

Y E A S T

A News Letter for Persons Interested in Yeast

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Many thanks to those who have contributed to this issue by sending in news items and accounts of research projects. The next issue will be published in Dec. 1971. A contribution of \$1.00 from those who have not contributed for some time would be appreciated to finance future editions of the News Letter. Many thanks to those who have contributed recently.

H. J. Phaff

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

Below follows a list of newly described species which have been received by the C.B.S. since the last issue of the News Letter.

Candida lactose CBS 6266 (= NRRL Y-7170)

D. Dwidjoseputro & F. T. Wolf, Mycopath. Mycol. Appl. 41:211-222 (1970).

Cryptococcus neoformans v. gatti CBS 6289 (Type), CBS 6290

R. Vanbreuseghem & M. Takashio, Ann. Soc. belge Med. trop. 50:695-702 (1970).

Hansenula malanga CBS 6267 (= NRRL Y-7175)

D. Dwidjoseputro & F. T. Wolf, Mycopath. Mycol. Appl. 41:211-222 (1970).

Pichia ambrosia CBS 6003 (Type), CBS 6004

J. P. van der Walt & D. B. Scott, A.v. Leeuwenhoek 37:15-20 (1971).

Pichia heimii CBS 6139 (Type)

M. C. Pignal, A.v. Leeuwenhoek 36:525-529 (1970).

Pichia spartinae CBS 6059 (Type), CBS 6077

D. G. Ahearn, D. Yarrow & S. P. Meyers, A.v. Leeuwenhoek 36:503-508 (1970).

Saccharomycopsis syneadendra CBS 6161 (Type), CBS 6186

D. B. Scott & J. P. van der Walt, Mycopath. Mycol. Appl. 44:101-106 (1971).

II United States Department of Agriculture, Northern Utilization Research and Development Division, 1815 North University Street, Peoria, Illinois 61604. Communicated by C. P. Kurtzman.

Below follow two abstracts of recent publications from this laboratory.

Kurtzman, C. P., R. Rogers, and C. W. Hesseltine. Microbiological Spoilage of Mayonnaise and Salad Dressings. Appl. Microbiol. May 1971. "Microbiological spoilage of mayonnaise and salad dressings has once again become a problem in various areas of the U.S. Saccharomyces bailii was isolated from two-thirds of the spoiled mayonnaise and salad dressing samples, and most of the remainder were spoiled by Lactobacillus fructivorans. Lactobacillus plantarum was isolated from one spoiled sample along with large numbers of Sacc. bailii. Two spoiled samples and one unspoiled sample contained small numbers of Bacillus spp. Spoiled dressings generally had a higher concentration of glucose and other monosaccharides than did the unspoiled dressings."

Wickerham, L. J. and C. P. Kurtzman. Two New Saturn-Spored Species of Pichia. Mycologia 1971.

In press. "Pichia mucosa, NRRL YB-1344, was isolated from topsoil in Illinois and is noteworthy for the large amount of extracellular polysaccharide which it produces. Pichia sargentensis, NRRL YB-4139, was isolated from the outlet of a small lake in New Hampshire. Both species are homothallic, ferment glucose, and produce pseudohyphae but not true hyphae."

III Agricultural Research Council, Food Research Institute, Colney Lane, Norwich Nor 70F, England. Communicated by James A. Barnett.

We are studying the number and kinds of yeast associated with fresh soft fruit. Such quantitative surveys have been done usually by assuming that surface colonies which look alike are identical, and picking for identification only those differing from each other. Because I do not think this procedure gives adequate evidence of identity, I have (i) guessed at the most likely species on fruit and (ii) studied the problem of how to select the minimum number of nutritional tests to establish the identity of the species. We have now isolated and identified over 1500 strains of yeast during the last 3 years and tested every strain. We have done a thorough survey of local marketed strawberries and preliminary work on blackcurrants and plums. This work has led to the following papers: (i) Selecting tests in diagnostic keys with unknown responses, J. C. Gower and J. A. Barnett, Nature (in the press); (ii) Selection of tests for identifying yeasts, J. A. Barnett, Nature New Biology (in the press); (iii) Torulopsis fragaria species nova, a yeast from fruit, J. A. Barnett and R. W. M. Buhagiar J. gen. Microbiol. (in the press); (iv) The yeasts of strawberries, R. W. M. Buhagiar and J. A. Barnett (not yet accepted for publication).

Connected with this fruit yeast work, I am following up my complaints [Nature 229: 578 (1971)] about the identification key in The Yeasts, A Taxonomic Study, ed. by J. Lodder (1970): using computer methods, I am trying to write a new key which would be based primarily on the physiological tests instead of on ascosporeulation.

In addition to this taxonomic work, I have been pursuing a little of my main interest, namely the utilization of pentoses and polyols by yeasts. In particular I have been examining the catabolism of D-ribose, ribitol, L-arabinose and D-xylose by species of Torulopsis and Pichia. I am looking for yeasts that utilize D-ribose strongly and ribitol not at all. The best I have is one of van Uden's strains of Torulopsis magnoliae; but this grows on D-ribose with a doubling time of only about 60 hr., compared with 4 hr. on D-glucose. Can anyone please send me one or more yeasts that grows fast on D-ribose and not at all on ribitol?

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

Below follows an abstract of a recent publication.

Studies on Himalayan yeasts and molds. IV. Several asporogenous yeasts including two new taxa of Cryptococcus. Shoji Goto and Tunta Sugiyama.

Summary

Thirteen strains representing six asporogenous yeast species were isolated from soil, dung, and forest humus samples collected in Bhutan. The following species, including two new taxa of Cryptococcus, were recovered: Cryptococcus albidus, Cr. bhutanensis sp. nov., Cr. diffluens, Cr. himalayensis sp. nov., Torulopsis candida, and Rhodotorula glutinis.

Cr. bhutanensis may be most closely related to Cr. diffluens. However, this yeast does not assimilate i-inositol and D-ribose. Cr. himalayensis is characterized by its ability to assimilate nitrate and its inability to assimilate maltose.

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

Dr. S. P. Meyers and Dr. D. G. Ahearn (Department of Biology, Georgia State University, Atlanta) presented the following papers at the ASM meeting in Minneapolis, Minnesota, May 4, 1971.

- 1) Occurrence of pulcherrimin-producing yeasts in Louisiana marshland sediments. Bacteriol. Proc., 71, G-74. (S. P. Meyers, P. Miles, and D. G. Ahearn).
- 2) Effect of oil on Louisiana marshland yeast populations. Bacteriol. Proc., 71, G-75. (S. P. Meyers, D. G. Ahearn, S. Crow and N. H. Berner).

Abstracts of these two papers follow:

- 1) A selective and diagnostic defined medium, enhancing production of pulcherrim, a ferric salt of pulcherriminic acid, was developed for isolation of Kluyveromyces drosophilorum from Spartina alterniflora marshland sediment in Southeastern Louisiana. Ready differentiation of K. drosophilorum from the associated sporogenous yeast, Pichia spartinae, is possible. At five random sites examined periodically over a 16 month period, total yeast populations as great as 90,000 viable cells/g wet sediment occurred, with K. drosophilorum comprising about 20% of the yeast standing crop. Random isolates demonstrated homogeneous physiological properties but diverse ascospore morphology. On yeast extract-malt extract agar, with trace amounts of iron, numerous strains formed deep maroon colonies. Large thick-walled pigment-bearing cells were present. On the selective galactose agar, containing 2.0% galactose in yeast nitrogen base, all strains consistently produced dark-rose colored colonies or a pinkish discoloration of the agar beneath the colony. Pigment production is independent of the nitrogen source used. The abundance of the yeast, and its active production of an iron-chelating compound, suggests a heretofore unsuspected ecological role for Kluyveromyces species in the marshland iron cycle.

2) Spartina alterniflora marshes in Southeastern Louisiana are characterized by dense (ca. 50,000 viable cells/g wet sediment) populations of yeasts of the sporogenous genera Pichia* and Kluyveromyces. Representative isolates initially are unable to utilize crude oil or its fractions as sole carbon sources for growth. In oil enrichment cultures, populations of P. spartinae eventually developed weak hydrocarbonoclastic activity. These organisms grew slowly and emulsified the gas-oil fraction of crude oil with optimal growth on simple alkanes (C₁₄-C₁₈). In oil-saturated marshland test plots, total populations of yeast increased slowly over a several month period with a shift in species density from the sporogenous forms to strains of Rhodotorula and Trichosporon. These latter yeasts utilized a broad spectrum of crude oil fractions and alkanes from C₉ to C₁₈. In contrast, areas with a history of oil pollution yielded, on initial isolation, strains of Trichosporon and Candida which rapidly utilized a range of hydrocarbons. The indigenous yeast biota of the Spartina marshland appears unable to readily biodegrade oil. Facilitation of oil biodegradation by seeding with appropriate hydrocarbonoclastic yeasts is suggested.

