin sectioning were the main topics of the course. The students were microbiologists from all over Czechoslovakia. As lecturers the most experienced electron microscopists were chosen. At the end of the course practical electron microscopy was demonstrated with observation of preparations prepared by students. Projection of films on electron microscopy summarized the main methods and procedures.

XXII. ALKO, Box 350, SF-00101 Helsinki 10, Finland. Communicated by Dr. Heikki Suomalainen.

Below follows a list of our work published or accepted for publication since April 1977.

SOME CHARACTERISTIC FEATURES OF THE ANAEROBIC METABOLISM OF YEAST

Erkki Oura

EUCHEM Conference on Metabolic Reactions in the Yeast Cell in Anaerobic and Aerobic Conditions, Helsinki 1977, pp. 23-25.

The following points of the effects of the absence of oxygen on yeast growth and metabolism are made:

1. Yeast grown under anaerobic conditions had nutritional requirements, besides those for sterol and unsaturated fatty acids, also for nicotinic acid, the biosynthesis of which requires the participation of molecular oxygen.

2. In the absence of oxygen, the oxidation-reduction reactions in yeast are balanced in the formation of glycerol.

3. In the absence of oxygen several TCA cycle enzymes are partially repressed and the glyoxylate cycle does not function.

BIOTIN AND THE METABOLISM OF BAKER'S YEAST

Heikki Suomalainen and Erkki Oura

EUCHEM Conference on Metabolic Reactions in the Yeast Cell in Anaerobic and Aerobic Conditions, Helsinki 1977, pp. 29-31.

A model for the metabolism is presented that explains the reasons for effects of biotin deficiency on baker's yeast metabolism, e.g.: the accumulation of citrulline during growth on limited amounts of biotin and the excretion of diazotizable amine and hypoxanthine from this yeast, the increased amounts of alanine and valine in the cellular pool, the excretion of pyruvate in biotin-poor yeast, the enhanced formation of ethyl acetate under biotin deficiency, and the largely insensitive biosynthesis of lipids and fatty acids.

In the studies of the biosynthetic pathway to biotin in biotin-dependent baker's yeast the following conclusion has been drawn. In this yeast the last part of biotin-path, that starting from 7-keto-8-aminopelargonic acid, does function. The block in the reaction sequence to biotin exists either in the formation of the active form of pimelic acid, pimelyl-CoA, or in the next step, the formation of 7-keto-8-aminopelargonic acid.

SOME ASPECTS OF CO_2 FIXATION BY BAKER'S YEAST IN ANAEROBIC AND AEROBIC CONDITIONS

Sampsa Haarasilta and Erkki Oura

EUCHEM Conference on Metabolic Reactions in the Yeast Cell in Anaerobic and Aerobic Conditions, Helsinki 1977, pp. 51-52.

In yeast grown under anaerobic conditions 5.5% of the yeast carbon was found to be derived from fixed Co_2. The presence of excess aspartate in the glucose medium reduced this amount to 1.6%. These values were obtained using the formula: 100 x specific activity of yeast carbon/specific activity of evolved CO_2. However, results were obtained that indicated that the cellular CO_2 and the evolving CO_2 were not, in fact, in complete equilibrium, but that the ratio of the specific 14C activities of the evolved and cellular CO_2 was 1.3. Using this as a correction factor, the adjusted value for assimilated CO_2 will be 7.2% for yeast grown anaerobically on glucose and 2.1% when grown in the aspartate-supplemented glucose medium.

The reasons for these differences in the amount of assimilated CO_2 by yeast under different growth conditions are discussed.

A RAPID METHOD FOR THE QUALITATIVE ANALYSIS FOR ENZYMES IN YEAST

Anssi Saura, Juhani Lokki, Marja-Liisa Lokki, Erkki Oura and Heikki Suomalainen

EUCHEM Conference on Metabolic Reactions in the Yeast Cell in Anaerobic and Aerobic Conditions, Helsinki 1977, pp. 57-59.

Using a special method for electrophoretic separation of enzymes, which has not been used until now for separation of enzymes in yeast, 18 yeast enzymes are surveyed for possible differences between samples representing yeasts grown from semiaerobic to completely aerobic conditions. The enzymes tested are mainly those participating in glycolysis or the TCA cycle.

THE ESTERASES OF BAKER'S YEAST. I. ACTIVITY AND LOCALISATION IN THE YEAST CELL

Elke Parkkinen, Erkki Oura and Heikki Suomalainen

Journal of The Institute of Brewing (in press).

The activity of esterase in baker's yeast cells and in cell fractions was estimated using 2-oxoglutaric acid diethyl ester, p-nitrophenyl acetate and α- and β-naphthyl acetate as substrates.

The esterase hydrolyzing 2-oxoglutaric acid ester was shown to be located inside the cell, both directly by an enzymatic method and by indirect evidence. The activities of the esterase

hydrolyzing aryl esters were found to vary greatly with the different substrates and estimation methods used. The presence of esterase activity towards phenyl and naphthyl esters in both cell wall digests and sphaeroplast lysates confirms the localisation of at least one esterase on both sides of the plasma membrane barrier. Depending on the method of evaluation, values between 80 and 40% of the total were obtained for the proportion of esterase activity located outside the plasma membrane, the most reliable values being about 50-65%.

INCORPORATION OF OLEIC ACID INTO THE PLASMA MEMBRANE OF BAKER'S YEAST AND ITS EFFECT ON THE PERMEATION OF PYRUVIC ACID

Kaija Konttinen and Heikki Suomalainen

YEAST 12-77

Journal of The Institute of Brewing 83:251-253 (1977).

The permeability of both natural and artificial membranes depends, among other things, on the nature of the fatty acyl chains of the phospholipids. A higher level of unsaturated fatty acids in the plasma membrane increases the permeability as a result of the increased mobility of the chains that results from a less tight packing (van Deenen, 1972).

The proportion of unsaturated fatty acids in the yeast and in the neutral lipids of plasma membrane isolated from it is higher in aerobically cultivated commercial baker's yeast than in more anaerobically cultured yeast or in brewer's yeast (Suomalainen & Keränen, 1968; Nurminen, Konttinen & Suomalainen, 1975). This difference in the composition of the plasma membrane may explain why pyruvic acid penetrates more rapidly into the cells of intact commercial baker's yeast than into more anaerobically cultured yeast cells (Suomalainen & Oura, 1958; Suomalainen, Konttinen & Oura, 1969).

Baker's yeast cannot synthesize unsaturated fatty acids under anaerobic conditions (Andreasen & Steir, 1954), but it can utilize exogenous fatty acids for lipid synthesis during anaerobic cultivation (Alterthum & Rose, 1973).

In the present work, we have studied the incorporatioan of oleic acid added as Tween 80 (polyoxyethylene sorbitan monooleate) into the plasma membrane of anaerobically grown baker's yeast (Saccharomyces cerevisiae) and the effect of the altered membrane composition on the permeation of pyruvic acid. A preliminary reference to these results has been made before (Suomalainen & Nurminen, 1976).

CARBON DIOXIDE IN YEAST GROWN UNDER GLUCONEOGENETIC CONDITIONS

Erkki Oura and Sampsa Haarasilta

Abstract of paper presented at the Eleventh FEBS Meeting, August 14-19, 1977, Copenhagen, Denmark.

In yeast grown under gluconeogenetic conditions with ethanol as substrate the usually important CO_2 assimilation reaction catalyzed by pyruvate carboxylase seems to be of little use to the yeast. Consequently, in these circumstances less CO_2 would be expected to be fixed than in yeast grown on glucose. In growth experiments using continuous cultivation and a continuous $^{14}CO_2$ feed the ^{14}C-activity indicated that 3.37% of the cellular carbon was derived from CO_2. The corresponding value for yeast grown anaerobically on glucose (where the pyruvate carboxylase reaction is the only anaplerotic mechanism) was 5.53%, and for yeast grown aerobically on glucose (where the second anaplerotic mechanism, the glyoxylate cycle, functions partially) it was 2.64%. The pyruvate carboxylase activity in yeast grown on ethanol was only slightly less than in yeast grown aerobically on glucose, whereas the phosphoenolpyruvate carboxykinase activity was nearly 30 times higher in yeast grown on ethanol than in yeast grown on glucose. The existence of the futile cycle: pyruvate → oxaloacetate → phosphoenolpyruvate → pyruvate could not satisfactorily explain the results obtained, e.g. the substantial radioactivity incorporated into carbohydrates and alanine and glycine. A better explanation was obtained by considering the CO_2-exchange reaction mediated by phosphoenolpyruvate carboxykinase. Thus the higher amount of ^{14}C in ethanol-grown yeast compared with yeast grown aerobically on glucose does not reflect a higher assimilation of CO_2, it is merely an indication of the exchange of CO_2 with phosphoenolpyruvate. The amounts of CO_2 assimilated by yeast grown on glucose either anaerobically or aerobically agreed closely with the amounts predicted by the CO_2 assimilation reactions.

ENZYMATIC DETERMNNATION OF BIOTIN

Sampsa Haarasilta

Submitted for publication in Analytical Biochemistry

An enzymatic method for the quantitative determination of biotin has been developed. The method involves the enzymatic binding of biotin *in situ* to the pyruvate carboxylase apoprotein of biotin deficient baker's yeast and the subsequent estimation of the pyruvate carboxylase activity by a $^{14}CO_2$ fixation method. The method is specific for biotin. Several biotin analogs and precursors were tested and only biocytin was found to interfere. Biotin amounts of less than 5 pg can be estimated.

