Y E A S T

A News Letter for Persons Interested in Yeast

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Many thanks to those who have contributed to this issue by sending in news items and accounts of research projects. The next issue will be published in May 1970. A contribution of $1.00 from those who have not contributed for some time would be appreciated to finance future editions of the News Letter. Many thanks to those who have contributed recently. The Editors extend their warmest wishes to the readers of the readers of the News Letter for a productive and happy New Year.

H. J. Phaff

SPECIAL NOTE

Through the courtesy of Dr. R. C. von Borstal of the Oak Ridge National Laboratory, we have obtained copies of the recent Yeast Genetics Supplement to volume 31 of the Microbial Genetics Bulletin, and are mailing them with this issue of YEAST. This supplement proposes a uniform genetic nomenclature for yeast, and includes a compilation of the presently known products of a large number of genes of Saccharomyces. Since genetic studies of yeast are progressing rapidly, it is very desirable that some standard nomenclature be used, and the editors therefore urge all readers of YEAST to follow the recommendations in their future communications.

I Centraalbureau voor Schimmelcultures (Netherlands), Delft, Julianalaan 67a. Communicated by Dr. D. Yarrow.

The following strains have been received in the collection:

Candida beechii CBS 4261 (= IGC 3423)
" blankii CBS 1898 (= IGC 3410)
" cacaoi CBS 2020 (= IGC 3422)
" freyschussii CBS 2612 (= IGC 3566)
" melibiosica CBS 5814 (= IGC 2515)
H. R. Buckley and N. van Uden, Mycopath. Mycol. Appl. 36: 256-266 (1968).

Candida guilliermondii var. japonica CBS 6021
" ishiwadae CBS 6022
" terebra CBS 6023
Rhodotorula terrea CBS 6020
J. Sugiyama and S. Goto, J. Fac. Sci. Univ. Tokyo Sec. 3, 10: 97-116 (1969).

Candida steatolytica CBS 5839 (type), 4075, 5923
D. Yarrow, A. v. Leeuwenhoek 35: 24-28 (1969).

Hansenula dimennae CBS 5762 (= NRRL YB-3239)
" glucozyma CBS 5766 (= NRRL YB-2185)
" henricii CBS 5765 (= NRRL YB-2194)
" nonfermentans CBS 5764 (= NRRL YB-2203)
" saturnus var. subsufficiens CBS 5763 (= NRRL YB-1657)
L. J. Wickerham, Mycopath. Mycol. Appl. 37: 15-32 (1969).

Saccharomyces cordubensis CBS 6007
" gaditensis CBS 6006
J. Santa Maria, Proc. 3rd. Int. Symp. Yeasts 1969.

Selenotila intestinalis Krassilnikov CBS 5946

Sterigmatomyces elviae CBS 5922

Trichosporon fennicum CBS 5928 (type), 6027, 6028
C. E. Sonck and D. Yarrow, A. v. Leeuwenhoek 35: 172-177 (1969).

Torulopsis mannitofaciens CBS 5981
J. Onishi and T. Suzuki, A. v. Leeuwenhoek 35: 258-260 (1969).

Trichosporon aquatile CBS 5973 (type), 5988
" eriense CBS 5974
L. R. Hedrick and P. D. Dupont, A. v. Leeuwenhoek 34: 474-482 (1968).

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

The following list summarize our recent activities concerning yeasts and yeast-like organisms at P.R.L.

We have continued our study of the mannose-containing polysaccharides of yeast by p.m.r. spectroscopy. The following papers have appeared recently, have been accepted or submitted for publication, or are in preparation:

1. "Systematics of the genera Saccharomyces, Schizosaccharomyces, Endomycopsis, Kluyveromyces, Schwanniomyces and Brettanomyces: proton magnetic resonance spectra of the mannans and mannose-containing polysaccharides as an aid in classification." J. F. T. Spencer and P. A. J. Gorin. Antonie van Leeuwenhoek. 35: 361 (1969).

2. "Systematics of the genera Debaryomyces and Metschnikowia: proton magnetic resonance spectra of their mannans as an aid in classification." J. F. T. Spencer and P. A. J. Gorin. Antonie van Leeuwenhoek. In press.

The proton magnetic resonance spectra of the mannans of a number of Debaryomyces and Metschnikowia species (Endomycetales) were determined. The spectra of all of the mannans had several similar characteristics, regardless of the species from which they originated.

Pichia vini, a species originally classified as Debaryomyces vini, formed a mannan with a spectrum almost identical with those of Metschnikowia (Candida) reukaufii and Pichia haplophila. Debaryomyces vanriji, originally placed in the genus Pichia, formed a mannan with a spectrum identical with those of Pichia robertsii and Candida (Pichia) guilliermondii mannans.

3. "Torulopsis bombicola sp.n." J. F. T. Spencer, P. A. J. Gorin and A. P. Tulloch. Antonie van Leeuwenhoek. In press.

A new yeast species, Torulopsis bombicola, is described, that produces extracellular hydroxy fatty acid sophorosides. It utilizes relatively few carbon compounds. It forms a mannan having a proton magnetic resonance spectrum similar to the spectra of the galactomannans of Torulopsis apis, Torulopsis nodaensis and T. magnoliae, but differing from that of T. gropengiesseri galactomannan.

4. "Yeasts insolated from bumblebee honey from western Canada: proton magnetic resonance spectra of their mannans and mannose-containing polysaccharides as an aid in classification." J. F. T. Spencer, P. A. J. Gorin, G. A. Hobbs and D. A. Cook. Can. J. Microbiol. Submitted for publication.

5. "Systematics of the genus Candida Berkhout: proton magnetic resonance spectra of the mannose-containing polysaccharides of some further species of Candida." J. F. T. Spencer and P. A. J. Gorin. In preparation.

6. "Systematics of the genus Torulopsis: proton magnetic resonance spectra of the mannose-containing polysaccharides as an aid in classification." J. F. T. Spencer and P. A. J. Gorin. In preparation.

7. "Systematics of the genera Ceratocystis and Graphium: proton magnetic resonance spectra of the rhamnomannans and glucomannans as an aid in classification." J. F. T. Spencer and P. A. J. Gorin. In preparation.

8. "Enzymic degradation of yeast cell-wall mannans and galactomannans to polymeric fragments containing α-(1→6)-linked D-mannopyranose units." P. A. J. Gorin, J. F. T. Spencer and D. E. Eveleigh. Carbohydrate Research. In press.

The cell-wall mannans of Candida parapsilosis, Endomycopsis fibuliger, Saccharomyces rouxii, Torulopsis apicola (Hajsig strain) and Torulopsos bombicola were degraded with an exo α-D-mannosidase from Arthrobacter GJM-1 to their α-(1→6)-linked D-mannopyranose mainchains, as demonstrated by proton magnetic resonance spectroscopy.

Galactomannans from Candida lipolytica, Torulopsis gropengiesseri, Torulopsis lactis-condensi, Torulopsis magnoliae and Trichosporon fermentans could be degraded to polysaccharides containing mainly 6-O-linked α-D-mannopyranosyl units following preferential removal of their enzyme-resistant D-galactopyranosyl non-reducing end-units with acid. The mannans of Saccharomyces lodderi, Citeromyces matritensis and Pichia pastoris could also be enzymatically degraded to polysaccharides containing predominant α-(1→6)-linked D-mannopyranosyl units after removal of most of the β-D-linkages in their side chains with acid.

The exo α-D-mannosidase, as would be expected, produces β-D-mannose on splitting of an α-(1→2)-linked D-mannopyranose tetramer. It is, however, very selective in its action since it does not cleave D-Manp$^{1,2\alpha}$D-Manp$^{1,2\beta}$D-Manp$^{1,2\beta}$D-Manp$^{1,2\alpha}$D-Man. Apparently two consecutive α-linkages are required by the enzyme for cleavage of a substrate to take place.

9. "Structures of the L-rhamno-D-mannan from Ceratocystis ulmi and the D-gluco-D-mannan from Ceratocystis brunnea." P. A. J. Gorin and J. F. T. Spencer. Carbohydrate Research. In press.

10. "Comparison of proton magnetic resonance spectra of cell-wall mannans and galactomannans of selected yeasts with their chemical structures." P. A. J. Gorin, J. F. T. Spencer and R. J. Magus. Can. J. Chem. 47: 3569 (1969).

11. "Location of acyl groups on two partly acylated glycolipids from strains of Ustilago (Smut fungi)." S. S. Bhattacharjee, R. H. Haskins and P. A. J. Gorin. Carbohydrate Research. In press.

12. "Evidence for a 'block-type' structure in the main chain of the cell-wall mannan from a Candida species." P. A. J. Gorin and J. F. T. Spencer. Can. J. Chem. In press.

We have submitted a review of all aspects to Advances in Applied Microbiology, entitled "Proton magnetic resonance spectroscopy as an aid in identification and chemotaxonomy of yeasts."

III Microbiological Laboratory of the Institute of Chemistry of the Slovak Academy of Sciences, Bratislava, Dúbravská cesta, Czechoslovakia. Communicated by Dr. Anna Kocková-Kratochvílová.

The numerical taxonomy of the Genus Saccharomyces (Meyen) Reess was completed after evaluating more than 500 strains and three papers will be published in English under the title "The taxometric study of the Genus Saccharomyces (Meyen) Reess" in the Biological Edition, Publishing House of the Slovak Academy of Sciences, Bratislava, Klemensova 17/:

I. part: Saccharomyces carlsbergensis Hansen and related species, toward the end of 1969.

II. part: Saccharomyces cerevisiae Hansen and related species, in 1970.

III. part: Smaller species, in 1971.

In the beginning of the year 1969 the working team of the Laboratory was reconstructed. Dagmar Ondrušová prom. biol. increased the number of persons working in this Laboratory. She studies the genus Torulopsis Berlese according to its phenotype. Dr. Kocková-Kratochvílová visited the team of of Dr. H. Marquardt in Freiberg i.Br. and the Faculty of Brewing in Weihenstephan b. München in June 1969. Assoc. Prof. Kazuo Komagata from Tokyo University stayed three days in September and Assoc. Prof. G. Weide from Ernst-Moritz-Arndt University in Greifswald 14 days in October of this year in our laboratory.

IV Institut für Mikrobiologie und Infektionskrankheiten der Tiere, der Ludwig-Maximilians-Universität, Munich, West Germany. Communicated by Brigitte Gedek.

We have published the following papers on yeasts and yeast-like fungi this year:

Gedek, B.: Hefemastitiden beim Rind nach Antibiotika-Behandlung des Euters (Yeast mastitis in cattle following antibiotic treatment of the udder). Berl. Münch. Tierärztl. Wschr. 82, 241-244, 1969.

Gedek, B.: Beurteilung des kulturellen Nachweises von Hefen im Untersuchungsmaterial des Menschen (Assessment of positive yeast cultures in human material). Dtsch. Med. Wschr. 94, 1117-1119, 1969.

Schiefer, B. und B. Gedek: Geotrichum candidum als Krankheitserreger. Mykosen 12, 491-498, 1969.

One year ago two publications on Prototheca spp. came out, namely:

Frese, K. und B. Gedek: Ein Fall von Protothecosis beim Reh (A case of Protothecosis in a deer). Berl. Münch. Tierärztl. Wschr. 81, 174-178, 1968.