*described as a new species of Pichia (Pichia spartinae sp. n. from Louisiana marshland habitat. D. G. Ahearn, D. Yarrow and S. P. Meyers. Ant. van Leeuwenhoek 36: 503-508. 1970)

As a part of the LSU Sea Grant program and studies of Louisiana marshland productivity, a special Sea Grant issue of Coastal Studies Bulletin (No. 6), February 1971, has been prepared. This issue deals with the Barataria Bay marsh research project and the overall marine science research and teaching program at Louisiana State University. The following marine microbiological papers have appeared (reprints available):

Microbiological studies of Barataria Bay. Louisiana State Coastal Studies Bulletin, 6: 1-6 (S. P. Meyers).

Characterization of yeasts in Barataria Bay. Louisiana State University Coastal Studies Bulletin, 6: 7-15 (S. P. Meyers, D. G. Ahearn and P. Miles).

Dr. Meyers has graduate student support for applicants interested in estuarine yeast ecology and applied food-related areas. This program emphasizes development of marine food resources and combines both food and marine science training. Interested students may contact Dr. Meyers directly.

VI Lehrkanzel für Biochemische Technologie, Institut für Lebensmitteltechnologie, Hochschule für Bodenkultur, Vienna, Austria. Communicated by H. Klaushofer.

Since our last contribution the following publications have appeared:

K. Dorfwirth: "Versuche zur Ascosporenbildung auf saurem Würzeagar". (The formation of ascospores on wort agar of low pH).
Mitteilungen d. Versuchsstation f.d.

Gärungsgewerbe, Wien 24(11) 167-169
(1970).

K. Dorfwirth: "Zum gegenwärtigen Stand der Hefesystematik". (The present situation of yeast systematic).
Mitteilungen d. Versuchsstation f. d. Gärungsgewerbe, Wien, EBC-number 1971 (in press).
This paper is a short summary of the taxonomic study of J. Lodder's "The Yeasts" (1970). There are two diagrams presented, one dealing with the origin of yeasts and yeast-like organisms, the other showing the distribution of the species within the different genera.

At the I. International Specialized Symposium on Yeasts at Smolenice, CSSR, 1-4 June 1971, the following paper will be presented:

K. Dorfwirth: "Taxonomic studies of yeasts isolated from mead". Isolations of yeasts by membrane filter technique were made from mead brewed under various conditions and the organisms identified. Following the methods of Lodder the morphological and physiological characteristics were determined.

The strains were classified as Saccharomyces inusitatus, Sacch. cerevisiae, Sacch. bisporus var. mellis and Rhodotorula glutinis.

In order to indicate the degree of affinity among them and the type strain of the described species the methods of numerical taxonomy have been used. The computed percentage of similarity shows a clear differentiation of the four species. It was found that the coefficient of Sokal and Michener was the most suitable.

VII Leslie R. Hedrick, 14225 SW 150th Avenue, Portland, Oregon 97223.

Leslie Hedrick writes that the paper whose abstract follows below, is now in press in the Journal of General Microbiology.

Deoxyribonucleic Acid Base Composition and Numerical Taxonomy of Yeasts in the Genus Trichosporon

By P. F. Dupont and L. R. Hedrick
Biology Department, Illinois Institute of Technology
Chicago, Illinois 60616

SUMMARY

Of the 49 strains of Trichosporon yeasts studied, 40 were retained in the genus and assigned to 10 species. The others were transferred to the genera Candida and Endomycopsis. The DNA base composition was determined for a representative of each Trichosporon species. On the basis of guanine and cytosine content (% GC), the species were arranged into 4 groups (a) about 55% GC, T. aculeatum, T. aquatile, T. cutaneum, T. inkin and T. pullulans; (b) about 64% GC, T. eriense, T. fermentans and T. infestans; (c) 59% GC, T. capitatum and (d) 45% GC T. penicillatum.

For 25 strains of the genus, 81 characteristics were selected for inclusion in a numerical taxonomic analysis with the aid of a computer. The dendrogram of the similarity values arranged these strains into three major branches arising at the 66% S level. Branch I includes T. aculeatum, T. cutaneum, T. infestans, T. inkin and T. pullulans. Branch II is limited to T. aquatile. Branch III includes T. capitatum, T. eriense, T. fermentans and T. penicillatum. Species arising from branch I at differing S levels are T. aculeatum at 68% S, T. pullulans at 72% S, T. inkin at 75% S and T. infestans at 78% S. The remaining strains in branch I belong to the T. cutaneum group.

VIII Ajinomoto Co., Inc., Central Research Laboratories, Suzuki-cho, Kawasaki, Japan. Communicated by T. Nakase.

The following papers recently have been published:

Significance of DNA base composition in the classification of yeast genus Pichia. T. Nakase and K. Komagata. J. Gen. Appl. Microbiol., 16, 511-521 (1970).

Significance of DNA base composition in the classification of yeast genus Debaryomyces. T. Nakase and K. Komagata. J. Gen. Appl. Microbiol., 17, 43-50 (1971).

Further investigation on the DNA base composition of the genus Hansenula. T. Nakase and K. Komagata. J. Gen. Appl. Microbiol., 17, 77-84 (1971).

The following papers are now in press:

Significance of DNA base composition in the classification of yeast genus Saccharomyces. T. Nakase and K. Komagata. J. Gen. Appl. Microbiol.

Significance of DNA base composition in the classification of yeast genus Candida. T. Nakase and K. Komagata. J. Gen. Appl. Microbiol.

Significance of DNA base composition in the classification of the yeast genus Torulopsis. T. Nakase and K. Komagata. J. Gen. Appl. Microbiol.

Significance of DNA base composition in the classification of the yeast genera Cryptococcus and Rhodotorula. T. Nakase and K. Komagata. J. Gen. Appl. Microbiol.

DNA base composition of some species of yeasts and yeast-like fungi. T. Nakase and K. Komagata. J. Gen. Appl. Microbiol.

- IX Microbiology Research Group of the South African Council for Scientific and Industrial Research, P. O. Box 395, Pretoria, South Africa.
Communicated by J. P. van der Walt.

THE YEAST GENUS SACCHAROMYCOPSIS SCHIÖNNING

J. P. van der Walt and D. B. Scott
Microbiology Research Group, Council for
Scientific and Industrial Research, Pretoria,
South Africa

Mycopath. Mycol. Appl. 43: 279-288

Abstract

In order to eliminate the confusion resulting from the homonyms Saccharomycopsis Schiönnig and Saccharomycopsis Guilliermond and to eliminate the usage of the name "Endomycopsis" which has been perpetuated contrary to the International Code of Botanical Nomenclature, the authors in this article outline the history of the species Saccharomycopsis capsularis Schiönnig and Saccharomycopsis guttulata (Robin) Schiönnig and give their reasons for proposing the name Cyniclomyces nom. nov. for the genus to which the second species is assigned, a step which permits the use of the generic name Saccharomycopsis Schiönnig (type S. capsularis) to designate the genus currently cited as "Endomycopsis".

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NEW SPECIES

Descriptions of the following new species are to appear shortly:

"Pichia ambrosiae sp.n.; a new auxiliary ambrosia fungus", by J. P. van der Walt and D. B. Scott in Antonie van Leeuwenhoek.

"Pichia cicatricosa sp.n.; a new auxiliary ambrosia fungus", by D. B. Scott and J. P. van der Walt in Antonie van Leeuwenhoek.

"Hansenula dryadoides sp.n.; a new species from South African insect sources", by D. B. Scott and J. P. van der Walt in Antonie van Leeuwenhoek.

"Pichia xylopsoci, a new yeast from South African insect sources", by J. P. van der Walt and D. B. Scott in Mycopath. Mycol. Appl.

"Saccharomycopsis synnaedendra, a new yeast from South African insect sources", by J. P. van der Walt and D. B. Scott in Mycopath. Mycol. Appl.

- X Université de Lyon, Laboratoire de Biologie Végétale, 43, Boulevard du 11 Novembre 1918, 69 - Villeurbanne, France. Communicated by M. Pignal.

Below follows news from our laboratory.

1) Recent publications:

PIGNAL, M. C. 1970. A new species of yeast isolated from decaying insect-invaded wood. *Antonie van Leeuwenhoek* 36: 525-529.

PIGNAL, M. C. 1971. Quelques levures associées à des larves xylophages de Coléoptères Buprestides du Canada. *Naturaliste can.* 98:69-82.

JACOB, F. H. 1971. Les levures des liqueurs tannantes végétales. I. Discussion taxinomique. *Ann. Inst. Pasteur* 120:649-664.

2) In press:

FIOL, J. B. et PONCET S. Comparaison de Kluyveromyces aestuarii et de Kl. wikenii par application de critères nouveaux. *Ann. Inst. Pasteur*.

JACOB, F. H. Les levures des liqueurs tannantes végétales. II. Répartition qualitative et quantitative. *Ann. Inst. Pasteur*.

In preparation:

JACOB, F. H. et PIGNAL, M. C. Interactions levures-tanins. I. Croissance et survie dans des solutions tannantes.

Examination of the growth of numerous yeasts isolated from tanning liquor of plant origin and from xylophagous insects in the presence of various tanning liquors shows that the condensed tannins support growth better than hydrolysable tannins. About 20% of the strains studied have a certain hydrolytic activity on pyrogallic tannins and only a few among them show strong activity.