The following publications have appeared since the last communications. The abstracts of reports have been given in Yeast News Letter 26:1, 29, 32 (1977).

John Londesborough. Characterization of an adenosine 3$^-$:5$^-$ -cyclic monophosphate phosphodiesterase from baker's yeast. Its binding to subcellular particles, catalytic properties, and gel-filtration behaviour. Biochem. J. 163:467-476 (1977).

Erkki Oura. Glycerol and succinic acid as the main side reaction products during yeast fermentations. Process Biochem. 12:3, 19-21 (1977).

XXIII. Technical Research Centre of Finland, Biotechnical Laboratory, Box 192, SF-00121 Helsinki 12, Finland. Communicated by V. Mäkinen.

Effect of Oxygen on the Formation of Flavour Compounds in Beer Fermentation

by T-M. Enari and E. Pajunen

EUCHEM Conference on Metabolic Reactions in the Yeast Cell in Anaerobic and Aerobic Conditions, Helsinki 1977, p. 43.

The most important flavour compounds formed during beer fermentation are esters, higher alcohols, carbonyl compounds and carboxylic acids. All these are influenced by:

1. oxygen concentration of wort
2. wort composition
3. yeast strain
4. yeast concentration
5. temperature
6. pH
7. pressure

Yeast and Alcohol Concentrations in Semi-Continuous Cultivation of Baker's Yeast

by Maija-Liisa Suihko and V. Mäkinen

EUCHEM Conference on Metabolic Reactions in the Yeast Cell in Anaerobic and Aerobic Conditions, Helsinki 1977, p. 73.

The Laboratory-scale baker's yeast production process used in this laboratory consists of 3 anaerobic, 1 weakly aerated and 2 strongly aerated stages. In this research the weakly aerated stage was carried out on a semi-continuous basis. Different dilutions of molasses, complemented by certain essential nutrients, were used as growth media. A part of the culture was removed at regular intervals and new medium was fed continuously to the fermentor at such a rate that the original volume once more attained immediately prior to the following removal of culture. The inoculum obtained from the semi-continuous cultivation was grown in one or two strongly aerated stages to produce "commercial" yeast.
Control of yeast concentration was attempted so that the

yeast in the broth removed from the fermentor would be suitable as inoculum for the subsequent cultivation stage. -High alcohol concentrations (4.0% w/w) and correspondingly low biomass yields were easily obtained using dilutions of molasses containing up to 12% sugar. The cultivation maintained "steady state". High biomass yields could also be obtained easily using 15% molasses, but maintenance of "steady state" was in this case very difficult. "Steady state" was, however, attained when the alcohol concentration of the cultivation fell below 2% w/w, stabilizing at 1.5% w/w.
The use of the semi-continuous state, however, resulted in reduced fermentation activity. This in turn caused a linear reduction in the raising power of the "commercial" yeast to a minimum of 80% of the original value after 50 days.

Fatty Acids and Sterols of Brewery Bottom Yeasts.

by V. Mäkinen and J. Ahvenainen

EUCHEM Conference on Metabolic Reactions in the Yeast Cell in Anaerobic and Aerobic Conditions, Helsinki 1977, p. 71.

The fatty acids and sterols of different bottom yeast strains were analysed by gas chromatography. Yeast biomass was studied after cultivation with and without aeration. Sedimented yeast from primary fermentation was also studied. The fatty acid concentration of anaerobic pitching yeast varies, depending on the strain, between 2 and 6% (d.w). The total amount of fatty acids is usually higher in anaerobic cultivation, but different strains have different properties in this respect. The relative concentrations of the individual fatty acids also vary to some extent depending on the strain. The greatest single fatty acid component of the pitching yeast of most strains is palmitic acid (C_{16}) varying from 27 to 39% (of total fatty acids), but for some strains the major component is palmitoleic acid ($C_{16:1}$) varying from 29 to 50%. During anaerobic cultivation the proportion of unsaturated fatty acids usually increases, e.g. palmitoleic and particularly linoleic acid ($C_{18:2}$), which is not observed at all in pitching yeasts. The amount of oleic acid ($C_{18:1}$) remains constant. Some strains have a very exceptional chromatogram. In pitching yeast cultivated anaerobically the amount of squalene is about double that of ergosterol, and a similar proportion is found in sedimented yeast after 12 days' fermentation. In aerated yeasts, however, this proportion is reversed.

Ester Concentration during Beer Fermentation (in German).

by E. Pajunen, K. Jaaskelainen, H. Vehviläinen and V. Mäkinen

Brauwissenschaft 30 Nr. 5, 1977, 129...133

Volatile flavour compounds - esters and higher alcohols - considerably affect the total flavour characteristics of beer, especially light and pale beer. The yeast strain has a more pronounced effect on the concentration of fusel alcohols than of esters, although isoamyl acetate concentration may be critical because of the low taste threshold of this compound. Sucrose addition enhances the concentration of higher alcohols with the exception of propanol, which remains almost constant. The concentrations of esters, especially isoamyl acetate, are considerably raised as a result of sucrose addition. Pilot scale brewing trials have been shown to be a convenient method of testing the suitability of yeast strains for industrial scale fermentations. A reliable view of the formation of most of the flavour compounds is obtained. The ethyl acetate concentration in pilot scale fermentations tends to be higher than in the corresponding brewery fermentation.

XXIV. Lvov Branch of A. V. Palladin Institute of Biochemistry, Ukrainian SSR Academy of Sciences, 290005 Lvov, Drahomanova Str., 14/16, USSR. Communicated by George M. Shavlovsky.

The following papers on yeast have appeared recently:

1. Shavlovsky, G. M., Kashchenko, V. E., Koltun, L. V., Logvinenko, E. M. and Zakalsky, A. E. Regulation of synthesis of GTP-cyclohydrolase participating in yeast flavinogenesis by iron. Mikrobiologiya (Moscow) 46:578-580 (1977).

SUMMARY

Pichia guilliermondii, Schwanniomyces occidentalis, Torulopsis candida and several riboflavin-dependent mutants of T. candida were grown in iron-deficient medium. In these conditions the ability of GTP-cyclohydrolase, which catalyzes the first step of flavinogenesis, increases. The activity of the enzyme increases also when iron-rich cells of T. candida and P. guilliermondii are incubated with α, α'-dipyridyl which induces overproduction of riboflavins; this action of α, α'-dipyridyl is eliminated by cycloheximide. Therefore, iron deficiency in the cells of these yeasts causes derepression of GTP-cyclohydrolase participating in riboflavin biosynthesis. The activity of the enzyme is inhibited by FAD but not by FMN and riboflavin.

2. Sibirny, A. A., Shavlovsky, G. M., Kshanovskaya, B. V. and Naumov, G. I. Hybridization and meiotic segregation in the paraffin-utilizing yeast Pichia guilliermondii Wickerham. Genetika (Moscow) 13:314-321 (1977).

SUMMARY

A simple method for hybridization of auxotrophic mutants of Pichia guilliermondii with complementary nutritional requirements on the acetate medium was worked out. P. guilliermondii hybrids possess a low frequency of sporulation. Two diploids were obtained

which produce up to 40% asci on the medium with sodium acetate. Sporulation does not occur or occurs very rarely on other media tested. Asci of diploids contain preferentially two spores.

A method for random spore analysis using ethanol for elimination for vegetative cells is proposed. A study of meiotic segregation by means of this method with a diploid strain D19 (mat /mat, +/ade 2, +/rib 1, his x/+) have shown a segregation of 1:1 for loci ade 2/+ and his x/+. Rib 1 survivors segregated more seldom than riboflavin prototrophs. Another diploid, AR, segregated 1:1 for mating locus among auxotrophic survivors.

3. Shavlovsky, G. M., Sibirny, A. A. and Ksheminskaya, H. P. Permease and "excretase" for riboflavin in mutants of Pichia guilliermondii. Biochem. Physiol. Pflanzen (Jena) 171:139-145 (1977).

SUMMARY

By means of UV irradiation mutants of the yeast Pichia guilliermondii MS1 and MS1-3 were selected, which in contrast to the wild type strain are able to accumulate appreciable amounts of riboflavin (RF) in the cells. RF was transported against a concentration gradient. This process was depressed by inhibitors of the energy metabolism and depended on temperature and pH of the incubation medium. RF uptake by the cells of MS1-3 mutant was characterized by substrate specificity and followed saturation kinetics. The transport of RF or of its analogues was strongly inhibited by glucose and by some glucose analogues; this inhibition showed competitive character.

The MS1-3 mutant was able to excrete the accumulated RF into the medium by means of an "excretase", which in some properties is different from that of RF permease.

4. Sibirny, A. A., Shavlovsky, G. M., Ksheminskaya, H. P. and Orlovskaya, A. G. Transport of riboflavin into the cells of riboflavin dependent yeast mutants. Mikrobiologiya (Moscow) 46:376-378 (1977).

