Y E A S T

A News Letter for Persons Interested in Yeast

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I. Centraal Bureau voor Schimmelcultures (CBS), Julianalaan 67A, Delft, The Netherlands. Communicated by D. Yarrow.

The following is a list of recently described new species of yeast which have been deposited in the CBS collection.

Brettanomyces abstinens CBS 6055 (Type)
D. Yarrow and D. G. Ahearn, A. v. Leeuwenhoek 37: 296 (1971)
Candida citrea CBS 6374 (Type)
T. Nakase, J. Gen. Appl. Microbiol. 17: 383 (1971)
Candida dendronema CBS 6270 (Type), 6271
Candida entomophila CBS 6160 (Type), 6159
J. P. van der Walt, D. B. Scott and W. C. van der Klift, A. v. Leeuwenhoek 37: 449 (1971)
Candida fibrae CBS 6375 (Type)
= Pichia burtonii Boidin et al. Mates with CBS 2352
Candida fragicola CBS 6376 (Type)
T. Nakase, J. Gen. Appl. Microbiol. 17: 409 (1971)
Candida hylophila CBS 6226 (Type)
J. P. van der Walt et al., A. v. Leeuwenhoek 37: 445 (1971)
Candida paralipolytica nom. nud. CBS 6303
K. Yamada and Y. Ota, J. Agr. Chem. Soc. Japan 37: 449 (1971)
= Saccharomycopsis lipolytica (Wickerham et al.) Yarrow. Mates with CBS 6124-2
Candida rugopelliculosa CBS 6377 (Type)
T. Nakase, J. Gen. Appl. Microbiol. 17: 383 (1971)
Candida silvanorum CBS 6274 (Type)
J. P. van der Walt et al., A. v. Leeuwenhoek 37: 449 (1971)
Candida sorboxylosa CBS 6378 (Type), 2120, 2121
T. Nakase, J. Gen. Appl. Microbiol. 17: 383 (1971)
Cryptococcus himalayensis CBS 6293 (Type)
S. Goto and J. Sugiyama, Can. J. Bot. 48: 2097 (1970)
Filobasidium floriforme CBS 6240, 6241 mt A, 6242 mt a
L. S. Olive, J. Mitchell Soc. 84: 261 (1968)
Hansenula dryadoides CBS 6154 (Type), 6155
D. B. Scott and J. P. van der Walt, A. v. Leeuwenhoek 37: 171 (1971)
Kluyveromyces thermotolerans (Philippov) nov. comb. CBS 6340 (Type)
D. Yarrow, A. v. Leeuwenhoek 38: (1972)
Pichia abadiae CBS 6067 (Type)
Pichia adzetii CBS 6066 (Type)
F. Jacob, Bull. Soc. Mycol. France 85: 117 (1960)
Pichia besseyi CBS 6344 (Type)
C. P. Kurtzman and L. J. Wickerham, A. v. Leeuwenhoek 38: 49 (1972)
Pichia cicatricosa CBS 6157 (Type), 6158
D. B. Scott and J. P. van der Walt, A. v. Leeuwenhoek 37: 177 (1971)
Pichia crossotarsi CBS 6392 = ATCC 18855 (Type)
Pichia microspora CBS 6393 = ATCC 18856 (Type)
L. R. Batra, Mycologia 63: 994 (1971)
CBS 6392 is indistinguishable from Ambrosiozyma monospora (Saito) v.d. Walt and CBS 6393 from Botryoascus synnaedendrus (Scott and v.d. Walt) v. Arx
Pichia mucosa CBS 6341 (Type)
Pichia sargentensis CBS 6342 (Type)
L. J. Wickerham and C. P. Kurtzman, Mycologia 63: 103 (1971)

Saccharomyces castelli CBS 4309, 4310
A. Capriotti, Studi Sassaresi, III, Agric. 14: 457 (1966)
Saccharomyces florenzanii CBS 6339
W. Balloni, R. Materassi & M. C. Margheri, Zentr. Bakt. Parasitenk., Abt. II, 26: 386 (1971)
Saccharomyces servazzii CBS 4311
A. Capriotti, Ann. Microbiol. 17: 79 (1967)
This species and S. castelli are indistinguishable from S. dairensis Naganishi
Sympodiomyces parvus CBS 6147 (Type)
J. W. Fell and A. C. Statzell, A. v. Leeuwenhoek 37: 359 (1971)
Torulopsis auriculariae CBS 6379 (Type)
T. Nakase, J. Gen. Appl. Microbiol. 17: 409 (1971)
Torulopsis dendrica CBS 6151 (Type)
J. P. van der Walt et al., A. v. Leeuwenhoek 37: 461 (1971)
Torulopsis fragaria CBS 6254 (Type), 6253, 6255
J. A. Barnett and R. W. Buhagiar, J. Gen. Microbiol. 67: 233 (1971)
Torulopsis fructus CBS 6380 (Type)
T. Nakase, J. Gen. Appl. Microbiol. 17: 409 (1971)
Torulopsis insectalens CBS 6036 (Type), 6149
J. P. van der Walt et al., A. v. Leeuwenhoek 37: 461 (1971)
Torulopsis musae CBS 6381 (Type)
T. Nakase, J. Gen. Appl. Microbiol. 17: 409 (1971)
Torulopsis nemodendra CBS 6280 (Type)
Torulopsis philyla CBS 6272 (Type)
J. P. van der Walt et al., A. v. Leeuwenhoek 37: 461 (1971)
Torulopsis pignaliae CBS 6071
F. H. Jacob, Ann. Inst. Pasteur 118: 207 (1970)
Torulopsis silvatica CBS 6277 (Type)
J. P. van der Walt et al., A. v. Leeuwenhoek 37: 461 (1971)
Torulopsis tannotolerans CBS 6070 (Type)
F. H. Jacob, Ann. Inst. Pasteur 118: 207 (1970)
Trichosporon brassicae CBS 6382, T. Nakase, J. Gen. Appl. Microbiol. 17: 409 (1971)

II. Université de Lyon, Laboratoire de Biologie Végétale, 43 Boulevard du 11 Novembre 1918, 69 Villeurbanne, France. Communicated by S. Poncet.

Below follows the latest news from our laboratory.

In June 1971 we participated in the Symposium of Smolenice and we presented a communication. On August 24 we had a visit of Dr. Bridge-Cooke who made a mycological and tourist tour of Europe. In mid-October, Dr. Van der Walt spent 2 days with us and we were able to have a detailed discussion on various points of view dealing with the biology and taxonomy of yeasts.

Recent publications:

Fiol J. B. et Poncet S. Comparison de Kluyveromyces aestuarii et K. wikenii par application de critères nouveaux. Ann. Inst. Pasteur 121: 75-85. 1971.

Jacob F. Les levures des liqueur tannantes végétales. II. Repartition qualitative et quantitative. Ann. Inst. Pasteur 121: 49-67. 1971.

Jacob F. and Pignal M. C. Reciprocal action of various yeasts and certain vegetable tanning extracts. Communication at Smolenice. June 1971.

In Press:

Jacob F. et Pignal M. C. Interactions levures-tanins. I. Croissance et survie dans les solutions tannantes. (Accepted for publication in Mycopath. et Mycol. appl.)

Poncet S. et Fiol J. B. Taxonomy of Kluyveromyces:evaluation of GC content of DNA species. (Accepted for publication in Ant. van Leeuwenhoek.)

Fiol J. B. Recherche d'enzymes intracellulaires chez les Kluyveromyces (van der Walt). (β galactosidase, α glucosidase, β glucosidase,tréhalase):Conséquences taxinomiques. (Accepted for publication in Mycol. et Mycopath. appl.)

Work in Progress:

Hydrolysis of tannic acid by yeast in culture medium as a function of tannin concentration, pH, and temperature.

Respiration of various aromatic compounds which are constituents of tannin by yeasts isolated from vegetable tanning liquors and from xylophagus insects and the effect of these compounds on respiration.

Determination of the GC contents of Pichia species with spherical spores by the thermal denaturation procedure.

Research on intracellular enzymes (β-galactosidase, α-glucosidase, β-glucosidase, and trehalase) in species of the genera Pichia and Hansenula.

Application of factorial analysis in the classification of the genus Kluyveromyces.

III. Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh, Scotland. Communicated by I. Campbell.

1. J. H. Duffus and C. S. Penman are studying the metabolism of Schizosaccharomyces pombe and Kluyveromyces fragilis with reference to the cell cycle. The histones of K. fragilis differ from those of S. pombe and are synthesized at a point in the cell cycle between the S period and the time of nuclear division. This appears to be the first organism in which a clear distinction has been observed between the S period and the period of histone synthesis.

2. I. Campbell has a paper entitled "Simplified Identification of Yeasts by a Serological Technique" in press in Journal of the Institute of Brewing; a summary follows.

A scheme is described for comparison of yeast colonies isolated on normal or selective media or by membrane filtration. By streak culture of each colony, or of a selection of each type of colony, followed by serological and morphological testing of the cultures, the different species present are distinguished with minimal effort. Colonies of the same color and morphology, with cells of the same morphological and serological properties, are of the same species; therefore if complete identification is desired, only one colony need be tested of each type. This saves the effort of tests on a large number of colonies, but the results of morphological and serological tests alone often provide sufficient information for identification. Three examples of the use of the method are described: identification of yeasts of the sherry flor complex, and of samples of clean and polluted water.

IV. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852. Communicated by Sally Ann Meyer.

The following is the abstract of the paper presented at the Second Specialized Symposium on Yeasts and Yeast-like Microorganisms. Kyoto, Japan. March 19-25, 1972.

## TAXONOMY OF CANDIDA AND TORULOPSIS

A. Stenderup, Sally A. Meyer, A. Leth Bak and C. Christiansen
Institute of Medical Microbiology
University of Aarhus, Aarhus, Denmark

Candida and Torulopsis, two imperfect yeast genera of great heterogeneity, accommodate those yeasts which lack finite characteristics necessary for classification in other asporogenous genera. They, in turn, are separated on the ability to form pseudomycelium. In Torulopsis, pseudomycelium development is absent or rudimentary, whereas Candida species generally produce a well-developed pseudomycelium. Difficulties arise in evaluating those yeast strains which form pseudomycelium that consists of sparse or reduced branched chains of cells.

The artificial basis of this generic separation is further manifested in the variability of pseudomycelial development exhibited among strains within a species and in the variation observed in cultures after prolonged maintenance.

Currently, in our laboratory more than 100 species of Candida and 50 species of Torulopsis are being studied in a combined manner using molecular biological techniques (DNA base composition, DNA-DNA hybridization, genome size, amount of repetitive DNA) to clarify their correct taxonomic position and to establish concrete criteria for differentiation.

The results obtained here, together with those reported by other investigators, are being evaluated to obtain an overall picture of the systematics of the Candida and Torulopsis species.

The DNA base composition ranges from 32-63% GC. Both genera show a similar extent in range (32-61% for Torulopsis and 33-63% for Candida), reflecting both their heterogeneity and possible overlapping nature. Most species have GC values below 50% which is consistent with values found for the Hemiascomycetidae. Species compared with their perfect counterparts reveal nearly identical GC% (C. pelliculosa-Hansenula anomala, 37%; C. pulcherrima-Metschnikowia pulcherrima, 48%; T. sphaerica-Kluyveromyces lactis, 40%). Some Candida and Torulopsis species which are incapable of spore formation but are identical in other respects with described sporogenous yeasts also show similar GC contents (T. holmii-Saccharomyces exiguus, 36%; C. pseudotropicalis-K. fragilis, 41%).

Approximately 10% of the yeasts examined have GC contents greater than 50%. Some of these may be expected to be Heterobasidiomycetes since several (C. gelida, C. frigida, C. scottii and T. capsuligenus) have been shown to possess heterobasidiomycetous life cycles (1).

Preliminary studies (2) indicate that repetitive DNA is present in amounts varying from 5 to 16% of the total nuclear DNA. Genome sizes range from approximately $8 \times 10^9$ to $1.4 \times 10^{10}$ daltons.

DNA-RNA and DNA-DNA hybridization experiments (3,4) have already demonstrated to be the most useful tool in assessing relatedness. Such results show that C. albicans is not closely related to C. tropicalis but has a high degree of relatedness with C. stellatoidea and C. claussenii; C. brumptii and C. catenulata possess a close relationship with one another but not with C. zeylanoides; C. obtusa can be considered synonymous with C. lusitaniae, and C. salmonicola with C. sake; the proposal that Lodderomyces elongisporus is the perfect stage of C. parapsilosis is not accepted on the basis of an insignificant degree of DNA homology.

References.


1. Fell, J. W., A. Statzell, I. L. Hunter and H. J. Phaff. 1969. Leucosporidium gen. n., the heterobasidiomycetous stage of several yeasts of the genus Candida. Antonie van Leeuwenhoek 35:433-462.
2. Christiansen, C., A. L. Bak, A. Stenderup and G. Christiansen. 1971. Repetitive DNA in yeasts. Nature New Biol. 231:176-177.
3. Bak, A. L. and A. Stenderup. 1969. Deoxyribonucleic acid homology in yeasts. Genetic relatedness within the genus Candida. J. Gen. Microbiol. 59:21-30.
4. Meyer, S. A. and H. J. Phaff. 1971. DNA base composition and DNA-DNA homology studies as tools in yeast systematics. Yeasts as Models in Science and Technics. First Specialized International Symposium on Yeasts. Smolenice, Czechoslovakia. June 1-4. Slovak Academy of Science.


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The following article has recently been published: Evolution of DNA Base Compositions in Microorganisms. A. L. Bak, J. F. Atkins and S. A. Meyer. Science 175:1391-1393, 1972.

In January I assumed my position at the American Type Culture Collection. I am here to develop the yeast collection and to continue research on the systematics of yeasts, particularly in the area of DNA base composition and homology, which I began under the direction of H. J. Phaff and continued in the laboratory of A. Stenderup, Institute of Medical Microbiology, University of Aarhus, Denmark. My research here at ATCC is supported by a grant from the National Institutes of Health.

Up to now, my time has been occupied totally with collection work: accession of new strains and characterization of the existing cultures. The facilities for preservation at the ATCC are excellent. Cultures are both lyophilized and stored in liquid nitrogen. Yeast workers who wish to preserve particularly important yeast strains are encouraged to take advantage of these conditions and deposit their cultures for preservation at the ATCC. I would be pleased to include in the collection yeasts that are of significant or general interest to the yeast community; in particular, type cultures; medically, industrially and ecologically important strains; assay organisms and

pertinent research strains should be deposited.

On May 5, Mrs. Marilyn Hale retired from the ATCC. In her last few years at the Collection she had been maintaining the yeast collection. Her work, interest and devotion to the yeasts are deeply appreciated and greatly missed.

V. Department of Biology, McMaster University, Hamilton, Canada. Communicated by J. J. Miller.

1. Summary of a manuscript by N. S. Grewal and J. J. Miller (based on M.Sc. thesis of N. S. Grewal) submitted to the Canadian Journal of Microbiology. "Non-reductional nuclear divisions in asci of two-spored yeasts".

Giemsa stains were made of sporulating cells of 15 yeast strains which had been selected from a group of 150 sporogenic yeasts because they produced predominantly 2-spored asci. The asci of 12 of the strains contained 4 nuclei of which usually only two became enclosed in spores. In the remaining 3 strains there were never more than 2 nuclei per ascus and the progeny of the spores were capable of sporulation without conjugation. DNA determinations gave evidence that both vegetative cells and spores were diploid. It was concluded that nuclear division in the asci of these yeasts is not reductional.

Subcultures will be supplied to laboratories wishing to study these unusual strains of S. cerevisiae. Also available are two strains with 3-4 spores per ascus in which the spores in most of the asci are seriate.

2. Ph.D. thesis completed by M. Banerjee in October, 1971. "The role of carbohydrate in the germination of yeast ascospores".

The carbohydrate content of a strain of Saccharomyces chevalieri was determined at different stages of the life cycle. The structural carbohydrates (mannan and glucan) in vegetative and sporulated cells were not significantly different in amount. The reserve carbohydrates (trehalose and glycogen) increased during sporulation and decreased during spore germination. These changes were particularly marked with trehalose. Dry weight increased during sporulation but showed no change during the first six hours in germination medium.

In spite of their high content of reserve carbohydrate, the spores required an exogenous source of carbon for germination induction and, in fact, the reserves were not significantly utilized in the absence of exogenous carbon. The carbon source had to be present in the medium continuously and at substrate level for completion of germination, which took approximately six hours. Glucose, fructose, mannose and sucrose supported germination but nine other carbon compounds (galactose, maltose, lactose, cellobiose, D-arabinose, L-arabinose, trehalose, 2-deoxy-D-glucose, acetate) did not do so. Respiration during germination was mainly fermentative, and experiments with inhibitors indicated carbon metabolism via the anaerobic pathway to be essential for germination. Exposure to high or low temperatures did not induce germination. It is suggested that the carbohydrate reserves of the spore function mainly to maintain endogenous respiration in the resting stage or to improve resistance to dessication.

3. Summary of a paper by P. V. Patel and J. J. Miller. "Stimulation of yeast sporulation by glycerol". To appear soon in Journal of

Applied Bacteriology.

Sporulation of S. cerevisiae was stimulated by glycerol especially when the cells were precultured in a complex growth medium instead of a chemically-defined medium. Highest spore yields were obtained in 1-4% glycerol. Sporulation in glycerol was much less sensitive to ammonium sulphate inhibition than it was in acetate. Growth occurred in a defined medium with glycerol as sole carbon source and glutamic acid as sole nitrogen source, but not with ammonium sulphate as sole nitrogen source. In these respects glycerol resembles dihydroxyacetone as a sporulation inducer. Stimulation of sporulation by the latter compound has been reported on earlier (Can. J. Microbiol. 3:81, 1957; and 9:259, 1963).

VI. Ecole Nationale Supérieure Agronomique de Montpellier - Laboratoire de Recherches de la Chaire de Génétique - Montpellier, France. Communicated by P. Galzy.

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

ARNAUD A., VEZINHET F. et GALZY P. Contribution à l'étude du métabolisme respiratoire de Saccharomyces cerevisiae HANSEN au cours de la sporulation. Rev. Ferment. Ind. Aliment. 1971, 26, 113-136.

Saccharomyces cerevisiae sporulates when there is no growth, when oxygen is present, when the pH is equal or greater than 7, when a convenient carbon source is present (acetic acid).

Cells during exponential growth give a higher percentage of sporulation than cells during late log phase of growth. During induction of sporulation, reserve oxidation rate increases. Carbohydrate and lipid reserves accumulate. The acetate metabolism of sporulating cells is different from acetate metabolism of growing cells.

GALZY P., VEZINHET F., ARNAUD A. et CHASSANG-DOUILLET A. Metabolic modifications during sporulation in Saccharomyces cerevisiae HANSEN. 4th International Fermentation Symposium - Kyoto 19/25 - 3 - 1972.