FIOL, J. B. Etude des caractères d'assimilation du lactose, du cellobiose, du maltose et du tréhalose en tant que critères systématiques pour le genre Kluyveromyces.

23 Strains, representing 19 species of Kluyveromyces have been studied to verify if the absence of ability to assimilate lactose, of cellobiose, of maltose and of trehalose corresponds to the lack of a transport system or to the absence of an enzyme able to hydrolyze the sugar inside the cell. The results show that the non-assimilation of these sugars can be the result of either one of these causes.

Work in progress:

A study of the tannase of several yeasts: induction, kinetic studies, specificity of action on different hydrolysable tannin extracts.

A study of the effect of tannins and of certain of their constituents on the respiration and the fermentation of yeasts.

Numerical analysis applied to the genus Kluyveromyces.

In Oct. 1970 we had the pleasure of receiving in our laboratory Dr. Umezumi from Japan, University of Tottori. On April 26, 1971 we had a visit from Dr. Karassevich, of the Institute of Microbiology, of the Academy of Sciences, Moscow, USSR with whom we had very interesting discussions on the problems of adaptation in microorganisms.

- XI Animal Pathology Division, Health of Animals Branch, Canada Department of Agriculture, Animal Disease Research Institute, P. O. Box 1400, Hull, Quebec, Canada. Communicated by K. L. Malkin.

A case report by Beauregard and Malkin in the Canadian Journal of Public Health, Vol. 61, p. 547-549, 1970, describes a case of Cryptococcosis in a domestic cat near the Ontario-Quebec border on the St-Lawrence River. This appears to be the second report of this condition in domestic animals in Canada. Smith, et al. described the first case in a dog (C. Med. Ass. J. 72:18, 1955).

Recently a third case in a domestic dog was encountered from Maidstone near Windsor, Ont. Cryptococcus neoformans was isolated from the brain of the animal and also from the brains of mice inoculated intracranially with a suspension of the dog brain.

- XII Tropical Mycology Laboratory, University of Puerto Rico at Mayaguez, Department of Biology, Mayaguez, Puerto Rico 00708. Communicated by Luis A. Roure.

Below follows a summary of my last paper now in press to be published in "The Caribbean Journal of Science".

"An epidemic of tinea pedis and tinea cruris caused mainly by Candida albicans and Geotrichum candidum is described among 112 subjects inmates of a boy's Center of Studies and Work in a rural area near Mayaguez, Puerto Rico. The average age of the boys was 15.71.

Only 10 subjects out of 112 were free of any clinical signs of probable superficial and/or cutaneous mycoses. The above mentioned yeasts were responsible for 75.60% of the infections, while dermatophytes were responsible for only 23.14% of the infections. Sixty-six of the subjects, that is, 58.92% were positive to tinea versicolor. Ten were assumed to suffer from erythrasma. The possible reasons for such a high incidence of superficial and cutaneous mycoses are described."

- XIII Atomic Energy of Canada Limited, Chalk River Nuclear Laboratories, Biology and Health Physics Division, Chalk River, Ontario, Canada. Communicated by M. M. Shahin.

Following is the summary of my thesis completed at the Institute of Biophysics, Free University of Berlin, 1 Berlin 33, Habelschwerdter Allee 30, Germany:

Formation and regeneration of yeast protoplasts

Protoplasts from cells of different ploidy in Saccharomyces were prepared using a snail enzyme treatment. No differences were found between the haploid, diploid and tetraploid strains which were almost isogenic and homozygous. The results showed that the rate of protoplast formation is correlated with the conditions of the pre- and main-cultures and the presence of glucose in the nutrient medium. Cells which grow in a medium with a low concentration of glucose, or when ethanol was used as a carbon source instead of glucose, did not form protoplasts. The data seem to indicate that the pH of the culture had no effect on protoplast formation. $MgSO_4$ was found to be a more suitable stabilizer than $(NH_4)_2SO_4$. Protoplasts prepared from cells of different phases of the cell cycle showed different regeneration rates. In addition to the phase of main-culture from which the cells were harvested for the preparation of protoplasts, gelatin concentration, solution used for the nutrient medium and incubation temperature influenced also the regeneration of protoplasts.

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The following papers have been submitted for publication in Nature:

- 1) Spheroplasts formation and growth phases in Saccharomyces
M. Shahin
W. Laskowski
- 2) Factors influencing regeneration of spheroplasts in Saccharomyces
M. Shahin
W. Laskowski

I have now joined the Biology and Health Physics Division of Atomic Energy of Canada Limited, Chalk River, Ontario, Canada, on a post-doctorate fellowship from the National Research Council of Canada, to work with Dr. A. Nasim.

The present research projects are mainly concerned with:

- 1) Preparation and study of spheroplasts from stationary phase cells of Schizosaccharomyces pombe
(Manuscript in preparation)
- 2) Dark repair, liquid holding recovery and spontaneous cell death in the normal and radiation-sensitive strains of Schizosaccharomyces pombe.

XIV Ecole Nationale Supérieure Agronomique de Montpellier, Chaire de Génétique, France. Communicated by P. Galzy.

The following papers will be published shortly.

- P. GALZY, P. DUPUY. Techniques utilisables pour l'amélioration génétique de la levure dans une optique industrielle. Agence Internationale de l'Energie Atomique. Colloque de Vienne (Autriche) 29/4 au 1/4/71.
- P. GALZY, C. PLAN, J. ALBERT. Remarques sur la sélection des micro-organismes dans les industries agricoles.

- P. GALZY, A. ARNAUD, Françoise VEZINHET et Aline CHASSANG. Un modèle de morphogénèse: la sporulation de la levure.
Bull. Soc. Botan. Colloque Orléans France, 1/4/71.

Sporulation is a process of morphogenesis which includes at least three stages: meiosis, differentiation of the ascus wall and formation of spores. A few factors acting on the sporulation have been studied: checking of growth, pH, carbon source, oxygen, physiological state. The main morphological stages are described. Metabolic changes have been pointed out: increase in dry weight and in protein, accumulation of carbohydrate and lipidic reserves, particularly of sterols. The study of the genetic control of sporulation has been considered.

- Roselyne LUMARET, P. GALZY. Estimation de la fréquence d'une souche dans une population microbienne.
Ann. Technol. Agric.

- P. GALZY, M. GALZIN. Les levures du fromage de Roquefort.
Rev. Ind. Agric. Aliment.

- Françoise VEZINHET, A. ARNAUD, P. GALZY. Etude de la respiration de la levure au cours de la sporulation.
Canad. J. Microbiol.

During the process of sporulation, the oxidation rate of exogenous carbon source, acetic acid, is high during a period lasting from one to four hours. Then it falls rapidly and the metabolism of acetic acid seems to be deflected. Endogenous oxidation rate rises rapidly at the beginning of the process and reaches its maximum after a twelve to sixteen hour contact with the sporulation medium. Then it falls regularly.

- A. ARNAUD, Françoise VEZINHET, P. GALZY. Remarques sur le métabolisme de l'acide acétique au cours de la sporulation.
Mycopath. Mycol. Appl.

We have noticed important metabolic modifications during the first 15 hours of the sporulation process of yeast in contact with acetic acid. Carbohydrate reserves, dry weight and proteins increase. On the other hand, the activity of fumarase and isocitritase in cell homogenates are hardly altered. The results prove true the hypothesis according to which the metabolism of acetic acid is altered during sporulation.

- Françoise VEZINHET, A. ARNAUD, P. GALZY. Relations entre l'état physiologique et l'aptitude à la sporulation chez la levure.

Cells harvested during logarithmic growth give a higher percentage of asci than cells harvested during stationary phase. This observation is valid regardless of the carbon source. When the culture is grown on yeast extract 0.5%, glucose 0.5%, cells during logarithmic growth have a weak acetate Q_{O_2} . The activities of fumarase and isocitritase are also weak. All these characteristics are not incompatible with an abundant sporulation.

-Monique PELLECUER, P. GALZY, J. PASERO. Use of mutations in marking industrial yeast strains.

Two yeast strains, a Candida tropicalis able to grow on hydrocarbons, and a Saccharomyces cerevisiae cultivated on glucose, were tested for their resistance to some antibiotics and ions reputed to be toxic for yeast. Acidione and canavanine were tentatively checked but the best results were obtained with cadmium, cobalt, copper and arsenate. It has been possible to isolate ion-resistant mutants which could be used as marked strains to follow the behaviour of a given organism within the relatively mixed population of an industrial culture. Preliminary results showed that this mutation did not generally impair the viability of the organisms under investigation.

-Aline CHASSANG, M. ROGER, Françoise VEZINHET, P. GALZY. Variations de la teneur en lipides des cellules de levures au cours de la sporulation.

The fluctuation of the lipids in yeast cells during sporulation has been studied. The rate of lipid synthesis increases during sporulation. The unsaponifiable fraction represents a very high percentage of the lipids extracted at the very time the process of the sporulation starts.