5. Sibirny, A. A., Shavlovsky, G. M., Ksheminskaya, H. P. and Orlovskaya, A. G. Active transport of riboflavin in the yeast Pichia guilliermondii. Detection and some properties of the cryptic riboflavin permease. Biokhimiya (Moscow) 42:1841-1851 (1977).

6. Sibirny, A. A., Shavlovsky, G. M. and Goloshchapova, G. V. Mutants of Pichia guilliermondii with multiple sensitivity to antibiotics and antimetabolites. I. Selection and some properties of mutants. Genetika (Moscow) 13:872-879 (1977).

7. Logvinenko, E. M., Trach, V. M., Kashchenko, V. E., Zakalsky, A. E., Koltun, L. V. and Shavlovsky, G. M. Study of activities of some yeast flavinogenic enzymes in situ. Biokhimiya (Moscow) 42:1649-1654 (1977).

8. Sibirny, A. A., Zharova, V. P., Kshanovskaya, B. V. and Shavlovsky, G. M. Breeding of a genetic line for the yeast Pichia guilliermondii capable of forming a large number of spores. Tsitologiya i genetika (Kiev) 11:330-333 (1977).

9. Shavlovsky, G. M. and Fedorovich, D. V. The activity of enzymes involved in synthesis and hydrolysis of flavin adenine dinucleotide in Pichia guilliermondii studied at different levels of flavinogenesis. Mikrobiologiya (Moscow) 46:904-911 (1977).

XXV. Queen Elizabeth College, University of London, Microbiology Department, Atkins Building, Campden Hill, London W8 7AH, England. Communicated by R. K. Poole.

Since the last contribution to the Yeast Newsletter from this laboratory, a number of papers have been published or prepared.

1. Journal of General Microbiology (1976) 93:241-250.

Fractionation by Differential and Zonal Centrifugation of Spheroplasts Prepared from a Glucose-repressed Fission Yeast Schizosaccharomyces pombe 972 h$^-$.

By R. K. Poole and D. Lloyd

A method is described for the preparation of spheroplasts in high yield from Schizosaccharomyces pombe by treating cells grown in the presence of glucose and deoxyglucose with snail digestive enzymes. Gentle disruption of such spheroplasts yielded homogenates, from which marker enzymes for nuclei (NAD pyrophosphorylase) and mitochondria (cytochrome c oxidase activity and spectroscopically-detectable cytochromes a + a_3) could be quantitatively sedimented by low-speed centrifugation. In contrast to previous findings with Saccharomyces carlsbergensis, cytochrome c oxidase and another mitochondrial enzyme, succinate dehydrogenase, were completely sedimentable by zonal centrifugation in sucrose gradients in the presence of either 2mM-$MgCl_2$ or 0.4 mM-EDTA. Mitochondria were apparently smaller and of lower buoyant density in gradients containing EDTA. The bulk of the total units of malate dehydrogenase and NADH-cytochrome c oxidoreductase sedimented with mitochondria, whereas NADPH-cytochrome c oxidoreductase was located in fractions containing no mitochondria. The distributions of mitochondrial enzymes were heterogeneous in populations of mitochondria separated on the basis of size or density. The possible origins of mitochondrial heterogeneity in extracts of S. pombe are discussed with special reference to changes in the enzyme activities of cells during cell cycle.

2. Proceedings of the Society for General Microbiology (1977) 4:75.

Termination of the Cell Cycle of Schizosaccharomyces pombe by Inhibition of Mitochondrial Protein Synthesis.

By R. K. Poole

During the cell cycle of S. pombe, certain components of the mitochondrial electron transfer chain including cytochrome c oxidase, whose synthesis probably requires mitochondrial protein synthesis, are synthesized periodically. In a study of the interactions between mitochondrial biogenesis and the cell cycle, the effects of chloramphenicol, an inhibitor of mitochondrial protein synthesis, on the progress of the cell cycle have been investigated.

Synchronous cultures of glycerol-grown cells were prepared by glycerol density-gradient centrifugation and the effect of the time of addition of chloramphenicol on residual cell division (final cell number/cell number prior to the first synchronous division) was determined. When chloramphenicol (2 mg/ml) was added between 0.3 and 0.8 of the cycle the subsequent synchronous division was not inhibited, i.e. residual division was 2. No further division occurred but cells continued to increase in volume.

The effect of chloramphenicol added in the last 0.2 of the cycle could not be measured directly due to the presence of both incipient and neonatal cells in synchronous cultures approaching the mid-point of doubling in cell numbers. However, the residual division observed (> 2) when chloramphenicol was added to such cultures is consistent with a model in which residual division is 2 throughout the cycle, except for a fraction at the start of the cycle (< 0.3) in which residual division is 1. This evidence for an early "transition point" for sensitivity to chloramphenicol was substantiated in experiments in which the addition of chloramphenicol to an exponential culture allowed approximately one doubling in cell numbers.

3. FEMS Microbiology Letters (1977) 1:305-307.

Preparation of synchronous cultures of microorganisms by continuous-flow selection: which cells are selected?

By R. K. Poole

Continuous flow centrifugation is a new, useful method for rapidly preparing synchronous cultures of micro-organisms. It has been assumed that it is the smallest cells in the exponential culture entering the rotor that fail to sediment, pass through the rotor and are collected to form the synchronous culture. In this paper, it is shown that density fluctuations during the cell cycle of Schizosaccharomyces pombe cause cells late in the cell cycle to have sedimentation coefficients lower than those even of neonatal cells. Thus for this organism the cells collected in the effluent are late in the cycle and consequently divide soon after establishment of the synchronous culture. A similar situation seems to apply for E. coli and Candida utilis.

4. Journal of General Microbiology. In the press.

Development of Respiratory Activity during the Cell Cycle of Schizosaccharomyces pombe 972h. Respiratory Oscillations and Heat Dissipation in Cultures Synchronized with 2'-Deoxyadenosine.

By R. K. Poole

The rates of oxygen uptake and heat dissipation were measured in cultures of Schizosaccharomyces pombe that had been induced to divide synchronously by adding 2 mM-2'-deoxyadenosine and then removing the inhibitor after 4 h. Respiratory oscillations occurred during the last 1.5 h of treatment with deoxyadenosine and throughout the subsequent period of synchronous growth. Before completion of the first synchronous division three peaks of oxygen uptake occurred, the third peak being coincident with cell division. These peaks were less sensitive to the rate-stimulating effect of the uncoupler, carbonyl-cyanide m-chloromphenyl hydrozone, than were the troughs, so that in the presence of the uncoupler the oscillations were attenuated. In the absence of uncoupler, heat dissipation of the culture increased linearly, during and after deoxyadenosine treatment, with sharp increases (approximate doublings) in the rate of dissipation occurring at intervals similar to the mean generation time of an exponential culture. Heat dissipation also increased continuously in samples removed from such a culture and incubated with the uncoupler. The possible modulation of oxygen uptake rates by respiratory control, and the implications of linear increases in heat dissipation are discussed.

5. Journal of General Microbiology. In the press.

The Induction of a Shortened, Synchronous Cell-Cycle by Removal of Chloramphenicol from an Inhibited Culture of the Fission Yeast Schizosaccharomyces pombe.

By A. Quinton and R. K. Poole

Adding chloramphenicol to exponential cultures of the fission yeast Schizosaccharomyces pombe, growing on glycerol as carbon source, results in a population of cells of abnormally large and heterogeneous volumes. After removal of the inhibitor and resuspension in fresh medium, these cells increase in volume, during which time cell volumes become more normally distributed. Cells then undergo two synchronous divisions separated by 5 h, which is significantly shorter than the doubling times for culture extinction and total cell volume (both about 7h). This chloramphenicol-induced cell cycle is also shorter than the first complete cell-cycle in synchronous cultures prepared by size-selection. The result is an overall decrease in mean cell volume. The induction of synchronous divisions is not caused by harvesting and washing procedures. Some factors that influence the ability of chloramphenicol-inhibited cells to recover are described.

6. Proceedings of the Society for General Microbiology (1976) 3:86.

Persistence of Respiratory Oscillations in Deoxyadenosine-induced Synchronous Cultures of Schizosaccharomyces pombe.

By R. K. Poole and A. M. Pickett

In addition to presenting the results in (4) above in a preliminary form, this abstract reported that respiratory oscillations in these cultures occurred with the same relative timing in either defined or complex growth media.

XXVI. Instituto de Enzimologia del Consejo Superior de Investigaciones Cientificas, Facultad de Medicina de la Universidad Autónoma, Madrid 34, Spain. Communicated by R. Serrano.

Energy Requirements for Maltose Transport in Yeast
Ramón Serrano

Eur. J. Biochem. 80:97-102. 1977.

Maltose transport in yeast (Saccharomyces cerevisiae) is inhibited by uncouplers under conditions where the intracellular concentration of the sugar is lower than in the medium. The uncouplers did not deplete the ATP content of the yeast cells and a 50-100-fold reduction in ATP caused by antimycin and 2-deoxyglucose had no effect on maltose transport. In ATP-depleted cells, the maltose transported is partially hydrolyzed to glucose but not further metabolized and therefore a mechanism of transport involving phosphorylation can be discarded. One proton is cotransported with every maltose molecule. The fact that maltose transport is inhibited by KCl but not by NaCl, Tris·Cl or KSCN suggests that the electroneutrality during maltose and proton uptake can be maintained by the exit of K^+ from the cells or by the entry of a permeable anion as SCN^-. These results indicate that the translocation of maltose across the yeast plasma membrane is not dependent on ATP and is coupled to the electrochemical gradient of protons in this membrane. When this gradient is abolished by uncouplers, the transport system is not able to function even in favour of a concentration gradient of the sugar.