This paper deals with a study of the metabolism of acetic acid during sporulation.

The respiratory metabolism was investigated. Acetate oxidation is necessary during meiosis; nevertheless a drop of acetate $Q_{O2}$ is observed during spore formation.

Sporulation is promoted by a high rate of endogenous oxidation. An increase in total lipids is observed during sporulation, with a particular increase in sterol content.

JEANBART F., BIZEAU C. et GALZY P. Influence de la mutation des gènes de la série PL sur le volume cellulaire de Saccharomyces cerevisiae HANSEN. C. R. Soc. Biol. (in press).

LADET J., GALZY P., JOUX J. L., BIJU-DUVAL. Comparaison des rendements de croissance sur lactose de quelques Kluyveromyces. Le Lait (in press).

ARNAUD A., VEZINHET F. et GALZY P. Remarques sur le métabolisme glucidique au cours de la sporulation. Arch. Mikrobiol. (in press).

The action of pH on respiratory metabolism, evolution of carbohydrate and sporulation was studied.

Experiments were done with cells harvested during the early log phase or during the late log phase.

Carbohydrate reserves are synthesized from acetate only when there is sporulation. During sporulation, the most important carbohydrate fraction is trehalose. At the end of sporulation, glycogen is very abundant in asci. Physiological state, pH and sporulation relations, are discussed.

SAQUET H., ARNAUD A., GALZY P. et COLSON-GUASTALLA H. Inhibition par des composés à deux carbones de la fumarase et de l'isocitritase chez Saccharomyces cerevisiae HANSEN. FEBS Letters (in press).

Km and Vm values for fumarase and isocitritase were determined. The inhibitory effect of glycolic acid, acetic acid and ethanol, on these two enzyme activities was studied; determination of inhibition; determination of type of inhibition and values of the inhibition constants Ki.

VII. Microbiological Unit, Department of Applied Biochemistry and Nutrition, University of Nottingham, LE12 5 RD, England. Communicated by R. V. Chudyk.

## Report on Yeast Research at the University of Nottingham

R. V. Chudyk*, A. Seaman and M. Woodbine

Over the past four years effects of stresses on yeast ascospores and vegetative cells have been studied. The stresses investigated include low temperatures, high temperatures and microwave irradiation. In general yeast spores were more resistant than vegetative cells. Much more interesting, however, is that yeast spores proved to be versatile test cells for stress studies since they could be subjected to various post-treatment analyses. This versatility provided an insight into the nature of the injury resulting from the stress in question.

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We present the summary of a Ph.D. thesis on low temperatures effects:

Chudyk, R. V. (1971) Low Temperature Studies on Yeast Ascospores. Ph.D. Thesis. University of Nottingham, England.

The literature is reviewed concerning post-sporulation events in yeast ascospores. The review points out the problems in studying heterogeneous suspensions of sporulated yeast cells.

An approach is developed for studying the effects of stress on yeast spores, the stress in question being low temperature. Yeast spores proved versatile for cryobiological investigations since they could be subjected to several post-treatment analyses such as viability, dye exclusion and cell leakage.

As a result of low temperature (0 to -78°) treatments, cells which showed manifestations of injury (e.g. leakage, internal structural changes, and loss of dye exclusion ability) were dead, but all dead cells did not necessarily show manifestations of injury. This indicated the complexity of factors involved in low temperature mortality and suggested that over a graded series of treatments cells may die or be injured for different reasons.

Results indicated that direct detrimental effects of sub-zero temperature occur during warming. Most of the results favor the idea that detrimental effects are due to chemical changes and not physical cell disruption. The most significant finding in this work was that lethal effects during warming occurred during a two to three degree

interval between -35 and -25°.

Rapidly cooled sporulated and vegetative cells were similarly susceptible to slow warming, but in the case of rapid warming sporulated cells were more resistant and this resistance was probably due to the ascospore per se as well as the multi-target nature of the two-spored ascus.

A suggestion is put forward that lethal narrow temperature intervals during slow warming be investigated as a possible sterilizing or sanitizing agent for frozen food products.

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*Last year Dr. R. V. Chudyk left the University of Nottingham and became a research scientist, with the Horticultural Research Institute of Ontario at Vineland Station, Ontario involved with a number of projects including wine microbiology, freezing and freezedrying of commercial wine yeasts, vineyard yeast ecology and microbiology of frozen horticultural products. In a future newsletter some yeast research projects will be outlined.

Publication:

Chudyk, R. V., P. K. Knight, Jill Rudland and M. Woodbine (1969-70). Effects of high and low temperatures on yeast ascospores. Rep. Sch. Agric. Univ. Nott. 1969-70: 111-114.

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

1. Our work in the area of DNA base composition and homology is continuing. Dr. Leda C. Mendonça is doing a systematic study of the psychrophobic enteric yeasts. Dr. Stephen Douglass is working on improved techniques for DNA extraction from yeast, estimation and separation of the mitochondrial and the nuclear DNA, determination of repetitive sequences in nuclear yeast DNA, and better labeling techniques of DNA. We are also comparing the DNAs of a number of isolates which key to certain species in the 2nd edition of The Yeasts (J. Lodder editor, 1970), but which differ in a number of biochemical properties from described species. It is becoming increasingly clear that species differing in one or two physiological traits may have nearly identical base sequences in their DNAs.

2. The following is an abstract of a paper which will appear in the August issue of the Journal of Bacteriology.

Deoxyribonucleic Acid Base Composition of Species in the Yeast Genus Kluyveromyces van der Walt emend. van der Walt.
Alessandro Martini, Herman J. Phaff and Stephen A. Douglass.

The deoxyribonucleic acid base composition (% guanine + cytosine) was determined for 29 strains, representing 18 species of the genus Kluyveromyces. It was concluded that on the basis of guanosine + cytosine (GC) content (47.4%) and other properties K. veronae occupies an uncertain position in the genus Kluyveromyces. The GC content of the remaining 17 species ranged from 35.3-43.4% and three groups of species were recognized. The %GC of the first ranged from 35.3-38.0; that of the second group from 39.5-41.7; that of the third group from 42.4-43.4. Several species revealed a nearly identical GC content. The GC contents do not correspond in all instances with the five groups of species

proposed by van der Walt.

3. H. J. Phaff presented a special lecture on yeast during the 4th International Fermentation Symposium, Kyoto, Japan, March 19-25, entitled "A comparative study of the yeast florae associated with trees on the Japanese Islands and on the West Coast of North America" (authors are H. J. Phaff, M. W. Miller, M. Yoneyama, M. Soneda). The full text will appear in the Proceedings of the Symposium.

4. The following paper will appear in the next issue of the Canadian Journal of Microbiology.

Dimorphism in a New Isolate of Saccharomycopsis Schiönning. Edward J. Buecher and Herman J. Phaff.

Two strains of Saccharomycopsis guttulata Robin (Schiönning) were isolated from two out of four specimens of Lepus californicus, the wild jack rabbit of California. One isolate was similar to typical strains with budding cells occurring in domestic rabbits. The second strain was atypical in being dimorphic. Colonies of the yeast form, consisting of budding cells, were smooth and glistening, while those of the filamentous form were irregular, rough, and dull. The filamentous form consisted of septate, branched hyphae, radiating from a central origin of growth. New filaments generally were initiated by fragmentation. The two new strains were similar to domestic strains in assimilation and fermentation reactions and grew only between 30 and 40C. Both strains formed ascospores at 18C. The filamentous strain, unlike other S'opsis guttulata strains, was not carbon dioxide dependent at 37C but did require oxygen. At 30C it became carbon dioxide dependent. During the stationary phase, a morphogenetic change occurred from the filamentous form to a yeast form, which was stimulated by anaerobic conditions. The budding form of the filamentous strain was relatively stable in culture and at either 30 or 37C it was carbon dioxide dependent. Spontaneous reversions from the budding to the filamentous form appear to occur sporadically.

5. H. J. Phaff will present a course dealing with "Selected Topics in Yeast Biology" from July 24 to August 5 at the Departamento de Microbiologia, Escuela Nacional de Ciencias Biologicas in Mexico City. The course is sponsored by the Latin American Visiting Professor Program of the American Academy of Microbiology.

In October he will give a paper "Enzymes involved in the lysis of cell walls of Schizosaccharomyces" during the Third International Symposium on Yeast Protoplasts, 2-5 October, 1972. Salamanca, Spain.

6. Professor V. D. Kostov from the Bulgarian Academy of Sciences, Sofia, is spending three months in our laboratory to study various techniques of yeast research. The visit is sponsored by the Scientific Exchange Program between the National Academy of Sciences of the U. S. A. and the Bulgarian Academy of Sciences.

In the latter part of July Dr. N. P. Elinov, Deputy Director of the Chemical-Pharmaceutical Institute, Leningrad, USSR, will visit the Davis Campus of the University for about one week. Dr. Elinov is a Fellow of the World Health Organization.

IX. Department of Serology and Bacteriology of the University of Helsinki, and from the Department of Pulmonary Diseases of the Helsinki University Central Hospital, Helsinki, Finland. Communicated by A. Kahanpää.

Below follows an abstract of the dissertation "Bronchopulmonary Occurrence of Fungi in Adults Especially According to Cultivation Material". The dissertation (147 pp) is published as Supplement 227 to Section B of Acta Pathologica et Microbiologica Scandinavica, 1972.

This investigation was undertaken for the purpose of studying by means of fungal cultures the bronchopulmonary occurrence of fungi in the adult population in Finland, and particularly in patients with pulmonary disease. The material studied consisted of a total of 8 290 specimens from which fungal cultures were made in the Department of Serology and Bacteriology of the University of Helsinki in 1963-1970. Of these specimens 5 280 were from the bronchopulmonary area. Additionally there are presented certain previously unpublished results of fungal cultures. Parallel with the above, about 2 500 bronchopulmonary specimens were examined by direct microscopy.

A review is first presented of the literature on isolation of fungi from bronchopulmonary specimens and on these fungi as cause of mycotic diseases. Major attention was paid to the most common bronchopulmonary mycoses in Scandinavia and Finland, particularly to candidosis and aspergillosis.

In the basic material, 436 bronchial secretion specimens from which fungal cultures were made yielded one species of fungus in 24.8 per cent and more than one species in 1.5 per cent. These incidences for 3 729 sputum specimens were 60.0 per cent and 12.0 per cent, respectively. The incidence of yeast growth from the bronchial secretion specimens was about 20 per cent and from the sputum about 70 per cent, and filamentous fungi grew from about 7 per cent and 8 per cent, respectively. A total of 4 539 yeast strains, belonging to 41 identified species, were isolated from the respiratory tract specimens.

The fungus most frequently isolated from the bronchopulmonary specimens was Candida albicans, as was to be expected. Its incidence in morning sputum varied between about 60 and 70 per cent in the different groups studied. In cultures of bronchial secretion the incidence was about 17 per cent, and in those of lung tissue about 20 per cent. In the control group Candida albicans was isolated from the sputum in 53-55 per cent.

The fungus ranking second in frequency was also a yeast, Torulopsis glabrata, which was statistically significantly more often present in the sputa of 100 hospital patients with pulmonary disease (in 14-15 per cent of sputum specimens) than in the control group (4 per cent). Growth of this yeast was also more abundant from the sputum of the patients. Additionally, its incidence was statistically highly significantly greater in patients over 50 years of age (21.0 per cent) than in younger patients (4.8 per cent). In the basic material Torulopsis glabrata grew from 5.3 per cent of 3 729 sputum specimens (which corresponds to 6.9 per cent of all the yeast strains isolated and is about 20-fold the incidence in a similar Finnish series studied some 10 years earlier), from 0.9 per cent of 436 bronchial secretion specimens, and from 2.2 per cent of 93 patients' lung tissue specimens. This yeast species was also present in 10 per cent of 646 gastric juice specimens from out-patients.

The most commonly encountered filamentous fungus, Aspergillus fumigatus, ranked next, with an incidence of 2-3 per cent in sputa and bronchial secretions of pulmonary disease patients. Geotrichum candidum was isolated from 2.2 per cent of sputum specimens and only 0.2 per cent of bronchial secretion specimens in the basic material, but in different groups of the material it grew from 3.7-20.2 per cent of faeces and 2.5 per cent of gastric juice specimens.

The differences between males and females and between the groups of non-mycotic diseases with respect to occurrence of bronchopulmonary fungi were small and those between certain parts of the material even conflicting, and with a few exceptions none were statistically significant. Women with bronchial asthma showed more growth of fungi and Candida albicans was more frequently encountered in them than in men. In general, differences in the incidences of occurrence of fungi are seen rather between age groups, growth of at least certain species of fungi apparently being more general in elderly persons.

A detailed model for performance of fungal cultures from bronchopulmonary specimens is presented. The addition of gentamycin to certain fungal culture media used gave results that must be regarded as favorable.

Bronchopulmonary mycotic diseases are not infrequent and, especially in the form of opportunistic infections, are showing an increase in incidence as well as in severity with the longer lifespan of debilitated patients. Also in the present study, bronchopulmonary mycosis was diagnosed in four of the 100 almost randomly selected patients with pulmonary disease who were submitted to a more thorough fungal culture examination.

Deep visceral mycoses should be given more consideration than generally has been the case so far. Fungal cultures of good specimens are, in the author's opinion, an important tool in the diagnosis of bronchopulmonary mycotic diseases. Correct interpretation of the cultures is indispensable and generally calls for close cooperation between the clinic and the fungus laboratory. Sputum specimens can also contribute to the information obtainable, although a large part of the species growing from them are contaminants from the upper respiratory tract. Sputum cultures in particular should be repeated a number of times to bring out the predominant fungal flora, and the quantity of growth of the different species present should also be reliably clarified. Simultaneous culture of the fungal flora in, e.g., the oral cavity must not be omitted. As a source of supplementary information, direct microscopic examination of unstained and stained specimens is to be recommended. The results of fungal culture of bronchopulmonary specimens in the present material were positive for fungi about twice as often as the results of direct examinations.

In addition to fungal cultures and clinical observations, the diagnosis of mycotic disease requires possibilities for, e.g., histopathological and serological examinations of fungi, both of which are gaining importance in this connection. All these steps call for more laboratories with adequate facilities for reliable fungal examination.

(811 full references are listed.)

X. University of Illinois at the Medical Center, Department of Microbiology, Chicago, Illinois 60680. Communicated by A. Widra.

A Combination Rapid and Standard Method for Candida albicans Identification. Grace Schaar, Isabel Long, and Abe Widra.

MATERIALS:

Media: Zein-Lactose- Tween 80 Agar.
1% Tween overlay solution.

PREPARATION OF MEDIA:

Weigh 40 grams of zein powder into a liter of distilled water. Prepare the zein extract by allowing the zein mixture to stir with a magnetic stirrer overnight in the refrigerator. Then steep the zein mixture for one hour on a boiling bath and filter the insoluble protein from the extract by the use of filter paper. A clear transparent extract of zein should result.

Add 20 grams of lactose, 15 grams agar and 10 cc of Tween-80. Bring the resulting mixture of extract to a liter with distilled water and autoclave for 15 minutes at 15 lbs. pressure. Pour into petri plates. The resulting media should be a clear transparent material. The media should be stored in the refrigerator until used.

METHOD OF INOCULATION OF YEAST SPECIMEN

Divide petri plate into 5 or 6 divisions with a marking pencil on the under side. Make a one stab inoculum from a needle touched lightly to an isolated colony from the patient's Blood or Sabouraud primary isolation, onto the area marked off on the test media. Drop a very small drop of Tween (1% Tween 80) over the inoculum with a 25-30 gauge needle. Cover with coverslip and incubate for 2-3 hr. at 37°C. Place the petri plate on the microscopic stage and examine the edge of the coverslip for filament "tubes". At this time report a preliminary result. Place the petri dish overnight at 25°C and read the final result of terminal chlamydospores which is diagnostic for C. albicans. (The complete paper will be published later this year.)

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

Most of the research on psychrophobic (thermophilic) enteric yeasts has been recently reviewed (Thermophilic Enteric Yeasts, by L. R. Travassos and A. Cury, Annual Review of Micro., 25: 49-74, 1971).

Papers in press:

Metabolism of ethionine in ethionine-sensitive and enthionine-resistant cells of the enteric yeast Candida slooffii.
Leda C. S. Mendonça and Luiz R. Travassos.
SUMMARY (paper will appear in the next issue of the Journal of Bacteriology)

In a defined medium with added ethionine + low methionine, phenylalanine, tryptophan, tyrosine, adenine and additional methionine, reversed inhibition of the enteric yeast Candida slooffii by ethionine. Isoleucine and 7-methylguanine restored half-maximal growth. Choline but not triethylcholine inhibited C. slooffii. 6-Mercaptopurine reversed ethionine inhibition and also synergistic inhibition by ethionine + choline. Protection against ethionine by adenine + aromatics was also evident with log-phase cells in the absence of methionine. Incorporation of ethionine-ethyl-1-$^{14}$C by resting cells was partially inhibited by aromatic amino acids and methionine. Ethionine depressed incorporation of phenylalanine-$^{3}$H but not of adenine-$^{3}$H. Ethionine-resistant mutants were isolated which incorporated ethionine efficiently and degraded it to yet unidentified substances not including 5'-ethylthioadenosine. Ethionine-sensitive cells accumu-

lated more S-adenosylethionine (SAE) than resistant mutants. Adenine was a good precursor of SAE. Radioactivity from ethionine-ethyl-1-$^{14}C$ was recovered from cell fractions of ethionine-sensitive cells with the following distribution: cold-TCA-soluble > hot-TCA-insoluble > lipids > DNA > RNA. Total radioactivity recovered from ethionine-sensitive cells was twice as much as that from ethionine-resistant mutants.

Comparison of the surface structures of choline-less Torulopsis pintolopesii grown on defined media with choline or methionine.
Jayme Angluster and Luiz R. Travassos.
SUMMARY (paper will appear in the next issue of the Archiv für Mikrobiologie)

The chemical composition of cell walls from choline-less Torulopsis pintolopesii grown with choline or with methionine was studied. Methionine-grown cells synthesized a weakened cell wall compared to normal choline-grown yeast. The ethylenediamine fractionation procedure yielded three fractions - A, B, and C - with different solubilities. Glucose and mannose were detected in hydrolysed unfractionated cell walls from yeasts grown under both conditions as well as in all fractions. Glucose content was greater in fractions B and C from methionine-grown cells; the mannose content was about the same. Walls from choline-grown cells ($W_c$) had 25% more protein than walls from methionine-grown cells ($W_m$). The amino acid composition of the proteins of $W_c$ and $W_m$ was not qualitatively altered. Seventeen amino acids were identified; glutamic and aspartic acids and valine predominated. $W_c$ had 3.5 times more lipid than $W_m$. The amount of phosphorus was the same. Yeasts grown on methionine synthesized more ergosterol than choline-grown cells. The rate of formation of spheroplasts was higher in methionine-grown cells. Rates of incorporation of adenine, glutamic acid, and uracil were similar in cells grown on methionine or choline; incorporation of phenylalanine and tyrosine was depressed in methionine-grown cells.