XV University of Kentucky, College of Medicine, Department of Biology, Lexington, Ky. 40506. Communicated by John Gorman.

Genetic Analysis of a Gene Required for the Expression of Allele Specific Missense Suppression in Saccharomyces cerevisiae, Gorman, Jessica A. and John Gorman, Genetics, in press.

Three allele specific suppressors of the his2-1 mutation have been isolated and analyzed genetically. The suppressors are not linked to the his2 locus, and are specific for the his2-1 allele, failing to suppress any other of several mutations tested. These suppressors appear to be relatively unstable during long stationary phase maintenance and give rise to mixed cultures of histidine independent and histidine requiring cells. The stable revertants of one suppressed strain were found to be cytoplasmic petites, and were therefore analyzed to see if there were any correlation between the loss of suppression and the loss of respiratory sufficiency. Genetic analysis demonstrated that the loss of suppression was not due to the loss of the suppressor, but rather to the presence of a second mutation which restricts the suppressed phenotype. The locus involved has been designated sin1 (suppressor interacting). This gene segregates independently of both the his2 gene and the suppressors. No explanation of the apparent correlation between the sin1-1 mutation and petitness can be given, as both the suppressors and sin1 have been shown to be nuclear genes, and segregants of crosses involving the sin1-1 allele are stable grandes. Aside from the restriction of suppression of the histidine requirement, this mutation has no apparent phenotypic effect on the cell.

XVI Institut für Biochemie der Universität Würzburg, Würzburg, West Germany. Communicated by E. Schweizer.

The following abstract was submitted to the 7th Meeting of the European Biochem. Societies at Varna, 1971.

GENE - ENZYME RELATIONSHIPS IN THE FATTY ACID SYNTHETASE SYSTEM OF SACCHAROMYCES CEREVISIAE.
E. Schweizer and L. Kühn.

Fatty acid synthetase (fas) mutants of S. cerevisiae have been reported to be encoded by at least five different cistrons (E. Schweizer, L. Kühn and H. Castorph, Hoppe-Seyler's Z. physiol. Chem. 352, 377 (1971)). Complementation data suggested the close linkage as well as the polar transcription of two and three of the cistrons, respectively. These findings have now been confirmed by extensive tetrad analysis data. Any cross between mutants of the same cluster resulted, after sporulation, in tetrads of an almost completely parental ditype segregation pattern. Apparently, the two clusters extended over a chromosome region of about 1-2 map units, each. According to the meiotic segregation of alleles from different clusters they were clearly unlinked. The purified fatty acid synthetase complexes from representative mutants of various fas-cistrons have been studied. So far, structural gene mutations of four different component enzymes of the complex have been demonstrated among them. Within the first cluster, the structural genes for the dehydratase and the 2nd reductase, in the second cluster those for the 1st reductase and the condensing enzyme were found. In both clusters polar mutations exhibit pleiotropic enzymatic lesions. In some cases, an impairment of other component enzyme activities in addition to the primary mutational defect could be observed. It is suggested that this is the result of allosteric interactions within the complex and probably reflects the spatial proximity of the enzymes involved.

XVII Institute of General Genetics, University of Oslo, P. O. Box 1031, Blindern, Oslo 3, Norway. Communicated by Ø. Strømnaes.

Dr. B. A. Siddiqi who is a visiting scholar from Pakistan, will shortly publish a paper on yeast technique (summary below). A paper on the effect of EMS is in preparation, and his present research project deals with repair of UV and X-ray damage.

Random-spore analysis in *Saccharomyces cerevisiae*

An efficient method for separating ascospores and diploid cells in a mixed suspension has been developed. The sporulating culture is

treated with snail enzyme to digest the ascus wall; the mixed suspension is treated with ultrasound which separates haploid ascospores and selectively kills diploid cells. The remaining portion of diploid cells can be further separated from the ascospores by a liquid paraffin phase. The ascospores being lipophilic tend to move into the paraffin phase and can, therefore, be separated from the hydrophilic diploid cells. Purified random-spore preparations have been obtained containing about 97% spores from an original population containing about 62% spores.

The reliability and efficiency of the new method were evaluated by comparing the genetic linkage map of five genes obtained using this method with the map obtained by the method of tetrad analysis of the same five genes by Hawthorne and Mortimer (1963, 1966).

The results of this work show that the random-spore suspension obtained by the new technique known as the ultrasonic-paraffin technique, can be used for genetic analysis of Saccharomyces cerevisiae. The technique as such introduced no factors that affected the viability of spores, at least, not those genotypes that were segregating from the diploid strains used in these experiments. The complete paper will be published shortly.

XVII University of California, Berkeley. Department of Genetics, Berkeley, California. Communicated by S. Fogel.

Below follow the contributions from the yeast genetics group within the Department of Genetics at Berkeley.

We are developing techniques for obtaining Saccharomyces cerevisiae populations containing a high percentage of zygotes. We expect to use these techniques to study the physiological events associated with zygote formation and the mitotic and meiotic processes which may be induced immediately after mating.

Elissa Sena
David N. Radin

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Mutants of Saccharomyces cerevisiae have been isolated which require a saturated fatty acid for growth. In defined media, saturated fatty acids 14, 15 and 16 carbons long support growth but unsaturates such as oleic and palmitoleic do not. These mutants fail to incorporate radioactive acetate into fatty acids though they elongate the desaturate preformed fatty acids twelve carbons and longer. Most probably, these mutants are similar to Schweizer and Bolling's fas mutant (Proc. Nat. Acad. Sci. 69: 660-666) in which they detected an altered fatty acid synthetase complex. Fatty acid synthetase is a complex enzyme aggregate involving seven distinct proteins; one is a carrier molecule, acyl carrier protein (ACP); the other six proteins catalyse reactions involved in the synthesis of long chain saturated fatty acids (F. Lynen, 1969. Methods in Enzymology, Academic Press 14: 17). None of the mutations are centromere-linked. They fall within two or possibly three independently assorting loci and may be arrayed in twelve complementation groups with three clusters. One cluster is represented by a single mutant. A mutant category within each of the larger groupings fails to complement any mutants in the same cluster. Preliminary

evidence suggests that these non-complementing mutants may be suppressible. Vigorous complementation occurs between other mutants in the same cluster. Undoubtedly some complementation reactions may be interallelic, though the complementation patterns suggest two or three complex loci encompassing several genes.

Fatty acid requiring mutants are expected to be valuable in studying the effect of fatty acid composition on the physical properties of membranes. Experiments using nitroxide electron spin labels have shown that a phase transition occurs in the membranes at characteristic temperatures dependent upon the fatty acid supplementation. Double mutant strains incorporating saturated fatty acid mutants with desaturase mutants of yeast should allow us to control the organism's fatty acid composition to the limits of tolerance. Approaches such as the above promise to enhance our experimental capabilities by bringing sophisticated biophysical techniques to bear on the relationship of genetic control to structure and function on the molecular level.

Susan Henry
Seymour Fogel

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The Sterol Composition and Genetic Analysis of Nystatin Resistant Mutants

Resnick and Mortimer (J. Bacteriol. 92, 597, 1966) reported the existence of a class of mutants that required for growth either an unsaturated fatty acid or ergosterol and in addition they were petite. Further investigation shows that these mutants (ol-2) were resistant to the fungal antibiotic, nystatin, as well as that these three phenotype attributes are ascribable to a single genetic locus. Revertants of this tripartite phenotype abolish the lipid requirement and petite phenotype but leave a diminished residue of nystatin resistance.

Lampen (Symposium Soc. Gen. Microbiol. 16, III, 1966) has suggested that nystatin resistance stems from the antibiotic interfering with sterols in the cell membrane. This results in pore formation such that essential metabolites leak out of the cell, with cell death ensuing. The phenomenon of nystatin resistance was further investigated. Nystatin resistant mutants were identified by plating wild type yeast on a minimal medium plate containing a low dose of nystatin (10-60 units/ml). The five resistant mutants reported in the present study display a variety of properties. Their growth rates are generally slower than wild type. Some mutants show greater sensitivity to ethanol, distilled water, while others are resistant to lysis by the detergent, tergitol, and some others show a capacity to accumulate the indicator from thymol blue. Such phenomena are expected of mutants affecting the structure and composition of membranes. The sterol patterns of these mutants, as well as Resnick's petite lipid requirer ol-2, showed altered sterol patterns as established by TLC, the Lieberman-Burchard colorimetric reaction, UV scanning of ergosterol, and GLC. Most resistant mutants had no ergosterol though ergosterol is the principal sterol in Saccharomyces cerevisiae. Some of the less resistant mutants contained ergosterol or a very closely related sterol precursor. In addition, genetic

analysis of the ol-2 revertant demonstrated that reversion was at the original mutant site and therefore not attributable to a non-allelic suppressor gene. One of the independently isolated nystatin resistant mutants, NR2, was found to be allelic to ol-2 and its revertant.