XXVII. Université de Nancy I , Biochimie Microbienne, 5 rue Albert Lebrun B. P. 403. 54001 Nancy Cedex, France. Communicated by R. Bonaly.

In our laboratory we have carried out investigations on yeast cell walls.

A. Some studies were concerned with the chemical structure of the cell walls as a function of the culture conditions of the yeasts.

A comparative study with a Torulopsis strain cultivated on methanol and ethanol indicates that the methanol source favours the synthesis of mannan polymers while the ethanol source favours the elaboration of glucan and glucomannan. Chitin and protein are more important after culture on methanol than on ethanol. (Phytochemistry, 1977, 16:1167-1170)

An investigation of the chemical structure of the walls of a Candida strain cultivated on paraffin substrate has been undertaken. The cell walls contain 43.5% of neutral sugar, 23% of protein and 26% of glucosamine. Chitin represents 22% of the cell wall. The high content of protein seems characteristic of all microorganisms grown on n-paraffin substrates. (Mycopathologia, 1977, 61:49-54)

A mannoprotein has been isolated from cell walls of a Torulopsis strain cultivated on methanol. In this compound, the protein and the mannan are attached to each other by an "Asparaginyl-N-acetylglucosamine structure in which chitobiose seems involved, and by O-glycosidic linkages." Long mannan chains are attached by the N-glycosidic linkage, while short mannan chains are bound by the O-glycosidic linkage. (Biochimie, 1977, in press)

B. Other studies are more specific and concern some studies of antifungal antibiotic actions on yeast cell walls.

A culture of Rhodotorula yeasts in the presence of hydroxy-2-biphenyl does not affect the glucosidic composition of the cell walls, but the protein content is increased and the glucosamine (chitin) content is diminished. Similar results were obtained by the use of "resting-cells" yeasts.

Griseofulvin had no action on yeast development, but inhibited fungal development. One of the hypotheses of the antifungal action is its inhibition of chitin synthesis. We have tested this compound on Rhodotorula yeasts, which may contain 11% chitin in their cell walls, and have detected a decrease of this polymer.

The action of cycloheximide was studied on Rhodotorula yeasts. The cell wall alterations observed are as follows: decrease of chitin-protein and galactose. It was not possible to establish a decrease of mannose by action of this antibiotic as it was shown to occur in Saccharomyces cell walls. (Mycopathologia, 1977, in press)

XXVIII. University of Edinburgh. Department of Zoology, West Mains Road, Edinburgh EH9 3JT, Scotland. Communicated by J. M. Mitchison.

Below follow abstracts of four papers from our laboratory.

1. P. Nurse and P. Thuriaux. Controls over the timing of DNA replication during the cell cycle of fission yeast. Experimental Cell Research 107 (1977) 365-375.

SUMMARY

The controls acting over the timing of DNA replication (S) during the cell cycle have been investigated in the fission yeast Schizosaccharomyces pombe. The cell size at which DNA replication takes place has been determined in a number of experimental situations such as growth of nitrogen-starved cells, spore germination and synchronous cultures of wee mutant and wild-type strains. It is shown that, in wee mutant strains and in wild type grown under conditions in which the cells are small, DNA replication takes place in cells of the same size. This suggests that there is a minimum cell size beneath which the cell cannot initiate DNA replication and it is this control which determines the timing of S during the cell cycle of the wee mutant. Fast growing wild-type cells are too large for this size control to be expressed. In these cells the timing of S may be controlled by the completion of the previous nuclear division coupled with a requirement for a minimum period in G1. Thus, in S. pombe, there are two different controls over the timing of S, either of which can be operative depending upon the size of the cell at cell division. It is suggested that these two controls may form a useful conceptual framework for considering the timing control over S in mammalian cells.

2. P. Fantes and P. Nurse. Control of cell size at division in fission yeast by growth-modulated size control over nuclear division. Experimental Cell Research 107 (1977) 377-386.

SUMMARY

In the fission yeast Schizosaccharomyces pombe, nutritional reduction of growth rate by supplying poor nitrogen, carbon or phosphate sources causes a decrease in cell size. The effect on cell division following three different nutritional shifts-up has been investigated. In all cases, about 20% of the cells divide at the original cell length, and then cell division stops for a period. Cell division then resumes at the new faster rate, cell length at division being characteristic of the new medium. Further investigation reveals that the first effect of the shift is to inhibit nuclear division rapidly and completely. These results are strongly suggestive of the operation of a cell size requirement for entry into nuclear division. The cell size necessary for nuclear division is set, or modulated, by the prevailing growth conditions. This model is confirmed by a nutritional shift-down, where nuclear division and cell division are stimulated after the shift. Cell length at division falls rapidly until the new shorter length is attained, when a new steady state is assumed at a slower growth rate. The control system is compared with that in bacteria, and its implications for various models proposed for the control of timing of mitosis are discussed.

3. K. A. Nasmyth. Temperature sensitive lethal mutants in the structural gene for DNA ligase in the yeast Schizosaccharomyces pombe. Cell (in press) 1977.

SUMMARY

cdc 17-K42 was isolated as a ts cdc mutant of the fission yeast Schizosaccharomyces pombe after nitrosoguanidine mutagenesis. The ts phenotype segregates 2:2 in tetrad analyses and it is recessive to the wild type allele. The pattern of cell division in this mutant on temperature shift implies that its defective function is usually completed by the end of S phase. Cells of cdc 17-K42 enter S phase and undergo a complete round of DNA synthesis at the restrictive temperature (r.t.), but mitosis does not follow. The nascent DNA accumulated at the r.t. is exclusively composed of short (Okazaki) fragments. After a 20 min pulse label, the main peak of labelled DNA ranges from 70 to 450 nucleotides long. DNA ligase assays, involving the formation of covalently closed Lambda DNA circles, show that the mutant has low levels of DNA ligase activity (less than 20%) when assayed at the permissive temperature and none detectable when assayed at the r.t. This implies that the cdc 17 locus codes for the structural gene for DNA ligase. cdc 17-K42 also has a temperature enhanced UV sensitivity, suggesting that the same enzyme is involved in DNA repair. Two other independent, mutant alleles in the same gene have also been isolated (M75 and L16). They share many of the above properties.

4. J. M. Mitchison. Enzyme synthesis during the cell cycle. In "Cell Differentiation in Microorganisms, Plants and Animals" (Eds. L. Nover and K. Mothes). pp. 377-401. VEB Gustav Fischer Verlag: Jena. 1977.

SUMMARY

Out of nineteen enzymes examined in synchronous cultures of the fission yeast Schizosaccharomyces pombe, nearly all show continuous increases in activity through the cell cycle. Only one, TMP kinase, an enzyme of DNA synthesis, shows a clearcut pattern of periodic synthesis. Asynchronous controls of the gradient selection technique show that some enzymes can be perturbed by the synchronising technique and that these perturbations can resemble periodic synthesis once per cycle. A short review of other systems emphasizes the importance of carrying out controls of the synchronising procedures.

Some of the enzymes in S. pombe show a linear pattern of activity increase with a doubling in rate once per cycle. This pattern is also shown by rRNA, mRNA and CO_2 production. The rate changes differ in their timing in the cycle and in their dependence on DNA synthesis and division.

Theories for the control of periodic enzyme synthesis do not explain the large number of cases where synthesis appears to be continuous. Gene dosage may be important. There is evidence for translational control and stable mRNA in yeast. The control of unstable enzymes needs to be considered.

XXIX. Department of Marine Microbiology, Botanical Institute, University of Göteborg, CarlSkottsbergs Gata 22, S-413 19 Göteborg, Sweden. Communicated by Birgitta Norkrans.

The following papers have been published or are in press:

L. Adler (1976). Purification of the alkaline phosphatase in the halotolerant yeast Debaryomyces hansenii. Acta Chem. Scand., 30, 43-48.

L. Adler (1977). Properties of alkaline phosphatase of the halotolerant yeast Debaryomyces hansenii. (Submitted for publication in BBA).

SUMMARY

The alkaline phosphatase of D. hansenii from cells cultivated at different salinities (6 mM & 2.7M NaCl) was characterized with respect to several chemical and enzymatic properties as: molecular weight (110,000 at gel filtration), isoelectric point (pH 4.4 at electrofocusing). It is a metalloenzyme and behaves with regard to a number of properties as a typical alkaline phosphomonoesterase. Arsenate, molybdate, and orthophosphate acted as competitive inhibitors. Various metalbinding agents inhibited enzyme activity. A Zn^{2+} addition almost completely reversed the EDTA inhibition. Mg^{2+} stimulated the enzyme activity and was required for its maintenance at high ionic strength in the presence of Na^{+}. (D. hansenii produces glycerol in increasing amounts at increasing cultural salinity). Glycerol in high concentration increased the K_m-values of the alkaline phosphatase and decreased its maximum velocity (V). As enzyme extracted from cells cultured at low salinity was indistinguishable from that of cells grown in the presence of 2.7 M NaCl with respect to several criteria, no salinity dependent modification of the enzyme seems to occur.