Schiefer, B. und B. Gedek: Zum Verhalten von Prototheca-Species im Gewebe von Säugetieren (Behavior of Prototheca species in mammalian tissue). Berl. Münch. Tierärztl. Wschr. 81, 485-490, 1968.

V The Mycology Laboratory, Royal Dental College, Copenhagen, Denmark. Communicated by Jessie Bodenhoff.

The following is an English summary of my doctoral dissertation (published in Danish 1969) and a list of publications resulting from this work.

Introduction

The aim of the present study has been to investigate the development of resistance to antimycotics in yeasts, with particular emphasis on Candida albicans and Cryptococcus neoformans.

Chapter 1

This chapter briefly reviews the relevant literature on mycotic infections with oral manifestations, particularly candidosis. On the basis of this review of the literature it is concluded that the exact incidence of Candida in man is unknown.

Chapter 2

The most important literature concerned with the development of resistance in strains of Candida to nystatin, amphotericin B and candidin is reviewed. Hitherto there have been no reports of the development of resistance to antimycotics in Cryptococcus neoformans. The experimental results reported in the literature show that it is possible, although difficult, to induce resistance to antimycotics in Candida albicans and certain other yeasts in vitro. It is furthermore apparent from these results that the development of resistance in Candida is of a different type to, and not produced as rapidly as, the resistance induced in bacteria to antibiotics. Increased resistance to antimycotics is associated with changes in the morphology and rate of growth of the colonies, demonstrable cross-resistance, and a decrease in virulence in mice.

Chapter 3

Chapter 3 describes the antimycotics used in the study with regard to their physicochemical characteristics, metabolism, toxicity, antimycotic spectrum, mode of action, chemoresistance and clinical application.

According to the reports in the literature, induction of resistance in Candida albicans and other yeasts to nystatin and amphotericin B has been demonstrated in experiments in vitro, but no resistance to Trichomycin® and polymyxin B has hitherto been demonstrated. Strains of Candida albicans with natural resistance to Trichomycin® have been observed. There are two reports in the literature of presumptive in vivo induction of resistance to amphotericin B in Candida parapsilosis. The mode of action of the antimycotics is elucidated on the basis of the most important literature. As yet no details of the pharmacodynamic action of the antimycotics have been elucidated.

Chapter 4

In this chapter the in vitro development of resistance to antimycotics in the yeasts named is reviewed. Seven strains of Candida and two of Torulopsis were exposed, by means of Szybalski's gradient plate technique, to increasing concentrations of nystatin, amphotericin B, Trichomycin® and polymyxin B.

In the majority of the strains resistance to the antimycotics named could be induced. With a few exceptions, the degree of resistance was low. Resistance to polymyxin B developed quickly, that to amphotericin B very quickly, but many passages were required for the development of resistance to nystatin and Trichomycin®.

Cross-resistance to nystatin and amphotericin B was demonstrated in Candida tropicalis and Torulopsis glabrata.

Chapter 5

Case histories of two patients who received long-term treatment with anitmycotics are described. Analysis of the sensitivity determinations emphasizes important factors which suggest the induction of resistance in vivo.

Chapter 6

An amphotericin B resistant and a polymyxin B resistant strain induced from the same parent strain of Cryptococcus neoformans have been investigated for virulence in the mouse. The amphotericin B resistant strain was obviously less virulent than the parent strain. The polymyxin B resistant strain showed a complete loss of virulence.

In the polymyxin B resistant strain the ability to form capsules in vivo was found to be obviously affected.

Chapter 7

A description is given of the strange and interesting clinical course of the infection in mice infected intravenously with a strain of Cryptococcus neoformans of low virulence. This chronic cryptococcosis in the mouse shows a certain resemblance to chronic cryptococcosis in man.

Chapter 8

In this chapter the results of the present study dealing with the experimental development of resistance to antimycotics in yeasts and the results of sensitivity determinations in Candida albicans isolated from two patients, who had received long-term antimycotic treatment, are discussed.

The results obtained are compared with the results of corresponding experiments reported in the literature. The change in virulence in strains of Cryptococcus neoformans during the development of resistance in mice is discussed, and it would seem justifiable to associate the change in capsule production with the loss of virulence. The resemblence of chronic cryptococcosis in the mouse to chronic cryptococcosis in man is emphasized.

Chapter 9

Conclusions:

Publications:

I. Resistance Studies of Candida albicans, with Special Reference to two Patients Subjected to Prolonged Antimycotic Treatment. Odont. T. 76: 279-294, 1968.

II. Development of Strains of Cryptococcus neoformans Resistant to Nystatin, Amphotericin, Trichomycin® and Polymyxin. Acta path. microbiol. scand. 73: 572-582, 1968.

III. Alteration in Virulence in Strains of Cryptococcus neoformans Resistant to Amphotericin and Polymyxin. Acta path. microbiol. scand. 75: 153-168, 1969.

IV. Chronic Cryptococcosis in the Mouse. Acta path. microbiol. scand. 75: 169-176, 1969.

V. Development of Strains of Genus Candida and Genus Torulopsis Resistant to Antimycotics. Acta path. microbiol. scand. 1969.

VI Department of Dermatology, University of Turku, Turku, Finland. Communicated by C. E. Sonck.

The following papers have been published.

Sonck, C. E. and Yarrow, D. 1969. Two new yeast species isolated in Finland. Antonie van Leeuwenhoek 35: 172-177.

Two new yeast species, Sterigmatomyces elviae isolated from a male patient and Trichosporon fennicum isolated from a domestic cat, are described.

S. elviae differs from the other members of the genus, S. halophilus var. halophilus and S. halophilus var. indicus Fell, in several properties, among them the assimilation of lactose. All three give a positive result to the colour test based on the staining of colonies with diazonium blue B (o-dianisidine) described by Hopsu-Havu, Laiho and Lundell (1967).

Physiologically there is a close resemblance between S. elviae and Cryptococcus dimennae Fell et Phaff. However, they differ in the assimilation of rhamnose, erythritol and inositol.

Tr. fennicum closely resembles Endomycopsis chodatii (Nechitch) Wickerham et Burton both morphologically and physiologically. However, it ferments and assimilates lactose and has not been observed to conjugate and form spores with the mating types of E. chodatii.

The morphological features agree well with those of Sporotrichum Carougeaui Langeron (1922). Two of the characteristics mentioned by Langeron for this species are the striking whiteness of the culture and the large diameter of the mycelium. The culture of T. fennicum is persistently milky white and the diameter of its mycelium, 2-6μ on potato agar, agrees well with the measurements of 2-5μ given by Langeron. Strains of E. chodatii have the same cultural and morphological characteristics and S. Carougeaui was listed as a synonym of this species by Kreger-van Rij (1964). Langeron did not examine the physiological properties of his isolate so it is no longer possible to determine its taxonomical position and it must be regarded as a nomen dubium.

This strain gave a negative result in the colour test with diazonium blue B.

Sonck, C. E. and Tunnela, Elvi. 1969. Staining with Diazonium-Blue B as an Aid in Yeast Diagnostics. Antonie van Leeuwenhoek Vol. 35, Supplement: Yeast Symposium.

Staining of yeasts with Diazonium Blue B (O-dianisidine) as a possible aid in the yeast diagnosticwas suggested by Hopsu-Havu. The first test results from our clinic in Turku were in 1967 presented by Hopsu-Havu, Laiho and Lundell. The chemical background of the test is still not fully understood, it has probably to do with some tryptophan metabolites, such as 3-hydroxyantranilic acid and 3-hydroxykynurenic acid. All species of Cryptococcus and Trichosporon, and most Rhodotorulae are stained dark red to dark violet, while most species of Candida and Torulopsis, as well as many other yeasts, remain constantly unstained. Since 1966 this DBB test is being used as routine in our mycological laboratory. The age of the colony

is important. Colonies of Cryptococci younger than 14 days give mostly negative reactions. They will, however, show positive reactions if tested again some 2 or 3 weeks later. The strains to be tested should be grown on culture media containing beef-extract or peptone.

VII Louisiana State University, Department of Food Science and Technology, Baton Rouge, Louisiana 70803, Communicated by S. P. Meyers.

A manuscript has been submitted to Mycologia "Mycological Studies of Lake Champlain" (S. P. Meyers, D. G. Ahearn and W. Cook)

Summary

Analyses of the seasonal fluctuation of yeast populations in Lake Champlain demonstrated a standing crop composed predominantly of Cryptococcus albidus, Rhodotorula spp., Torulopsis spp., yeasts of the Candida krusei-Pichia membranaefaciens complex and the omnipresent black yeast-like fungus Aureobasidium pullulans. Cell concentrations generally were less than 20 cells/100 ml, and varied between 1 to over 400/100 ml. In sectors of the Lake affected directly by industrial or urban effluents, distinctive yeast populations regularly exceeded 300 cells/100 ml. These yeasts rarely were isolated from other regions of the Lake. In areas receiving heated wood-pulp wastes, thermoduric strains capable of growth at 45°C were isolated. Comparison of data on yeast populations with that obtained in earlier studies of sub-tropical waters, demonstrates the feasibility of using species distribution patterns as indicators of water quality.

Current research by S. P. Meyers, portions of which are in cooperation with Dr. D. G. Ahearn (Georgia State University, Atlanta) involve the following:

Amine utilization by marine-occurring yeasts.

Proteolytic activity of Candida lipolytica

Ecology of yeasts in marshland, Spartina alterniflora, habitats. (here we are finding striking populations, i. e., >50,000 cells/ml sediment of yeasts, predominantly a heretofore undescribed species of Pichia (?).)

Dr. Meyers is examining the overall biodegradation of Spartina and the role played by yeasts in the productivity of these marshlands.

"I always welcome hearing from people working in yeast ecology as well as any yeast investigators who have examined yeast for their ability to utilize amines."

Dr. Ahearn will be visiting Dr. Meyers at LSU in a continuation of their cooperative research program.

Stipends are available for graduate study in the Department of Food Science and Technology, L.S.U. Contact Dr. Meyers. Major emphasis will be in mycology and marine food resources, under auspices of the Sea Grant Program at L.S.U.

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

An informal colloquium "Recent Trends in Yeast Research" was held at the Miner Center and the State University of New York, College of Arts and Science at Plattsburgh on August 15-16, 1969. The participants who presented papers included: D. G. Ahearn, (Chairman) Georgia State University, Atlanta, W. L. Cook, (Co-Chairman) New York State College of Arts and Sciences, Plattsburgh, S. P. Meyers, (Vice-Chairman) Louisiana State University, Herman J. Phaff, University of California, Davis, L. J. Wickerham, U. S. Department of Agriculture, Peoria, Chun-Juan K. Wang, State University College of Forestry, Syracuse, Vladimir Munk, State University College, Plattsburgh, J. J. Miller, McMaster University, Hamilton, Ontario, Canada, James N. Bicknell, University of Washington, Seattle, Jack W. Fell, University of Miami, Cletus P. Kurtzman, U. S. Department of Agriculture, Peoria, Jean Shadomy, Medical College of Virginia, Richmond, John R. Forro, General Electric Co., Syracuse.

Proceedings of the Colloquium will be published in a special edition of the "Research Articles of the School of Arts and Sciences of Georgia State University. Publication is anticipated in early 1970.

The following papers have recently been published.

Fungi from the normal outer eye. Wilson, L. A., D. G. Ahearn, R. R. Sexton and D. Jones, Amer. J. Ophthal. 67: 52-56.