Martin Bard

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Auxotrophic mutants of structural genes are powerful tools for elucidating biosynthetic pathways and gene-polypeptide relations. A new category of mutants has now been isolated. These yield a different kind of information - information bearing on cellular structure and the correlation between this structure and function. The unsaturated fatty acid auxotrophs of *Saccharomyces cerevisiae*, ol-1 and ol-2, provide the first source of biological membranes with a homogeneous fatty acid composition for comparative biochemical and physical studies. Our research has revealed those fatty acids which may serve as replacements for the naturally-occurring fatty acids 16:1A⁹ cis and 18:1A⁹ cis. Current work centers on the relevance of the fatty acid specificity to membrane structure, function, and biosynthesis. With freeze-fracture electron microscopy, morphological alterations are monitored as a function of fatty acid supplement in conjunction with variations in carbon source, growth temperature, and strain (petite versus respiratory competent). Lipid-depleted cultures, when compared by this technique to lipid-enriched cultures, exhibit a characteristic vacuolar membrane surface containing many smooth, flat, particle-free areas interlaced with narrow, particle-rich bands. One possible explanation for this striking deviation in appearance from the vacuoles of lipid-enriched cells (smooth surfaces interspersed with particles) is that the lipids of cells depleted of unsaturated fatty acids must contain an excess of saturates which lead to the formation of rigid crystalline zones. These zones presumably prevent the development of fully spherical vacuoles. The type of membrane appearing after 48 hours of lipid depletion resembles a golfball. Another possibility is that protein incorporation into membranes is linked to the incorporation of lipids containing unsaturated fatty acids. When unsaturates are no longer available, smooth particle-free areas result.

Changes in the physical state of membrane components are monitored by electron-spin resonance (ESR) spectroscopy employing spin-labeled fatty acid analogues. Phase transitions in membranes monitored over a wide temperature range appear to be solely a function of fatty acid supplementation. Transitions are obtained by plotting the ESR-calculated motion index, correlation time, against temperature (an Arrhenius plot). Denaturing the membrane proteins by various treatments does not effect the transition temperature. Cells grown on stearolic acid, an 18-carbon fatty acid with a triple-bond between carbons 9 and 10, demonstrate a phase transition at about 25°C. When these cells are shifted from 30° to 18°, they clump into fairly uniform-sized aggregates (vmm.), but continue to grow. Also, these cells do not attain a normal density when grown with 1% lactate as the carbon source instead of 2% glucose, even at 30°, indicating that stearolic acid may not be compatible with proper mitochondrial formation or function. Since growth does reach normal levels with 2% glucose,

other membranes must in some way differ from mitochondrial membranes.

Bernadine J. Wisnieski
Robert James (Botany Department)
Alec D. Keith

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Fogel, Seymour, Robert Roth, assisted by Patricia Leslie, Carol Lax and Karin Lusnak, Department of Genetics, University of California, Berkeley, California: Mutants deficient in meiotic recombination.

Roth and Fogel have recently submitted a first report to Molecular and General Genetics concerning a rationale and system for detecting and recovering mutants deficient in meiotic recombination. The present report briefly reviews the system and presents further data on the analysis of several mutants.

The genetical device which signals the occurrence of a recessive mutation that blocks or impairs meiotic recombination is a disomic haploid strain of Saccharomyces cerevisiae. The disomic chromosome pair involves linkage group III. It is simultaneously heterozygous for mating type, thr4, Mal2, his4 and a heteroallelic pair at leu2. The latter serves, via enumeration of induced prototrophs, as a convenient recombination monitor.

Basic to the system's rationale are two experimental findings that bear on the nature of meiotic recombination. Sherman and Roman (Genetics 48, 255, 1963) discerned two periods of allelic recombination as a function of exposure time to sporulation medium. The first period yields mostly diploid recombinants and coincides as postulated with a major round of DNA replication, identified and shown by Roth and Lusnak (Science 168, 493, 1970) to occur only in diploids heterozygous at the mating-type locus. The second period, presumed to represent the time of chromosomal pairing, generates mostly haploid prototrophs associated with extensive outside marker recombination. We have shown that the disomic haploid described above behaves, on exposure to sporulation medium, in a manner wholly comparable to conventional diploids. Unlike a/a or α/α heteroallelic disomes or diploids homozygous at the mating-type locus, all of which fail to exhibit DNA doubling or heightened allelic recombination upon challenge to meiotic induction, the a/ α disomic haploids display a characteristic meiotic-like developmental sequence including DNA replication, enhanced allelic recombination, and incipient sporulation that in turn leads to the production of incompletely formed, inviable "ascospores".

After mutagenic treatment with ethylmethane sulfonate approximately 1,000 survivor clones were screened for acetate-induced enhanced allelic recombination. In the preliminary screening isolates were discarded when they exhibited a "recombinationless" (or reduced recombination) phenotype attributable to failure to grow on acetate medium, secondary blocks in the leucine pathway, X-ray or U.V. sensitivity, or homozygosity for mating-type or either heteroallele at leu2. A residuum of some 90 presumptive mutants were retained for further analysis. What are the primary attributes of these putative mutants and how might they be utilized in resolving genetical recombination into its separable pathways and component reactions?

Five independent isolates of the most extreme "recombinationless" phenotype were selected for a preview study aimed at evaluating the individual mutants' effects in the homozygous state on reciprocal and non-reciprocal recombination in both mitosis and meiosis. Since the mutants are isolated in an a/α disome, or a non-mater, analysis begins with isolating mitotic recombinants (between mating-type and centromere III) or secondary non-disjunctants. Both events, detected with equal ease as a confluent mating by appropriate testers are verified by subsequent tetrad analysis of the resulting hybrids. Each mutant Mendelizes in a conventional manner and is fully recessive to the wild type allele. Two non-allelic mutants M8-21 and M8-25 have been analyzed somewhat more extensively. They are fully complementary, assort independently of each other and neither is centromere-linked. In the homozygous state both mutants exhibit a significantly reduced capacity to sporulate, though in one mutant, M8-21, DNA duplication preparatory to meiosis proceeds unimpaired and reciprocal meiotic recombination is normal. It is plausible to suppose that the mutants' principal action is to modulate the extent of co-conversion so that on the average a longer informational segment is transferred in each recombinational event.

An impressive and challenging array of molecular models have been proposed to account for recombination. While these unified models differ significantly from each other in their postulated reaction sequences, extent of heteroduplexed DNA, and importance assigned to mis-matched base pairs, they all envision breaks in single-stranded DNA, unwinding, annealing and heteroduplex formation by hydrogen bonding between complementary base pairs, excision and resynthesis of some 1,000 nucleotides, and reattachment. The requisite enzymatic equipment is readily found in endonucleases, exonucleases, DNA polymerases and ligases. Mutants isolated in the present study may well provide excellent material for the analysis of enzyme mediated recombination in a fungal eucaryote.

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Mating-type dependent repair in yeast

Friis and Roman (*Genetics* 59, 33-36) have shown that at low doses the frequency of ultraviolet-light-induced recombination in diploid yeasts shows a mating-type dependence but survival does not. We have isolated a series of haploid strains disomic for Chromosome III and are investigating their responses to X-irradiation as a function of homozygosity or heterozygosity for mating type. Of strains hetero-allelic at leu2, the a/α isolates show a two- to five-fold increase in the frequency of induced prototrophs over the a/a and α/α isolates in the dose range 0-4 Kr. Survival in all strains decreases exponentially for doses up to 10 Kr, declining to about ten percent at this point. Inactivation of a/a and α/α cells continues at this rate up to doses of 20 Kr, when survival levels off at about one-tenth to one-half percent; a/α cells, on the other hand, show four to five percent survival for doses of 10 Kr and above. At doses of 80 Kr, the highest to which we have exposed these strains, the surviving a/a and α/α cell clones show gross morphological abnormalities, while the a/a clones appear nearly normal.

No clear conclusions can be drawn from these data, but some suggestions may be made. It seems that yeasts possess a mating-type

dependent repair system. The increased induction of prototrophy in a/a strains may be attributable to this system, or there may be a mating-type dependent recombination system as well. Such activity might be related to the presence of cells able to enter a parameiotic state; the frequency of these cells would be greater in a/a than in a/a or α/α strains. The System(s) may be inducible in heterozygotes and exist only at low levels in homozygotes; alternatively, it may be that repair and recombination in the homozygotes are mediated by a different mechanism. In the latter case, we might expect that a/a and α/α strains would not exhibit the same frequencies of flanking marker arrays in leucine prototrophs, and of coincident exchanges in other regions of the genome, as a/a strains. Studies aimed at resolving these alternatives have been initiated.

D. Maloney
S. Fogel

XIX Department of Biology of the City University of New York, Brooklyn, New York 11210. Communicated by Nasim A. Khan.