Vitamin requirements and induced nutritional imbalances as criteria in speciating psychrophobic yeasts.
Luiz R. Travassos and Lucia Mendonça.
SUMMARY (paper will appear in the next issue of the Antonie van Leeuwenhoek, J. Microbiol. Serol.)

Vitamin requirements, determined by two different methods, and other nutritional characteristics of 45 psychrophobic and 30 non-psychrophobic yeasts were investigated. Vitamin requirements and induced nutritional imbalances emerged as criteria for defining Candida slooffii. Strains of this species behaved uniformly. Variations in vitamin requirements of strains of Saccharomyces telluris, Torulopsis bovina and Torulopsis pintolopesii were confirmed. The choline requirement exhibited by several strains (by as many as 86% in S. telluris) was evident on a simple defined medium without methionine, but not on Wickerham's vitamin-free basal medium. Choline-requiring strains comprised strains responding similarly to choline or methionine, and some strains growing with choline but not with methionine on short incubation. Other frequently required vitamins were biotin, pantothenate, thiamine, nicotinic acid, pyridoxine, and inositol.

Recent publications:

Cruz, F. S. and Travassos, L. R. 1970 Physiology of a choline-requiring strain of Torulopsis pintolopesii. Archiv. für Mikrobiologie 73: 111-120.

Lima, M. E., Angluster, J. and Travassos, L. R. 1970 Ergosterol biosynthesis in psychrophobic yeasts. Revista de Microbiologia (São Paulo) 1: 61-69.

Current projects:

Development of a microbiological method for assaying carnitine by use of a spontaneous carnitine-requiring mutant of Torulopsis bovina.

Further studies on ethionine-sensitive and ethionine-resistant strains of Candida slooffii and on the action of ethionine as influenced by temperature.

Further identification of the product arising from the metabolism of choline in choline-less Torulopsis pintolopesii.

New address:

Luiz R. Travassos is presently at the Department of Dermatology, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, New York 10032.

Leda C. S. Mendonça is presently at the Department of Food Science and Technology, University of California, Davis, California 95616 in Dr. Phaff's laboratory.

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

Erkki Oura. The growth energetics of baker's yeast. Paper presented at 14th Nordiska Kemistmötet, Umeå 1971. Summary on p. 106.

Heikki Suomalainen. The α-glucosidase content and leavening of baker's yeast. Paper presented at 14th Nordiska Kemistmötet, Umeå 1971. Summary on p. 107. A preciser report is being published in Process Biochem., abstracted below.

α-GLUCOSIDASE AND LEAVENING OF BAKER'S YEAST
H. Suomalainen, J. Dettwiler, and E. Sinda
Process Biochem. (in press)

In tests with different brands of baker's yeast, no correlation has been found between the α-glucosidase activity (with p-nitrophenyl-α-D-glucoside (PNPG) as substrate) and either the leavening power or the ability to ferment maltose. The leavening power was not influenced by addition of either invertase or β-amylase, but glucoamylase clearly increases the evolution of carbon dioxide and thus markedly improved the results of proofing.

OCCURRENCE OF LONG-CHAIN FATTY ACIDS AND GLYCOLIPIDS IN THE CELL ENVELOPE FRACTIONS OF BAKER'S YEAST
T. Nurminen and H. Suomalainen
Biochem. J. 125 (1971), 963-969.

The total yield of fatty acids from the whole envelopes was markedly higher than that obtained from the ordinary cell walls. In both samples the major fatty acids were $C_{16}$ and $C_{18}$ acids. The whole envelopes contained $C_{18}$ acids and long-chain ($C_{19}$-$C_{26}$) fatty acids, in a higher proportion than did the ordinary cell walls. Fifteen fatty acids with more than 18 carbon atoms were identified, among which 2-hydroxy-$C_{26:0}$ and $C_{26:0}$ acids predominated. A complex sphingolipid containing inositol, phosphorus and mannose was isolated from the whole cell envelopes. The main fatty acids of this lipid were 2-hydroxy-$C_{26:0}$ and $C_{26:0}$ acids. It was concluded that this sphingolipid is present both in the ordinary cell wall and the plasma membrane of baker's yeast. The neutral lipids amounted to over 50% and the glycerophosphatides to about 30% of the total fatty acid content of the whole envelope. The

major fatty acids in these lipids were $C_{16:1}$, $C_{18:1}$ and $C_{16:0}$ acids. The proportion of fatty acids with more than 18 carbon atoms was lowest in the neutral lipids, whereas the neutral glycolipids contained the highest percentage of these fatty acids. Acidic glycolipids amounted to 14% of the total fatty acid content of the whole envelope. The presence of a cerebroside sulfate in this lipid fraction was demonstrated, whereas the high content of 2-hydroxy-$C_{26:0}$ acid found is caused by the complex inositol- and mannose-containing sphingolipid.

THE ORIGIN OF n-PENTANOL, n-HEXANOL, AND n-HEPTANOL IN THE FUSEL OIL OF FERMENTED SULFITE WASTE LIQUOR

H. Suomalainen, P. Ronkainen, and S. Brummer
Amer. J. Enol. Viticult. 22 (1971), 118-120.

The presence of n-hexanol and n-heptanol in sulfite fusel oils has been difficult to explain as the corresponding keto acid precursors of the fusel alcohols have not been identified in fermentation solutions. On analysis of the sulfite waste liquor utilized as raw material in sulfite spirit production, it has been found that the higher straight-chain aldehydes, pentanal, hexanal, heptanal, and octanal, appear in the sulfite waste liquor in concentrations that are sufficiently large to explain the formation of the corresponding alcohols.

Heikki Suomalainen and Timo Nurminen, Isolation and properties of the plasma membrane of the yeast cell. Paper presented at the 4th International Fermentation Symposium, March 19-25, 1972, Kyoto, Japan.

After enzymic removal of most of the carbohydrates from isolated cell envelopes of baker's yeast, the plasma membrane fraction can be isolated by differential centrifugation. It contains mainly protein and lipids, although an appreciable amount of carbohydrate is still present. In the plasma membrane preparation, the prominent enzyme is a $Mg^{2+}$-dependent ATPase active at neutral pH. Also present are small amounts of saccharase, various acid phosphatase activities, and most of the cell envelope lipase and phospholipase. The cell envelopes lack short-chain fatty acids and instead contain principally $C_{16:1}$ and $C_{18:1}$ acids, of which $C_{18:1}$ acid predominates in the plasma membrane. The major very long-chain fatty acids found were 2-hydroxy-$C_{26:0}$ and $C_{26:0}$ acids, which also were the major fatty acids of a complex sphingolipid isolated. This also contained long-chain bases, inositol, phosphorus and mannose, and is present in both the true cell wall and the plasma membrane. There are three groups of neutral glycolipids. The acidic glycolipids contain a sulphatide and the complex sphingolipid mentioned above. The main glycerophosphatides present in the plasma membrane are phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol. Neutral lipids, principally triglycerides and sterols, are a large part of the plasma membrane lipids. The distribution of various marker enzymes, proteins, nucleic acids and some lipids after zonal zentrifugation in continuous density gradients of sucrose and Ficoll has been studied. The fragments of plasma membrane are mainly found in the median density range 1.10, slightly contaminated with cytoplasmic material. Thus, zonal centrifugation is a promising possibility for the larger scale isolation of plasma membranes from yeast.

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

E. Oura and H. Suomalainen. Das Verhalten der Bäckerhefe bei verschiedenen Belüftungsstärken in Laboratoriumsversuchen. Spiritusindustrie-Konferenz, Szeszipar 19 (1971), 5-8.

Heikki Suomalainen and Erkki Oura. Yeast nutrition and solute uptake. In The Yeasts, ed. by A. H. Rose and J. S. Harrison, vol. 2, Academic Press, London-New York 1971, pp. 3-74.

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

1. Recent publications:

A. Einsele and A. Fiechter: Liquid and Solid Hydrocarbons. In "Advances in Biochemical Engineering", Vol. I, Ed. T. K. Ghose and A. Fiechter, p. 169-194. Springer Verlag, Berlin, 1971.

M. Küenzi: Ueber den Reservekohlenhydratstoffwechsel von Saccharomyces cerevisiae. Thesis. Juris Druck + Verlag, Zürich, 1970. Summary I:

On the reserve carbohydrate metabolism of Saccharomyces cerevisiae

The carbohydrate composition of Saccharomyces cerevisiae cultivated aerobically in the chemostat at different growth rates was investigated and compared with the specific activities of enzymes of the reserve carbohydrate pathways. Under glucose limitation the synthesis of reserve carbohydrates increased strongly with increasing limitation of substrate supply (equal to decreasing growth rate). In contrast to the general opinion, an excess of energy and carbon source and a simultaneous limitation of the growth through other factors (for example nitrogen) is not a prerequisite for the synthesis at these reserves. Fermenting cells accumulate only glycogen, whereas more slowly growing organisms with a purely respirative metabolism contain trehalose in addition to high amounts of glycogen. During the budding process the respiratively growing cells degrade part of their reserves. In the presence of excess substrate, the reserve pool is low and remains unchanged during the cell cycle. The percentage of structural carbohydrates in the organisms shows only small changes over the budding cycle and is scarcely influenced by the growth rate. At a growth rate of 0.077 $h^{-1}$ the cells cultivated under glucose limitation have an even higher reserve carbohydrate content than those grown under nitrogen-limitation. The course of the specific activities of UDPG pyrophosphorylase and glycogen phosphorylase as a function of the growth rate follows that of the reserve carbohydrates. In the course of cultivations it was observed regularly, that in slowly growing cultures with a purely respiratory metabolism a gradual differentiation in two cell types took place, which was accompanied by changes in the carbohydrate composition. The two cell types are different from the fermentative organism and can be characterized as follows:

h-type (h = hell [german] i.e. light) appears light in the phase contrast microscope (the largest cells have a dark center), without large vacuoles, shape: round to oval, size: small to very large, length of the generation time. Populations with a high percentage of h-cells can grow synchronously in the chemostat (the older and larger cells determine the period of the synchronous oscillations).

The h-type arise at the beginning of a continuous cultivation in the respiratory range of the dilution rate.

d-type (d = dunkel [german] i.e. dark) appears dark in the phase contrast microscope, contains one to several large vacuoles; shape: irregular, round to elongate; size: small, length of the budding period increases with increasing generation time. This cell type partially displaces the h-cells during long-term cultivation. Simultaneously, the actual glucose concentration in the medium falls slightly.

The differences between the two types are clearest at growth rates of 0.1 $h^{-1}$. From both types fermenting cells arise when the substrate supply is increased above the critical value. Differentiation was observed in three different yeast strains tested, in fully synthetic and complex media. It is assumed that the development of d-cells is related to the formation of pseudomycelia.

The two cell types differ in their carbohydrate composition. At equal growth rates the d-cells contain markedly less trehalose and more mannan than the h-cells.

2. In press:

A. Einsele and A. Fiechter: Growth Behaviour of Candida tropicalis in Batch Fermentation Processes on n-Hexadecane as Substrate. Path. Microbiol.

A. Einsele, H. P. Knöpfel and A. Fiechter: Respiratory Activity of Candida tropicalis during Growth on Hexadecane and on Glucose. Arch. Mikrobiol.

A. Fiechter, F. A. Mian, H. Ris and H. O. Halvorson: Characterization of Insoluble Protein Fractions of Mitochondria from Saccharomyces cerevisiae. J. Bact.

J. R. Mor: Ueber den glucosesparenden Effekt von Glutaminsäure bei Saccharomyces cerevisiae. Juris Druck + Verlag, Zürich.

Summary II:

Effect of glutamic acid on glucose metabolism in Saccharomyces cerevisiae

An addition of glutamic acid (0.075%) to a synthetic growth medium results in an increased oxidative turnover rate in yeast cell populations. An analogous effect results from the addition of aspartic acid or alanine. The turnover of the tricarboxylic acid cycle is improved by eliminating a kinetic bottle neck (enhancement of the oxalacetic acid concentration). Glutamic acid will be assimilated by growing yeast cells in the presence of an exogenous carbon source (glucose). Part of the amino acid is directly assimilated, partially by transamination reaction and partially by oxidative deamination mediated through the corresponding dehydrogenases. The relative proportion of glutamic acid which will be transformed by the glutamate-dehydrogenase reactions increases with higher amino acid concentrations in the medium. The $NAD^+$-specific glutamate-dehydrogenase is regulated by the $NH_4^+$-concentration and shows no glucose repression.

Regardless if glutamate was present or not in the medium the intracellular concentrations of α-oxoglutarate and glutamate remained constant. However, enzymes related to the glutamic acid metabolism showed increased activities. The carbon skeleton of glutamic acid (as α-oxoglutarate) effects an additional feeding of the citric cycle. A direct demonstration, i.e. the substitu-

tion of glutamate by α-oxoglutarate was not possible because the anion as all the Krebs cycle intermediates are not taken up by growing cells of Saccharomyces cerevisiae. This replenishment of the TCA cycle implies a glucose conservation for synthetic reactions by relieving anaplerotic pathways. The Crabtree effect is thereby reduced.
Considering the distribution of substrate-carbon between oxidation for energy production and utilization as cell-carbon, it could be shown that in the presence of glutamic acid a greater proportion of substrate will be utilized for synthetic reactions of the cell.

XIV. Chemical-pharmaceutical Institute of Leningrad. 14 Prof. Popov-street. 197022, Leningrad, USSR. Communicated by N. P. Elinov.

1. The structural polysaccharides of cell walls from red and yellow yeasts (N. P. Elinov, G. A. Vitovskaya, V. G. Kaloshin). The composition of structural (insoluble) polysaccharides of cell walls from some red and yellow species of yeasts were investigated. The structural polysaccharides from Rhodotorula glutinis strain 309, R. rubra VKM y 341, R. pilimanae 3-015, R. lactosa 3-018 contained mainly mannose (60-70% in terms of total quantity of sugars). Glucan was the insoluble carbohydrate component of cell walls from Cryptococcus laurentii IFO 0609, Cr. flavus VKM y 331 and others. The structural polysaccharides of Cr. macerans VKM y 1263 and Cr. infirmo-miniatus VKM y 1265 were also glucans.
Thus not only the ability to utilize i-inositol as a sole source of carbon by Cryptococcus but not by Rhodotorula constitutes another sound basis for their differentiation (Phaff, Spencer, 1969). The composition of the structural polysaccharides of cell wall is also an essential criterion for differentiating these organisms.

2. Chemical composition of preparations from Candida albicans cells (N. P. Elinov, V. G. Kaloshin). The chemical composition of preparations successively obtained by different methods (by using distilled water at room temperature, boiling water, glycerol, β-naphthol, TCA, phenol, 3% NaOH at room temperature, 3% NaOH at 100°C) from the same biological mass of cells of C. albicans strain 846 was investigated. Preparations contained polysaccharides (47-90%) consisting mainly of glucose and mannose. They included protein (5.7-12.5%), phosphorus (0.2-7.6%). The ratio of glucose to mannose in the preparations varied from 1:4 to 7:1.

3. The influence of the composition of nutrient media on the biosynthesis of polysaccharides by Candida tropicalis (N. P. Elinov, V. A. Galinki). The influence of some components of the nutrient medium on the biosynthesis of extracellular (EP), structural (SP) and intracellular (IP) polysaccharides by C. tropicalis was studied. All these polysaccharides contained mannose and glucose. To characterize the biosynthesis of polysaccharides the following ratio is proposed:

$$K = \frac{\text{quantity of glucose in polysaccharide}}{\text{quantity of mannose in polysaccharide}}$$

An investigation of the dynamics of polysaccharide formation in Lodder and Kreger van Rij's medium containing 5% glucose revealed

the maximal accumulation of EP, SP, IP on the 4th day of the fermentation when the ratio $K_{EP}$ = 0.6, $K_{SP}$ = 0.9 and $K_{IP}$ = 2.0 remained constant without dependence on the age of the culture. When glucose was changed to mannose, xylose, sucrose, maltose, fructose or mannitol as well as ammonium sulfate to glycine, asparagine, glutamine or peptone we confirmed a lability of the synthesis rate of EP, IP as well as $K_{EP}$ and $K_{IP}$, but SP and $K_{SP}$ remained constant.
These findings have been confirmed in experiments with C. viswanathii ($K_{IP}$ = 4.2, $K_{SP}$ = 1.7) and C. albicans ($K_{IP}$ = 2.0, $K_{SP}$ = 0.9).

XV. Departamento de Microbiología, Facultad de Ciencias, Universidad de Salamanca, Salamanca, Spain. Communicated by J. R. Villanueva.

Our main projects are the isolation and characterization of yeast lytic enzymes, a study of the fungal structure and more recently a study of membranes (lipids) as well as the process of spore germination. We are also interested in glycoprotein synthesis and yeast invertase synthesis and secretion.

Several glucanases produced by different microorganisms have been purified to a considerable extent and we hope this will help us to elucidate some aspects of the yeast cell wall structure.

Drs. R. Sentandreu and M. W. Elorza have recently moved from the Department of Biochemistry in Madrid to the Department of Microbiology of the Institute of Cell Biology at Salamanca. Their main interest is still the study of yeast cell wall glycoproteins.

The following papers from this laboratory related to yeast have appeared during the past year or are in progress.

Beteta, P. and Gascón, S. Localization of invertase in yeast vacuoles. FEBS Letters 13, 297-299, 1971.

In this paper we report our studies on the localization of invertase inside the yeast protoplasts. We have found that the internal invertase is localized in small vesicles or vacuoles. Both heavy and light invertases are present in the vesicles at similar concentrations. We present a scheme for the formation and secretion of yeast invertase. According to this working hypothesis the light invertase is probably synthesized in the rough endoplasmic reticulum or by ribosomes attached to the surface of the vesicles. Next the addition of carbohydrate would take place and reversed pinocitosis would be the final stage for the liberation of the heavy invertase into the extracellular space.

Liras, P. and Gascón, S. Biosynthesis and secretion of yeast invertase. Effect of cycloheximide and 2-deoxy-D-glucose. Eur. J. Biochem. 23, 160-165 (1971).

The effect of cycloheximide and 2-deoxy-D-glucose on the synthesis and secretion of invertase by yeast cells and protoplasts has been studied. Two yeast strains were used: Saccharomyces 303-67, whose invertase synthesis is repressed by glucose, and a mutant of it, Saccharomyces FH4C, which is only partially affected by high levels of glucose in the incubation media. For both strains the optimum glucose concentration is approximately 10mM.