Abstract: Samples from the eyelid margins and conjunctival sacs of both eyes of 158 people with no clinical evidence of ocular disease were cultured twice to determine the incidence and persistence of fungi. Fungi, predominantly yeasts, were obtained from 47 eyes of 35 individuals, 22 women and 13 men. Candida parasilosis, the most common species isolated, occurred in 12 out of 47 eyes. Fungi in the healthy outer eye appeared to depend on random seeding from the environment and to be transient. In two cultures at a one-week interval of samples from all eyes, only three subjects had the same fungus in the same eye. Following alteration of environment of two of these three subjects, a third culture was negative for fungi. The introduction of high numbers of adventitious pathogenic fungi into the eye or in the surrounding region, by cosmetics or ophthalmic medications is potentially hazardous.

Fungal flora of the normal human small and large intestine. R. Cohen, F. J. Roth, E. Delgado, D. G. Ahearn and M. H. Kalser. New Eng. J. Med. 280: 638-641.

Abstract: Cultures of 86 specimens (including 23 oropharyngeal, 26 jejunal, 20 ileal and 17 fecal samples in 27 normal adults showed Candida albicans to be the most frequent fungus in high concentrations in all areas sampled. Both the frequency and concentration of C. albicans increased progressively from the oropharynx to the colon: 30% in the oropharynx, 54% in the jejunum, 55% in the ileum and 65% in the fecal specimens. C. albicans, in concentrations of 10^2 colonies per ml or greater, were encountered in 27% of oropharyngeal, 43% of jejunal, 50% of ileal and 59% of colonic specimens. The

stability of the mycofloral pattern of the small intestine was demonstrated in five subjects who were resampled five to nine months after the initial studies; the fungal pattern was qualitatively and quantitatively unchanged.

The occurrence of Sporotrichum schenckii on a cold stored meat product. Ahearn, D. G. and W. Kaplan. Amer. J. Epidemiol. 89: 116-124.

Ecology of yeast from Lake Champlain. Ahearn, D. G. Meyers, S. P., Cook, W. L. & G. Hansen. Ant. van Leeuwenhoek. 35 Supplement Yeast Symposium: D-19-20.

IX Istituto di Patologia Vegetale e di Microbiologia Agraria e tecnica, Università di Pisa, Italy. Communicated by O. Verona.

The papers listed below were completed since our last communication to the News Letter (28 November 1968):

Aldo A. Lepidi and Giovanni Picci (°)

On the Yeast microflora of the Gastro-enteric cavity of the snail (Helix aspersa Müller): III - Persistence of Saccharomyces cerevisiae in such an environment.

The persistence of Saccharomyces cerevisiae, added to sterile food, in the gastro-enteric cavity of the snail decreases if antibiotics are present. The gastro-enteric bacterial flora, after Saccharomyces is supplied is abundantly constituted by Proteus vulgaris, which causes a decrease of viable Saccharomyces cells. On the other hand, crude bacterial liquid of Proteus contains substance which may cause lysis of yeast cell walls at suitable temperatures and pH.

(submitted to Archiv für Mikrobiologie).

(°) - Present address: Istituto di Microbiologia Agraria, Università di Napoli-Portici (Na) (Italia).

G. Picci, S. Coppola and A. A. Lepidi

Further research on the antilipogenic effect of penicillin. Antibiotic action on some yeasts.

Penicillin reduces not only total lipids of some yeast, but also the per cent of sterides. Cephalosporin N and actitiazic acid also cause a lipid decrease.

Such a penicillin, cephalosporin N and actitiazic acid effect is quite reduced or reverted in the presence of suitable doses of biotin.

A discussion of the mechanism is proposed on the basis of molecular similarities of the compounds used.

(XV National Congr. Ital. Microbiol., Torino, 1969).

X U. S. Army Medical Research Laboratory, Fort Knox, Kentucky. Communicated by W. A. Maxwell and Edward Spoerl.

Uptake of Mannitol by Saccharomyces erevisiae

Reports in the literature indicate that mannitol is not transported into cells of the yeast Saccharomyces cerevisiae. These results are based primarily upon the observation that optical density changes are not detectable when yeast spheroplasts are suspended in mannitol, presumably because intracellular osmotic changes are not induced when mannitol enters the cell. Results in our laboratory also show that changes in optical density do not occur when spheroplasts of Saccharomyces cerevisiae are suspended in a mannitol solution, but radiochemical analyses have shown that mannitol is taken up by the cells. Various kinetic and biochemical measurements have been made which lend support to the belief that mannitol does enter the cell. The following are some of these observations.

(1) As the temperature of incubation during the uptake decreased the rate of uptake also decreased. These data indicated that mannitol may have entered the cell by a carrier system and was not merely adsorbed on the cell surface, which should be a temperature independent process. (2) Uranyl nitrate, a compound known to inhibit sugar transport in yeasts, blocks mannitol uptake. (3) Glucose inhibited the uptake of 0.1M mannitol at concentrations as low as 0.005M. Concentrations below this level stimulated mannitol uptake. (4) Mannitol counter flow occurred with glucose added at concentrations as low as 0.003M. (5) A Lineweaver-Burk plot showed a Michaelis' constant (K_m) of 0.6M for mannitol uptake. Glucose present at a 0.01M concentration competitively inhibited mannitol uptake since the V_{max} remained unchanged while the K_m was increased. Glucose at a 0.001M concentration acted as a non-competitive activator in that the K_m remained unchanged while the V_{max} was increased. (6) Energy inhibitors such as iodoacetic acid, dinitrophenol and sodium azide all inhibit mannitol uptake. (7) The presence of energy sources such as low levels of glucose or ethanol stimulate mannitol uptake indicating an energy involvement as shown by the use of energy inhibitors.

All of the results obtained by radiochemical measurements show that mannitol was taken up by Saccharomyces cerevisiae. The transport appears to be carrier mediated with some relationship to the glucose carrier system and to require a supply of metabolic energy. The lack of optical density changes in spheroplasts suspended in mannitol would be explained if mannitol were osmotically inactive inside the cell. Preliminary results have indicated that mannitol is bound within the cell. This result is consistent with other reported observations which show that mannitol has a strong tendency to form hydrogen bonds with protein.

Recent Publications:

Spoerl, Edward. Membrane changes in yeast cells caused by sulfhydryl reagents and accompanied by a selective release of sugar. Accepted for publication, J. Membrane Biol.

C. O. Chichester and W. A. Maxwell, 1969. The effects of high intensity visible and ultraviolet light on the death of microorganisms. In Life Sciences and Space Research, VII; 11-18.

W. A. Maxwell and C. O. Chichester, 1969. Free radical production photodynamically inactivated cells of Rhodotorula glutinis. Presented at the 12th Plenary COSPAR meeting in Prague, Czech., 11-24 May 1969. To be published in Life Sciences and Space Research Vol. VIII, 1970.

XI State University of New York at Stony Brook, Stony Brook, New York 11790. Communicated by V. P. Cirillo.

We have continued our investigation of galactose transport in Saccharomyces over the past year and two aspects of our studies are worth noting:

(1) The role of phosphorylation in galactose transport.

Genetic evidence indicates that D-galactose and its nonmetabolized analogues, D-fucose and L-arabinose, depend for their transport on the product of the Ga 2 gene (Cirillo 1968, J. Bacteriology 95, 1727-1731). In Ga 2 positive cells, irrespective of the presence or absence of the galactose pathway enzymes, the nonmetabolized (and nonphosphorylated) analogues are transported with identical kinetics by facilitated diffusion. The Ga 2 gene product would appear to be a galactose "carrier." However, the rate of galactose uptake is very low in cells in which the Ga 2 gene is not present together with an active Ga 1 gene which produces galactokinase. The apparent rate of galactose uptake in Ga 2 positive, galactokinase-positive cells is also very low when galactose phosphorylation is inhibited by iodoacetate treatment. Furthermore, the apparent rate of galactose uptake in either iodoacetate treated or galactokinaseless cells is far below the rate at which galactose is metabolized in noninhibited, wild type cells.

Two alternative explanations have been offered to account for the low rate of galactose uptake when it cannot be phosphorylated: (1) Galactose has a high affinity for the carrier and the V_{max} for transport is very high. If the external galactose concentration is higher than the K_m, the net rate of galactose uptake in nonmetabolizing cells decreases constantly as the intracellular concentration of free sugar rises. This would result in underestimating the initial rate of uptake even when the first sample is taken as early as 30 seconds after sugar addition. (2) Alternatively, galactose is proposed to have a low affinity for the carrier. In cells unable to phosphorylate galactose, galactose is transported as a low affinity sugar. However, in the presence of a specific phosphotransferase and a phosphoryl donor, the sugar-carrier complex is phosphorylated thereby converting the low affinity carrier to a high affinity carrier which results in either the active transport of free galactose or the phosphorylative transfer of galactose as galactose phosphate.

Experiments designed to determine the apparent affinity of galactose for the carrier in the absence of its metabolism did not seem to support the first hypothesis. Measurement of the apparent initial rate of galactose uptake by galactokinaseless cells over the concentration range, 10^{-5} to 10^{-1} M, showed no evidence of saturation. However, no evidence could be obtained that galactose has a higher apparent affinity in metabolizing cells. The apparent K_i of galactose inhibition of L-arabinose uptake is the same in metabolizing, iodoacetate treated and galactokinaseless cells. These results seemed to favor a direct role of galactokinase in a chemical transfer mechanism similar to that of the PEP-phosphotransferase system in the uptake of certain sugars by bacteria.

What appears to be a final resolution of the conflicting hypotheses resulted from a suggestion by Alberto Sols that galactose uptake be measured in galactokinaseless cells which were preloaded with nonradioactive sugar. If the low net rate of galactose in these cells is due to saturation of exit rate at low internal concentrations, preloading cells with a high concentration of galactose (10^{-1} Molar) followed by washing in the cold and exposure to a low external concentration of ^{14}C-labelled galactose (10^{-3}M) should result in a much higher initial rate of uptake than in unloaded cells. If the high rate of galactose uptake during metabolism is due to a chemical transfer mechanism, preloading should have little if any effect. The rate of galactose uptake in preloaded cells was found to be 20-50X greater than in unloaded cells. From an analysis of the rate and level of equilibration of the external ^{14}C label with the internal pool, the reason for the low net rate of uptake of galactose into unloaded cells is not due primarily to its high rate of affinity, but to the fact that galactose uptake involves two phases associated with two cell "compartments." The first phase of uptake is rapid and involves only a small fraction of the cell volume, about 5% of the total cell water. Equilibration of the external sugar with this small compartment is achieved so quickly that it was over by the time the first measurements were made to determine the net rate of uptake (30 seconds). Uptake into the rest of the cell water by the much slower process is the "nonsaturable" process which was being measured in previous studies. Credit for these studies goes to Dr. Shou-Chang Kuo.

(2) The apparent gratuitous induction of the galactose pathway by L-arabinose.