CLUSTERING OF MALTOSE AND SUCROSE GENES IN YEAST

N. A. Khan and N. R. Eaton

In Saccharomyces several sugar fermenting genes have been reported to be very closely linked. For example the maltose gene MAL₁ is closely linked to the sucrose gene SUC₁, and MAL₃ is linked to SUC₃. We have found that there is a sucrose gene closely linked to the maltose gene MAL₄. Thirty-four asci were dissected from a cross of (MAL₄ SUC_x/mal suc) and ascospore clones were analyzed for maltose and sucrose fermentation. The maltose gene segregated in a 2:2 ratio, and all of the maltose fermenters were sucrose fermenters. Failure to find a tetratype ascus suggest that sucrose and the maltose genes are closely linked. A virtually homogeneous preparation of maltase from the fermenter strain used in the above cross can also split sucrose. An analogous situation exists in strains carrying the MAL₃ and SUC₃ genes. A purified maltase preparation from MAL₃ can also hydrolyze sucrose. However, we have found a tetratype ascus between MAL₃ and SUC₃ with a reciprocal product. Thus, although maltase can hydrolyze sucrose in vitro, it is not responsible for the fermentation of sucrose in vivo. Moreover, MAL₁ and SUC₁ also can exist in repulsion.

On the basis of these observations we suggest that there is a sucrose gene closely linked to the maltose MAL₄.

I am working on the regulation of MAL1 and MAL3 gene activity in Saccharomyces cerevisiae. Strains carrying either one of these genes form appreciable amounts of maltase activity only in the presence of an appropriate inducer, usually maltose. This induction can be prevented by addition to the medium of glucose. Maltase activity was assayed using cells lyophilised over phosphorous pentoxide in a vacuum, determining the rate of splitting p-nitrophenyl-alpha-D-glucoside. Cells were grown in a YEP (1% yeast extract, 2% bacto peptone) medium. To this was added the desired carbon source. Cells were always taken from logarithmic phase cultures and transferred to new media at a density allowing for at least three rounds of cell division. MAL3 was investigated in two strains: EK-6B and 1412-4D, the latter being able to ferment alpha-methylglucoside. MAL1 was investigated in strain A1327A, a strain unable to ferment sucrose.

Induction of maltase activity in both MAL3 strains required maltose concentrations higher than 0.1%. In contrast to this, the MAL1 strain would form increased amounts of maltase with as little as 0.04% maltose in the medium. Induction of maltase synthesis in both strains could be inhibited by glucose. To measure this inhibition more accurately, cells were induced in a 2% maltose YEP for 90 min, a period long enough to initiate maltase synthesis. Glucose was then added, and the continuation of maltase synthesis followed. In strain EK-6B MAL3 inhibition was observed at glucose concentrations higher than 0.2%. In A1327A 0.1% glucose was already inhibitory for MAL1. Still lower concentrations promoted induction, 0.02% being already very stimulatory in both strains. The two genes apparently had different requirements for inducer and inhibitor concentrations. The stimulatory effect of glucose was the same, and probably due to a supply of additional energy by glucose breakdown.

Addition of glucose during various stages of induction stopped further increases in maltase activity within about 15 min, and this was the same for both strains. The inhibitory effect of 2 ppm cycloheximide was tested on induction of MAL1; it stopped synthesis within 15 min like glucose. In another type of experiment cells were removed from the inducing maltose medium and resuspended in YEP with maltose or with other carbon sources. In MAL3 in 1412-4D removal of maltose had the same immediate effect as addition of glucose. In MAL1 there was a continuation of synthesis, at a reduced rate though in comparison to the maltose control, for about 75 min when neither maltose nor glucose were present.

The working hypothesis has been adopted that the activity of the maltose genes in yeast is subject to autoregulation: Maltase in the absence of maltose represses its own synthesis. Maltose interacts with maltase so as to prevent repression. Glucose interacts with maltase so as to maintain the enzyme in a repressing form even in the presence of maltose. This concept of autoregulation is supported by the observation that measurable levels of maltase activity are found in cells grown in the absence of maltose and in the presence of glucose. Moreover, two alleles of MAL4 have been studied by Khan and Eaton (Bact. Proc. G. P45, 1967; see also previous report). Strains carrying one of those alleles show very high levels of maltase in the absence of inducer, and even in the presence of as much as 8% glucose.

Those strains were crossed against nonfermenting strains. The progeny from such crosses showed a 2:2 segregation for maltase synthesis which in all cases was very high even after growth on glucose. No regulatory properties could be separated from the ability to synthesise maltase. Further work is carried on to prove to disprove the autoregulatory control of the yeast maltose genes.

XXI Stanford Research Institute, Life Sciences Division, Menlo Park, California 94025. Communicated by W. A. Maxwell.

The following is an abstract from a paper which appeared in the journal Photochem. Photobiol. by W. A. Maxwell and C. O. Chichester. 1971. Photodynamic responses in *Rhodotorula glutinis* in the absence of added sensitizer. 13:259-274.

The lethal effect of a continuous spectrum of light at wavelengths longer than 300 nm has been examined for the yeast *Rhodotorula glutinis*. A survival curve consisting of a shoulder, an exponential phase of death, and a "tailing off" phase was shown to occur when cells were irradiated in the absence of a sensitizing dye. An action spectrum showed that the portion of the light used for irradiation which is lethal to the cells lies between 310 and 430 nm. It was found that carotenoid pigments were not able to provide any protection against this endogenously photosensitized oxidation. Exponentially growing cells were the most sensitive to killing and, as cells approached and entered into the stationary phase of growth, they became more resistant until a constant level of sensitivity was reached. A primary temperature effect on the length of the shoulder prior to the death of the cell has been observed. The sensitivity was greatest at low temperatures with an optimum resistance occurring at 22.5°C and an increasing sensitivity occurring at higher temperatures. Various sites have been examined for cellular damage which might lead to the death of the cell. Damage was found to impair the function of the plasmalemma, the glycolytic and respiratory system, the nucleus, and to be observable cytologically.

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The following articles have been recently published. Early phases of some of this work has already been communicated in past Yeast News Letters:

1. W. A. Maxwell and Edward Spoerl. 1971. Mannitol uptake by *Saccharomyces cerevisiae*. J. Bacterial. 105:753-758.
2. W. A. Maxwell, R. Metzler, and Edward Spoerl.

1971. Uranyl nitrate inhibition of transport systems in Saccharomyces cerevisiae.
J. Bacterial. 105:1205-1206.

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I would also like to communicate the following for Dr. Edward Spoerl of the U. S. Army Medical Research Laboratory, Biophysics Division, Ft. Knox, Kentucky:

Abstract from Edward Spoerl. 1971. Disruption of yeast membranes by methylphenidate. J. Bacterial. 105:1168-1174.

Methylphenidate blocked sorbose uptake and loss by yeast spheroplasts and, at higher concentrations (30 mM), disrupted the spheroplasts. At still higher concentrations (70 mM), methylphenidate also ruptured the membranes of whole yeast cells; sorbose and materials absorbing at 280 nm were lost from the cells, and methylene blue stained them. Intracellular structures were extensively affected, as shown by electron micrographs, and were more sensitive to disruption by methylphenidate than the external membrane. N-ethylmaleimide and Ca^{2+} enhanced the rupture of external membranes by methylphenidate.

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

1) Alcool- et aldéhyde-déshydrogénases particulières de *Candida tropicalis* cultivé sur hydrocarbures.

J. M. Lebeault, B. Roche, Z. Duvnjak et E. Azoulay
Biochim. Biophys. Acta, 220 (1970) 373.

Some alcohol and aldehyde dehydrogenases of *Candida tropicalis* cultivated on hydrocarbons.

Cell-free extract of a strain of *Candida tropicalis* cultivated on hydrocarbon catalyze in the presence of NAD^+ the oxidation of higher alcohols to the corresponding fatty acids; two enzymes, an alcohol dehydrogenase (alcohol: NAD^+ oxidoreductase, EC 1.1.1.1) and an aldehyde dehydrogenase (aldehyde: NAD^+ oxidoreductase, EC 1.2.1.3), have been studied.

Assay of these enzymes (340 nm spectrometry) fails with substrates barely soluble in water. The assay is therefore carried out with emulsions prepared with Tween-80 or with deoxycholate. Alternatively the substrates were solubilized with dimethyl formamide. K_m values obtained with 1-decanol using the modifications described were 31 mM, 7 mM and 0.7 mM, respectively. Kinetics properties and particularly the variation of the K_m and v_{max} as a function of chain length of the substrates are reported.

Alkane and the corresponding alcohol and aldehyde are inducers of these enzymes.

- 2) Oxydation des alcools supérieurs chez Candida tropicalis cultivé sur hydrocarbures.
J. M. Lebeautl, F. Meyer, B. Roche et E. Azoulay.
Biochim. Biophys. Acta, 220 (1970) 386.

Oxidation of higher alcohols in Candida tropicalis cultivated on hydrocarbons.

A soluble NAD⁺-linked alcohol dehydrogenase (alcohol:NAD⁺ oxidoreductase, EC 1.1.1.1) induced by hydrocarbon has been studied in cell-free extract of Candida tropicalis cultivated on n-tetradecane. Kinetic properties, such as the variation of K_m and v_{max} as a function of chain length, and substrate specificity might involve hydrophobic interactions between the substrate and the enzyme.

This inducible enzyme is different from the particulate previously described.

- 3) Activation des acides gras à chaîne moyenne et des diacides chez Candida tropicalis.
E. Azoulay et Z. Duvnjak
Biochimie, 53 (1971) 113.