Cycloheximide inhibited the synthesis of invertase in cells and protoplasts. The secretion of the enzyme by the protoplasts was inhibited to a similar extent. Parallel results were obtained with 2-deoxy-D-glucose.

Two forms of invertase are present in yeast protoplasts; a light form devoid of carbohydrate and a heavy form which contains carbohydrate and is readily secreted. In all experimental conditions studied the relative amount of both forms remained constant. This indicates that although cycloheximide and 2-deoxy-D-glucose exert their action at two different points in the biosynthesis of glycoproteins, any of them can stop the process of synthesis and secretion of yeast invertase.

Gascón, S. and Ottolenghi, P. Influence of the medium on the invertase content of a strain of Saccharomyces bearing the SUC 2 gene. Compt. Rend. Trav. Lab. Carlsberg (in press).

The cellular content of invertase (β-D-fructofuranoside fructohydrolase, EC.3.2.1.26) under the control of the SUC 2 gene of Saccharomyces was studied in continuous increase as the glucose concentration in the culture supernatant decreases. With the strain used, and at pH 5.25, a fifty-five fold difference in the amount of cellular invertase could be obtained by varying the glucose concentration of the culture. The pH of the culture also influenced the amount of invertase per unit weight of cells: the enzyme content was lowest at pH 5.2-5.5 and markedly higher both at lower and at higher pH (4 and 6, respectively).

The work described in this paper was carried out at the Department of Physiology, Carlsberg Laboratorium, Copenhagen, Denmark.

Ballesta, J. P. G. and Villanueva, J. R. "Cell wall components of various species of yeasts". Trans. Brit. Mycol. Soc. 56, 403-410 (1971).

Nombela-Cano, C. and Peberdy, J. F. "The lipid composition of Fusarium culmorum mycelium". Trans. Brit. Mycol. Soc. 57, 342-344 (1971).

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

THE ROLE OF TRACE METALS IN DEREPRESSION OF HAPLOID GLYCOPROTEIN MATING FACTORS IN DIPLOID YEAST OF HANSENULA WINGEI
(Lecture presented at the 4th International Fermentation Symposium, Kyoto, Japan, March 19-25, 1972)

The purpose of this investigation was to study a genetic regulatory system involving mating function in the yeast Hansenula wingei. This yeast was isolated by Wickerham in 1956 and the two mating types are called strains 5 and 21. These haploid strains are heterothallic and the first step in conjugation between 5 and 21 is a strong sexual agglutination reaction in which the cells clump together. Agglutination enhances mating and Brock has found that typically 80% conjugation is obtained in a glucose salts buffer which will not support growth. Herman has shown that the mating types and the complementary agglutination types segregate 2:2 at meiosis; thus, they are either identical genes or very closely linked. The diploid is nonagglutinative even though it carries the genes for both agglutination factors.

The agglutination reaction is due to complementary glycoprotein agglutination factors which are present on the cell walls of the opposite mating types. The agglutination factor from strain 5 was characterized independently by Taylor and Brock and was shown to agglutinate cells of strain 21. Work by Crandall and Brock demonstrated that the 21-factor is univalent and inhibits or neutralizes the agglutination activity of solubilized 5-factor.

The 5-factor and the 21-factor are synthesized constitutively in the respective haploids. However, neither factor nor the neutralized 5-factor:21-factor complex can be detected in cytoplasmic extracts or

on the cell wall of the diploid. Therefore, Dr. T. D. Brock and I proposed in 1968 that the synthesis of both factors is repressed in the diploid. This model proposes that each haploid carries a structural gene for its agglutination factor and a repressor gene for the complementary factor. For example, strain 5 carries the gene for 5-factor and the gene for the repressor of 21-factor synthesis. This repression of agglutination and mating activities in the diploid is teleologically compatible with the fact that these functions are not necessary in the diplophase.

Physiological conditions have been found which allow for derepression or synthesis of one or the other factor in the diploid. Normally the diploid is nonagglutinative when grown in glucose synthetic broth. However, when yeast extract is added, the diploid will synthesize 5-factor and become agglutinative with strain 21 in stationary phase. This is called the diploid → 5 transition. The assay for agglutination is to shake equal numbers of washed diploid cells and heat-activated haploid tester cells together in saline. The results of agglutination assays of the diploid → 5 cells with strain 21 cells are scored ±, +, or ++. The concentration of yeast extract which results in maximal 5-factor synthesis in the diploid is 0.7%. However, different batches of yeast extract give different degrees of induction. The concentration of glucose which results in maximal 5-factor induction in diploid cultures is 2 to 3%. This diploid → 5 transition is a physiological change and is reversible by inoculating the cells into fresh medium. Aeration is required for this 5-factor synthesis as would be expected in an obligate aerobe. The substances present in yeast extract which induce 5-factor synthesis in the diploid are trace metals: vanadium, molybdenum, iron, copper, zinc and nickel.

Vanadium ion is active alone. Weak agglutination occurs between diploid cells grown in its presence and strain 21 tester. Induction of 5-factor synthesis by sodium meta-vanadate occurs maximally with $0.3 - 1.0 \times 10^{-3}$ M.

Addition of molybdenum synergistically enhances the derepression caused by vanadium but before maximal induction (++ agglutination) occurs; the same concentration dependence is observed with both metal ions as with vanadium alone. Maximal induction of 5-factor synthesis by $V^{+5}$ plus $Mo^{+6}$ occurs in stationary phase as when yeast extract is used as the inducer.

Derepression of 21-factor synthesis occurs if 0.7% yeast extract plus $10^{-3}$ M EDTA are added to the glucose synthetic broth. This diploid → 21 transition is maximal in early stationary phase and is induced by a substance present in yeast extract but evidently inhibited by metal ions.

Specificity of induction was demonstrated by treating agglutinative diploid cells with reagents which selectively destroy the agglutinability of only one of the haploid testers. β-Mercaptoethanol destroyed the agglutinability of strain 5 cells and diploid → 5 cells whereas trypsin destroyed strain 21 cells and diploid → 21 cells.

This work has shown that metal ions have a differential effect on the synthesis of the two agglutination factors because metal ions derepress 5-factor synthesis but must be removed by EDTA to allow for 21-factor synthesis. Since metal ions or EDTA are maximally effective in the $10^{-3}$ Molar concentration range, it seems reasonable to assume that they act at the same site in derepression. If this eucaryotic regulatory mechanism can be compared to that in bacteria, then it is possible that the repressor of 5-factor synthesis is inactivated by vanadium and other metal ions, whereas the aporepressor of 21-factor

synthesis is activated by metal ions but inactivated by a substance present in yeast extract.

Derepression of 5-factor synthesis in the diploid is inhibited by cycloheximide, azide, nystatin and polymixin if added in early stationary phase. Inhibitors of RNA, DNA or carbohydrate synthesis when added in exponential phase either had no effect (because the cell was impermeable to them) or inhibited growth and, hence, the diploid → 5 transition. Similarly, since 21-factor synthesis is initiated early in exponential phase the effect of growth inhibitors is not meaningful since growth is required for derepression.

The proposed regulatory mechanism which accounts for these results involves the combination of vanadium and molybdenum with the 5-Repressor thus allowing 5-factor messenger RNA synthesis. Synthesis of the 5-factor protein would require ATP, and would occur on cytoplasmic ribosomes. This step would be inhibitable by azide and cycloheximide. After conjugation of the mannan moiety with the nascent polypeptide, the glycoprotein agglutination factor must traverse the cytoplasmic membrane. This step would be inhibitable by nystatin and polymixin.

Not excluded from consideration is the possibility that metal ions could play a role in the synthesis or attachment of the carbohydrate moieties to these agglutination factors.

XVII. Brooklyn College, Department of Biology, Yeast Laboratory, Brooklyn, New York 11210. Communicated by N. R. Eaton.

The following is a communication from the Yeast Laboratory at Brooklyn College (F. K. Zimmermann, Nasim Khan, N. R. Eaton).

1. The role of MAL genes in sucrose fermentation

Crude extracts derived from strains fermenting maltose but unable to ferment sucrose show a strong sucrose activity. This activity has been shown to be inseparable from maltase activity during enzyme purification (Khan and Eaton, 1968, 1971). Strains carrying MAL4 are constitutive for maltase synthesis and ferment sucrose even if no separable SUC gene is present in such a strain nor any sucrose activity separable from maltase activity. This raises the question as to why other maltose genes do not allow for sucrose fermentation. MAL1 and MAL2 strains which do not ferment sucrose cannot be induced for maltase synthesis by sucrose. This suggested that it is the inability of sucrose to induce maltase synthesis which prevents sucrose fermentation in MAL1 and MAL2 strains. Only the constitutive MAL4 gene can act as a sucrose gene. MAL1 and MAL2 strains ferment sucrose only if cells are taken out of a culture growing on maltose. However, if such cells were transferred out of this sucrose medium where they fermented sucrose into fresh sucrose medium no further fermentation occurred indicating a rapid loss of the ability to ferment sucrose. To study this effect, a MAL2 strain was grown on maltose medium, transferred into glucose medium, and at 20 min intervals after transfer cells were again transferred into sucrose fermentation tubes. Maltase activity was measured in samples removed at the same time. No loss of maltase activity per culture volume was to be observed for 3 h. However, the sucrose fermenting capacity decreased very fast. Fermentation of sugars in yeast leads to acid and $CO_2$ production. Acid production can be monitored by a change of brom thymol blue from green to yellow, $CO_2$ is trapped

in Durham tubes. At 0 time (based on the time cells were in the glucose medium) and after 20 min the Durham tube became full of $CO_2$, after 40 min only a few gas bubbles were visible and after 60 min no gas was formed. After that time not enough acid was formed to turn the color to full yellow and after 100 min no color change was to be observed. This shows that sucrose fermentation by MAL genes does not only require a constitutive maltase synthesis but also some uptake system which can neither be induced nor maintained by sucrose.

2. Identification of a gene which specifically interferes with sucrose fermentation in yeast strains carrying the MAL4 allele.
We have recently described a strain of Saccharomyces (strain 1403-7A), which carries the MAL4 gene and produces maltase constitutively (Khan and Eaton, MGG, 112, 317-322, 1971). This strain, or any other strain in our stock which carries the MAL4 allele, can also ferment sucrose (see Khan and Eaton, Yeast News Letter, vol. XX(2), 1971). This ability to ferment sucrose is a function of the constitutive character of the MAL4 allele. (See 1. "The Role of MAL genes in sucrose fermentation".)
However, we have identified a specific gene which interferes with sucrose fermentation by strains carrying MAL4 alleles but does not affect maltose fermentation.
The following crosses illustrate the above conclusion:
Cross 1. M4-20-R9: (a MAL4 $SUC^+$ trp1 ura3) X Z1-2D ($:\alpha$-$mal^-suc^-$ leu2 lys1). Twelve asci were dissected and analyzed for maltose and sucrose fermentation. The maltose gene segregated 2:2 and all the maltose fermenters were sucrose fermenters.
Cross 2. M4-20-R9: (a MAL4 $SUC^+$ trp1 ura3) X EK-7C ($:\alpha$-$mal^-suc^-$ leu thr ade1 ade8). Sixteen asci were dissected from this cross; maltose fermentation segregated 2:2, but sucrose fermentation gave 9 asci 2:2 segregation and 7 asci 1:3 segregation. However, all sucrose fermenters were maltose fermenters.
Cross 3. M4-20-R9: (a MAL4 $SUC^+$ trp1 ura3) X 1403-17A ($\alpha$-MAL4 $suc^-$ trp1 ura3 ade2 ade8). In the above cross parent 1403-17A was derived from a cross of 1403-7A and Ek- 7C and carries the gene which interferes with sucrose fermentation. Sixteen asci were dissected, and, as expected, maltose fermentation segregated 4:0 and sucrose fermentation 2:2. To check whether this gene has any effect on sucrose fermentation by strains carrying sucrose genes, we made the following cross:
Cross 4. 1412-4D (a MAL3 SUC3 ade2) X EK-7C ($\alpha$ $mal^-$ $suc^-$ leu thr ade2 ade8). Sixteen asci were dissected and both maltose and sucrose fermentation segregated in 2:2 ratios. Thus there was no affect of this gene on sucrose fermentation by the strains carrying SUC3.
These studies demonstrate the presence in strain EK7C of a single recessive gene which affects sucrose fermentation in strains carrying the MAL4 allele. Extracts of such strains are able to catalyze the hydrolysis of sucrose, however, and preliminary studies suggest that the defect is in sucrose uptake.

3. Mutants preventing sucrose fermentation by a MAL4 strain
Mutants have been isolated which still form maltase and ferment glucose but not maltose nor sucrose. 8 mutants were subjected to genetic analysis. In all cases maltose and sucrose fermenting haploid progeny was obtained from crosses of such mutants against

a sucrose and maltose non-fermenting strain showing that the mutations did not affect either a structural or a regulatory gene required for maltase synthesis. These mutants were crossed against each other and no complementation was observed for 3 mutants, #8, 18 and 21. Allelism was established for #8 and 18 by tetrad analysis. Mutants 6, 7, 17, 24 and 28 were not allelic amongst each other nor to the group 8, 18 and 21. Consequently there are at least 6 loci the function of which is required for maltose and sucrose fermentation by constitutive MAL4. Mutant #7 when crossed to any other mutant except for leaky mutant #28 gave fermenting progeny only in incomplete tetrads indicating that double mutants are inviable. Mutant #7 would not ferment α-methylglucoside within 6 weeks; the other mutants did so within 3 weeks and the parent strain 1403-7A in 3 days. Crosses of the mutants 21 and 24 against MAL, MAL2 and MAL3 strains revealed a general effect on maltose fermentation but not on sucrose fermentation in a SUC3 strain. All mutants were crossed against a MAL2 and a MAL3 strain. It could be shown by extensive tetrad analysis that all mutants affect maltose fermentation mediated by other genes. Moreover, it could be shown that none of those genes had an effect on galactose fermentation. These mutants are therefore called dsf mutants (disaccharide fermentation). Uptake of radioactive maltose was studied in a variety of strains carrying MAL2, MAL4 and no maltose gene. All strains growing on maltose, sucrose or 0.3% glucose YEP showed uptake of radioactivity which leveled off after about 4-8 min. Only MAL4 strains or MAL2 strains growing on maltose showed an increase in uptake beyond this period of time. Of the dsf mutants all but dsf 6 and leaky dsf 28 showed an uptake beyond this brief period which was less than in 1403-7A parent strain. This indicates that the dsf mutants interfere with maltose uptake into the cell or with transport through an intracellular compartment barrier.

4. Ultraviolet light induction of new SUC genes

Fermentation of sucrose by Saccharomyces cerevisiae is under the control of a number of genes any one of which is sufficient for fermentation of this sugar. Strains carrying a MAL2 gene and unable to ferment sucrose were exposed to ultraviolet light (about 60% killing) and plated onto yeast extract peptone medium with 2% sucrose and brom thymol blue as an indicator for acid formation. There was considerable residual growth on those plates, however, no acid formation as judged by the lack of color change. Out of about 3000 viable cells plated on that medium a few yellow papillae of fast growing cells were formed which eventually turned the entire medium yellow. Cells of such papillae were restreaked on the same medium and isolated colonies from such streaks analyzed for their ability to ferment sucrose. All isolates fermented sucrose if directly transferred from sucrose plates to sucrose fermentation tubes but this ability was not retained in all cases after an intermittant growth on glucose. Stable "de novo" sucrose fermenters were crossed against strain E-2A which ferments neither maltose nor sucrose. The resulting diploid fermented sucrose showing that the new trait was dominant. A fast and a slow fermenting diploid were sporulated and tetrad analysis performed. In all cases sucrose fermentation and maltose fermentation segregated 2:2 but the ability to ferment sucrose and maltose segregated independently

(M = maltose fermentation, S = sucrose fermentation).

| Mutant 22 | | | Mutant 36 | | | |
|---|---|---|---|---|---|---|
| PD | TT | NPD | PD | TT | NPD | Irregular |
| M S | M S | M- | MS | MS | M- | M S |
| M S | M- | M- | MS | M- | M- | M S |
| - - | -S | S- | - - | -S | -S | M- |
| - - | - - | S- | - - | - - | -S | - - |
| 2 | 7 | 5 | 1 | 23 | 5 | 1 |

To establish possible allelism, segregants of those crosses fermenting sucrose only but no maltose derived from mutants 22 and 36 were crossed against each other and the resulting diploid sporulated and tetrad analysis performed. The following types of tetrads were obtained in respect to sucrose fermentation.

| PD | TT | NPD |
|---|---|---|
| S | S | S |
| S | S | S |
| S | S | - |
| S | - | - |
| 2 | 7 | 3 |

This result shows that at least two of the newly arisen sucrose genes were not allelic.

XVIII. Institut du Radium - Biologie, Batiment 110, Faculté des Sciences, Orsay (91), France. Communicated by E. Moustacchi.

The following articles have been recently published:

A UV-supersensitive mutant in the yeast Schizosaccharomyces pombe. Evidence for two repair pathways. F. FABRE. Molec. Gen. Genetics, 110, 134-143 (1971).

The synthesis of mitochondrial DNA during the cell cycle in the yeast Saccharomyces cerevisiae. D. H. WILLIAMSON and E. MOUSTACCHI. Biochem. Biophys. Res. Commun., 42, 195-200 (1971).

ADN satellite $\gamma$ et molécules circulaires torsadées de petite taille chez la levure Saccharomyces cerevisiae. B. J. STEVENS and E. MOUSTACCHI. Exp. Cell Res., 64, 259-266 (1971).

A procedure for rapidly extracting and estimating the nuclear and cytoplasmic DNA components in yeast cells. D. H. WILLIAMSON, E. MOUSTACCHI and D. FENNELL. Biochim. Biophys. Acta, 238, 369-374 (1971).

Photoreactivation in the yeast Schizosaccharomyces pombe. F. FABRE. Photochem. Photobiol., 15, 367-373 (1972).

Evidence for nucleus independent steps in control of repair of mitochondrial damage. I. UV-induction of the cytoplasmic "petite" mutation in UV-sensitive nuclear mutants of Saccharomyces cerevisiae. E. MOUSTACCHI. Molec. Gen. Genetics, 114, 50-58 (1972).

Lethal and mutagenic effects of elevated temperature on haploid yeast. I. Variation in sensitivity during the cell cycle. A. C. SCHENBERG-FRASCINO and E. MOUSTACCHI. Molec. Gen. Genetics, 115, 243-257 (1972).

The following articles are in the press:

Lethal and mutagenic effects of elevated temperature on haploid yeast. II. Recovery from thermolesions. A. C. SCHENBERG-FRASCINO. Molec. Gen. Genetics (1972).