L. Gustafsson and B. Norkrans (1976). On the mechanism of salt tolerance. - Production of glycerol and heat during growth of Debaryomyces hansenii. Arch. Microbiol. 110:177-183.

L. Gustafsson and B. Lindman (1977). A flow microcalorimetric cell for studies of aerobic yeast growth. FEMS Microbiol. Letters 1:227-230.

B. Norkrans and I. Tunblad-Johansson (1977). Cellular content of Krebs cycle keto-acids in yeasts during growth on different nitrogen sources including hydroxylamine. Arch. Microbiol. (In press).

SUMMARY

The cellular pool of Krebs cycle keto-acids was followed as a function of growth in three yeasts. The keto-acids were analyzed as silylated methoximes by quantitative gas chromatography with capillary glass columns. The 2-oxoglutaric acid content was strikingly

high in the hydroxylamine (HA)-tolerant, HA-utilizing Endomycopsis (Saccharomycopsis) lipolytica when compared to that in the nitrate-utilizing yeast Cryptococcus albidus and in Saccharomyces cerevisiae, requiring fully reduced nitrogen for growth. The content in E. lipolytica increased throughout the log phase to maxima of about 200-250 µg per g dry weight in HA and ammonia media. These amounts are 20 to 25 times greater than those attained in the two other yeasts. The cellular content of pyruvic acid was at a maximum early in the log phase, amounting to 50-70 µg per g dry weight for all yeasts. The oxalacetic acid content never exceeded 9 µg per g dry weight in any of the yeasts. Oxime formation, for which keto-acid production is a prerequisite, is discussed as part of the HA-tolerance.

I. Tunblad-Johansson (1977). Quantitative determination of free amino acids by gas-liquid chromatography with special reference to yeasts. Acta path. microbiol. Supplement: Applications of Gas and High pressure Liquid Chromatography in Microbiology (In proof).

SUMMARY

A gas-liquid chromatographic method for quantitative determination of free amino acids in yeasts is described. The amino acids are extracted with boiling water and separated from interfering substances on a cation-exchange resin. They are analyzed as their n-propyl N-acetyl esters. The best separation of the derivatives was achieved when using glass columns containing a mixture of three polar phases.

By this method the amino acid pool is now followed for the yeasts used in the above given study of Krebs cycle keto-acids during growth on different nitrogen sources.

XXX. Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan. Communicated by Kenji Soda.

We have investigated the metabolism of nitroalkanes by yeasts (A), purification and characterization of 2-nitropropane dioxygenase of Hansenula mrakii (B), and production of L-lysine by Candida pelliculosa (C). The following papers along these lines have been published.

(A). T. Kido, T. Yamamoto and K. Soda: Microbial Assimilation of Alkyl Nitro Compounds and Formation of Nitrite. Arch. Microbiol. 106: 165-169 (1975).

ABSTRACT

Sixty-six representative strains of bacteria, yeasts and fungi were tested for their ability to grow in a semidefined medium containing 0.5% nitroethane as a nitrogen source. About half of them were found capable of growing in the medium. Hansenula beijerinckii, Candida utilis, and Penicillium chrysogenum were most active in assimilating nitroethane. 2-Nitropropane inhibited growth of most of the microorganisms tested in a medium containing 0.2% peptone and 0.2% glycerol. Hansenula mrakii was found to grow

rapidly in the nitroethane-peptone medium after a lag phase. Nitrite accumulated in the culture fluid after the phase of logarithmic multiplication, and increased with growth, followed by a decline after the maximum growth. The alkyl nitro compounds were oxidatively denitrified to form nitrite by a crude enzyme from Hansenula mrakii. Nitroethane was generally a poor substrate, but was the best inducer to produce the nitro compounds' oxidizing enzyme. 2-Nitropropane and nitroethane were enzymatically oxidized to nitrite, and acetone and acetaldehyde, respectively, which were isolated as 2,4-dinitrophenylhydrazones and identified. The nitrite formed was found to be reduced to ammonia by intact cells and also by the crude enzyme.

(B)-1. T. Kido, T. Yamamoto and K. Soda: Purification and Properties of Nitroalkane-Oxidizing Enzyme from Hansenula mrakii. J. Bacteriol. 126-3: 1261-1265 (1976).

ABSTRACT

A nitroalkane-oxidizing enzyme was purified about 1,300-fold from a cell extract of Hansenula mrakii (grown in a medium containing nitroethane as the sole nitrogen source) by ammonium sulfate fractionation, diethylaminoethylcellulose column chromatography, hydroxyapatite column chromatography, and Bio-Gel P-150 column chromatography. The enzyme was shown to be homogeneous upon acrylamide gel electrophoresis and ultracentrifugation. The enzyme exhibits absorption maxima at 274, 370, 415, and 440 nm and a shoulder at 470 nm. Balance studies showed that 2 moles of 2-nitropropane are converted into equimolar amounts of acetone and nitrite with the consumption of 1 mol of oxygen.

$$2CH_3CH(NO_2)CH_3 + O_2 \longrightarrow 2CH_3COCH_3 + 2HNO_2$$

Hydrogen peroxide is not formed in the enzyme reaction. In addition to 2-nitropropane, 1-nitropropane and nitroethane are oxidatively denitrified by the enzyme, but nitromethane is inert to the enzyme. The nitroalkanes are not oxidized under anaerobic conditions.

(B)-2. T. Kido and K. Soda: A New Oxygenase, 2-Nitropropane Dioxygenase of Hansenula mrakii. J. Biol. Chem. 251-22: 6994-7000 (1976).

ABSTRACT

2-Nitropropane dioxygenase, purified to homogeneity from Hansenula mrakii (IFO 0895), has a molecular weight of approximately 62,000 and consists of two subunits nonidentical in molecular weight (39,000 and 25,000).

Stoichiometrical studies and the results obtained with $^{18}O_2$ showed that 2 atoms of molecular oxygen are incorporated into 2 molecules of acetone formed from 2-nitropropane. In addition to 2-nitropropane, nitroethane, 3-nitro-2-pentanol, and 1-nitropropane are oxidatively denitrified.

The enzyme, which exhibits absorption maxima at 274, 370, 415, and 440 nm and a shoulder at 470 nm, contains 1 mol of FAD and 1 g-atom of non-heme iron per mol of enzyme. The enzyme-bound FAD is reduced by 2-nitropropane under anaerobic conditions, but the enzyme-bound Fe^{3+} is not affected. The introduction of oxygen to the reduced form of enzyme causes reoxidation of the enzyme. The bound FAD and Fe^{3+} are reduced by the addition of nitromethane, which is not a substrate, under anaerobic conditions. The aerobic dialysis of the enzyme treated with nitromethane causes reoxidation of only the Fe^{2+}. Sodium dithionite also reduces both the enzyme-bound FAD and Fe^{3+} under anaerobic conditions. When the enzyme is dialyzed against 10 mM potassium phosphate buffer (pH 7.0) immediately after reduction by dithionite, the absorption spectrum similar to that of the native enzyme appeared with concomitant restoration of approximately 80% of the activity.

The enzyme activity is significantly inhibited by pyrocatechol-3,5-disulfonate disodium salt, 8-hydroxyquinoline, reducing agents such as 2-mercaptoethanol, and $HgCl_2$. The Michaelis constants are as follows: 2-nitropropane (2.13×10^{-2} M), nitroethane (2.43×10^{-2} M), 3-nitro-2-pentanol (6.8×10^{-3} M), 1-nitropropane (2.56×10^{-2} M), and oxygen (3.03×10^{-4} M, with 2-nitropropane).

(C). E. Takenouchi, D. K. Nikolova, K. Awano, K. Soda and H. Tanaka: Excretion of Lysine by Lysine Sulfur Analogue Resistant-mutants of Candida pelliculosa. Agric. Biol. Chem. 41: 615-616 (1977).

ABSTRACT

The mutants of Candida pelliculosa resistant to S-(β-aminoethyl)-L-cysteine (SAEC), a sulfur analog of L-lysine, excreted remarkable amounts of lysine in the medium. SAEC inhibits the growth of C. pelliculosa depending on the concentrations of SAEC, but L-lysine and L-α-aminoadipate restored the growth effectively. SAEC-resistant mutants (1726 strains) were induced from the wild-type strain of C. pelliculosa by UV irradiation or N-methyl-N'-nitro-N-nitrosoguanidine treatment, and lysine productivity of the mutants was examined. Although the wild-type strain did not produce appreciable concentration of lysine extracellularly, almost all the resistant mutants obtained did more or less. The several potent lysine producers excreted more than 2.2 mg/ml of lysine in the medium.

XXXI. Janssen Pharmaceutica - B-2340 Beerse - Belgium. Communicated by Hugo van den Bossche.

The following is an abstract of a paper presented at the 10th International Congress of Chemotherapy, Zürich, Switzerland, September 18-23, 1977 and of a paper submitted for publication in Chemico Biological Interactions.