Earlier this year we reported that L-arabinose selectively inhibits galactose metabolism by inactivating UDPGlucose-4-epimerase (Azam, Rosen and Cirillo 1969, Bacteriol. Proc.). Last year we reported that L-arabinose is a gratuitous inducer of the galactose pathway, and furthermore, that the inducibility by L-arabinose is a strain variable characteristic (Cirillo 1968, op. cit.). We have now discovered that the induction by L-arabinose is an artefact due to its contamination by less than 1% by weight of D-galactose. Ordinarily this low level of galactose in an inactive inducer would not lead to detectable induction since L-arabinose was used at 0.2% which represents 0.001% galactose. Such a low level of galactose would be metabolized so rapidly that deinduction would begin almost as rapidly as it began. However, because L-arabinose inhibits D-galactose metabolism about 95%, the small amount of galactose present as a contaminant persisted about 12 hours in the induction medium leading to significant induction. If the L-arabinose is treated with galactose induced cells prior to recrystallization, it does not act as an inducer. The presence of D-galactose was demonstrated in Sigma Chemical Co. (St. Louis, Mo.) L-arabinose by the galactose oxidase test.

XII Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh 9, Scotland. Communicated by J. M. Mitchison.

Research topics and papers, mostly on the biochemistry and physiology of the cell cycle of Schizosaccharomyces pombe.

J. M. Mitchison and J. Creanor

Enzyme synthesis during the cell cycle.

Effects of various agents (e.g. hydroxyurea phenylethanol, temperature) which distort the cycle.

Recent Papers:

J. M. Mitchison and J. Creanor. "Linear synthesis of sucrase and phosphatases during the cell cycle of Schizosaccharomyces pombe." J. Cell Sci. 5, 373 (1969).

Patterns of enzyme synthesis suggest that there may be a delay in the cell cycle between the time when a new genome is synthesised and the time when it is expressed.

J. M. Mitchison. "Enzyme synthesis is synchronous cultures." Science 165, 657 (1969).

A short review of current facts and theories, in which yeasts play an important part.

J. M. Mitchison, J. E. Cummins, P. R. Gross and J. Creanor. "The uptake of bases and their incorporation into RNA during the cell cycle of Schizosaccharomyces pombe in normal growth and after a step-down." Exp. Cell Res. (In press).

Data from autoradiographs and synchronous cultures on the rate of synthesis of RNA during the cell cycle, changes in pool size, and changes in base ratio.

J. M. Mitchison. "Physiological and cytological methods for Schizosaccharomyces pombe." Methods in Cell Physiology (ed. D. M. Prescott). 4, (In press).

A laboratory guide to techniques together with a bibliography of recent work on S. pombe. Microphotographs of nuclear staining by C. F. Robinow. There will be an accompanying guide to genetical methods by U. Leopold in the same volume.

J. H. Duffus

Nuclear isolation. Characteristics of nuclear proteins and "histones", and their behaviour during the cell cycle.

Recent Papers:

J. H. Duffus. "The isolation of nuclei from Schizosaccharomyces pombe." Biochim. Biophys. Acta. (To be published November 1969).

Technique for mass isolation of nuclei using the Eaton press.

J. H. Duffus and C. J. Mitchell. "The growth of Schizosaccharomyces pombe in media of high osmotic pressure." Antonie van Leeuwenhoek 35, Suppl. H 25 (1969).

Growth in high glucose concentrations affects the DNA cycle and delays the S period.

W. H. Wain

Synthesis of specific proteins during the cell cycle. Using a pulse labelling technique, there is no evidence of deviations during the cycle from an exponential pattern of synthesis in the peaks of soluble protein separated by acrylamide gel electrophoresis. This is in sharp contrast to the results with a similar technique using mammalian cells (Kolodny and Gross 1969 Exp. Cell Res. 56, 117).

C. J. Bostock (now at Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado, U.S.A.)

DNA synthesis during the cycle. Effects of phenylethanol in distorting the DNA cycle.

Ph.D. thesis, University of Edinburgh. Papers in preparation.

N. Stebbing (now at The Cell Research Laboratory, High Wycombe, Berks)

Change in the amino-acid pool during the cell cycle. Patterns of synthesis of dry mass, protein and RNA in synchronous cultures.

Ph.D. thesis, University of Edinburgh. Papers in preparation.

A. A. Robinson (now at Department of Biochemistry, Pharmaceutical Division, I.C.I. Ltd., Mereside, Alderley Park, Macclesfield, Cheshire)

Patterns of synthesis of "step enzymes" during the cycle. Effects of cycle distortion.

Ph.D. thesis, University of Edinburgh. In preparation.

A. Herring (research student)

Division delay with various inhibitors (mitomycin C, cycloheximide).

J. Robinson (research student)

Electron microscopy of the nucleus.

M. L. Sartirana (visitor from Institute of Botanical Science, Milan)

RNA synthesis during the cycle, ribosomal precursors.

XIII School of Biological Technology, University of New South Wales, Kensington, N.S.W. Australia. Communicated by F. J. Moss and Pamela A. D. Rickard.

The following are abstracts of three papers which have recently been submitted for publication:

THE RESPONSE BY MICROORGANISMS TO STEADY STATE GROWTH IN CONTROLLED CONCENTRATIONS OF OXYGEN AND GLUCOSE. II. SACCHAROMYCES CARLSBERGENSIS.
by F. J. Moss, Pamela A. D. Rickard, F. E. Bush and P. C. Caiger.

The growth of Saccharomyces carlsbergensis in continuous culture has been studied when dissolved oxygen and glucose concentrations were held constant at a series of steady state levels. Both oxygen and glucose controlled the degree of aerobic catabolism and ethanolic fermentation. Increased oxygen enhanced oxygen uptake and, if glucose was limiting, strongly inhibited ethanolic fermentation. On the other hand, increased glucose enhanced ethanolic fermentation and, unless oxygen was very high, depressed oxygen uptake.

The extent to which catabolism proceeded by an anaerobic or aerobic pathway, as judged by ethanol production, was controlled more by the uptake of glucose than of oxygen. Although increases in oxygen between zero and air saturation caused ethanolic fermentation to contribute a lesser proportion to total catabolism if glucose was limiting, this pathway continued to predominate throughout the complete oxygen range if glucose was in excess. The concentrations of A-, B- and C-type cytochromes were depressed by high glucose concentrations. The concentrations of A- and C- types varied directly with the dissolved oxygen concentration when glucose was low.

THE EFFECTS OF GLUCOSE AND OXYGEN ON THE CYTOCHROMES AND METABOLIC ACTIVITY OF YEAST BATCH CULTURES. I. SACCHAROMYCES SPP.
by Pamela A. D. Rickard, F. J. Moss and Micheline Ganez.

Saccharomyces cerevisiae and Saccharomyces carlsbergensis were grown in batch culture with and without oxygen control.

The concentrations of A-, B- and C-type cytochromes of both yeasts were dependent on the oxygen concentration during growth as well as on the initial glucose concentration of the growth medium. S. cerevisiae cytochromes were maximal after growth in low glucose and low oxygen; S. carlsbergensis cytochromes were maximal after growth in low glucose and high oxygen.

Except when glucose was in very low concentration, its catabolism by S. carlsbergensis was directed predominantly toward ethanolic fermentation regardless of the oxygen concentration.

Growth rate, total cell mass and yield were maximal and anabolism was closely balanced with catabolism when glucose and oxygen of S. carlsbergensis cultures were both high. Under these conditions neither catabolism, respiratory or ethanolic, nor glucose uptake were maximal.

THE EFFECTS OF GLUCOSE AND OXYGEN ON THE CYTOCHROMES AND METABOLIC ACTIVITY OF YEAST BATCH CULTURES. II. CANDIDA UTILIS.
by Pamela A. D. Rickard, F. J. Moss, D. Phillips and T. C. K. Mok.

Candida utilis was grown in batch culture with and without oxygen control.

The concentrations of A-, B- and C-type cytochromes were found to vary with the initial glucose concentration, with the dissolved oxygen concentration and with time. A-type was the most sensitive.

After glucose was essentially exhausted, the yeast catabolized ethanol, if it had been growing in a relatively low initial glucose concentration, or non-glucose carbohydrate, including some of that previously accumulated within the cell, if it had been growing in a high initial glucose concentration. This difference in metabolic pattern could explain why cytochrome derepression was initiated soon after glucose uptake ceased only if initial glucose had been relatively low.

The effects of glucose and dissolved oxygen concentrations on yeast cytochromes and respiratory activity are discussed.

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

1. The carbohydrate composition and the specific activity of the trehalase of synchronised yeast populations have been investigated. The cells accumulated the reserve carbohydrate during the single cell phase between two buddings. The rapid degradation of part of these reserve began shortly before the swelling of the bud. The specific activity of the trehalase changed during the budding cycle and indicates that the synthesis of this enzyme is linked to the growth cycle (M. T. Küenzi and A. Fiechter: Arch. Mikrobiol. 64, 396-407, 1969).
2. An automatically working test arrangement for the continuous analysis of O_2 and CO_2 in microbiol cultures is described. The measuring principle is based on the paramagnetic properties of oxygen and on

the absorption of infrared by carbon dioxyde. A formula of correction was computed and a nomogram for routine work was established. The advantage of the described systems was illustrated by two biological examples of growth experiments on Sacch. cerevisiae (A. Fiechter and H. K. v. Meyenburg: Biotechn. & Bioeng. 10, 535-549, 1968).

3. The Thesis of H. K. v. Meyenburg was published under the title: Katabolit-Repression und der Sprossungszyklus von Sacch. cerevisiae [Diss. ETH No. 4279 ETH, Zürich, 1969]. Published also in "Vierteljahresschrift der Naturforschenden Gesellschaft in Zürich, Heft 3 (September), Jahrgang 114, 1969. A large number of experiments with batch and continuous cultivation has been undertaken and a short description of a synchronization method in chemostat technique is given. The results of activity measurements of several enzymes of the glycolytic and respiratory pathways are summarised in a hypothesis on sequential gene activation in fermenting-respiring systems of Sacch. cerevisiae. Some aspects are published separately e.g.

H. K. v. Meyenburg: Der Sprossungszyklus von Sacch. cerevisiae, Path. Microbiol. 31, 117-127, 1968.

Energetics of the budding cycle of S. cerevisiae during glucose limited aerobic growth. Arch. Mikrobiol. 66, 289-303, 1969.

XV Rutgers. The State University of New Jersey, Institute of Microbiology, Waksman Hall, New Brunswick, New Jersey 08903. Communicated by J. O. Lampen.

Two papers related to yeast appeared from this laboratory during the past year:

(1) The glycoprotein structure of yeast invertase. N. P. Neumann and J. O. Lampen. Biochemistry 8:3352-3356 (1969).

It had earlier been shown that the invertase of yeast (Saccharomyces cerevisiae) exists in two forms; one intracellular and devoid of carbohydrate; the other localized externally to the cell membrane in the cell wall. Unlike the internal enzyme this is a glycoprotein which contains approximately 50% carbohydrate. From an examination of glycopeptides obtained after proteolytic digestion of the external invertase, it was estimated that each invertase molecule contains 25-30 chains of polysaccharide (mannan) of molecular weights ranging between 2,000 and 10,000. The carbohydrate-protein linkage appears to involve a glycosylaminyl-asparagine bond. There is evidence that some glucosamine is present at other sites in the mannan than in the carbohydrate-protein link. This invertase may contain a few O-glycosidic linkages as well, but obviously to a much more limited extent than in the preparation (of unstated purity) studied by Greiling et al. (Z. Physiol. Chem. 350: 517 (1969)).

(2) Amphotericin B and other polyenic antifungal antibiotics. J. O. Lampen. Am. J. Clinical Pathology 52:138-146 (1969).