When after heat treatment at 52°, yeast cells are held in water at 28° with aeration for 24 hours to 5 days before plating, their survival is

greatly enhanced in comparison to what is obtained on immediate plating. Moreover, the frequency of induced nuclear (canavanine-sensitivity to canavanine-resistance) and cytoplasmic ($\rho^+$ to $\rho^-$) mutants decreases in these conditions of liquid holding. This recovery from potentially lethal and mutagenic damage, induced by heat treatment, is blocked if the cells are stored at 4° or in the presence of protein synthesis inhibitors. This implies that active metabolism is required for the reversion of thermolesions.

Relation between repair mechanisms and UV-induced mitotic recombination in the yeast Schizosaccharomyces pombe. Effects of caffeine. F. FABRE. Molec. Gen. Genetics (1972).

Two different pathways A and 1 are known to control the repair of UV lesions in the yeast Schizosaccharomyces pombe. The relation between the UV-induced intergenic mitotic crossing over (MCO) and the repair of prelethal lesions controlled by these pathways were studied in the following strains: $UVS1_{,1}/UVS1_{,1}$, where pathway A acts; UVSA/UVSA where pathway 1 acts, $UVS^+/UVS^+$ (wild type) and UVS1A/UVS1A (double mutant). The analysis of the survival and MCO induction curves, and the comparison, as a function of the dose and as a function of survival, of the MCO induction curves corresponding to the different strains, show that the repair pathway 1 controls a mechanism involving recombination, and that the repair pathway A controls a mechanism which removes prerecombinational lesions. Studies were done with $UVS1_{,1}/UVS1_{,1}$ cells in different physiological conditions affecting the repair efficiency of prelethal lesions (Irradiation during the logarithmic growth phase, liquid holding). In all cases the more efficient the repair of prelethal lesions is, the smaller is the recombination inducibility. This is expected if pathway A controls an excision repair mechanism. The effect of the repair inhibitor, caffeine, was studied. It inhibits only the repair of UV prelethal lesions controlled by pathway 1. The involvement of recombination in the repair of UV lesions in $UVS^+/UVS^+$ and UVSA/UVSA cells is also shown by the fact that the sensitization to the lethal effect of UV by caffeine in these strains is correlated with a decrease in UV MCO inducibility. Caffeine has no effect either on the UV survival, or on the MCO inducibility in $UVS1_{,1}/UVS1_{,1}$ cells. It is concluded that it inhibits the recombinational repair pathway and not the excision repair pathway.

The MCO induction observed in UVS1/UVS1 and UVS1A/UVS1A cells could be due to the presence of a second recombinational pathway, not sensitive to caffeine. At least a fraction of the prerecombinational lesions would not be prelethal, and they are repairable by the excision repair mechanism.

Work in progress:

The study of the interaction between nuclear and mitochondrial functions with respect of repair of mitochondrial genetic damage (UV induction of the cytoplasmic "petite" mutations or $\rho^-$) was continued by the isolation of mutants with a normal sensitivity to nuclear UV damage but much more sensitive to $\rho^-$ induction. These mutants denominated uvs$\rho$ are characterized by an enhanced spontaneous frequency of $\rho^-$ cells and a modified sensitivity not only to UV but also to other mutagenic treatments such as ethidium bromide, temperature (52°C), etc... These mutants appear to be defective in repair mechanisms as demonstrated by the modification in response to liquid holding after UV and the fractionated dose technique as compared to the wild

type.

Among the five independent uvsρ mutants found, three of them demonstrate a mendelian pattern of segregation of the uvsρ marker, where two mutants show a cytoplasmic pattern of segregation. In this last case, the uvsρ character appears to be associated to the ρ factor since a $\rho^-$ mutant deprived of mitochondrial DNA and derived from a "grande" uvsρ have lost the uvsρ marker. On the other hand, the UV induction of "petites" in diploids homozygous for the uvsρ marker behaves like a diploid obtained by a cross between a uvsρ strain and a haploid $\rho^-$ without mitochondrial DNA.

The existence of this novel class of mutants taken together with the analysis of the response of the nuclear rad mutations (Molec. Gen. Genetics, 1972, 114, 50-58) points to the following overall picture of interaction between the nuclear and mitochondrial genomes for the repair of mitochondrial UV damage:

a) Some nuclear Rad genes govern simultaneously some of the steps of both nuclear and mitochondrial repair (Rad 2, 7, 10, 11, 17, 18, 6 and 5).
b) Some nuclear genes control specifically the repair of mitochondrial damage (uvsρ 5, 6 and 34).
c) Some cytoplasmic determinants are involved in the specific control of repair of mitochondrial lesions (uvsρ 72, 13).

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R. Chanet and M. Heude are following the cyclic variations to lethal and cytoplasmic genetic damages induced by UV light in synchronized and random cultures of wild type and UV-sensitive strains. The influence of the cell stage in the cycle and of the carbohydrate source in the medium on the ability of the cells to recover from the UV damage is analyzed. Their results points to a dissociation between the nuclear and mitochondrial responses both in timing, in the influence of the sugars used and in the genetic factors involved.

XIX. The University of Texas at Dallas. Box 30365. Division of Biology, Dallas, Texas 75230. Communicated by H. Gutz.

The former "Southwest Center for Advanced Studies" is now the University of Texas at Dallas. The following constitutes the annual report of the Division of Biology.

MOLECULAR GENETICS OF THE YEAST SCHIZOSACCHAROMYCES POMBE
H. Gutz, F. Doe and S. Goldman

Meiotic gene conversion. The ade6 mutant M26 of S. pombe shows a pronounced marker effect with respect to intragenic recombination and gene conversion. The behavior of M26 can be best explained by assuming that this mutant influences breakage of DNA rather than correction of mismatched bases in hybrid DNA. I suppose that, during meiotic prophase, the DNA at the M26 site preferentially undergoes single-strand breakage followed by exonucleolytic degradation towards both ends of the locus. It is then assumed that one free end of the broken and partially degraded strand triggers events in the homologous chromatid which finally result in a conversion tetrad (see the gene conversion model of Paszewski, 1970). The extraordinary feature of M26 appears to be that, if this allele is present in a cross, an additional point of preferential breakage is initiated within the ade6 locus. Thus, the original mutation not only blocks translation of the ade6 gene (M26 is a nonsense mutation), but also seems to

change the DNA so that this region of the gene now behaves differently with respect to initiation of gene conversion (Gutz, 1971a).

To get further evidence for the above hypothesis, M26 was crossed with non-identical ade6 alleles which map either to the left or to the right of this mutant. Asci with a prototrophic spore were selected. M26 was the minority parent in all crosses, regardless of the position of the second site. If normal (in contrast to M26) ade6 mutants are crossed with each other, the left-hand allele is predominantly the minority parent. Thus M26 reverses the polarity in the ade6 locus with regard to mutants mapping to its left. This result is additional evidence for our working hypothesis. Other ade6 nonsense mutations, which respond to the same supersuppressors as M26 and map close to this mutant, do not show the marker effect. Thus the influence of M26 on gene conversion does not seem to be a property of the nonsense codon itself, but appears to be an inherent feature of the M26 site in conjunction with the adjacent base-pair sequence. The marker effect of M26 is lost if mutational changes are induced in the nonsense codon (Gutz, 1971b).

Results from intragenic three-factor crosses indicate that, in the presence of M26, some hybrid DNA is also formed in the ade6 locus, which is exposed to excision-repair processes (Goldman and Gutz, 1971).

In my theoretical work on gene conversion, I have discussed some quantitative implications of the conversion models of Holliday and of Whitehouse and Hastings. These models predict two kinds of mispairing when a heterozygous mutant is in hybrid DNA. It was pointed out that the latter model, in contrast to statements in the literature, does not require identical repair rates in both kinds of mispairing. It was found that, in the Holliday model, a minimum results for the frequency of asci that show a normal 4:4 segregation, but that have originated by repair from meioses with hybrid DNA, if the frequency of repair to wild type is the same in both kinds of mispairing (Gutz, 1971c).

Studies of the mating type of S. pombe. In our experiments with S. pombe strains of different geographical origin, we have found some new mating-type factors which differ from those of Leupold's (1958) strains.

Goldman, S., and H. Gutz (1971) Genetics 68, s23 (abstract).
Gutz, H. (1971a) Genetics 69, 317-337.
Gutz, H. (1971b) Genetics 68, s26 (abstract).
Gutz, H. (1971c) Genet. Res., Camb. 17, 45.
Leupold, M. (1958) Cold Spring Harbor Symp. Quant. Biol. 23, 161.
Paszewski, A. (1970) Genet. Res., Camb. 15, 55.

XX. Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario, Canada. Communicated by A. Nasim.

GENETIC CONTROL OF RADIATION SENSITIVITY AND DARK REPAIR IN SCHIZOSACCHAROMYCES POMBE.

Our current research projects are related to the isolation and characterization of radiation-sensitive mutants in the haploid fission yeast S. pombe. Since this organism shows a very marked degree of resistance to both ultraviolet and ionizing radiations and many highly sensitive mutants have been isolated, it is more than likely to possess very efficient dark repair system(s). A large number of uv-sensitive mutants are being tested for allelism to

determine the number of independent loci controlling radiation sensitivity. These include 10 non-allelic mutants from M. Schupbäch, 1 mutant from F. Fabre, 20 mutants obtained from R. Megnet and 24 additional mutants isolated in our laboratory. All these mutants have been tested for cross-sensitivity to ionizing radiations and some chemical mutagens and those showing a widely different sensitivity compared to the wild type are being further analyzed.

Dark repair inhibitors

Many substances known to inhibit dark repair in both bacterial and mammalian systems have been tested in S. pombe. The compounds tested include acridine orange, chloroquine, quinacrine, hydroxyurea, acriflavine and caffeine. A marked inhibition of growth at very low concentrations of the drug indicates that all these compounds are taken up by the cell, yet none of these except caffeine reduce colony-forming ability when present in the post-irradiation plating medium. Caffeine is now being used in all subsequent experiments to test the response of all known radiation-sensitive mutants. This should give an indication of the possible defect in these mutants as well as show which step in the repair pathway is being preferentially inhibited by caffeine.

Radiation-sensitive and conditional lethal mutants

The 2-deoxyglucose enrichment method for auxotrophs has been used to get an enrichment for temperature-sensitive mutants by growing a mutagenized population at the restrictive temperature. The technique proved useful and led to nearly 10% enrichment of conditional lethals, many of them showing enhanced radiation sensitivity. Extensive genetic analysis has however shown that a majority of these are only double mutants. However, in mutant UVS4-138 isolated by Schupbäch which is also a conditional lethal the two phenotypes have resulted from the same mutation and these failed to show independent segregation in $5 \times 10^3$ random spores and many tetrads. This mutant is being further characterized.

Based on these studies the following manuscripts are in preparation:

1) A. Nasim and B. P. Smith. Dark repair inhibitors in S. pombe.
2) A. Nasim and B. P. Smith. Radiation sensitive and conditional lethal mutants of yeast.

XXI. Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario, Canada. Communicated by Norman E. Gentner.

RECOVERY OF SCHIZOSACCHAROMYCES POMBE FROM RADIATION-INDUCED DAMAGE.
Norman E. Gentner and Mary M. Werner

The following is a report on a project concerned with determination of the time course and requirements for recovery from radiation-induced inactivation in the haploid fission yeast S. pombe.

The presence in the post-irradiation medium of caffeine, an inhibitor of dark repair in bacterial and mammalian systems, is known to reduce the colony-forming ability of S. pombe following uv- or $\gamma$-irradiation. The requirements of, and the effect of various parameters on, the repair process can be investigated by looking at the recovery of irradiated S. pombe from susceptibility to the effect of caffeine. For example, S. pombe 972h$^-$, after a uv dose of 1200 ergs/mm$^2$, yields $\sim$10% survival if plated on YPG (1% yeast extract, 0.5% peptone, 2% glucose) and $\sim$0.3% survival if plated on YPG - 0.1% caffeine. If irradiated cells are incubated in liquid YPG medium for various periods

of time, and then plated and scored for colony-forming ability on YPG-0.1% caffeine, one can observe the time course of recovery between these two levels of survival. The overcoming of the inhibitory effect of caffeine represents the recovery or repair process (the 1200 erg/mm$^2$ dose induces a division delay of >8 hours in logarithmic phase cells; we are concerned with recovery events before this first post-irradiation division). This technique, i.e., the overcoming of the block by a dark repair inhibitor, should be adaptable to any organism (and any effective inhibitor of dark repair therein) in order to allow examination of the recovery process.

This approach has led to the following conclusions concerning the recovery process in S. pombe: 1) After either uv- or $\gamma$-irradiation, recovery was 80-90% accomplished within 3 hours of incubation at 30°C in YPG. 2) Recovery required a complete growth medium; either 1% yeast extract or 2% glucose, by itself, gave only a slight degree of recovery. No recovery was observable with non-nutritive solutions; this agrees with the known absence of any liquid holding recovery process in S. pombe 972h$^-$. The time course of recovery in minimal medium was somewhat more rapid than in YPG. 3) The optimal temperature of incubation for recovery in the liquid YPG medium was 25°C to 30°C. At lower temperatures (15°C and 7.5°C) the recovery process proceeded more slowly, and maximal recovery was not attained; at higher temperatures (36°C), the initial rate of recovery was higher, but the final extent of recovery was slightly lower than at 30°C. In these experiments, the YPG-0.1% caffeine plates were all incubated at 30°C.

This system is currently being used to examine the question of whether new protein synthesis is necessary for the recovery process, or whether the cell utilizes its pre-irradiation complement of enzymes to accomplish repair. Preliminary experiments in which protein synthesis has been inhibited in the post-irradiation liquid YPG medium indicate that new protein synthesis may be required for recovery.

XXII. Groupe Euratom de L'Universite'de Louvain, Laboratoire D'Enzymologie, de Croylaan 46, 3030 Heverlee, Belgium. Communicated by A. Goffeau.

Below follows some information concerning our laboratory.

F. FOURY is studying two mutants of a "petite-negative" yeast, Schizosaccharomyces pombe, which seem to be affected in their response to glucose repression. Respiration of mutant COB5 is more repressed than that of the wild strain, whereas the strain COB6 is totally insensitive to glucose repression. Respiration of the "superrepressed" strain COB5 can be derepressed in the absence of cellular division. This derepression is chloramphenicol, cycloheximide and ethidium-bromide-sensitive. On the other hand, growth and respiration of the derepressed strain COB6 are resistant to ethidium bromide.

A. GOFFEAU, Y. LANDRY and A. M. COLSON are studying mutants of S. pombe affected in their mitochondrial ATPase. Several types of deficiencies were found. Most of them concern the loss of oligomycin and DCCD sensitivity. All mutants tested so far are of nuclear heredity.

XXIII. Department of Genetics, The University of Sheffield, Sheffield S10 2TN, England. Communicated by R. A. Woods.

Current research on yeast in this department is centered on (a) the

genetic control of purine metabolism (b) the use of polyene resistant mutants in studies on sterol metabolism and physiology. We are approaching purine metabolism from both ends; Dr. T. S. Gross has been studying the control of de novo synthesis and shown that activity of PRPP amido transferase is subject to feed-back inhibition and repression. The "alternative first step" of purine synthesis, Ribose-5-phosphate + $NH_3$ → phosphoribosylamine, is catalysed by cell free extracts of yeast, including mutants of ade4 which are auxotrophic and lack activity of PRPP amido transferase. We infer that this alternative step has little or no physiological significance in vivo. We have recently found that mutants of ade3 and ade8 accumulate glycineamide ribonucleotide (GAR). This is consistent with the defect in folate metabolism in ade3 and suggests that ade8 may specify GAR transformylase. Biochemical studies to confirm this are in progress. Mr. W. R. Pickering has been investigating a series of mutants resistant to 4-amino-pyrazolo (3,4d) pyrimidine. He has identified 7 genes, app-1 to app-7, which confer resistance. He has found that yeast has two constitutive purine permeases, the adenine permease which accepts adenine, hypoxanthine and their analogues, and a guanine permease which accepts guanine, guanine analogues and hypoxanthine. Mutants of app-1 have an altered adenine permease which no longer accepts 4-APP and several other analogues as substrates. Mutation at all of the other app genes have pleiotropic effects on purine uptake, pyrophosphorylation and de novo synthesis.

Mr. W. J. Gardner is studying mutants affecting GMP synthesis. He has isolated a number of guanine requiring mutants and is currently engaged in genetic and biochemical analysis.

Recent publications:

C. A. Lomax and R. A. Woods. Prototropic regulatory mutants of adenylosuccinate synthetase in yeast. Nature, New Biology 229, 116, 1971.

C. A. Lomax, T. S. Gross and R. A. Woods. New mutant types at the ade3 locus of Saccharomyces cerevisiae. J. Bacteriol, 107, 1-7, 1971.

T. S. Gross and R. A. Woods. Identification of mutants defective in the first and second steps of de novo purine synthesis in Saccharomyces cerevisiae. Biochim. et Biophys. Acta 247, 13-21, 1971.

W. R. Pickering and R. A. Woods. The uptake and incorporation of purines by wild-type Saccharomyces cerevisiae and a mutant resistant to 4-amino pyrazolo (3,4-d) pyrimidine. Biochim. et Biophys. Acta 264, 45-58, 1972.

Communications:

T. S. Gross and R. A. Woods. Regulation of de novo purine nucleotide synthesis by enzyme repression in Saccharomyces cerevisiae. Heredity 28, 275. 1972.

W. R. Pickering and R. A. Woods. Genetic and biochemical studies on resistance to the purine analogue 4-amino pyrazolo (3,4-d) pyrimidine (4-APP) in Saccharomyces cerevisiae. Heredity 28, 275-276. 1972.

W. R. Pickering and R. A. Woods. Altered purine permease activity in a purine analogue resistant mutant of Saccharomyces cerevisiae. Biochemical Journal, in the press.

R. A. Woods, T. S. Gross, W. R. Pickering and I. E. Jackson. Some features of the control of purine synthesis in Saccharomyces cerevisiae. Heredity, in the press.

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We are studying resistance to polyene antibiotics both for its intrinsic interest and also because resistant mutants have all proved

to have altered cell sterols. We think that these mutants may enable us to investigate the biochemical genetics of sterol metabolism in yeast. We have been joined by Dr. Martin Bard from the Department of Genetics, Berkeley. Dr. Bard did his Ph.D. on nystatin resistance and is in Sheffield for three years on a Medical Research Council Grant. Mr. S. W. Molzahn has isolated mutants resistant to etruscomycin, filipin, nystatin, pimaricin and rimocidin and identified 5 genes, pol1 to pol5. pol1 and pol3 are allelic to the previously reported nys1 and nys3. He has analysed the sterols in mutants of these five genes by U.V. spectrophotometry and T.L.C. (Dr. Bard is analyzing them on G.L.C.) Tests for functional allelism between our mutants and Dr. Bard's indicate that there are 7 genes for polyene resistance in yeast and that all affect sterol metabolism.