In addition to its mitochondrial acyl-CoA-synthetases, Candida tropicalis possesses other activation systems (Acid:CoA ligase (AMP, EC. 6213). The latter which are cytoplasmic and soluble, act specifically on short or middle chain fatty acids. Maxima are observed with heptanoate and octanoate and among dicarboxylic acids only with dodecane dicarboxylic acid. These cytoplasmic systems exhibit some interesting differences in behaviour toward both short chains fatty acids and dicarboxylic acids, namely in their kinetic parameters, ionic effects and their more or less specific dependence on ATP. These differences suggest that, like mitochondrial systems, these cytoplasmic systems consist in fact, of several enzymes.

XXIII Bath University of Technology, School of Biological Sciences, Claverton Down, Bath, BA2 7AY, England. Communicated by R. V. Brunt.

Below follow several titles and abstracts of publications originating in our laboratory.

The Effect of H-Ion Concentration Upon Aerobic Polysaccharide Synthesis by Resting Cells of Saccharomyces cerevisiae. C. J. Barwell and R. V. Brunt. Arch. Mikrobiol. 64, 315-318 (1969).

The Regulation of Aerobic Polysaccharide Synthesis in Resting Cells of Saccharomyces cerevisiae. C. J. Barwell and R. V. Brunt. Arch. Mikrobiol. 66, 59-62 (1969).

The Effect of 3-Deoxy-3-fluoro-D-glucose on Saccharomyces cerevisiae. B. Woodward, N. F. Taylor and R. V. Brunt. Biochem. J. 114, 445-447 (1969).

Quantitative Determination of Fluorinated Carbohydrates and Related Compounds in Biological Materials. B. Woodward, N. F. Taylor, and R. V. Brunt. Analytical Biochemistry 36, 303-309 (1970).

Regulation of Pyruvate Kinase by Fructose 1,6-diphosphate in Saccharomyces cerevisiae. Clive J. Barwell, Brian Woodward, and Rodney V. Brunt. *Eur. J. Biochem.* 18, 59-64 (1971).

The concentrations of glycolysis intermediates, citric acid and adenine nucleotides were measured in resting cells of Saccharomyces cerevisiae during aerobic glucose metabolism. Addition of glucose resulted in a decrease in the intracellular content of phosphoglyceric acids and phosphoenolpyruvate. As extracellular glucose became depleted the level of these intermediates increased. Thus changes in their intracellular content were regulated by factors other than the supply of extracellular substrate.

Cross-over behaviour of phosphoenolpyruvate and pyruvic acid together with the inverse relationship which existed between the concentration of phosphoenolpyruvate and the rate of the pyruvate kinase reaction, indicated that regulatory changes in the activity of pyruvate kinase occurred. These activity changes were compared with those of the intracellular content of reported in vitro effectors of the enzyme: fructose 1,6-diphosphate, citric acid, ATP and ADP. ATP and ADP did not appear to be involved in regulating changes in the activity of the enzyme. Although citric acid may have contributed towards some of the changes in enzyme activity, fructose 1,6-diphosphate appeared to be the primary regulator, under the conditions of these experiments.

Comparison of the results with the properties reported for the isolated enzyme, suggested that, in vivo, fructose 1,6-diphosphate affects the affinity of pyruvate kinase for phosphoethanolpyruvate. This, in turn, appears to regulate the intracellular content of phosphoenolpyruvate and phosphoglyceric acids. The significance of the results is discussed in relation to both glycolysis and gluconeogenesis.

The Preparation and Some Properties of Yeast Mitochondria. Carole Spencer, Susan A. Symons, and R. V. Brunt. *Arch. Mikrobiol.* 75, 246-259 (1971).

A comparison of the structural and functional integrity of yeast mitochondria prepared by a mechanical disintegration method with that reported for mitochondria prepared by enzyme methods has been made.

The mitochondria prepared by mechanical disruption show at least as great a degree of functional integrity as that reported for enzymically prepared mitochondria. This was judged by the substrates oxidised, the rates of these oxidations, the respiratory control and ADP:O ratios and difference spectra.

The respiration of citrate and of ethanol by the mechanically prepared mitochondria has been shown to be stimulated by exogenous NAD; in the case of the latter substrate a degree of respiratory coupling became apparent as a consequence of this stimulation.

The penetration of NADH₂, and of citrate or of ethanol in the presence of exogenous NAD, into the mitochondria has been examined.

The results are consistent with the reported existence of a NADH₂ dehydrogenase system located in the outer area of the inner mitochondrial membrane. The NAD stimulation of ethanol respiration also suggests the occurrence of an external alcohol dehydrogenase in the mitochondrial preparation; similar results with citrate suggest external forms of aconitic hydratase and isocitric dehydrogenase to be present.

Unlike animal mitochondria the rate of penetration of oxidisable substrates in uninhibited suspensions appeared to exceed respiration and/or outward diffusion rates in this preparation.

Evidence of a malate stimulated citrate and isocitrate transport system similar to that reported for animal mitochondria is presented, but the specificity of the system for tricarboxylate anion appears to differ, at least in respect of propane 1.2.3 tricarboxylate (tricarbalylate).

XXIV Station de Technologie, Des Produits Végétaux, 7, Rue Sully, 21-Dijon, France. Communicated by P. Brechot and P. Dupuy.

Oleanolic acid as a growth factor for wine yeasts in anaerobiosis

P. Brechot, J. Chauvet, P. Dupuy, Madeleine Croson and Arlette Rabatu

This work is the result of a cooperation between Institute Pasteur (Paris) and Institute National de la Recherche Agronomique (Dijon). It has been submitted for publication to Annales de Technologie Agricole.

Abstract

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 oleanolic acid an oxiterpenic acid. It crystallizes from hot alcohol and can be purified by preparative thin layer chromatography. The activity of this product has been compared with that of ergosterol either in presence of oleic acid or not. Anaerobic cultures of S. cerevisiae give more growth and ferment more sugar in presence of oleanolic acid than in presence of ergosterol which has been reported as a growth factor by Andreasen and Stier (1954).

This finding can explain why abundant growth of yeasts occurs even when the grapes are placed under anaerobic conditions.

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Extraction of soluble nitrogen compounds from Candida utilis

Eva Szechenyi and J. N. Morfaux

From Candida utilis ^C14, release of soluble nitrogen compounds was examined. As for Saccharomyces cerevisiae, there was passive and nonpreferential release of free amino acid from a buffered suspension of resting cells.

Excretion of nucleotides was also studied; it was not modified when glucose (and other energy source) or general metabolic inhibitors were added. All of the soluble nitrogen lost by the cell probably comes from amino acids and nucleotides. It was not possible to show any peptides excretion. Practical consequences of this nitrogen

release are discussed. (This work was conducted during the time Eva Szechenyi from the Central Institute of Research in Food Industry, Budapest, Hungary, spent in the laboratory of P. Dupuy.)

XXV Gesellschaft für Strahlenforschung M.B.H., Abteilung für Biophysikalische Strahlenforschung, Paul-Ehrlich-Strasse 15 u.20, 6 Frankfurt/Main, West Germany. Communicated by V. K. Jain.

The following is an abstract of a recent paper appearing in Arch. Mikrobiol. 72, 252-259 (1970).

Relationship between Energy Metabolism and Growth
I. Glucose Dependence of the Exponential Growth
Rate of Saccharomyces cerevisiae

A biphasic dependence of the exponential growth rate on the glucose concentration of the medium was observed in batch culture experiments for a strain of S. cerevisiae and one of its petit mutants. The data can be fitted to an equation of the Michaelis-Menten type with two sets of values of the growth parameters; the switch-over occurs at a glucose concentration of 4 mM. Another petit mutant did not show the biphasic character.

Regulation of the energy metabolism in relation to the cell cycle is discussed. It is suggested that the observed shift in the growth parameters may be due to a change in the control point of glycolysis from phosphofructokinase to pyruvate kinase at higher glucose concentrations. This could reduce the duration of the G₁ phase by permitting a faster synthesis of reserve carbohydrates required as intracellular energy reservoirs for DNA synthesis.

XXVI Chemical-Pharmaceutical Institute, Department of General and Industrial Microbiology, Leningrad, U.S.S.R. Communicated by N. P. Elinov.

1. The polysaccharides of Rhodotorula species (N. P. Elinov, G. A. Vitovskaya). The composition of water-soluble polysaccharide preparations obtained from cells of 17 species of Rhodotorula by extraction with 0.1 N HCl and also extracellular ones were investigated. The distinction between the preparations isolated from the same yeast species by different methods was shown. Water-soluble and extracellular polysaccharides of red species were different. The main component of cell polysaccharides of these organisms is glucose. R. rubra, R. pilimanae, R. longissima, R. pallida, R. glutinis, R. mucilaginosa produce extracellular mannans containing β -1,3 and β -1,4 mannopyranose residues alternately arranged in straight or slightly branched chains.