INHIBITION OF ERGOSTEROL BIOSYNTHESIS IN *CANDIDA ALBICANS* BY MICONAZOLE

H. van den Bossche, G. Willemsens and W. F. J. Lauwers

Sterol biosynthesis from ^{14}C-acetate was studied in *Candida albicans* grown in a casein hydrolysate-yeast extract-glucose medium in the presence or absence of the antifungal agent miconazole. A time- (1, 4, 16 and 24h) and dose- (2.10^{-10} - 10^{-4}M) dependent inhibition of ^{14}C-acetate incorporation into ergosterol was observed. Fifty percent inhibition of this incorporation was found after 1h incubation in the presence of 10^{-9}M miconazole. Simultaneously, accumulation of radioactivity into 4,4'-dimethyl-and 4α-methylsterols was observed. Further identification of the lipid fractions of miconazole-treated cells (using thin-layer-, gas-lipid chromatography and mass spectroscopy) revealed the presence of 24-methylenedihydrolanosterol, lanosterol, obtusifoliol, 4,14-dimethylzymosterol and 14-methylfecosterol. The major sterol in cells, grown for 16h (end of logarithmic phase of growth) in the presence of 10^{-7}M miconazole was 24-methylenedihydrolanosterol. In these cells 14-methylfecosterol instead of ergosterol was found.

The accumulation of 14α-methyl sterols suggests that miconazole is a potent inhibitor of one of the metabolic steps involved in the demethylation at C-14.

Miconazole also intervenes in the ^{14}C-acetate incorporation into fatty acids and triglycerides. However, in all circumstances studied, ergosterol synthesis was affected at lower doses than those interfering with fatty acid and triglyceride synthesis.

It is suggested that the miconazole-induced inhibition of the C-14 demethylation may be involved in the previously observed permeability changes in miconazole treated *C. albicans*.

XXXII. University of Pittsburgh, Department of Life Sciences, Pittsburgh, PA 15260. Communicated by Terrance G. Cooper.

Below follow abstracts of two manuscripts representing work from our laboratory.

BASIC AMINO ACID INHIBITION OF CELL DIVISION AND MACROMOLECULAR SYNTHESIS IN *SACCHAROMYCES CEREVISIAE*

by Roberta Sumrada and Terrance Cooper
(submitted for publication)

Growth of *Saccharomyces cerevisiae* on poor nitrogen sources such as allantoin or proline was totally inhibited by addition of lysine or homoarginine to the medium. The same result was observed with ornithine and arginine if their catabolism was prevented by using appropriate mutant strains. Cells treated with lysine contained greatly reduced quantities of histidine and arginine. Conversely, lysine and histidine were severely reduced in arginase-minus cells treated with arginine. When all three basic amino acids were present

in the culture medium, growth was normal, suggesting that synthesis of all three basic amino acids can be halted by an excess of any one of them. Inhibition of growth was accompanied by a five-fold increase in the observed ratio of budded to unbudded cells. These morphological changes would be expected if DNA synthesis was inhibited. Therefore, cells growing with proline or allantoin as sole nitrogen source were tested for their ability to carry out DNA, RNA, and protein synthesis in the presence and absence of lysine or ornithine. Addition of a basic amino acid to the culture medium substantially reduced the ability of the cells to incorporate ^{14}C-uracil into alkali-resistant, trichloroacetic acid-precipitable material (presumably DNA). RNA and protein synthesis, although decreased, were less sensitive to the effects of such additions.

FACTORS INFLUENCING THE OBSERVED HALF LIVES OF SPECIFIC SYNTHETIC CAPACITIES IN SACCHAROMYCES CEREVISIAE

by Terrance G. Cooper, Gene Marcelli and Roberta Sumrada

(in press)

We have identified a variety of factors affecting the stability of allophanate hydrolase-specific and gross cellular protein synthetic capacities. These synthetic capacities have been extrapolated by many laboratories to represent functional messenger RNAs. Synthetic capacity turnover rates that we measured were greater in diploid organisms than in haploid strains and were proportional to the temperature of the culture medium. The stability of allophanate hydrolase-specific synthetic capacity was not influenced by alterations in the nitrogen source provided in the culture medium, but was increased up to 15 fold by the total inhibition of protein synthesis. Cultures in which protein synthesis was inhibited as little as 20 percent exhibited hydrolase-specific synthetic capacities more than two-fold greater than those observed in the absence of inhibition.

XXXIII. Universidad Nacional Autonoma de Mexico, Instituto de Biologia, Departamento de Biologia Experimental, Apartado Postal 70-600, Mexico 20, D. F. Communicated by A. Peña.

In the last issue of the Yeast Newsletter, we informed the readers that we had sent a paper on the preparation of yeast mitochondria to be published in BBRC; the paper was finally published in FEBS Letters 80:209-213, 1977.

With this mitochondrial preparation, we have obtained the following information:

THE ACTION OF MONOVALENT CATIONS ON OXIDATIVE PHOSPHORYLATION OF YEAST MITOCHONDRIA

By M. Tuena de Gómez-Puyou, S. Uribe, J. R. Mattoon, A. Gómez-Puyou and A. Peña.

The effect of K^+ and other monovalent cations on the oxidative phosphorylation of mitochondria from two strains of yeast (D-311-3A and baker's yeast) has been examined. In the

presence of ethanol, K^+ increases the rate of respiration and phosphorylation; with succinate as oxidizable substrate, K^+ enhances the ADP:O ratios of mitochondria. K^+ enhances the rate of electron transport in the NADH-CoQ span of the respiratory chain. In addition, K^+ and other monovalent cations augment the ATPase activity of intact mitochondria by inducing changes in the activity of the F_1 component. K^+ stimulates the hydrolytic activity of souble F_1 by changing the kinetic characteristics of F_1 towards Mg-ATP. The results are consistent with the idea that K^+ favors oxidative phosphorylation by an action at the level of F_1.

XXXIV. Ecole Nationale Supérieure Agronomique de Montpellier, Chaire de Génétique et de Microbiologie, 34060 Montpellier Cedex, France. Communicated by P. Galzy.

The following articles represent recent work from our laboratory.

1. G. Moulin, J. F. Arthaud, R. Ratomahenina, P. Galzy.

   Remarques sur la production de Kéfyr.
   Industries Agricoles et Alimentaires 5:495-497. 1977.

   First we describe principal metabolic characteristics (respiration and fermentation) of the yeast Candida kéfyr. Then we make remarks about some organic compounds of the traditional kéfyr and about a possible production by fermentation of an industrial drink, like traditional kéfyr.

2. G. Moulin, P. Galzy. Etude de l'α- amylase de la paroi de Pichia burtonii Boidin. Zeitschrift für Allgemeine Mikrobiologie Vol. 18, 1978.

   The presence of α-amylase in the wall of Pichia burtonii Boidin has been investigated. Two forms of enzyme were found. A loosely-bound one required only the aid of a 0.20 mM phosphate buffer for its solubilization. The principal properties were presented.

3. G. Moulin, P. Galzy. Remarque sur la régulation de la biosynthèse de l'α-amylase de Pichia burtonii Boidin. Zeitschrift für Allgemeine Mikrobiologie Vol. 18, 1978.

   The synthesis of amylolytic enzymes by Pichia burtonii strain CBS 6141 requires the presence of starch, maltose and saccharose. Glucose exerts a strong repression which completely inhibited enzyme induction.

XXXV. University of Illinois at Chicago Circle, Department of Biological Sciences, Box 4348, Chicago, Illinois 60680. Communicated by F. Schlenk.

The following publications have appeared:

The Biochemistry of Adenosylmethionine (1977). F. Salvatore, E. Borek, V. Zappia, H. G. Williams-Ashman, eds. (Symposium Report). Columbia

University Press, New York. 588pp.

Members of this Department have contributed as follows:

F. Schlenk: Recent studies on the chemical properties of adenosylmethionine and related compounds, pp. 3-17.

K. D. Spence, A. J. Ferro, and T. F. Petrotta-Simpson: Transport and intracellular balance of adenosylmethionine in yeast, pp. 77-82.

S. K. Shapiro and A. J Ferro: Metabolism of adenosylmethionine during the life cycles of Enterobacter aerogenes and Saccharomyces cerevisiae, pp. 58-76.

H. de Robichon-Szulmajster, K. D. Nakamura, and F. Schlenk: Active transport of adenosylmethionine in yeast, pp. 83-86.

Law, R. E., and A. J. Ferro: Effect of S-adenosylmethionine and cyclic adenosine 3', 5'-monophosphate on RNA synthesis during glucose derepression in Saccharomyces cerevisiae. FEBS Letters 80:153-156 (1977).

Choih, S. -J., A. J. Ferro, and S. J. Shapiro: The relationship between polyamines and macromolecules in germinating yeast ascospores. J. Bacteriol. (in press) (1978).

Schlenk, F., C. H. Hannum, and A. J. Ferro: Biosynthesis of adenosyl-D-methionine and adenosyl-2-methylmethionine by Candida utilis. Arch. Biochem. Biophys. (in press) (1978).

Sun-Jim Choih has been awarded the Ph.D. degree; he is now at the Institute for Dental Research, University of Minnesota.

Dr. Susan Liebman, formerly at the University of Rochester, N. Y., has joined the Department as Assistant Professor. She will continue her research on yeast genetics.

XXXVI. Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, Communicated by A. L. Demain.

Below follows an abstract of some yeast work from this laboratory.