Publications:

R. A. Woods. Nystatin-resistant mutants of yeast: Alterations in sterol content. J. Bacteriol. 108, 69-73. 1971.

S. Molzahn and R. A. Woods. Polyene resistance and the isolation of sterol mutants in Saccharomyces cerevisiae. J. Gen. Microbiol., in the press.

Communication:

S. Molzahn and R. A. Woods. Selection of sterol mutants of Saccharomyces cerevisiae by exposure to polyene antibiotics. J. Gen. Microbiol. 68, vii-viii. 1971.

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Dr. Bard has submitted a paper based on the work for his Ph.D. thesis to J. Bacteriol.

XXIV. Centre National de la Recherche Scientifique, Centre de Génétique Moléculaire, 91, Gif-sur-Yvette, France. Communicated by Mario Luzzati.

MITOTIC RECOMBINATION IN SACCHAROMYCES CEREVISIAE STRAINS CARRYING ADE3 MUTATION

Alain Henaut and Mario Luzzati

ade3 mutants of Saccharomyces cerevisiae require adenine and histidine; it has been shown by E. W. Jones that this double requirement is due to the deficiency of some enzymes related to tetrahydrofolate metabolism.

In a diploid strain heteroallelic at ade3 locus, the mitotic intragenic recombination frequency is enhanced ten-fold when the cell population is starved for histidine. By studying simultaneous recombinational events at two independent loci, we were able to show that the effect of histidine starvation is most simply explained in terms of an increase in the frequency of cells capable of recombination. In these competent cells, intragenic recombination frequency is not affected by histidine. Our working hypothesis is that histidine starvation in ade3 diploid strains stimulates chromosome pairing in cells during the vegetative phase (Mol. Gen. Genetics 111, 120-137; 111, 138-144; 116, 26-34).

We started studying the characteristics of the spontaneous mitotic recombination. From our preliminary results it seems that this recombination is due only to gene conversion, crossing over being absent. Cytologically we looked for the presence of the synaptinemal complex, but, unfortunately, we were unable to show it even during meiosis. However we find a puzzling correlation between mitotic

recombination and invagination of the nuclear membrane.

Our system should allow a study of the biochemical relationship between chromosome pairing and the metabolism of tetrahydrofolate co-enzymes. This work is in progress.

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

THE EFFECT OF 5-BROMOURACIL ON DIPLOID CELLS HETEROZYGOUS AT THE ade2 LOCUS OF SACCHAROMYCES CEREVISIAE

B. A. Siddiqi

In Saccharomyces cerevisiae a thymine requiring mutant is not available. Therefore, a synthetic medium containing aminopterin (Apt) was used to inhibit the formation of thymine triphosphate.

Some biological systems can tolerate the specific replacement of some of the thymine in the DNA by a sterically similar synthetic base analogue. In this work Apt was used to create a condition of thymine starvation and 5-Bromouracil (5-BU) and thymine (Th) were added to the blocked system to overcome the inhibition.

A heterozygous diploid of the constitution ade2/± is white and stable, but following treatment with well known mutagens like EMS and UV, the frequency of red colonies increases significantly among the survivors. It was expected that in using a heterozygous ade2 diploid an effect of 5-BU incorporation on mitotic crossing over and/or gene conversion would result in an increase in sectoring of colonies derived from treated cells. The change in titre of cell suspensions was studied during 40 hr. Adding Apt to the suspension was expected to stop cell division and further addition of 5-BU or Th was expected to overcome this inhibition. Growth was, therefore, taken as an indication of 5-BU incorporation.

In the first series of experiments, the final concentration of Apt, 5-BU or Th was 100 µg/ml. In the second series of experiments two different concentrations of 5-BU were tested viz. 50 µg/ml and 100 µg/ml. The treated suspensions were aerated for 40 hr at 28C.

The results demonstrate that Apt caused inhibition of DNA synthesis as anticipated. No significant increase in growth was observed between suspensions with Apt alone and with Apt and 5-BU or Th added. This was interpreted to mean that neither 5-BU nor Th were incorporated into the cells of Saccharomyces cerevisiae when Apt was present.

Another important observation was that Apt-treated cells plated on agar medium formed significantly higher frequencies of sectored colonies than cells from control suspensions.

Holliday who treated cells of Ustilago maydis with the DNA inhibitors, Mitomycin C and 5-fluorodeoxyuradine, obtained an increase in mitotic recombination. It was therefore, not surprising that Apt known to inhibit DNA synthesis increased the frequency of sectoring in colonies derived from cells treated with it. Further studies on the effect of Apt on diploid cells are being carried out.

XXVI. University of Strathclyde, Department of Applied Microbiology, Royal College, George Street, Glasgow C1, Scotland. Communicated by John Johnston.

Recent publications are as follows:

Thornton, R. J. and Johnston, J. R. Rates of spontaneous mitotic recombination in Saccharomyces cerevisiae. Genet. Res. 18, 147, 1971.

Johnston, J. R. Genetic analysis of spontaneous half-sectored colonies of Saccharomyces cerevisiae. Genet. Res. 18, 179, 1971.

Johnston, J. R. Genetic analysis of X-ray induced half-sectored colonies of Saccharomyces cerevisiae. Radiat. Res. (in press).

MacKinnon, J. M. and Johnston, J. R. Mitotic segregation in polyploid strains of Saccharomyces cerevisiae. Heredity (in press).

The following is an abstract of a paper read at the Genetical Society meeting in Leeds in March:

GENETIC CHANGES IN CONTINUOUS CULTURES OF YEAST
J. R. Johnston, R. J. Thornton and E. McDermott

Reduced temperatures induce high frequencies of petite mutants in some strains of yeast (Ogur, Ogur and St. John, Genetics, 45, 189, 1960). In continuous culture, dramatic increases in the proportion of respiratory-deficient mutants occur at 15, 18, and 21°C. Similar results using either glycerol or glucose as carbon-sources show that this effect is due to mutation-induction and crosses show that induced petites are cytoplasmic. Other respiratory-deficient mutants, both capable and incapable of reversion to wild-type and having growth rates approximately equal to wild-type, have been detected. These are both cytoplasmic and segregational mutants.

Continuous culture selects a respiratory-sufficient line in which petites are not induced by reduced temperatures. The genetic change in cells of this line is not yet known but presumably they contain mitochondrial DNA insusceptible to low-temperature mutation. Selection of stable lines explains why commercial brewing strains show no induction of petites by reduced temperatures.

An increase in the proportion of mitotic segregants of heterozygous diploid strains is expected to occur during prolonged culture. This has been observed.

XXVII. Laboratoire de Génétique Physiologique, Universite' Louis Pasteur, 8 rue Goethe, 67 Strasbourg, France. Communicated by F. Lacroute.

Current activities in our laboratory are as follows:

REGULATION OF THE PYRIMIDINE PATHWAY:

Study of the salvage pathway especially of the utilization and the conversion of cytidine and cytosine (R. JUND).

Research of deletions in the ura 2 gene (M. L. BACH). Genetic study of an extra chromosomal non-mitochondrial mutant allowing ureidosuccinic acid uptake (M. AIGLE).

Metabolism and physiology of mendelian and extra chromosomal mutants allowing ureidosuccinic acid uptake (R. DRILLIEN).

Study of regulatory and structural mutations concerning cytosine and uracil permeases (M. R. CHEVALLIER, F. LACROUTE).

Mapping of the ura 2 region (M. DENIS).

Channeling of ureidosuccinic acid in the yeast cell (C. T. KORCH).

MECHANISM OF CHROMOSOME REPLICATION:

Research of a chromosomal mutation causing a temperature sensitive loss of one chromosome in a disomic strain (F. EXINGER).

MEMBRANE FUNCTIONS:

Study of an organic solvent dependant mutant; research of mutants impaired in sterol biosynthesis different from Nystatin resistant mutants (F. KARST).

RELAXED MUTANTS:

The search for relaxed mutants has been stopped because no

relaxed mutants have been found. Those mutants thought to be relaxed (as discussed at the last Yeast Genetic Conference) were found to be impaired in uracil uptake.

RECENT PUBLICATIONS:

JUND R. and F. LACROUTE. Genetic and Physiological Aspects of Resistance to 5-Fluoropyrimidines in Saccharomyces cerevisiae. J. Bacteriol. 102, 607-615, 1970.

LACROUTE F. Non-Mendelian Mutation Allowing Ureidosuccinic Acid Uptake in Yeast. Jour. of Bacteriol., 519-522, 1971.

DENIS M. and LACROUTE F. Complementation of the Ura 2 Locus of Saccharomyces cerevisiae. M.G.G. 112, 354-364, 1971.

LACROUTE F. et EXINGER F. Note avec comptes rendus: Recherche de mutants de découplage entre la synthèse d'ARN et la synthèse protéique chez Saccharomyces cerevisiae. C. R. Acad. Sc. PARIS, t. 273, p. 1573-1575, 1971.

BACH M. L. and F. LACROUTE. Direct Selective Techniques for the Isolation of Pyrimidine Auxotrophs in Yeast. M.G.G. 115, 126-130, 1972.

DRILLIEN R. and F. LACROUTE. Ureidosuccinic Acid Uptake in Yeast and Some Aspect of Its Regulation. J. Bact. 109, 203-208, 1972.

JUND R. and F. LACROUTE. Regulation of orotidylic pyrophosphorylase in Saccharomyces cerevisiae. J. Bacteriol. 109, 196-202, 1972.

XXVIII. Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan. Communicated by Haruhiko Mori.

Below follows an abstract of a recent publication.

INDUCTION OF AUXOTROPHIC MUTANTS IN SACCHAROMYCES ROUXII BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE
Haruhiko Mori
J. Ferment. Technol. 50(3), 218-221 (1972)

Induction of auxotrophic mutants of Saccharomyces rouxii has been tried by N-Methyl-N'-Nitro-N-Nitrosoguanidine (MNNG). S. rouxii, an industrially important yeast for soy sauce and miso brewing, characteristically spends the vegetative growth in the haploid phase. However, appearance of mutants from the vegetative cells was very low and showed no correlation with incubation period, pH in a reaction mixture with MNNG and temperature of incubation. Probably, it resulted from the tendency of cell-aggregation. In order to avoid the complexity caused by cell-aggregation, a free-spore suspension was prepared and treated with MNNG. Incubation with 0.5 mg/ml of MNNG for 80 min or 1.0 mg/ml of MNNG for 60 min in pH 5.0 solution at 30°C gave good results in which about 3 to 4 per cent of survivors could be isolated as auxotrophic mutants. The spectrum of MNNG induced mutants showed high frequencies of adenine, leucine, lysine and arginine requiring mutants. Although the mechanism of the high mutagenic action of MNNG for free-spores is still obscure, this method will be useful in isolating auxotrophic mutants of such sporulation microorganisms as S. rouxii, of which auxotrophic mutants are difficult to induce from the vegetative cells.

XXIX. Allied Breweries (Production) Limited, The Brewery, Burton-on-Trent, England. Communicated by G. A. Howard.

Below follows a paper given at the Am. Soc. of Brewing Chemists Convention in May, 1972:

YEAST HYBRIDISATION
E. Clayton, G. A. Howard and P. A. Martin

SUMMARY:

Hybridisation has been investigated as a possible technique for improving traditional brewery yeast strains and for introducing properties required by modern brewing technology whilst retaining characteristic product flavours.

Mass mating techniques were adopted as being particularly suited to the breeding of industrial yeasts where a general improvement was required by increasing the total level of genetic material rather than the development of an isolated property.

Ale and particularly lager yeasts, in regular brewery use, sporulated poorly and gave few fertile mating strains. In contrast baking and distilling yeasts of high fermentation rate, originally from other breeding programmes, sporulated well and gave many fertile maters but in extensive cross mating with brewing strains gave few hybrids of interest.

Some hybrids from brewing yeast strains had shorter lag phases, better fermentation rates and attenuating properties than their parents but lacked flocculence. The hybrids formed sporulated much more readily than their parents and gave a high proportion of fertile mating strains for further hybridisation.

Hybridisation with mating strains from flocculent yeasts generally resulted in non-flocculent hybrids or the loss of other desirable properties and our data disagrees with the simple genetic theory of flocculence advanced by other workers.

XXX. Labatt Breweries of Canada Limited, Box 5050, 150 Simcoe Street, London, Ontario, Canada. Communicated by G. G. Stewart.

The following is an abstract of a paper that was presented at the recent meeting of the American Society of Brewing Chemists:

SOME OBSERVATIONS ON CO-FLOCCULATION IN SACCHAROMYCES CEREVISIAE
G. G. Stewart and I. F. Garrison

The flocculation characteristics of a number of Saccharomyces cerevisiae strains have been surveyed using the Helm Sedimentation Test and a novel micro test. It has been found that, although some strains are flocculent when cultured in any complete medium, some require a special "inducer" that is present in wort but absent from a defined medium. It is proposed that this "inducer" substance is a polypeptide with a molecular weight less than 12,000.

The flocculation characteristics of two Saccharomyces cerevisiae strains have been studied in some detail. These two strains were non-flocculent when cultured in wort; however, when they were mixed together after growth (or grown together), they were flocculent. This phenomenon of two strains exhibiting non-flocculence alone but flocculence when mixed together has been termed co-flocculation. For co-flocculation to occur the presence of an "inducer polypeptide", in the growth medium, has been found necessary.

Cell wall analysis has revealed certain differences between co-flocculent and non-flocculent cultures; however, conclusions are that it is not simply variations in gross composition of the cell wall that influence the development of flocculation characteristics in a particular strain. The special arrangement of cell wall components, with relation to one another, is of paramount importance.

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

This is the shortened summary of a paper recently published in the Yearbook of the Institute "Progress in the Research of Viticulture and Enology" 6, 263-275, Publishing House of the Slovak Academy of Sciences, Bratislava 1971: Ecology of natural yeast species in the wine region of Hlohovek - Trnava.

Extended investigations on the yeast flora of natural habitats in Czechoslovakia have been made in western Slovakia in the region of Hlohovec-Trnava. The most frequently occurring species in spontaneously fermenting musts is Saccharomyces cerevisiae. The fermentation is started in nearly all cases by Kloeckera apiculata, sometimes in association with Candida pulcherrima (Metchnikowia pulcherrima). The predominant yeast species of refermentations of sweet table wines is Saccharomyces cerevisiae and Saccharomyces oviformis (Saccharomyces bayanus). Film forming yeasts of the genus Candida (C. vini, C. zeylanoides) often occur in young dry wines with low alcohol level causing wine spoilage ("flor"). Saccharomyces uvarum is a frequent accompanying species in fermenting musts and young wines. Other species occurring sporadically on grapes and in musts have no technological importance.

Another paper published on yeast ecology in "Progress in the Research of Viticulture and Enology" 5, 305-320, Bratislava, 1971: Yeasts and yeast-like microorganisms in marginal vine regions of Czechoslovakia.

XXXII. Laboratorios Nacionales de Fomento Industrial, Departamento de Bioquímica, Apdo. Postal 41-537, Mexico 10, D. F. Communicated by O. Paredes-Lopez.

The following is a research report on single cell protein going on in our Institute.

PRODUCTION OF SINGLE CELL PROTEIN USING ALFALFA RESIDUAL JUICE
O. Paredes-Lopez and A. Fernandez-Arias

The aim of these studies is the production of single cell protein using alfalfa residual juice as substrate. Alfalfa plants were pulped and expressed. The juice obtained was coagulated by steam injection. The coagulum was separated for leaf protein concentrate purposes and the remaining juice supplemented with two mineral salts was utilized for the propagation of yeasts.

Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida utilis, Candida tropicalis, Candida sp and Rhodotorula glutinis were tested, selecting Candida sp because of its highest cell concentration. The optimum pH and temperature for growth were 5.0 and 30°C, respectively. At fermentor level a cell growth of 9.2 g/l was obtained. The amino acid profile of the yeast protein is comparable to that of soybean oil-meals, as well as to the FAO standard-reference protein.

XXXIII. Department of Microbiology, Orman Post, Giza, Egypt. Communicated by Amin El-Nawawy.

Below follow abstracts of 5 papers presented during the 2nd Conference of Microbiology, Cairo, 1970. The complete texts are in press.

1) El-Nawawy, Amin S.: Studies on some factors affecting the commercial production of bakers yeast in ARE.

Studies were conducted to increase the yield of commercially produced baker's yeast. Pattern, total yield and the turn-over of the yeast in connection with the fermented molasses and the nitrogen consumption were the factors investigated. The amount of molasses used for fermentation was reduced from four to three tons/batch. Experiments were made to feed this amount exponentially considering the hourly modulus for yeast being 1.167. The results showed that an increase of 6.6 to 23.3% in the crop was obtained when molasses and salts were added exponentially up to the tenth hour (the batch lasts 12 hr). The sugar consumption by the yeasts reached its maximum during the first 6 or 7 hours, it declined thereafter. Decreasing the amount of molasses to three tons instead of four resulted in higher efficiency in utilizing it without any decrease in yeast yield. The turn-over of the yeast crop was 76.8% of the molasses used in the presence of three tons of molasses and 56.38 kg of nitrogen. The conditions kept the alcoholic fermentation to a minimum and this will help to obtain high quality baker's yeast.

2) El-Nawawy, Amin S., M. A. Fouda and Abdel-Kader S. Ahmed: Utilization of petroleum paraffinic fractions for the production of fodder yeast.

Candida tropicalis was adapted to grow on two paraffinic fractions obtained from Morgan wells having pour points of 70 and 110°F. After adaption and selection of one strain, its efficiency on growth and reduction of the pour point of residual paraffinic fraction were studied. The organism was allowed to grow for 7 days on a medium containing 10% paraffinic fractions as the sole source of carbon under vigorous aeration (v/v/m·). From the total volume of 7 l. fermentation liquor, 2.5 l. were withdrawn every 24 hr, and the fermentor replenished with fresh 2.5 l. of new medium. The yeast from each withdrawal was separated by centrifugation, washed 3 times with 1% teepol and once with petroleum ether. The daily yield showed an increase up until the fourth day when it reached 57% of the utilized petroleum fractions. This represented 35% of the total paraffinic fraction added. The same result was obtained on the 7th day when the experiment ended. The pour point of the daily recovered oil fractions showed a decrease from 70°F to 55°F on the second day and to about 40°F from the fourth day on. This pour point brings the used raw fractions to have the quality of "solar" which represents a good and promising result. Analysis of dried yeast showed the following constituents: total carbohydrates (26.8%), total proteins (49.3%), lipids (10.1%), and ash (4.5%). The chromatograms showed the presence of the following classes: phospholipids, free sterols, free fatty acids, triglycerides and hydrocarbons. The hydrocarbons represented the greater part of the lipid content and were identical with the original paraffinic fractions which was attributed to either penetration of paraffinic fractions into cells, or adherance to cell walls.