A short summary of studies on the mechanism of the antimicrobial action of polyenes. The recent work on the

alteration of sterol and steroid metabolism by feeding certain polyenes to animals has also been summarized.

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

Timo Nurminen and Heikki Suomalainen, Enzymatic properties of the isolated cell walls and plasma membrane preparation of baker's yeast. FEBS, Sixth Meeting, Madrid 1969, Abstr. Commun. p. 66.

A study of the distribution of various enzymes among the subcellular fractions of yeast has disclosed the absence of hexokinase, alkaline phosphatase, esterase and the $NADH_2$ oxidase system in the isolated cell walls. Noticeable amounts of saccharase, phosphatases hydrolysing p-nitrophenyl phosphate, ATP, ADP, thiamine pyrophosphate and inorganic pyrophosphate, with optimum activity at pH 3-4 and an activity of Mg^{++}-dependent ATPase at neutral pH, were found in the isolated cell walls. During enzymatic digestion, the other activities apparent in the isolated cell walls were mostly released into the medium, but the bulk of Mg^{++}-dependent ATPase remained in a sedimentable preparation obtained by centrifugation after digestion. It is concluded from the chemical composition that this fraction contains fragments of plasma membrane. It may accordingly be assumed that the enzymes released into the medium during digestion are located in the cell wall outside the plasma membrane, whereas the Mg^{++}-dependent ATPase is an enzyme of the plasma membrane. When the cell walls were isolated in the presence of Mg^{++}, an increase occurred in the activity of Mg^{++}-dependent ATPase, and to some extent also that of saccharase remaining in the cell walls. Thus it can be concluded that Mg^{++} promotes preservation of the plasma membrane during the course of preparation.

See also: T. Nurminen and H. Suomalainen, Behaviour of some enzymes of the cell envelope of baker's yeast during the digestion of the cell wall. Second Meeting of the North West European Microbiological Group, Stockholm 1969, Abstracts, p. 17.

Heikki Suomalainen and Timo Nurminen, Phospholipid and fatty acid composition of the cell surface region of baker's yeast. FEBS, Sixth Meeting, Madrid 1969, Abstr. Commun. p. 65.

Comparison between the content of phospholipids and fatty acids in isolated cell walls and in a plasma membrane preparation of baker's yeast, and the content in the whole cells and in the cell interior of yeast shows that the cell walls contain neutral lipids and phospholipids. The lipid phosphorus is present in the membrane preparation to a greater extent than the other phosphorus compounds of the cell walls. In all the yeast preparations investigated, the main phospholipid compounds were phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine. It was found that the whole cells, and the cell interior, contain phosphatidylcholine in a

larger proportion than do the cell walls, in which again the amount of phosphatidylinositol, in addition to phosphatidylserine, is present in a larger proportion. When the cell walls were digested enzymatically to such an extent that their original lipid content had risen to four-fold, it was found that the original distribution of phospholipids in the cell walls remained about the same. This indicates that the phospholipid composition of the plasma membrane corresponds mainly to that of the isolated cell walls.

See also: T. Nurminen and H. Suomalainen, Changes in chemical and enzymatic composition during enzymatic digestion of isolated yeast cell walls containing plasma membrane. Antonie van Leeuwenhoek Vol. 35, Suppl.: Yeast Symposium 1969, p. I 13.

Erkki Oura, The behaviour of baker's yeast towards oxygen. FEBS, Sixth Meeting, Madrid 1969, Abstr. Commun. p. 125.

Baker's yeast has been cultured in a chemostat, in a synthetic medium with carbohydrate limitation. Not only the temperature and pH, but also the growth rate of the yeast was held constant. To change the oxygen tension in the medium, the partial pressure of oxygen in the aerating gas was varied from 50% to nearly zero but the degree of ventilation was kept constant. Samples of yeast in steady-state conditions were analysed for some cell components, and determination was made of the activities of enzymes involved in glycolysis, the tricarbonic acid cycle, and the glyoxylate pathway. The anaerobic metabolism was reflected in the activity of alcohol dehydrogenase. A good indicator of the shift towards aerobic metabolism is provided by the cytochromes and, in regard to the enzymes tested, the malate dehydrogenase.

E. Oura, Aeration and the biochemical composition of baker's yeast. Antonie van Leeuwenhoek 35, Suppl.: Yeast Symposium 1969, p. G 25.

A series of cultures of baker's yeast has been made in continuous cultivation with a synthetic medium limited in respect of the carbon source. Samples representing yeast in steady-state conditions were analysed for some cell components, and the activities of several enzymes were determined. The cell components studied included adenosine and pyridine nucleotides, riboflavin compounds and cytochromes. The enzymes examined were so selected that they represented glycolysis, the tricarbonic acid cycle, the glyoxylate pathway, and the pentose phosphate shunt. Tests were also made of some enzymes in link positions between the tricarbonic acid cycle and amino acids or fatty acids.

Aeration affects the yeast metabolism, and accordingly the yield of cell mass calculated per carbon source in the cultivation medium. When the yeast was aerated with a gas containing 10-50% oxygen, less than 0.01% alcohol was detected in the media and the yield was found to be 52% of the carbon source

given. On aeration with 5% oxygen, the medium contained more than 0.01% alcohol, and the yield was only 46%.

The total amounts of adenosine phosphates, and the distribution of individual nucleotides, did not clearly reflect the aerobicity of the metabolism of yeast. This applied also to the behaviour of riboflavin components but the degree of reduction in pyridine nucleotides increased abruptly when the oxygen concentration had diminished to a given level. The amounts of cytochromes were very clearly characteristic of the degree of aeration of the medium. For the sum of cytochromes aa_3, b and c, a clear maximum was observable when the aerating gas contained about 5% oxygen.

The ability of the yeast to respire sugar and ethanol diminished with decreasing oxygen level, and conversely the fermentation of glucose increased. In many cases this is clearly apparent in the activities of glycolytic and TCA-cycle enzymes. The anaerobic metabolism was very clearly reflected in the activity of alcohol dehydrogenase, whereas it appeared that malate dehydrogenase activity is a good indicator of a shift towards aerobic metabolism. It was found that some of the enzyme activities tested changed very distinctly when ethanol was used instead of sugar as the limiting substrate.

A. J. A. Keränen, On the synthesis of biotin in yeast. Antonie van Leeuwenhoek 35, Suppl.: Yeast Symposium 1969, p. H 7.

Biotin is most effective growth factor for baker's yeast, although the intermediate stages of the biosynthesis are not known definitely.

In this study, an examination has been made of the influence of aspartic acid, alone and together with oleic acid, on the biotin synthesis of the yeast C. utilis and S. cerevisiae. Oleic acid added to the growth medium of C. utilis induced an accumulation of desthiobiotin. When biotin was added to the medium, it partly blocked the synthesis of the biotin in C. utilis. In 7 hours, C. utilis grown without biotin produced four times as much biotin as the yeast grown with a biotin addition and considerable amounts of biotin analogues were observed in the alcohol extracts of the yeasts grown without biotin. When cultivated with biotin, there were no more than traces of biotin analogues. After the addition of aspartic acid, the total amount of biotin in S. cerevisiae increased and on addition of aspartic acid together with oleic acid, the biosynthesis of biotin increased by many times.

Heikki Suomalainen and Kaija Konttinen, The penetration of halogenated acetic acids into cells of baker's yeast. Suomen Kemistilehti 42B, 255-256 (1969).

Monohalogenated acetic acids penetrate into the cells of baker's yeast less rapidly than acetic acid, the penetration rate decreasing in the order monoiodo-, monobromo-, monochloro- and monofluoroacetic acid. The penetration rates of these acids thus increase with decreasing acid strength. On the other hand, the size of the molecule is not observed to influence the rate of penetration.

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

AUXIN-INDUCED EXPANSION GROWTH OF CELLS AND PROTOPLASTS OF YEAST. By N. Yanagishima and C. Shimoda, Physiol. Plantarum 21, 1122, 1968.

Using an auxin-responsive mutant of Saccharomyces ellipsoideus, expansion growth of cells caused by auxin was studied especially in comparison with that of protoplasts.

1. Indole-3-acetic acid induced detectable cell expansion growth in 3 hours in a buffered simple solution where no cell division occurred.
2. The auxin-induced expansion growth was inhibited by an antiauxin, transcinnamic acid.
3. Actinomycin D, chloramphenicol and cycloheximide inhibited the auxin-induced cell expansion growth.
4. Protoplasts did not expand in response to auxin under the condition where intact cells did.
5. The stability of protoplasts was not changed by the low auxin concentration (20 mg/l) which induced cell expansion.
6. High concentrations (100-1000 mg/l) of auxin caused protoplasts to burst even under an osmotically stable condition.

STRAIN DEPENDENCE OF THE CELL-EXPANDING EFFECT OF β-1,3-GLUCANASE IN YEAST. Chikashi Shimoda and Naohiko Yanagishima, Physiol. Plantarum, 21, 1163-1169, 1968.

The effect of β-1,3-glucanase on the cell expansion was studied with diploid strains of Saccharomyces ellipsoideus and S. cerevisiae, and their mutants differing in the response to auxin. The following results were obtained.

Cell expansion was induced by β-1,3-glucanase only in auxin-responsive or potentially auxin-responsive strains.

β-1,3-Glucanase induced cell expansion more rapidly than auxin.

The cell wall of the auxin-responsive strain was more susceptible to digestion by β-1,3-glucanase than that of the auxin-unresponsive one.

The sensitivity of yeast cells to auxin action is discussed in relation to the nature of cell wall.

XVIII Macaulay Institute for Soil Research. Aberdeen, Scotland. Communicated by J. S. D. Bacon.

The following publications have appeared in recent years from our laboratory.

(1) Features of the cell-wall structure of yeast revealed by the action of enzymes from a non-fruiting myxobacterium (Cytophaga johnsonii). By J. S. D. Bacon, Beatrice D. Milne, Irene F. Taylor and D. M. Webley, Biochem. J. 95, 28C, 1965.
(2) The location of chitin in the yeast cell wall. By J. S. D. Bacon,

Elizabeth D. Davidson, D. Jones and Irene F. Taylor, Biochem. J. 101, 36C, 1966.

(3) Lysis of the cell walls of yeast (Saccharomyces cerevisiae) by soil fungi. By D. Jones and D. M. Webley, Trans. Br. mycol. Soc. 50 (1), 149-154, 1967.

(4) A comparison of the lytic action of Cytophaga johnsonii on a eubacterium and a yeast. By D. M. Webley, E. A. C. Follett and I. F. Taylor, Antonie van Leeuwenhoek 33, 159-165, 1967.

(5) A new enrichment technique for studying lysis of fungal cell walls in soil. By D. Jones and D. M. Webley, Plant and Soil 28, 147, 1968.

(6) The occurrence of α(1-3)glucan in Cryptococcus, Schizosaccharomyces and Polyporus species, and its hydrolysis by a Streptomyces culture filtrate lysing cell walls of Cryptococcus. By J. S. D. Bacon, D. Jones, V. C. Farmer and D. M. Webley, Biochim. Biophys. Acta 158, 313-15, 1968.

(7) The presence of a predominantly β(1→6) component in preparations of yeast glucan. By J. S. D. Bacon and V. C. Farmer, Biochem. J. 110 (3), 34-35, 1968.