Enzymatic basis for tryptophan overproduction in a methanol-utilizing yeast, Hansenula polymorpha

Emmanuel Denenu and Arnold L. Demain

Using a sequential resistance development program, mutants resistant to increasing concentrations of 5-fluorotryptophan (5-FT) were derived following ethylmethane sulfonate (EMS) mutagenesis. This 3-step mutation sequence resulted in a 20-30 fold increase in L-tryptophan production. Tryptophan accumulation by the wild type strain is minimal and occurs mostly after exponential growth whereas it is growth-associated in the mutant strains, indicating successful

deregulation of the mutants. In situ (permeabilized cells) enzyme studies were carried out to determine the basis of tryptophan overproduction by the mutants. The first two mutants possess anthranilate synthetase activity which is partially desensitized to feedback inhibition. The degree of desensitization to tryptophan inhibition is proportional to the degree of overproduction. The most recent mutant does not show increased desensitization in comparison to its parent but it does have an increased specific activity of anthranilate synthetase and probably is partially derepressed.

XXXVII. Allied Breweries (Production) Limited, The Brewery, Station Street, Burton-on-Trent, DE14 1BZ. England. Communicated by Dorothy A. Lovett and P. A. Martin.

The following is an abstract of a paper which was read to the Annual Convention of the American Society of Brewing Chemists in May 1977. The full text will be published shortly in the Journal of the American Society of Brewing Chemists.

USE OF IMMUNOFLUORESCENCE AND VIABILITY STAINS IN QUALITY CONTROL

M. J. Chilver, J. Harrison, T. J. B. Webb

Immunofluorescence has been evaluated as a rapid quality control technique for the detection of low levels of wild yeasts in culture yeast or other brewery samples. Antisera have been prepared against antigenic groups A to F, pooled and absorbed with culture yeast. Using the indirect staining method and fluorescein isothiocyanate or rhodamine as fluorochrome, detection levels as low as 10 wild yeasts per million cells can be detected in 3 hours. Combined immunofluorescence and viability staining allows the differentiation of live and dead, culture and wild yeast cells on the same slide by alternating the light sources. The preferred method combines fluorescein isothiocyanate excited by incident blue light for wild yeast detection with methylene blue viewed by transmitted light for viability differentiation. Fluorescein diacetate is a useful viability stain for yeasts, although agreement between results comparing it with methylene blue and slide culture viabilities is not exact for heat-stressed cells. Some nonbrewing yeasts require heating for 2 min at 50°C before consistent results are obtained with fluorescein diacetate. An examination of the mechanism of action of methylene blue as a viability stain has suggested that it is one of permeability rather than permeability followed by enzymic reduction.

XXXVIII. Department of Food Science and Technology, University of California, Davis, California 95616. Communicated by Eric Johnson.

Johnson, Eric A., Douglas E. Conklin and Michael J. Lewis. 1977. The yeast Phaffia rhodozyma as a dietary pigment source for salmonids and crustaceans. J. Fish. Res. Board Canada 34:0000-0000. (In press).

Abstract

The red yeast Phaffia rhodozyma contains astaxanthin as its principal carotenoid pigment. Incorporation of this yeast into the diet (15% w/w) of rainbow trout induced pigmentation within 43 days. However, lobsters fed the same diet did not readily accumulate the carotenoids of the yeast, though they became pigmented on a diet of live brine shrimp. The primary pigment isolated from the bodies of the trout and lobsters fed the red yeast was astaxanthin. We conclude that P. rhodozyma is an excellent source of astaxanthin for cultivated salmonids.

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

E. Minárik and A. Navara: Occurrence of Saccharomycodes ludwigii Hansen in sulphited low alcoholic young wines. Mitteilungen Klosterneuburg 27: 1-3 (1977).

The large-celled apiculate yeast Saccharomycodes ludwigii Hansen could be temporarily isolated and identified in strongly sulphited young wines of low alcohol content prior to racking. This yeast species, which is resistant to sulphur dioxide, reveals an unfavourable metabolism for wine quality. It may be effectively eliminated by racking and/or kieselgur filtration.

xxx

E. Minárik: 13th Conference of the Subcommission "Microbiology of Wine". Vinohrad 1977 (submitted).

The Annual Meeting of the Subcommission of the Office International de la Vigne et du Vin (O.I.V.) was held in Paris May 11, 1977. Delegates from 18 countries attended the meeting. The following papers were presented:

1. V. Kovács (Jugoslavia): Moulds from grapes and wine browning.
2. F. Radler (GFR): Microbiological studies on active dry yeasts.
3. W. A. Agenbach (Rep. South Africa): Use of active dry yeasts in wine making.
4. E. Minárik (Czechoslovakia): Metabolism and formation of sulphur compounds by yeasts.
5. Ch. Vialatte (France): Yeasts responsible for film-formation on wine.
6. N. Goranov (Bulgaria): Application of gas chromatographic methods for the characterization of yeasts.

7. P. Bidan (France): The Yeast Catalogue of the O.I.V.

xxx

E. Minárik: Microbiology Research Results. 1965-1970 and 1971-1976 (in German). VITIS No. 3 and 4, 1977 (in press).

A world survey of important publications of the last 12 years on wine microbiology and related subjects. The survey includes the following fields of scientific activity and studies: systematics, taxonomy, ecology, physiology and biochemistry of wine yeasts, acid decomposition by yeasts of the genus Schizosaccharomyces, pure yeast starters, yeast collections, inhibitors of grape juice fermentation lactic and acetic bacteria of the wine. The survey is completed by nearly 500 bibliographic quotations.

XXXX. NATIONAL AND INTERNATIONAL YEAST MEETINGS

1. The Fifth International Protoplast Symposium will be held July 9-14, 1979 in Szeged, Hungary. Persons interested in receiving the various circulars with details on this meeting should write to Dr. L. Ferenczy, Dept. of Microbiology, Attila Jozsef University, H6701, Szeged, P. O. Box 428, Hungary.

2. The XII International Congress of Microbiology, Mycology Section, will be held in Munich, Federal Republic of Germany, Sept. 3-8, 1978. Persons wishing to attend this meeting should write for further information to Kongresszentrum, München-Messeglände, XII ICM, Postfach 12 10 09, D-8000, München 12, West Germany. The deadline for submitting a paper/poster is 15 April 1978. Simultaneous with this congress there is a Bacteriology Section and an Intersectional Meeting. The latter will consist of lectures by distinguished scientists.

3. BRITISH YEAST GROUP

In 1975 and 1976, meetings to discuss aspects of yeast biology were held in the University of Bath in Great Britain, sponsored respectively by the British Mycological Society and the Cell Surfaces and Membranes Group of the Society for General Microbiology. At these meetings, considerable enthusiasm was expressed for the setting up of an informal British yeast group. The first meeting of this group was held on September 26-27 1977, again in the University of Bath. The principal topics discussed at the meeting were the yeast cell envelope and the yeast nucleus. Approximately 50 people attended on each of the two days. It was agreed that the meeting should be an annual event in Great Britain and it is likely that the meeting in September 1978 will be held at the Brewing Research Foundation, Lyttel Hall, Nutfield, Surrey. Visitors from abroad would be very welcome at meetings of the British Yeast Group.

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4. The Tenth Annual Meeting of Yeast Genetics Conference-Japan

The Tenth Annual Meeting of Yeast Genetics Conference - Japan was held from September 8 to 10, 1977 at Rakuyu-Kaikan, Kyoto, Japan. Around eighty yeast researchers met and the following topics were presented and discussed.

Session 1: Mutation and Radiation Genetics (Chairperson - C. Shimoda)

S. Mori, H. Nakanishi, M. Murakami (Lab. Crop Sci. & Plant Breeding, Fac. Agr., Kyoto Pref. Univ., Shimogamo, Sakyo-ku, Kyoto 606) and T. Shigenobu (Fac. Agr., Kyoto Univ.) - UV- and γ-ray-induced mitotic crossing-over in Saccharomyces cerevisiae.

Session 2: Metabolism and Biochemistry (Chairpersons - M. Hayashibe and A. Kimura)

M. Okuda, Y. Tatsutomi and A. Kimura (Res. Inst., Food Sci., Kyoto Univ., Uji, Kyoto 611). Phosphorylation of nucleotides by yeast energy (ATP). Relationship between hexokinase isozymes and mitochondria.

N. Miyazaki (Inst. Molec. Biol., Fac. Sci., Nagoya Univ., Furoh-cho, Chigusa-ku, Nagoya 464). Nucleotide sequences of 5S ribosomal RNA from several yeast species.

T. Kamihara, Y. Nishikawa, I. Nakamura, M. Noda and S. Fukui (Lab. Indust. Biochem., Dept. Indust. Chem., Fac. Eng., Kyoto Univ., Yoshida-honmachi, Sakyo-ku, Kyoto 606). Effects of thiamine and pyridoxine on sterol metabolism in yeast.

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M. Hayashibe and H. C. Bhandari (Dept. Biol., Fac. Sci., Osaka City Univ., Sugimoto-cho, Sumiyoshi-ku, Osaka 558). Hexose transport in Schizosaccharomyces pombe.