3) El-Nawawy, Amin S. and M. A. Foda: Utilization of Egyptian Molasses in the production of single cell protein (SCP). A comparative study of three different yeast species.

A comparative study was carried out with three species of yeast, S. cerevisiae, C. utilis, C. pelliculosa, for the pro-

duction of yeast protein when grown on Egyptian blackstrap molasses. The experiments were carried out in three 14-liter fermentors. The starter was propagated in 4 steps in molasses medium (10% molasses, 0.85% ammonium sulphate, 0.2% $K_2HPO_4$). The yields of yeast produced were 43.3, 51.2, 57.0% of the sugar consumed, respectively. From one kg of molasses it was possible to obtain 82.1, 111.6, 128.9 grams of protein in the same order.

4) El-Nawawy, Amin S., M. Abdel Akher and Thereza G. Gabra: Studies on Yeast Polysaccharides.

The present work includes studies on the polysaccharides of different types of yeast produced industrially in ARE i.e. baker's yeast, fodder yeast and brewer's yeast.

The total carbohydrates in yeast differed according to the strain and method of production. It was 33.0, 23.5, 19.5 in baker's yeast, brewer's yeast and fodder yeast, respectively. Baker's yeast contained the highest percentage of trehalose, glycogen and glucan; while fodder yeast contained the highest mannan content. The glycogen was hydrolyzed completely in the presence of 1.0 N $H_2SO_4$ at 134°C for 120 min; while glucan hydrolysis was obtained when treated by 1.0 N $H_2SO_4$ at 100°C for 120 min. The mannan was partially hydrolyzed (88.9%) by 0.3 N HCl at 121°C for 30 min. Increase of acid concentration, temperature, or time of hydrolysis higher than mentioned above resulted in sugar degradation especially with mannan.

5) El-Nawawy, Amin S., S. M. Taha, S. A. Z. Mahmoud and M. H. El-Kattan. Microbiological assay of some vitamins in fermentation raw materials: l-Pantothenic acid content in molasses and other raw materials.

The microbiological assay of the true pantothenic acid was carried out by the turbidimetric method using Lactobacillus casei NRRL B 1445.

It was found that the concentration of pantothenate in different molasses samples was not the same, the average concentration in molasses from Kom-Ombo, Edfu, Koose, and Nagh Hamadi were 36.6, 33.4, 26.5 ppm respectively. There was a noticeable effect of heat treatment. The vitamin content in beet molasses, refinery cane molasses, malt extract, rice bran, corn steep precipitate, and sugar cane syrup was comparatively low. The vitamin balance in baker's yeast production was followed up in four batches.

## XXXIV. INTERNATIONAL AND NATIONAL YEAST MEETINGS

### 1. Commission on Yeasts and Yeast-like Organisms of the International Association of Microbiological Societies (IAMS). Communicated by the Chairman Torsten Wikén.

The following resolution was adopted by the IAMS Commission on Yeasts and Yeast-like Microorganisms on behalf of the participants of the Second Specialized International Symposium on Yeasts held in connection with the Fourth International Fermentation Symposium at Kyoto, March 19-25, 1972.

1. The participants of the Second Specialized Symposium on Yeasts at Kyoto wish to express the most sincere thanks to their Japanese Hosts for a meeting characterized by a perfect organization, a high scientific level and a rare personal

thoughtfulness. This made the meeting extremely profitable and pleasant.

2. The following specialized symposia will be held in the future in accordance with the resolution adopted in Delft and The Hague in 1969:

   a. In Tokyo, August 7-10, 1972, on the yeasts and yeast-like microorganisms of importance in medical science.
   b. At Aulanko (a tourist and congress center 100 km north of Helsinki), Finland, June 4-8, 1973, on the metabolism and regulation of cellular processes in yeasts.

3. The next General Symposium on Yeasts will be held at Vienna, Austria, July 8-12, 1974.

4. Dr. T. Uemura, Japan, and Dr. H. Heslot, France, were nominated as Members of the Commission, while Drs. K. Komagata, H. Kuraishi, Y. Nunokawa and Y. Yamamoto were nominated as Observers.

5. Finally, the participants of the Kyoto Symposium like to express their appreciation of the excellent work done by Professor Dr. H. J. Phaff as editor of the Yeast News Letter.

2. Speech given by Professor Dr. T. O. Wikén, Chairman of the IAMS Commission on Yeasts and Yeast-like Microorganisms, at the banquet in the Kyoto Hotel on Friday, March 24, 1972, on behalf of the Participants of the Second Specialized Symposium on Yeasts held in connection with the Fourth International Fermentation Symposium at Kyoto, March 19-25, 1972.

Ladies and Gentlemen,

Because of the fact that the programme of the Closing Session on Saturday morning is very crowded, I have as Chairman of the IAMS Commission on Yeasts and Yeast-like Microorganisms been asked to announce the following at the banquet:

Specialized Symposia on Yeasts will in the future be held at Tokyo in the period of August 7-10, 1972, and at Aulanko, Finland, in the period of June 4-8, 1973.

The Symposium in Tokyo will deal with yeasts and yeast-like microorganisms of significance in medical science, a field of rapidly increasing importance. The Symposium at Aulanko will as subject have the metabolism and regulation of cellular processes in yeasts.

The next General Symposium will take place at Vienna, Austria, in the period of July 8-12, 1974.

After these announcements I would on behalf of the IAMS Commission on Yeasts and Yeast-like Microorganisms and the Participants of the Second Specialized Symposium on Yeasts here in Kyoto like to say a few words to Dr. Ochi, President of the Science Council of Japan, and to Dr. Terui, Chairman of the Organizing Committee of the Fourth International Fermentation Symposium.

We all highly appreciate that we were allowed to join our meeting with the Fourth International Fermentation Symposium. This was in accordance with the resolution adopted at our meeting in Delft and The Hague, The Netherlands, in 1969, and, furthermore, gave us the opportunity to learn much more about the new experiences in the fields of biochemistry and physiology

of microbes and their industrial application than if we had had only a limited yeast symposium.

We are fully aware of the fact that it was not an easy task to combine the two symposia and we admire the fruitful manner in which you, Dr. Ochi and Dr. Terui, have solved this problem in cooperation with Dr. Hasegawa, Dr. Nagai, Dr. Oshima and their Colleagues.

The symposia were characterized by a perfect organization, a high scientific level and a rare personal kindness and thoughtfulness. This made the scientific sessions extremely profitable and pleasant.

After having listened to the excellent lecture given by Dr. Sakaguchi on the "Historical Backgrounds of the Industrial Fermentations in Japan" we were allowed to study the industries concerned in their present highly developed state.

In addition, we have had the opportunity to admire the beautiful Japanese landscapes and sceneries and to enjoy Japanese theatre, the Japanese food so appealing to the palate of man and the Japanese beverages used for enhancing the pleasure of life.

The meetings undoubtedly represent an important contribution to international understanding and we are all eagerly looking forward to symposia in Japan in the future.

We are sure that the symposium on yeasts of importance in medical science that is now being organized in Tokyo by Dr. Iwata and Dr. Fukazawa and their Colleagues will become as great a success as the present symposium in Kyoto.

For all that we have enjoyed and, I like to add, have survived, we, Participants of the Second Specialized Yeast Symposium, wish to express the most sincere thanks to our distinguished Japanese Hosts. May all of you for all future belong to the group of men which, according to a Japanese religion, do not suffer themselves and save others from suffering or which, according to another classification in the same religion, pass from dark to light or go from light to light!

Thank you.

3. Third International Specialized Symposium on Yeasts. "Metabolism and Regulation of Cellular Processes". Aulanko, Finland, June 4-8, 1973.
Prof. T. O. Wikén - Honorary Chairman (Delft)
Prof. Heikki Suomalainen - Chairman (Helsinki)

MEMBERSHIP

Membership will be open to all persons interested in scientific work on yeasts. Persons accompanying members may apply for Accompanying Membership.

DATE AND PLACE

The Symposium will be held at Aulanko June 4-8, 1973. Aulanko is a Tourist and Congress Center including lodging accommodations, restaurant and cafeteria, saunas, swimming pool, conditionhall etc. It is situated 4 km from the town Hämeenlinna (100 km north of Helsinki) at the lake Vanajavesi. Good connections to Helsinki airport (80 km).

PROGRAM

The program will comprise invited lectures and short reports. As

a rule, each session will be opened by an invited lecturer.

LANGUAGE TO BE USED - PROCEEDINGS OF THE SYMPOSIUM
The official language for the Symposium will be English and all Symposium literature will also be published in English. No simultaneous translation service will be available. On arrival the members will receive the first part of the Proceedings with the abstracts of the papers except the invited lectures. The latter will be published in full a few months after the Symposium.

PROGRAM FOR ACCOMPANYING MEMBERS
An enjoyable program will be organized for the accompanying members.

ACCOMMODATION
Participants will be lodged in Hotel Aulanko at the Congress Center. The capacity of the hotel is, however, limited to 250 beds and, thus, we beg all those wishing to participate to send the application form not later than by May 15, 1972. If the number of participants exceeds the resources of the Congress Center, the Organizing Committee will arrange lodgings in the nearby city, Hämeenlinna. However, the number of participants may have to be limited.

ROOM RATES (estimated)

| | | | |
|---|---|---|---|
| Double room with bath | $20 | Single room with bath | $15 |
| Double room without bath | $17 | Single room with shower | $10 |
| | | Single room without bath | $ 8 |

Breakfast and service included.

REGISTRATION FEE

| | | | |
|---|---|---|---|
| Full member | $60 | Accompanying member | $30 |

PREREGISTRATION AND GENERAL INFORMATION
Those wishing to attend this Symposium are requested to apply for a registration form from the Secretary, Mrs. Christine Waller, M.Sc., c/o Alko, Box 350, SF-00101 Helsinki 10, Finland, as soon as possible. As the forms are to serve as the basis for our card system no further announcements and communications will reach you unless you return this form with your name and full address promptly. Please use typewriter or block letters. Additional copies of the preregistration form may be obtained from the Secretary, Mrs. Christine Waller, M.Sc., c/o Alko, Box 350, SF-00101 Helsinki 10, Finland. (The Editor of the Yeast News Letter has a limited number of application forms available.)

4. Third International Symposium on Yeast Protoplasts. October 2-5, 1972, Salamanca, Spain. President Prof. Julio R. Villanueva; General Secretary Dr. S. Gascón (Dept. of Microbiology, Faculty of Sciences, University of Salamanca, Spain).

Preliminary Program:
Prof. O. Necas, Czechoslovakia, "Regeneration of the cell wall in yeast protoplasts".
H. J. Phaff, U.S.A., "Enzymes involved in the lysis of cell walls of Schizosaccharomyces".
J. S. D. Bacon, Great Britain, "The contribution of β-glucanases to the lysis of fungal cell wall".

J. R. Villanueva, J. M. Sierra and M. Gacto, Salamanca, "Characterization of microbial lytic enzymes active on fungal cell walls".
S. Bartnicki-Garcia, U.S.A., "Wall ontogeny by a natural protoplast".
L. M. Carbonell, Venezuela, "Regeneration of protoplasts from Histoplasma capsulatum".
E. Cabib, U.S.A., "Yeast septum formation as a model system for morphogenesis".
J. O. Lampen, U.S.A., "Control of synthesis and secretion of exoenzymes by yeast protoplasts".
S. Gascón, S., Salamanca, "Biosynthesis and secretion of invertase by yeast protoplasts".
H. Suomalainen, Finland, "Structure and function of the yeast cell envelopes".
R. Sentandreu, Madrid, "Yeast mannan: Structure and biosynthesis".
A. H. Rose, Great Britain, "Modifications to the sterol and fatty acid composition of yeast protoplast membranes".
I. S. Kulaev, USSR, "The enzymes of polyphosphate metabolism in protoplasts and some subcellular structures of Neurospora crassa".
M. D. Berliner, U.S.A., "The induction and regeneration of protoplasts on defined media without external enzymes".
I. Garcia-Acha and F. Uruburu, Salamanca, "Mechanisms of liberation of fungal protoplasts".
J. G. H. Wessels and O. M. H. de Vries, Holland, "Wall degradation, protoplast liberation and wall regeneration in Schizophyllum commune".
I. Potrykus, Germany, "Isolation, fusion and culture of protoplasts from Petunia".
E. C. Cocking, Great Britain, "Isolation, fusion and development of protoplasts of higher plants".
A. W. Linnane, Australia, "The influence of mitochondrial membrane organization on mitochondrial activity and the synthesis of its nucleic acids".
Free Communications.

5. Suita Laboratory, Brewing Science Research Institute, Asahi Breweries Ltd., 5-3 Deguchi-cho, Suita 564, Japan. Communicated by Toshiaki Takahashi.

The fourth meeting of the Yeast Genetics Conference-Japan was held on March 27 and 28 in the Parlor Room, Rakuyu Hall, Kyoto University as a postcongress meeting of the Fourth International Fermentation Symposium. Eight researchers from five foreign countries and 43 domestic researchers attended the meeting.

The morning session on March 27 was chaired by T. Takahashi (Suita) and S. Nagai (Nara). S. Doi and N. Yanagishima (Osaka) reported the isolation of auxin-induced large cell mutants of haploid yeast and the trisomic segregation of hybrids between the mutants and normal haploids. I. Takano (Osaka) described the extraction of cell elongation factor in yeast mating. Nagai induced rho-minus mutants by the use of acid dyes, Rose bengal and Phloxine B. F. Tabusa (Nara) discussed the unstableness of ribosomes in copper-resistant yeast. T. Miura (Tokyo) reported the degeneration of mitochondria in stationary and

senescence phases and the characters of oleic acid requiring mutants obtained by UV irradiation. An oleic acid mutant was also reported by A. W. Linnane (Australia). K. Wakabayashi (Tokyo) demonstrated genetic and biochemical studies of a new segregational petite mutant, pet-431.

H. O. Halvorson (U.S.A.) and N. Yanagishima were chairmen of the afternoon session. Linnane discussed the antibiotics resistances in Saccharomyces, especially mikamycin resistance. This character was controlled by a mitochondrial gene in mitosis and controlled by a nuclear gene in meiosis. H. B. Lukins (Australia) described the recombination and segregation of mitochondrial genes based on bud analysis of the hybrid. M. Crandall (U.S.A.) reported the meiotic and mitotic segregation of ethidium bromide resistance in Hansenula wingei, and the correlated events were also discussed. N. Gunge (Yokohama) demonstrated the mitotic recombination of chromosome III in the production of polyploid yeast between heterozygote on mating type and haploid or homozygote on mating type.

On March 28, N. Yanagishima and C. Shimoda (Osaka) were chairmen of the morning session. Y. Oshima (Osaka) discussed the new model of homothallism controlling system in Saccharomyces. His gene symbols HO and HM were changed to $HM_{\alpha}$ and HO, and $HM_{a}$ gene was newly assumed as a mutator for a type. Takahashi suggested the complex structure of homothallism gene(s) based on the appearance of homothallic segregants in tetrads of UV-irradiated heterothallic diploid. Takano found the $\alpha$ locus, which was not affected by the mutator action of the homothallism gene, in a strain of Sacch. diastaticus, and the $\alpha$ temporarily termed non-convertible $\alpha$ (NC$\alpha$). A. Toh-e (Osaka) and Oshima isolated many mutants on acid phosphatase formation and discussed the regulatory mechanism of the enzyme formation. H. Mori (Noda) described the life cycle of Sacch. rouxii, soya sauce yeast, and lysine-requiring mutants of the yeast.

The afternoon session of the second day was chaired by C. Shimoda and A. W. Linnane. M. Tsuboi, S. Kamisaka (Osaka) and Yanagishima reported the effect of cyclic AMP on glucose repression in yeast sporulation. Halvorson described the existence of one hundred and forty ribosomal RNA cistrons in Saccharomyces and the studies on distribution of the cistrons by the use of disomic strains. Finally, C. Robinow (Canada) demonstrated the nuclear cytology in yeasts by showing many microphotographs.

The organizing committee of the Yeast Genetics Conference-Japan wishes to express many thanks to foreign participants in the fourth meeting for their interesting lectures and valuable comments to the domestic researchers.

The next meeting will be held after the Sixth International Conference on Yeast Genetics and Molecular Biology, in fall 1972.

6. New York Metropolitan Yeast Club, Stevens Institute of Technology, Hoboken, New Jersey 07030. Communicated by L. S. Baskin and I. R. Lapidus.

The first meeting of the New York Metropolitan Yeast Club was held at Stevens Institute of Technology on February 16, 1972. Yeast workers from Rutgers University, Columbia University, Brooklyn College, Albert Einstein College of Medicine, New York

Blood Center, and Stevens Institute of Technology attended.

The purpose of the Yeast Club is to provide an opportunity for persons carrying out research with yeast to meet and exchange ideas. The scientific sessions are informal. Presentations were made by Dr. John Blamire (Albert Einstein College of Medicine), Dr. Joseph Lampen (Institute of Microbiology, Rutgers University), Dr. Frederick Zimmermann (Brooklyn College), and Dr. Leonard Baskin (Stevens Institute of Technology). There was considerable discussion and exchange among the other participants.

The second meeting of the New York Metropolitan Yeast Club took place at Stevens Institute of Technology on May 12, 1972. Yeast workers from Albert Einstein College of Medicine, Brandeis University, Brooklyn College, Merck Institute, Rutgers University, and Stevens Institute of Technology gathered to discuss a number of research reports and exchange ideas.

The program included:

1. C. A. Michels, Barbara Goldfinger and Julius Marmur, Albert Einstein College of Medicine, "Do Suppressive Petites Actually Suppress?"
2. A. K. Bose and B. L. Hungund, Stevens Institute of Technology, "Role of Amino Acids in Yeast Steroid Biosynthesis".
3. B. L. A. Carter, S. Sogin and H. O. Halvorson, Brandeis University, "Temporal Events During the Cell Cycle of S. cerevisiae".
4. N. Eaton, N. Khan and F. Zimmermann, Brooklyn College, "Genetics of Disaccharide Fermentation in Yeast".
5. S. J. Sogin, J. Haber and H. O. Halvorson, Brandeis University, "Synthesis of Sporulation Specific RNA by S. cerevisiae".
6. L. S. Baskin and J. Savarese, Stevens Institute of Technology, "Germination of Saccharomyces cerevisiae Spores".

The next meeting of the Yeast Club will take place at Stevens in September, 1972. In addition to the informal presentations an invited speaker will give a more formal talk.

For further information regarding the activities of the Yeast Club, inquiries should be sent to Dr. Leonard S. Baskin or Dr. I. Richard Lapidus, New York Metropolitan Yeast Club, Stevens Institute of Technology, Hoboken, New Jersey 07030 (Tel.: 201-792-2700).

7. Laboratorio di Mutagenesi e Differenziamento - C.N.R., c/o Instituto di Genetica dell-Universita, 56100 Pisa - Viale Matteotti, 1/A, Italy. Communicated by Prof. N. Loprieno.