(8) Lysis of cell walls of Mucor ramannianus Möller by a Streptomyces sp. By D. Jones, J. S. D. Bacon, V. C. Farmer and D. M. Webley, Antonie van Leeuwenhoek 34, 173-182, 1968.

(9) The glucan components of the cell wall of baker's yeast (Saccharomyces cerevisiae) considered in relation to its ultrastructure. By J. S. D. Bacon, V. C. Farmer, D. Jones and Irene F. Taylor, Biochem. J. 114, 557, 1969.

(10) A study of the microbial lysis of the cell walls of soil yeasts (Cryptococcus spp.). By D. Jones, J. S. D. Bacon, V. C. Farmer and D. M. Webley, Soil Biol. Biochem. 1, 145-151, Pergamon Press, 1969.

SUMMARY - The difference in the ability of lytic microorganisms to bring about dissolution of the cell walls of the soil yeasts Cryptococcus albidus and C. terreus has been examined. It was found that the composition of the cell walls, which varied according to the cultural conditions employed, determined the extent to which the walls were lysed. Thus walls from cells of C. albidus grown under conditions favourable for growth contained α- and β-glucans and chitin as major components and were lysed by two Streptomyces spp. but not by a non-fruiting myxobacterium Cytophaga johnsonii. Significant lysis of C. albidus walls by the myxobacterium as well as by the Streptomyces sp. occurred, however, when the α-glucan component was considerably reduced by growing the yeast under unfavourable conditions.

Ultrastructural studies showed the absence of definite layers in the wall. The chitinous residue after chemical extraction of the walls retained the general shape of the cell and was composed of microfibrils, in contrast to the granular chitinous residues from other yeasts e.g. Saccharomyces spp.

XIX Laboratoire de Recherches de la Chaire de Génétique - Ecole Nationale Supérieure Agronomique - C.R.A.M. (34) Montpellier, France. Communicated by Pr. P. Galzy.

The following publications have appeared or will be published shortly.

- Variation phénotypique de S. cerevisiae HANSEN au cours d'une culture prolongée sur acide pyruvique.

P. GALZY, Francoise VEZINHET, Jacqueline DURAND.
C. R. Soc. Biol. T. 162, n° 8-9, 1968.

- Remarques sur le bilan d'oxydation du glucose et de l'acide pyruvique par la levure.
A. ARNAUD, J. ALBERT, P. GALZY, R. BRU, in press.
Ann. technol. Vég. 3/1969.

- Contribution à l'étude de la sporulation chez S. cerevisiae HANSEN.
Francoise VEZINHET, in press.
Mycopathologica et Mycologia Applicata - U.S.A.

- Observations sur l'oxydation de l'éthanol et de l'acide acétique par S. cerevisiae HANSEN.
P. GALZY, A. ARNAUD, Francoise VEZINHET, J. ALBERT, in press.
C. R. Colloque oenologique de Tours 27-30 Mai 1969.

- Modifications métaboliques de la levure au cours de la sporulation.
Francoise VEZINHET, in press.
Bulletin de la Soc. de Chimie Biologique.

- Remarks on the sporulation of Saccharomyces cerevisiae HANSEN.
Francoise VEZINHET, P. GALZY, in press.
Third International Symposium on Yeasts, Delft - The Hague, 2-6/6/69.

- Studies of the mode of action of the gene pl_5 in Saccharomyces cerevisiae HANSEN.
C. BIZEAU, in press.
Third International Symposium on Yeasts, Delft - The Hague, 2-6/6/69.

- La génétique des levures. Techniques et méthodologie.
P. GALZY, C. PLAN, J. ALBERT, in press.
Revue des Fermantations et des Industries Alimentaires. Bruxelles.

XX Centre National de la Recherche Scientifique, Laboratoire d'Enzymologie, 91-Gif-sur-Yvette, France. Communicated by Dr. H. de Robichon-Szulmajster.

The following publication just appeared:

"Allosteric behavior of yeast threonine deaminase under partially inactivating conditions", A. BRUNNER and H. de ROBICHON-SZULMAJSTER, FEBS letters, 5 (1961) 141-144.

A manuscript will be sent very shortly to J. of Bacteriol.:

"The role of homocysteine synthetase in an alternate route for methionine biosynthesis in Saccharomyces cerevisiae", H. CHEREST, G. TALBOT, and H. de ROBICHON-SZULMAJSTER, with the following summary:

In vivo studies have been shown that in the absence of homoserine-O-transacetylase activity (locus met_2), the C_4-carbon

moiety of ethionine is utilized (provided the ethionine resistance gene eth-2r is present), by methionine auxotrophs with the exception of met_8 mutants (homocysteine synthetase deficient). Concomitant utilization of sulfur and methyl group from methylmercaptan or S-methylcysteine has been demonstrated. In the absence of added methylated intermediates, the methyl group of methionine formed from ethionine is derived from serine.

In vitro studies, with crude extracts of Saccharomyces cerevisiae have demonstrated that this synthesis of methionine occurs by the following reactions.

(a) CH_3-SH + Ethionine $\rightleftharpoons$ Methionine + C_2H_5SH

(b) S-methylcysteine + Ethionine $\rightleftharpoons$ Methionine + S-ethylcysteine

In the forward direction the second product of reaction b was shown to be S-ethylcysteine. Reaction b has also been found reversible, leading to ethionine formation.

Genetic and kinetic data have shown that homocysteine synthetase catalyses these two reactions, at 0.3% of the rate it catalyses direct homocysteine synthesis:

(c) O-Ac-homoserine + Na_2S → Homocysteine + Acetate

The three reactions are lost together in a met_8 mutant, and recovered to the same extent in spontaneous prototrophic revertants from this strain. Methionine mediated regulation of enzyme synthesis affects the three activities, and is modified to the same extent by the presence of the recessive allele(eth-2r) of the regulatory gene eth-2.

Affinities of the enzyme for substrates of both type of reactions are of the same order of magnitude. Moreover, ethionine, substrate of reaction (b) inhibits reaction (c), while O-acetyl-homoserine, substrate of reaction (c), inhibits reaction (b).

An enzymatic cleavage of S-methylcysteine leading to methylmercaptan production has been shown to occur in crude yeast extracts.

It is concluded that the enzyme homocysteine synthetase participates to the two alternate pathways leading to methionine biosynthesis in S. cerevisiae, one involving O-acetyl-homoserine and H_2S, the other involving the 4-carbon chain of ethionine and a mercaptyl donor. Participation of the two types of reactions catalysed by homocysteine synthetase, to in vivo methionine synthesis, has been shown to occur in a met_2 partial revertant.

XXI Laboratorio di Mutagenesi e Differenziamento del C.N.R. and Istituto di Genetica dell'Università, Viale Matteotti 1/A, 56100-Pisa, Italy. Communicated by Dr. N. Loprieno.

Drs. C. Bauer and Pina Dibenedetto have recently joined the former group of people working with the yeast Schizosaccharomyces pombe (N. Loprieno, R.

Guglielminetti, A. Abbondandolo and S. Bonatti): both are presently interested in the study of the genetics and the biochemistry of acid phosphatase enzymes in this fission yeast.

During the last week of September, U. Leupold and M. Schüpbach from the Institut für Mikrobiologie, Bern (Switzerland), M. A. Resnick from the National Institute for Medical Research, London (England), F. Fabre from the Institut du Radium, Biologie, Orsay (France) have spent two days in Pisa discussing with their guests of the Laboratorio di Mutagenesi e Differenziamento about the following topics:

1) genetics of the radiation sensitive mutants of S. pombe and their influence on intragenic and intergenic recombinations;
2) induced and spontaneous mutations in the radiation sensitive mutants of S. pombe;
3) lethal sectoring, liquid holding recovery, growth rate and sensitivity of G1 and G2 cells to radiations;
4) DNA synthesis and radiation sensitivity of different steps during copulation and meiosis of S. pombe cells;
5) influence of caffeine on mutation, radiation and chemical sensitivity, recombination in wild type and radiation sensitive mutants of S. pombe.

Present researches in progress with S. pombe refer to:

1) repair processes and mutations;
2) mosaicism and genetic instability;
3) conditional lethal mutations;
4) acid phosphatase mutations.

The following papers have recently been published or presented as communications to meetings:

1) N. Loprieno: UV-mutability of the wild type and of a UV-sensitive strain of S. pombe. Atti Ass. Genet. Ital., 14, 185-187 (1969).
2) N. Loprieno and R. Guglielminetti: Repair of prelethal and premutational damages in S. pombe. Antonie van Leeuwenhoek, 35, Suppl.; Yeast Symp. (1969).
3) N. Loprieno, S. Bonatti, A. Abbondandolo and R. Guglielminetti: The nature of spontaneous mutations during vegetative growth in S. pombe. Mol. Gen. Gen., 104, 40-50 (1969).
4) N. Loprieno, R. Guglielminetti, S. Bonatti and A. Abbondandolo: Evaluation of the genetic alterations induced by chemical mutagens in S. pombe. Mutation Res., 8, 65-71 (1969).
5) N. Loprieno: Negative interallelic complementation of ad-6 temperature dependent mutants of S. pombe. Acc. Naz. Lincei (1969) in press.
6) R. Guglielminetti and M. Schüpbach: Mechanisms of dark repair and photoreactivation in S. pombe. Atti Ass. Genet. Ital., 14, 182-183 (1969).
7) A. Abbondandolo and S. Bonatti: The production, by nitrous acid, of complete and mosaic mutations during defined nuclear stages in cells of S. pombe. Mutation Res. (1969) in press.
8) P. Dibenedetto, C. Bauer and N. Loprieno: Aspecific acid phosphatases in S. pombe: general biochemical and genetic properties. Atti Ass. Ital. Biol. Sperim. (1969) in press.

XXII Department of Genetics, Edinburgh University, Scotland. Communicated by B. J. Kilby.

Mutation Group

The M.R.C. Mutagenesis Research Unit officially ceased to exist in October 1969 but we wish to make clear that mutation work will still be continuing in Edinburgh.

Our interests include the analysis of cellular processes which influence the expression of newly induced mutations, the influence of mutagenic treatments on these cellular events and on mutagen specificity. We are also interested in photobiological problems and are in the process of installing facilities for the monochromatic irradiation of biological material.

The group comprises Charlotte Auerbach, Douglas Ramsay, Clara Queiroz, Sheena Smith, Anne Ferguson and Brian Kilbey. We have space for, and would welcome, visitors and Ph.D. students interested in mutagenesis and related subjects. Facilities are available for work with Saccharomyces cerevisiae and other microorganisms.

Some recent publications are the following:

Auerbach, C. & Ramsay, D. (1968). Molec. Gen. Genetics 103, 72. Analysis of a case of mutagen specificity in Neurospora crassa. I. Dose Response Curves.

Auerbach, C. (1969). CIBA Symposium on Mutation as a Cellular Process. Analysis of a case of mutagen specificity (in press).

Kilbey, B. J. (1969). Mutation Res. 8, 73. Diepoxybutane pre-treatment effects on U.V. inactivation and photoreactivation of Saccharomyces cerevisiae.

Kilbey, B. J. & Smith, S. M. (1969). M. G. G. 104, 253. Similarities between a U.V.-sensitive mutant of yeast and bacterial mutants lacking excision-repair ability.

Kilbey, B. J. (1969). CIBA Symposium on Mutation as a Cellular Process. Allele specific responses to factors that modify U.V. mutagenesis (in press).