H. Tamaki (Doshisha Women's Col., Imaidegawadori, Kamigyo-ku, Kyoto 602). Purification of glucoamylase in Saccharomyces.

K. Mitsushima, A. Shinmyo and T. Enatsu (Dept. Ferment. Technol., Osaka Univ., Yamadakami, Suita 565). Control of citrate formation in mitochondria prepared from Candida lipolytica.

H. Miwa (Ctr. Res. Lab., Ajinomoto Co., Inc., Susuki-cho, Kawasaki-ku, Kawasaki 210). Breeding of rapid-growth variants in Saccharomyces yeast.

Session 3: Recombination and Mapping (Chairperson - Y. Oshima)

T. Takahashi (Ctr. Res. Lab., Asahi Brew. Ltd., Ohmorikita, Ohta-ku, Tokyo 143). β-Phenyl ethanol sensitive genes.

T. Yamazaki (Dept. Ferment. Technol., Yamanashi Univ., Takeda, Koh-fu 400) and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Attempt of mapping in Saccharomycodes ludwigii.

C. Fukazawa (Natl. Food Res. Inst., Shiohama, Kohto-ku, Tokyo 135) and K. Udaka (Tokyo Col. Domestic Sci.). Molecular weight determination of DNAs from several Candida species by optical Cot analysis.

Session 4: Structure and Function of Cell Organelles (Chairperson - M. Osumi)

K. Arima and I. Takano (Ctr. Res. Inst., Suntory Ltd., Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618). Protoplast fusion of Saccharomyces yeast.

N. Kawakami, H. Mondo (Dept. Ferment. Technol., Fac. Eng., Hiroshima Univ., Senda-cho, Hiroshima 730) and H. Kawakami (Dept. Nutrition, Suzugamine Women's Col.). Morphological evidence of fusion in yeast protoplasts.

M. Osumi (Dept. Biol., Japan Women's Univ., Mejirodai, Bunkyo-ku, Tokyo 112). DNA in yeast microbody.

Session 5: Cytoplasmic Inheritance and Drug Resistance (Chairpersons - S. Nagai and K. Suda)

Y. Miyata (Doshisha Women's Col., Imaidegawadori, Kamigyo-ku, Kyoto 602). On the stabilization of the killer factor produced by Sake yeast.

F. Miyamoto (Dept. Biol., Fac. Edu., Wakayama Univ., Masago-cho, Wakayama 640). Effect of chloramphenicol, NaN_3 and dinitrophenol on the RD induction of yeast.

S. Nagai (Dept. Biol., Fac. Sci., Nara Women's Univ., Nara 630). Induction of respiratory deficient mutants by ethidium bromide in Saccharomyces rosei.

K. Suda (Biol. Lab., Nara Univ. Edu., Takabatake, Nara 630) and A. Uchida (Biol. Div., Col. Gen. Edu., Kobe Univ.). Segregation of mitochondrial genes on the meiosis of primary zygotes.

N. Gunge and A. Tamaru (Ctr. Res. Lab., Mitsubishi. Chem. Indust., Kamoshida, Midori-ku, Yokohama 227). Genetic evidence for protoplast fusion in Saccharomyces cerevisiae.

Y. Arakatsu (Dept. Biol., Fac. Sci., Konan Univ., Okamoto, Kobe 658). Saccharomyces mutants resistant to the petite-inducing action of acriflavine.

T. Morita, I. Mifuchi (Dept. Microb., Shizuoka Col. Pharm., Kojika, Shizuoka 420) and K. L. Yielding (Lab. Mol. Biol., Univ. Alabama). Photolytic binding of azido analogs of ethidium to yeast mitochondrial DNA.

Session 6: Gene Regulation (Chairperson - Y. Oshima)

Y. Oshima (Dept. Ferment. Technol., Osaka Univ., Yamadakami, Suita 565). Genetic regulatory systems for enzyme systhesis. Revision of the models.

Session 7: Sexuality and Life Cycle (Chairpersons - N. Yanagishima, I. Takano and T. Takahashi)

H. Miyata (Biol. Inst., Fac. Sci., Nagoya Univ., Chigusa-ku, Nagoya 464). Relationship between cell length and cycle time.

C. Shimoda and K. Nichi (Dept. Biol., Fac. Sci., Osaka City Univ., Sugimoto-cho, Sumiyoshi-ku, Osaka 558). Germ tube formation and DNA synthesis during germination of Schizosaccharomyces pombe spores.

N. Sando, S. Satoh, T. Abe, M. Fugane and M. Sasaki (Lab. Appl. Microbiol., Fac. Agr., Yamagata Univ., Tsuruoka-shi, Yamagata 997). Aging and sporulating ability in Saccharomyces cerevisiae.

M. Tsuboi (Dept. Biol., Fac. Sci., Osaka City Univ., Sugimoto-cho, Sumiyoshi-ku, Osaka 558). Ribonucleases of sporulating cells in Saccharomyces cerevisiae.

M. Hagiya, K. Yoshida and N. Yanagishima (Biol. Inst., Fac. Sci., Nagoya Univ., Chigusa-ku, Nagoya 464). Purification and characterization of sexual agglutination substances of yeast.

H. Tohyama, M. Hagiya, Y. Yoshida and N. Yanagishima (Biol. Inst., Fac. Sci., Nagoya Univ.). Changes in sexual agglutinability during the mating process in Saccharomyces cerevisiae.

T. Shimizu, K. Yoshida and N. Yanagishima (Biol. Inst., Fac. Sci., Nagoya Univ.). Binding substance for α-substance-I.

I. Takano (Ctr. Res. Inst., Suntory Ltd., Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618). Genetic analysis of sporogenous diploid strains which show mating response in Saccharomyces.

T. Oshima and I. Takano (Ctr. Res. Inst., Suntory Ltd.). Conversion of mating-type and polyploidization of triploid cells by the action of homothallism genes in Saccharomyces.

S. Harashima, K. Sugamoto and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., Yamadakami, Suita 558). On the function of homothallism genes.

Session 8: Special Lecture (Chairperson - N. Yanagishima)

H. Fukuhara (Fondation Curie Instut du Radium, Biologie, Bât. 110, Orsay 91190, France). Genetic map of mitochondria in Saccharomyces yeast.

Yeast researchers interested in these topics are welcome to contact the author. The next Annual Meeting of the Yeast Genetics Conference-Japan will be held at Ohmori Plant of Asahi Brewing Co., Tokyo in

October, 1978.

Isamu Takano
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XXXXI. Brief News Items

1. I announce with deep regret to the readers of the Yeast Newsletter that Professor Maurice Ingram died in November 1977. He was the author of An introduction to the biology of yeast, published by Pitman in 1955. He presented the first plenary lecture at the Third General Symposium on Yeasts, the Hague, 1969. Formerly he was Director of the Low Temperature Research Station, Cambridge. Later he became the Director of the Meat Research Station in Bristol.

   J. A. Barnett
   School of Biology,
   University of East Anglia,
   Norwich, England.

2. Professor Akira Kimura has recently changed his position from the Dept. of Food Science and Technology, Faculty of Agriculture, Kyoto University 505 to: Professor of Applied Microbiology, Research Institute of Food Science, Kyoto University, Uji, Kyoto 611, Japan.

3. Below follow the titles of two recent publications from this laboratory.

   S. Harashima and Y. Oshima (1977). Frequency of twelve ascus-types and arrangement of three genes from tetrad data. Genetics 86:535-552.

   Y. Nogi, K. Matsumoto, A. Toh-e, and Y. Oshima (1977). Interaction of super-repressible and dominant constitutive mutations for the synthesis of galactose pathway enzymes. Molec. gen. Genetics 152:137-144.

   Y. Oshima, Dept. of Fermentation Technology, Osaka University
   Yamadakami, Suita-shi,
   Osaka 565, Japan.

4. M. Ciriacy (1977). Isolation and characterization of yeast mutants defective in intermediary carbon metabolism and in carbon catabolite derepression. Molec. gen. Genetics 154:213-220.

   M. Ciriacy, Institute for Microbiology, Technische Hochschule Darmstadt D-6100, Darmstadt, G.F.R.

5. Following is a list of recent publications from this laboratory:

Steele, S. D. and J. J. Miller (1977). Amino acid uptake and protein synthesis in germinating spores of Saccharomyces cerevisiae. Canadian Journal of Microbiology 23:407-412.

Ashraf, M and J. J. Miller (1977). Induction of multi-spored asci in two-spored strains of Saccharomyces cerevisiae by amitrole. Canadian Journal of Microbiology 23:690-694.

IN PRESS:

Ho, K. H. and J. J. Miller. Free proline content and sensitivity to desiccation and heat during yeast sporulation and spore germination. Canadian Journal of Microbiology.

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Hamilton, Ont. L8S 4K1,
Canada.

6. "PHYCOMYCES" NEWSLETTER

This newsletter is open to any aspect of research using Phycomyces or other Mucorales as experimental subjects.

It publishes concise communications of experimental results; recommendations on techniques and nomenclature; lists of available strains; bibliography, letters and comments.

"Phycomyces 1" appeared in April 1977. "Phycomyces 2" is due to be distributed at the beginning of 1978.

Persons interested in contributing to the Newsletter, or in receiving it, should contact E. Cerdá-Olmedo, departamento de Genética, Edificio de Biología, Universidad, Sevilla, Spain.