The Sixth International Conference on Yeast Genetics and Molecular Biology, sponsored by E.M.B.O., and C.N.R., will be held in Pisa, Italy, on September 5-9, 1972. Following the tradition of previous meetings, the Conference will be completely informal. There will be no official program and formal paper presentations. Participation will be limited to a small number of invited scientists.

A report of the Conference will be sent to the Yeast News Letter.

XXXV. Brief News Items

1. The following paper has recently been published:

ACIDE OLÉANOLIQUE, FACTEUR DE CROISSANCE ANAÉROBIE DE LA LEVURE DE VIN
P. Bréchot, J. Chauvet, P. Dupuy, Madeleine Croson and Arlette Rabatu
Laboratoire des Fermentations, Institut Pasteur, 75 Paris ($15^{e}$), France.

Ann. Technol. agric., 1971, 20(2), 103-110.

SUMMARY

OLEANOIC ACID AS A GROWTH FACTOR FOR WINE YEAST UNDER ANAEROBIC CONDITIONS.

The wax which covers the grapes contains a factor increasing the anaerobic growth of yeasts. The main constituent extracted by chloroform from fresh grapes is oleanoic acid, an oxyterpenic acid. It crystallizes from hot alcohol and can be purified by preparative thin layer chromatography. Whether in the presence of oleic acid or not, the action of this product has been compared with that of ergosterol. Anaerobic cultures of S. cerevisiae produce more growth and ferment more sugar in presence of oleanoic acid than in presence of ergosterol, which has been reported as a growth factor by Andreasen and Stier (1954).

This finding can explain why an abundant growth of yeasts occurs even when the grapes are placed in anaerobic conditions from the start of the wine making process.

2. Department of Dermatology, University Hospital, 59 Oostersingel, Groningen, The Netherlands.

The following publication is currently in press in the Canadian Journal of Botany:

N. J. W. Kreger-van Rij and M. Veenhuis, "Some features of vegetative and sexual reproduction in Endomyces species".

N. J. W. Kreger-van Rij

3. Massachusetts Institute of Technology, Department of Nutrition and Food Science, Cambridge, Massachusetts 02139.

The following is an upcoming publication, most of which deals with yeasts:

Arnold L. Demain, "Riboflavin Oversynthesis", Ann. Rev. Microbiol. 26, 1972 (in press).

A. L. Demain

4. Harvard School of Public Health, Department of Microbiology, 665 Huntington Avenue, Boston, Massachusetts 02115.

I am now working on defined minimal media for induction of protoplasts and should be reporting on this material shortly. In January of 1972 I gave a seminar on yeast protoplasts at Instituto Venezolano de Investigaciones Cientificas in Caracas, Venezuela, and have been promoted to Senior Research Associate here at Harvard.

The following two publications have appeared recently:

M. D. Berliner, "Induction of protoplasts of Schizosaccharomyces octosporus by magnesium sulfate and 2-deoxy-D-glucose", Mycologia 63, 819-825, 1971.

M. D. Berliner and M. E. Reca, "Studies on protoplast induction in the yeast phase of Histoplasma capsulatum by magnesium sulfate and 2-deoxy-D-glucose", Mycologia 63, 1164-1172, 1971.

Martha D. Berliner

5. Institute for Fermentation, Juso-Nishino-cho, Higashiyodogawa-ku, Osaka, Japan.

The cultures in I.F.O. Culture Collection have been charged for distribution since 1969. The following charge schedule for each culture is effective after May 1, 1972. There is no charge for postage of cultures sent to foreign organizations by air mail.

Distribution charges:

| | |
|---|---|
| For non-profit organization | $ 5.00 |
| For commercial firms | $13.25 |

Prices of publications (including postage):

| | |
|---|---|
| I.F.O. LIST OF CULTURES, 1972 | $3.00 |
| I.F.O. RESEARCH COMMUNICATIONS | $3.00 |

The institute has its own periodical that had been issued biennially up to the fourth publication under the title of "Annual Report". This title was changed to "Institute for Fermentation, Osaka, Research Communications" from the fifth edition in 1971. The serial contains original articles on mycology, bacteriology and microbial genetics. Back numbers are still available.

Takezi Hasegawa
Director-in-chief

## XXXVI. YEAST GENETICS STOCK CENTER CULTURE LIST

The Yeast Genetics Stock Center has been established for the purpose of maintaining and making available strains of Saccharomyces cerevisiae containing the most commonly studied genetic loci. The following stock list presents an inventory of the strains now on deposit with the Stock Center. We invite correspondence concerning the deposition of stocks that you believe should be available through the Stock Center. Supplements to the stock list will be published in forthcoming issues of the Yeast News Letter. Also, stock lists will be available on request.

1. Wild type strains:

| | |
|---|---|
| X2180-1A | a SUC2 mal gal2 CUP1 |
| X2180-1B | α SUC2 mal gal2 CUP1 |

2. Nutritional tester strains:

Adenine

| Locus | Strain | Genotype |
|---|---|---|
| ade1 | X464-1A | a ade1 his2 leu1 trp1 gal1 mal |
| | X464-20C | α ade1 his2 leu1 trp1 gal1 mal |
| ade2 | X1687-16C | a ade2-1 trp5-48 arg4-17 his5-2 lys1-1 leu1-12 met1-1 |
| | JB120 | α ade1 lys2 |
| ade3 | S1238D | a ura1 ade3 |
| | α ade3 HR | α ade3* |
| ade4 | a ade4 HR | a ade4* |
| | α ade4 HR | α ade4* |
| ade5 | a ade5 HR | a ade5* |
| | α ade5 HR | α ade5* |
| ade6 | S1795A | a his4 trp5 ade6 ura1 gal2 |
| | S1799D | α his4 trp5 ade6 gal2 |
| ade7 | a ade7 HR | a ade7* |
| | α ade7 HR | α ade7* |
| ade8 | X1056-2D | a his8 ser1 ade8 ade9 ura3 met1 leu1 lys7 trp1 trp5 |
| | X1056-1C | α his8 ade8 met2 leu1 lys7 trp1 |
| ade9 | S2649B | a gal1 ade9 trp1 ura3 hom3 |
| | S2649C | α gal2 ade9 leu1 his6 hom3 |

* Complete genotype not known.

## Arginine

| Locus | Strain | Genotype |
|---|---|---|
| arg1 | S1586C | a ura1 ade5 arg1 |
| | S1588C | α met1 ade5 trp3 arg1 |
| arg2 | ---- | a arg2 - not available |
| | S288C-22 | α arg2 |
| arg3 | --- | a arg3 - not available |
| | JA17 | α ade2 arg3 |
| arg4 | S2072A | a leu1 trp1 arg4 |
| | S2072D | α leu1 trp1 thr4 arg4 |
| arg6 | D160-4D | a ura3 hom3 his1 arg6 ilv1 trp2 ade1 |
| | D160-2C | α ura3 hom3 his1 arg6 ilv1 trp2 ade1 |
| arg7 | S2684D | a leu1 trp5 his2 his8 ade1 arg7 |
| | GA27 | α arg7 |
| arg8 | X1011-1A | a ade1 arg8 |
| | JB82 | α ade2 arg8 |
| arg9 | X1265-2A | a his4 leu2 thr4 ade6 his2 ura1 ade2 arg9 |
| | JB11 | α ade2 arg9 |
| arg10 | JD33 | a ade2 arg10 |
| | JB134 | α ade2 arg10 |

## Glutamic

| | | |
|---|---|---|
| glu1 | X1251-J1D | a his4 thr4 leu2 |
| | X1251-J2A | α leu2 |

## Histidine

| | | |
|---|---|---|
| his1 | S395D | a leu1 trp2 his1 |
| | S394B | α met1 his1 |
| his2 | X2181-1A | a gal1 trp1 ade1 his2 |
| | X2181-1B | α gal1 trp1 ade1 his2 |
| his4 | S1795A | a his4 ura1 trp5 ade6 |
| | S1799D | α his4 trp5 ade6 |
| his5 | S827A | a ade1 his5 trp1 |
| | S288C-26 | α gal2 his5 |

| Locus | Strain | Genotype |
|---|---|---|
| his6 | X901-35C | a hom2 aro1 trp5 leu1 ade6 lys1 his6 ura1 arg4-1 thr1 CUP1 |
| | X901-26A | α hom2 aro1 trp5 leu1 ade6 his6 ura1 arg4-2 thr1 CUP1 |
| his7 | S878C | a leu1 his7 |
| | N14 | α gal2 his7 |
| his8 | S1237B | a ura1 lys2 his8 |
| | DY117 | α gal2 his8 |

Leucine

| | | |
|---|---|---|
| leu1 | X901-35C | a hom2 tyr4 trp5 leu1 ade6 lys1 his6 ura1 arg4-1 thr1 |
| | X901-26A | α hom2 tyr4 trp5 leu1 ade6 his6 ura1 arg4-2 thr1 CUP1 |
| leu2 | X1069-1A | a ade1 his4 leu2 thr4 met2 ura1 trp5 |
| | X1069-2D | α ade1 his4 leu2 thr4 met2 ura1 trp5 |
| leu3 | X2203-13C | a leu3 |
| | | α leu3 - not available |

Tryptophan

| | | |
|---|---|---|
| trp1 | X2181-1A | a gal1 trp1 ade1 his2 |
| | X2181-1B | α gal1 trp1 ade1 his2 |
| trp2 | D160-4D | a ura3 hom3 his1 arg6 trp2 ade1 met1 |
| | D160-2C | α ura3 hom3 his1 arg6 trp2 ade1 met1 |
| trp3 | S1783D | a gal1 his6 thr1 arg4 ura2 ade1 ura1 lys1 trp3 |
| | S1788B | α ura2 ura1 lys1 trp3 |
| trp4 | X1011-1B | a trp4 ade1 ade2 |
| | X1011-6D | α his6 trp4 ura2 ade1 asp5 |
| trp5 | S1795A | a his4 trp5 ade6 ura1 |
| | S1799D | α his4 trp5 ade6 |

Tyrosine

| | | |
|---|---|---|
| tyr1 | S507C | a ade2 leu1 tyr1 |
| | AC39 | α tyr1 |
| tyr2 | S544A | a lys1 tyr2* |
| | AM26 | α tyr2* |

| Locus | Strain | Genotype |
|---|---|---|
| tyr3 | S2299B | a leu1 his4 tyr3* |
| | AQ14 | α tyr3* |
| tyr4 | S2626C | a hom2 tyr4 leu1 his2 trp1 arg4-1 thr1* |
| | S2621B | α hom2 tyr4* |

* tyr2, 4, 6 comprise part of a complex locus with the proposed designation aro1 ABCDE, tyr3 has been designated aro2. Yeast Genetics Supplement to Microbial Genetics Bulletin #31, Nov. 1969.

Threonine

| | | |
|---|---|---|
| thr1 | S960C | a gal1 gal2 trp1 thr1 |
| | AN33 | α thr1 arg1 |
| hom2* | S2614C | a hom2 aro1 lys1 ade1 trp1 arg4-1 |
| | S2615B | α hom2 aro1 leu1 ade5 ade1 |
| hom3* | S2206D | a gal2 his2 his6 hom3 ura3 leu met |
| | S2207A | α gal2 his6 hom3 ura3 met |
| thr4 | S2073B | a gal2 leu1 ade1 thr4 |
| | S2068B | α gal2 trp1 ade1 thr4 |
| asp5* | X1049-9C | a trp1 ura3 his8 asp5 arg8 |
| | X1049-2B | α his2 trp1 ura3 lys7 his8 ade2 asp5 arg8 |
| hom6* | | a hom6 - not available |
| | X1264-1A | α his4 ade1 ade6 ade2 ura1 hom6 |

* The designations hom2, hom3, asp5 and hom6 refer to thr2, thr3, thr5 and thr6 respectively. Yeast Genetics Supplement to Microbial Genetics Bulletin #31, Nov. 1969.

Isoleucine-Valine

| | | |
|---|---|---|
| ilv1 | D160-4D | a ura3 hom3 his1 arg6 ilv1 trp2 ade1 |
| | D160-2C | α ura3 hom3 his1 arg6 ilv1 trp2 ade1 |
| ilv2 | X869-JB2 | a ade2 ilv2 |
| | X869-JB5 | α ade2 ilv2 leu1 his |
| ilv3 | X3424-5D | a trp1 ilv3 lys1 gal2 |
| | X3424-6C | α leu2 his6 ilv3 ade2 arg4 lys1 gal2 |

## Lysine

| Locus | Strain | Genotype |
|---|---|---|
| lys1 | S720B | a trp4 lys1 his2 |
| | S288C-24 | α lys1 |
| lys2 | S856C | a ade1 lys2 |
| | P49 | α lys2 |
| lys4 | X3356-1A | a lys4 |
| | X3356-1B | α lys4 |
| lys5 | S1388B | a gal1 gal2 trp1 ade1 his2 thr1 lys5 |
| | AB9 | α gal2 lys5 |
| lys6 | X3355-1A | a lys6 pet |
| | X3355-1B | α lys6 pet |
| lys7 | X3127-2A | a leu2 leu1 trp1 pet17 ura3 thr1 arg4 lys7 his5 |
| | X3127-27D | α leu2 leu1 trp1 pet17 thr1 arg4 lys7 ade1 |
| lys8 | X815-1C | a lys8 his4 arg4 pet1 ade1 ura3 leu1 |
| | X815-1B | α lys8 his6 arg4 hom3 pet1 gal1 trp1 |
| lys9 | X1012-1D | a lys9 ade2 ade1 |
| | JB101 | α lys9 ade2 |
| lys10 | X3357-1D | a lys10 |
| | X3357-1C | α lys10 |

## Methionine

| | | |
|---|---|---|
| met1 | S416B | a met1 ura1 trp1 ade6 ade3 ade2 |
| | S416A | α met1 ura1 ade2 |
| met2 | X963-18B | a gal1 trp1 trp5 ura3 met2 lys7 |
| | X963-18C | α his8 met2 ade8 |
| met3 | 30-3 | a met3* |
| | | α met3 - not available |
| met4 | S1336B | a ade1 leu1 his2 met4 |
| | P65 | α met4* |
| met5 | S1731A | a trp1 leu1 his2 thr1 met5 |
| | AB2 | α met5 |
| met6 | 22B | a met6* |
| | Y16 | α met6 |
| met7 | S1896D | a trp1 leu1 met7 |
| | F33 | α met7 |

| Locus | Strain | Genotype |
|---|---|---|
| met8 | 35730 | a met8* |
| | EY9 | α met8 can1 |
| met10 | X1266-1C | a his4 leu2 ura1 met10 ade2 |
| | X1266-1D | α thr4 ade1 trp1 ura1 met10 ade2 |
| met13 | XS144-S19 | a met13 leu1 trp5 cyh2 tyr3 lys5 ade5 |
| | XS144-S22 | α met13 leu1 trp5 cyh2 tyr3 lys5 ade5 |
| met14 | X1914-32A | a met14* |
| | X1692-5A | α met14* |

* Complete genotype not known.

Uracil

| | | |
|---|---|---|
| ura1 | S1786B | a gal1 his6 ade1 ura1 trp3 |
| | S1780D | α gal1 arg4 ade1 ura1 lys1 trp3 |
| ura2 | S1780B | a gal1 thr1 ura2 ade1 lys1 ura4 |
| | S1780C | α his6 thr1 arg4 ura2 met1 ura4 |
| ura3 | S2021B | a trp5 leu1 his4 ura3 |
| | S2022D | α trp5 leu1 ade6 ura3 |
| ura4 | S2112D | a his4 ura4 ura2 |
| | S2115C | α trp5 leu1 hom3 ura4 ura2 |

3. Temperature sensitive lethals:

| Mutant | Locus-allele | Defect | Ref. |
|---|---|---|---|
| 187 | prt1-1 | initiation of protein synthesis | 1,4,5,10 |
| 241 | prt2-1 | translation | 4 |
| 275 | prt3-1 | translation | 4 |
| 19·3·4 | mes1-1 | methionyl-tRNA synthetase | 4,6 |
| 341 | ils1-1 | isoleucyl-tRNA synthetase | 3,4,8 |
| 437 | --- 1 | energy metabolism? | 4 |
| 132 | --- 1 | membrane? | 4 |
| 134 | --- 1 | membrane? | 4 |
| 441 | --- 1 | membrane? | 4 |
| 136 | rna1-1 | synthesis of all RNA species | 4,7,9,10 |

| Mutant | Locus-allele | Defect | Ref. |
|---|---|---|---|
| 368 | rna2-1 | synthesis of rRNA | 13 |
| 125 | rna3-1 | synthesis of rRNA | 13 |
| 339 | rna4-1 | synthesis of rRNA | 13 |
| 108 | rna5-1 | synthesis of rRNA | 13 |
| 166 | rna6-1 | synthesis of rRNA | 13 |
| 202 | rna7-1 | synthesis of rRNA | 13 |
| 219 | rna8-1 | synthesis of rRNA | 13 |
| 257 | rna9-1 | synthesis of rRNA | 13 |
| 261 | rna10-1 | synthesis of rRNA | 13 |
| 382 | rna11-1 | synthesis of rRNA | 13 |
| 369 | cdc1-1 | bud emergence | 11,17 |
| 370 | cdc2-1 | early nuclear division | 11,16 |
| 104 | cdc3-1 | cytokinesis | 11,17 |
| 314 | cdc4-1 | initiation of DNA synthesis | 15,18 |
| 473 | cdc5-1 | --- | --- |
| 327 | cdc6-1 | medial nuclear division | 16 |
| 124 | cdc7-1 | medial nuclear division | 16 |
| 198 | cdc8-1 | DNA synthesis | 1,15,18 |
| 244 | cdc9-1 | medial nuclear division | 16 |
| 17-12 | cdc10-1 | cytokinesis | 17 |
| 332 | cdc11-1 | cytokinesis | 17 |
| 471 | cdc12-1 | cytokinesis | 17 |
| 428 | cdc13-1 | medial nuclear division | 16 |
| 7-41 | cdc14-1 | late nuclear division | 16 |
| 17-17 | cdc15-1 | late nuclear division | 16 |
| 13-53 | cdc15-2 | late nuclear division | 16 |
| 269 | pop1-1 | wall or membrane | --- |
| 315 | pop2-1 | wall or membrane | --- |
| 186 | pop3-1 | wall or membrane | --- |
| 233 | pop4-1 | wall or membrane | --- |
| 254 | pop5-1 | wall or membrane | --- |
| 308 | pop6-1 | wall or membrane | |
| 333 | pop7-1 | wall or membrane | --- |
| 197 | pop8-1 | wall or membrane | --- |

PARENT STRAIN: A364A a ade1 ade2 ura1 his7 lys2 tyr1 gal1

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