XXIII Forstbotanisches Institut, Universität Freiburg, D-78 Freiburg Bertoldstr. 17, Germany. Communicated by F. K. Zimmermann.

The following publications have appeared:

F. K. Zimmermann: Mutation in diploid cells and its role in carcinogenesis. Arzneim.-Forsch. 19, 1046-1050 (1969).

F. K. Zimmermann, I. Schmiedt and A. M. A. ten Berge: Dominance and recessiveness at the protein level in mutant x wildtype crosses in Saccharomyces cerevisiae. Molec. Gen. Genetics 104, 321-330 (1969).

Reverse mutation is being studied by Messrs. E. Gundelach and K. L. Friedmann using isoleucine requiring mutant alleles of the locus $\underset{\sim}{is}_1$ (threonine dehydratase locus) which had been induced with the carcinogen 1-nitroso-imidazolidone-2 (see: Molec. Gen. Genetics 103, 348, 1969 and 104, 321, 1969). Spontaneous or chemically induced reverse mutants of 13 alleles were analysed by determining threonine dehydratase (TDH)-activity in crude extracts or else by genetic crosses and tetrad analysis. The results of the enzymatic tests are shown in table 1. The mutants are grouped as complementing and non-complementing mutants. In most cases TDH specific activity was much lower than in wildtype cells. Revertants expressing somewhat higher activities than the majority produced a TDH which was either partly or not at all feedback-inhibited by a concentration of L-isoleucine which completely inhibits TDH of wildtype cells. Consequently, none of the 66 revertants of 9 complementing and 4 non-complementing mutants had reverted to wildtype. Genetic analysis was performed with 2 revertants of the very efficient complementer allele 49 and allele 112. In all cases reversion was due to external suppressors unlinked in the case of 49 and linked (22 map units) in the case of 112. Two revertants of allele 61 and 4 revertants of 92 had also reverted by external suppressors. The results revealed a striking lack of true reverse mutants among 66 revertants from 13 different alleles.

Mitotic gene conversion has been shown to provide a simple, accurate and sensitive tool to detect genetic activity of many mutagens and carcinogens (cf. Zimmermann: Molec. Gen. Genetics 103, 11-20, 1968). Mr. D. Siebert is testing fungicides and other pesticides for genetic activity. A heteroallelic strain D4 is used which requires adenine ($\underset{\sim}{ad}_{2-1}/\underset{\sim}{ad}_{2-2}$) and tryptophan ($\underset{\sim}{tr}_{5-12}/\underset{\sim}{tr}_{5-27}$) for growth. Cells no longer requiring either adenine or tryptophan for growth are generated by mitotic gene conversion (Zimmermann and Schwaier: Molec. Gen. Genetics 100, 63-67, 1967). A commercial preparation of Ortho-Phaltan 50 containing N-trichloro-methyl-thiophtalimide (Folpet), as the active principle was suspended with cells of D4 in 0.1 M potassium phosphate buffer (pH 7.5) and incubated under shaking at about 22°C for 4 hr. After that, cells were spun down by centrifuging, washed three times in distilled water, and plated on media selective for convertants and on a complete medium to determine survival. As shown in table 2 Folpet induced mitotic gene conversion at both loci at sublethal doses already. Consequently this fungicide has to be considered as genetically hazardous to farmers and consumers.

Table 1: Specific activities of threonine dehydratase (ΔE_{366}/min per 0.1 ml crude extract) in isoleucine non-requiring revertants of is_1-mutants of Saccharomyces cerevisiae.

Complementing alleles	Specific activities of individual mutants						
15	52.5*	4	3	3.5	3.5	9*	
	14*	16*	11*	10*			
33	2	4	1	2	3		
36	<1	<1	<1				
48	1	5**	1	5*	1	1	
49	2	1	3	3	4	3	3
51	<1	1	<1	<1	1		
77	3						
83	2.5	2					
112	3	1	<1	1	<1		
Non-complementing alleles							
41	2	2					
42	26*	5**	14*	3	5**		
61	2	2	2	2	3	2	2
	3	2.5	1	1.5	1.5	<1	<1
92	2						
wildtype	50-90						

*activity partly, **activity completely inhibited by L-isoleucine. Feedback inhibition only tested when spec. activity higher than 5. Assay conditions: 1.7×10^{-2} M L-threonine, pH 7.9, 23°C; L-isoleucine final concentration 3.3×10^{-3} M. Assay after Holzer, Boll and Cennamo: Angew. Chem. 75, 895 (1963).

Table 2: Induction of mitotic gene conversion with Ortho-Phaltan 50 in Saccharomyces cerevisiae (ad_{2-1}/ad_{2-2}; tr_{5-12}/tr_{5-27})

Concentration in ppm	Convertants per 10^6 survivors		Number of convertant colonies per 5 plates	
	tr	ad	tr	ad
0	12,4	28,6	54	125
25	15,8	27,8	74	130
50	28,6	37,7	129	170
75	75,7	70,3	326	303
100	86,4	81,1	354	332

XXIV Division of Genetics, National Institute of Radiological Sciences, Chiba, Japan. Communicated by Sayaka Nakai.

PREFERENTIAL REPAIR OF UV-INDUCED MUTATIONS IN YEAST. Sayaka Nakai and Eiko Yamaguchi. Japanese Journal of Genetics (in press).

Experiments have been carried out using a UV-sensitive mutant of uvs 1 of Saccharomyces cerevisiae, presumably

lacking ability of dark repair, to investigate the genetic nature of UV-induced premutational damages leading to three-different types of mutation under both dark and light conditions. Yields of these types of mutation in uvs 1 are markedly higher than those in wild type at similar doses. Under dark conditions a logarithmically plotted dose-response curve of true-back mutation from arg 4-17, his 5-2 and lys 1-1 possessing nonsense codon in uvs 1 consists of two straight lines. Slopes of curves produced at high doses are steeper than those produced at low doses. The former curves are almost the same as those in wild type. The curves for induced mutation of super-suppressor and back mutation from addition-deletion types of his 1-1 consist of single straight lines. Slopes of these curves of both uvs 1 and wild-type strains are almost the same. Furthermore differential yields of true-back mutation between uvs 1 and wild-type strain at the same doses are greater than those for induced super-suppressor of back mutation of addition-deletion type. From these results it is postulated that preferential repair exists of the premutational damages leading to true-back mutation as compared to other types of mutations. Molecular nature of the premutational damages leading to these types of mutation is discussed.

XXV Case Western Reserve University, Developmental Biology Center, 2127 Cornell Rd., Cleveland, Ohio, 44106. Communicated by Elizabeth W. Jones.

The following is a summary of research recently completed and currently under investigation.

PHYSIOLOGICAL CONSEQUENCES OF A GENETIC LESION IN FOLIC ACID METABOLISM IN SACCHAROMYCES CEREVISIAE

Point mutations in the ad-3 locus of S. cerevisiae result in a simultaneous requirement for adenine and histidine. Analysis of enzymatic activity in crude extracts of ad-3 mutants reveals a total absence of activity for formyltetrahydrofolate synthetase and 5,10-methyltetrahydrofolate cyclohydrolase, and 10-15% of the wild type activity for 5,10-methylenetetrahydrofolate dehydrogenase. The result of this pleiotropic mutation is to deprive the cell of single carbon units at the formate level of oxidation and thus to interfere with the synthesis of purines both before and after the formation of the imidazole nucleus.

Accumulation studies indicate that intact cells of ad-3 mutants lack the function of phosphoribosylformiminophosphoribosylamino-imidazolecarboxamide ketolisomerase, the fourth enzyme of histidine biosynthesis, and thus have a histidine requirement. This malfunction is not due, however, to loss of this enzyme, for extracts of ad-3 mutants contain the full complement of this activity. The ketolisomerase has been partially purified and some of its properties have been examined in an attempt to clarify the nature of this lesion in histidine biosynthesis. A plot of V versus S for the enzyme reveals sigmoid kinetics. The enzyme is completely inhibited by 1.2×10^{-4} M 5,10-methylenetetrahydrofolate, a derivative which may accumulate in the mutant. In addition, 5,10-methyltetrahydrofolate, a derivative completely lacking in ad-3 mutants, appears to be an activator of the enzyme. These two

phenomena, the inhibition by 5,10-methylenetetrahydrofolate and the absence of activation due to the lack of 5,10-methyltetrahydrofolate, may be sufficient to explain the histidine requirement in the ad-3 mutants.

Current studies center on the kinetics of activation and inhibition, the physiological significance of controls located in the middle of a biosynthetic chain, and the genetic basis of point mutations which consistently result in loss of three enzymatic activities simultaneously.

XXVI The Research Laboratory, Arthur Guinness Son & Co. (Dublin) Ltd., St. James's Gate, Dublin 8, Ireland. Communicated by R. B. Gilliland.

The following are abstracts of two recent papers:

Gilliland, R. B. The Raffinose Fermentation of Saccharomyces pastorianus and Saccharomyces bayanus. Antonie van Leeuwenhoek, 35 (1969), 13-23.

> Using described quantitative methods four strains of S. pastorianus from culture collections were shown to be able to ferment two-thirds of the raffinose molecule, but they were unable to ferment melibiose in the absence of some other fermentable sugar. Two cultures of S. bayanus proved to be mixtures of two strains, one of which could ferment two-thirds raffinose and the other could ferment raffinose completely, mutation of the former to the latter could take place. The other three strains of S. bayanus fermented only one-third raffinose.

Gilliland, R. B. Yeast Strain and Attenuation Limit, European Brewery Convention Proceedings Interlaken 1969, in press.

> The following yeasts which have differing abilities to ferment the sugars present in brewery wort are described.
>
> 1. A maltotriose non-fermenting variant of Saccharomyces cerevisiae.
> 2. A variant of S. cerevisiae which was able to ferment isomaltose and panose.
> 3. A variant of S. cerevisiae which was able to ferment maltotetraose. Single gene inheritance of this character was observed.
> 4. S. diastaticus which fermented maltotetraose and longer straight-chain maltodextrins but which could not ferment isomaltose or panose.

XXVII Ecole Superieure de Brasserie, Malterie & Biochimie Appliquee, Université de Nancy, France. Communicated by C. Bourgeois.

Work in our laboratory is concerned with

- improving methods of microbiological analysis and strain selection in the brewing industry.

- uptake, metabolism and metabolic regulations of some aminoacids of great importance in brewing.

Our investigations about lysine effects in yeast, have just resulted, owing to the advices of Mme H. de ROBICHON-SZULMAJSTER in the publication of a paper entitled "Influence de la lysine sur la croissance de Saccharomyces cerevisiae".

(C. BOURGEOIS, BULL. SOC. CHIM. BIOL., 1969, 51, n° 5)

Evidence was presented that lysine, added to an ammonium sulfate medium considerably reduces the yield, when growth is limited by the concentration of nitrogen nutrient. The analysis of amino acid pools has shown that the presence of lysine provokes, at the end of exponential growth, an early loss of endogenous arginine, likely related to a regulatory action which is now being investigated.

XXVIII The Australian Wine Research Institute, Private Mail Bag No. 1, Glen Osmond, S. A. 5064. Communicated by B. C. Rankine.

Part of the research programme of the Institute has been concerned with the role of different yeasts in the winemaking process, and a paper summarizing the work over some years has now been published.

"The importance of yeasts in determining the composition and quality of wines" by B. C. Rankine, Vitis (1968) 7:22-49.

Further work has been concerned with the binding of sulphur dioxide in wine by fermentation products of wine yeasts (particularly α-ketoglutaric acid), and on the production of sulphur dioxide by reduction of sulphate during fermentation.

Two papers have recently appeared reporting the results of these investigations.

"Formation of α-ketoglutaric acid by wine yeasts and its oenological significance" by B. C. Rankine, J. Sci. Fd. Agric. (1968) 19:624-627.

"Influence of yeast strain on binding of sulphur dioxide in wines, and on its formation during fermentation" by B. C. Rankine and K. F. Pocock, J. Sci. Fd. Agric. (1969) 20:104-109.

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

Dr. Arnold L. Demain has moved from the Ferm. Res. Dept., Merck Sharp & Dohme Res. Labs., Rahway, N. J., to the Dept. of Nutrition and Food Science,

M.I.T., Cambridge, Mass. His new position is that of Processor of Applied Microbiology.

The following paper was presented by Drs. L. Kaplan and A. L. Demain of the Merck Laboratories at the symposium "Recent Trends in Yeast Research" at Plattsburgh, N. Y.: "Nutritional Studies on Riboflavin Overproduction by Ashbya gossypii". It was found that as much as 1 g of riboflavin per liter could be produced in a new defined medium. Of the components, biotin, thiamine, inositol and asparagine are required only for growth and these could be eliminated from incubation media designed for flavinogenesis by washed mycelial suspensions. Glycine, an aromatic amino acid, and a purine are required for riboflavin production. Glucose and tween 80 are needed for both growth and riboflavin synthesis. Riboflavin synthesis is very dependent on temperature. Although production of the vitamin by washed mycelia occurs at 28°C and 37°C and although growth proceeds equally at both temperatures, cells grown at 37°C do not overproduce riboflavin while 28°C grown cells are very active. Thus, low temperature appears to derepress the riboflavin overproduction process.

XXX Central Research Laboratory of Ajinomoto Co., Inc., Kawasaki, Japan. Communicated by Takashi Nakase.

Fermentation of η-Paraffins by Yeast. Part I. Fermentative Production of α-Ketoglutaric Acid by Candida lipolytica. By Ryuichiro Tsugawa, Takashi Nakase, Tadao Kobayashi, Koichi Yamashita and Shinji Okumura. Agr. Biol. Chem., Vol. 33, No. 2, p. 158-167, 1969.

Screening test for obtaining microorganisms which produce L-amino acids or organic acids from η-paraffins were carried out. Fourteen strains of microorganisms which seemed to belong to the yeast showed ability to produce α-ketoglutaric acid. A representative strain of these micro-organisms was identified as Candida lipolytica AJ 5004.

Optimal conditions for production of α-ketoglutarate using Candida lipolytica AJ 5004 were also studied. Under the condition thus obtained using a culture medium of 8 weight % of η-paraffins, the yeast accumulated 59% of a α-ketoglutarate to the substrate added after three days culture.

XXXI Meetings

Yeast Genetics Conference - JAPAN. Reported by T. Takahashi, Suita Laboratory, Brewing Science Research Institute, Asahi Breweries Ltd., 5-3 Deguchi-cho, Suita, Japan.

The first meeting of the "Yeast Genetics Conference-Japan", which was arranged in memory of the IVth International Yeast Genetics Conference held in Osaka, Japan in September, 1968, was held October 14 and 15, 1969, and twenty-seven investigators met at "Rakuyu-Hall", Kyoto University. Six general areas were discussed: mutation and radiation effects, sex controlling mechanism, cytoplasmic inheritance, regulation of gene action, recombination and fine- and macro-mapping, and cytology.

Nakai (Chiba) presented data on mutagenesis of UV induced foreward mutation. From 30 to 80% of the mutants were class I nonsense mutants. Mutation rate was higher in xs and lower in uvs. Takahashi & Utsumi (Suita)

described the relation of auxotrophic mutant induction and pH of preincubation medium. Hieda (Tokyo) supposed the denaturation of double helix of DNA in partially dehydrated yeast cell by the occurrence of UV induced pink colonies from $\frac{AD}{ad_1}$. The results were similar to the in vitro experiment. Ohuti (Tokyo) reported the isolation and the characters of the foamless mutant of "Sake"-yeast.

Takano & Oshima (Osaka) reported the genetic conversion from α to a' by the action of HO_α and from a to α' by the action of HO_α HM. They also discussed the fine structure model of the mating type locus and its connection to homothallism. Takahashi presented the loss and the recovery of homothallism by intragenic recombination in D locus (= Takano & Oshima's HO_α). Gunge (Yokohama) crossed haploid with unbalanced heterozygotes for mating type and obtained tri- to heptaploids. During the crossing, mutation of mating type occurred; for example, $aa\alpha \times \alpha = aaa\alpha$, $a\alpha\alpha\alpha \times a = \alpha\alpha\alpha\alpha a$ and so on. Yanagishima (Osaka) suggested the presence of at least three kinds of sex hormones (two steroids and a peptide-like substance) for the copulation of a and α cells. Shimoda (Osaka) studied the relation between gene constitutions of mating type and homothallism and cellular response to hormones. The response in non-mater or homothallic strains was irregular.

Nagai (Nara) induced the rho-mutants in high frequency with the use of nutritional deficient medium and various nutrients were tested to inhibit the rho-mutant induction. Saeki & Nakai (Chiba) described the high rho-mutant induction in uvs by UV irradiation and YEP-glycerol medium. Yamamoto has studied the base components of ribosomal RNA extracted from strontium resistant and found the deviations on electrophoretic patterns after methylation.

Tamaki (Kyoto) reported two inhibitor genes, which might be located on the same chromosome, for amylase synthsis based on studies using the starch fermenting yeast S. diastaticus. Oshima isolated a constitutive mutant of α-glucasidase formation. Cell free extract of the mutant showed α-glucosidase activity using p-nitrophenol-α-glucoside.

Oshima found four complementation units in MA_α, eight units in MA_β and five units in MA_γ. Takahashi studied the 1:3 segregation in tetrads of MET/met3. An abberant tetrad has occurred by gene mutation at a different locus which is concerned with homoserine synthesis, and another abberant tetrad has derived from the intragenic recombination of met3, which is composed of at least three alleles. Takahashi presented the linkage studies of new markers and found a linkage group having try53 and arg22.

Iguti (Mito) suggested the recovery of protoplasts produced by snail juice. Hirano (Tokyo) has studied the differentiation and development of cell organelles from cytoplasmic membrane by the use of electron microscope. Osumi (Tokyo) & Sando (Tsuruoka) reported the formation and morphological changes of mitochondrial structure during cell cycles. Yuasa (Tokyo) described studies on chromosome observations in various yeasts.

Nakai introduced the "proposal for a new system of genetic nomenclature for use in yeast genetics research" proposed by a committee of Mortimer, Sherman and von Borstel. In principle, proposed symbols were supported, but concerning the mating type, the present symbol was supported for its simplicity.

After the discussion, topic in the IIIrd International Symposia on Yeasts, the XIIth Congress of European Brewery Convention and the XIth International Congress of Botany were reported by Amaha (Tokyo), Hirano, Nagai, Osumi and Yanagishima.

This year is the 30th anniversary of the first yeast genetics research in Japan by Dr. Yukio Yamamoto (Genetical Investigation in Saccharomycetes. I. Segregations in Saccharomyces Sake Yabe. Bot. Magaz. Tokyo 53: 449-459, 1939: in Japanese with English resume). For the memory of his pioneering work and the starting of the yeast genetics conference-Japan, a flower vase was presented to Dr. Y. Yamamoto from the members of the conference.

T. Hirano (Tokyo Metropol. Isotope Res. Ctr.), S. Nagai (Nara Women's Univ.), S. Nakai (Natl. Inst. Radiol. Sci.), Y. Oshima (Ctr. Res. Inst., Suntry Ltd.), T. Takahashi (Suita Lab., Brew. Sci. Res. Inst.) and H. Tamaki (Doshisha Women's Univ.) were elected as members of the organising committee of the yeast genetics conference-Japan. The next meeting will be held after the International Yeast Genetics Conference in Canada, 1970.

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The Section of Yeasts of the Czechoslovak Society of Microbiologists (CzSM) held its Third Annual Meeting in the castle of Smolenice near Bratislava, Octobre 29 to 31. 65 members of CzSM of the whole Republic participated there. The presence of many young people starting to study yeasts grouped in new teams constituted a fine development. Prof. Dr. Kabelík, the honorary member of CzSM visited the conference, Prof. Dr. Dietrich and Dr. Würdig, two German specialists in wine technology and ecology of wine yeasts were the guests of CzSM. 30 papers on various problems were presented: 4 on ecology of wine yeasts; 3 on antimycotica of the allyl-sulphide and izothiocyanate groups and the mechanisms of their action; 3 on numerical taxonomy; 2 about new diagnostic methods; 5 on cytology and cell ultrastructure; 3 on immunology; 1 on bioengineering; 3 on mutational genetics and application in the industry; 6 on biochemistry.

The discussions were very extensive.

Since the functional period of the Executive Committee of CzSM terminated at the end of 1969 a new committee of the Section of Yeasts was elected for the period of 1970 to 1974: DrSc. A. Kocková-Kratochvílová, chairman, Prof. Dr. O. Nečas, vice-chairman, Doc. Ing. E. Minárik, secretary. Some changes happened in the Committee-membership. It was decided that the Committee will function as the Organizing Committee for the International Symposium "Yeasts as models in science" which will be held in the castle Smolenice near Bratislava, June 1971.

A. Kocková-Kratochvilová

XXXII Brief News Items

1. Research Institute for Viticulture and Enology, Bratislava, Czechoslovakia.

The following paper has been prepared and sent for publication in Mitteilungen Klosterneuburg/Austria: "Hyalodendron - a fungus occurring seldom on grapes and grape juice".

Several strains of a fungus resembling to yeasts and belonging to the genus Hyalodendron Diddens were repeatedly isolated and identified from Ortho-Phaltan (N-Trichlormethylthio-phtalimide) and Captan (N-Trichlormethylthio-tetrahydrophtalimide) treated grapes and grape juice. Morphological and physiological properties of the fungus are briefly described.

E. Minarik

2. We can supply to interested persons mating types and a sporulating diploid of Candida lipolytica.

I am planning to retire in September 1970 and my wife and I plan to move then to Southern Arizona.

L. J. Wickerham
Northern Regional Laboratory
U.S.D.A.
Peoria, Illinois 61604

3. The following two publications have now been published.

H. SAËZ et J. RINJARD - (1969) - Levures isolées du tube digestif de Mammifères sauvages, en captivité, à régime alimentaire piscivore. Revista Iberica de Parasitologia, vol. 29, n°I, p. 45-56.

H. SAËZ et J. RINJARD - (1969) - Candidose aviaire de l'oesophage associée à une helminthose. Economie et Médecine Animales, IO, n°2, p. 141-147.

Dr. Henri Saëz
Parc zoologique
53, Avenue de Saint-Maurice
Paris 12è, France

4. Our laboratory is studying the energetics of Schizosaccharomyces pombe and Candida lipolytica as well as the biogenesis of mitochondrial membrane of Saccharomyces cerevisiae.

The address below is my new one.

Dr. A. Goffeau
Laboratoire de Enzymologie
92 Avenue Cardinal Mercier
Héverlé, Belgium