The composition of water-soluble polysaccharide preparations of cells from "yellow" species of Rhodotorula are similar to extracellular polysaccharides of the same species. They contain glucose, mannose, xylose and glucuronic acid. Their main component is heteropolymer consisting of mannose, xylose and

glucuronic acid units. Heteropolymers of Rh. flava are xylo-mannans only.

2. The extracellular polysaccharide of Pullularia (Aureobasidium) pullulans (N. P. Elinov, A. K. Matvejeva). The composition and structure of extracellular polysaccharide produced by Aureobasidium pullulans have been studied. As shown by periodate oxydation, methylation, IR-spectroscopy and paper chromatography, pullulan is a homopolymer consisting of glucose units linked by C₁-C₄, C₁-C₆ and C₁-C₃ links. The possible structure of pullulan is presented.

3. Candida albicans plasmocoagulase and its comparison with staphylocoagulase (N. P. Elinov, N. A. Zaikina). The investigation of the specificity of the yeast and staphylococcal enzymes with respect to synthetic peptides has shown that both coagulases are very close to leucinaminopeptidase. Microbial plasmocoagulase and leucinaminopeptidase have common activators and inhibitors. Tissue leucinaminopeptidase was found to produce plasma clotting affecting prothrombin as the substrate.

Yeast plasmocoagulase possesses the activity of weak endotoxins. It inhibits the phagocytosis and when injected simultaneously with microbes complicates the infections independently of the etiologic agents. Fungal plasmocoagulase was found to represent a protein-carbohydrate complex with high contents of polysaccharide that is strongly bound to the protein. The polysaccharide part of the complex causes the stability of the enzyme.

Plasmocoagulase of Candida albicans was found to be immunologically active.

4. The distribution of some microbial polysaccharides in macroorganism (N. P. Elinov, G. Y. Arkadjeva, and I. P. Sokolova). The aim of this work was to study the localisation and the period of staining Candida, Rhodotorula and Pullularia (Aureobasidium) polysaccharides in tissues of white mice. Coon's method has been used in these experiments and also polysaccharides labelled by C¹⁴. Radioactivity is determined with the liquid-scintillation counter. We could establish that polysaccharide complexes and haptens injected intravenously and intraperitoneally are absorbed on erythrocytes and transported over macroorganism. The absorption of the named substances on erythrocytes takes place during 5 min after injection. It was established by the luminescent method and by the high radioactivity of erythrocytes.

Polysaccharide preparates remain in macroorganism for some months after one injection of 200-500 µg/mouth.

Polysaccharides are excreted by macroorganisms through kidneys and lungs.

5. The studying of cell-wall preparations of Candida viswanathii (N. P. Elinov and G. A. Narmatova). Preparing cell walls of C. viswanathii by the method of desintegration in tissue disintegrator with ballotini N 14 has been shown.

Preparations of cell walls contained carbohydrates (mostly), proteins and phosphorus.

XXVII Miller Brewing Company, Milwaukee, Wisconsin 53201. Communicated by J. R. Helbert.

Below follows the abstract of a paper presented before the American Society of Brewing Chemists, Montreal, Canada, May 2-6, 1971.

Identification of Dekkera intermedia
Isolated from Spoiled Beer

By T. A. Day and J. R. Helbert

Five beer spoilage yeasts (BSY) were isolated during the years 1964-1966 from unpasteurized, spoiled commercial beer. All five yeasts were identified as Dekkera intermedia, formerly Brettanomyces intermedius. The isolates were studied on malt agar slants. They were "slow growers" and had poor viability on the slants due to their high production of acetic acid.

The isolates were grown in malt extract broth and pasteurized beer and compared to Saccharomyces carlsbergensis. The BSY's exhibited varied morphology, ogival shape and pseudomycelium. Sporulation was induced only after a vitamin supplement [(van der Walt and Van Kerken, Antonie van Leeuwenhoek, 26, 292 (1960)] was added to malt extract yeast extract agar. The fermentation and assimilation profile, acid production from glucose, ability to split arbutin, assimilation of potassium nitrate, lysine utilization and the ability to grow in the presence of 100 ppm of cycloheximide all indicated that the five BSY's were Dekkera intermedia, as described by van der Walt [Antonie van Leeuwenhoek, 30, 273 (1964) and in Lodder, "The Yeasts," 2nd Ed., No. Holland Publ. Co., Amsterdam and London, 1970].

This is the first known report of Dekkera intermedia from American beer.

It is suggested that this organism may be much more common in American breweries than the paucity of American literature on the subject would indicate, since the microbiological methods routinely employed in breweries would generally not detect it. Therefore, it is also suggested that this yeast be considered when methods other than pasteurization are used to preserve beer.

XXVIII Statens SerumInstitut, Amager Boulevard 80, København S, Denmark. Communicated by Jessie Bodenhoff.

A New Antimycotic from Bayer Ag, Bay b 5097

In vitro Experiments with Strains of the Species
Candida, Torulopsis, and Cryptococcus

In vitro sensitivity determinations to Bay b 5097 were carried out on 38 yeast-like fungi of the species Candida, Torulopsis, and

Cryptococcus. The strains examined were clearly different from each other as regards their sensitivity to Bay b 5097. Some strains of Candida parapsilosis, Candida pseudotropicalis, and Cryptococcus neoformans, and a few strains of Candida albicans were clearly sensitive to Bay b 5097, while the majority of the Candida albicans and all the Candida tropicalis strains were among those that must be regarded as only slightly sensitive. The divergencies between previously published reports of sensitivity determinations on yeast-like fungi to Bay b 5097 and those in the present study are pointed out, and it would seem justifiable to demand that sensitivity determination should be carried out before any treatment with antimycotics is embarked upon.

XXIX Allied Breweries (Production) Limited, The Brewery, Burton-on-Trent, United Kingdom. Communicated by Miss D. A. Lovett.

One of our staff has recently had a paper published in the Journal of the Institute of Brewing, and another is due to read a paper at the European Brewery Convention this month. Abstracts of these papers follow below.

Use of Difco WLN agar for (a) detection of wild yeast in the presence of Sacch. cerevisiae, (b) demonstration of the instability of strains of Sacch. carlsbergensis. J. F. Hall. J. Inst. Brew., 1970, 75, 522-3.

After 72 h growth at 25°C on WLN agar, wild yeast (i.e. non-brewing) strains can be differentiated from brewing yeasts (Sacch. cerevisiae) by the growth, form and colour of the colonies. Sacch. carlsbergensis exhibits within one strain a wide range of colony coloration on WLN and this method cannot be used to differentiate between Sacch. carlsbergensis brewing yeasts and wild yeasts. The variation within Sacch. carlsbergensis may be indicative of strain instability.

The sulphur metabolism of brewing yeasts and spoilage bacteria. R. J. Anderson, G. A. Howard, J. S. Hough. Paper to be read at the European Brewery Convention, Estoril, May 1971.

In synthetic media containing sulphate both Sacch. cerevisiae and Sacch. carlsbergensis formed hydrogen sulphide and sulphur dioxide. The addition of methionine to the medium completely inhibited the formation of these compounds by Sacch. cerevisiae, but inhibited their formation by Sacch. carlsbergensis to a lesser extent. Addition of cysteic acid to the medium only slightly affected the production of these sulphur compounds. No volatile organic sulphur compounds were formed in the synthetic medium although both yeasts formed a small amount in wort. In wort Sacch. carlsbergensis, but not Sacch. cerevisiae, produced hydrogen

sulphide. The formation of volatile organic sulphur compounds by Enterobacter aerogenes, Obesumbacterium proteus, Zymomonas anaerobia and Acetobacter rancens alone and in mixed cultures with the yeasts was also studied.

XXX International Meetings

Specialized Symposium on Yeast. 1971. Yeasts as models in science and techniques. June 1-4 at the Home of Scientific Workers of the Slovak Academy of Sciences - Smolenice Castle, Czechoslovakia.

Proposed program:

- Wikén, T. O. (The Netherlands): On the physiology of the formation of ascospores in yeasts
- Santa-Maria, J. (Spain): Genera characterization in yeast classification
- Johanides, V., Markovič, I. (Jugoslavia): Effect of substitution of potassium by lithium in the medium on the morphology of Saccharomyces cerevisiae
- Norkrans, B. (Sweden): Yeast utilizing hydroxylamine as the sole nitrogen source
- Yamada, J. (Japan): Taxonomic significance of coenzyme Q system in yeasts and yeast-like fungi
- Dobolyi, C., Novák, K. E. (Hungary): Polyene sensitivity of various yeast species measured by conductometry
- Naumov, G. (USSR): Taxogenetics of Saccharomyces spp.
- Šilhánková, L. (CSSR): Some causes of the instability of colony characters in Saccharomyces cerevisiae
- Böttcher, F., Samsonova, I. A., Birnbaum, D. (GDR): Biochemical-genetical characteristics of Rhodotorula glutinis by auxotrophic mutants
- Samsonova, I. A., Birnbaum, D., Böttcher, F. (GDR): Characteristics of some revertants of the type Nič mutants of Rhodotorula glutinis
- Birnbaum, D., Böttcher, F., Samsonova, I. A. (GDR): Contribution to the nicotinic acid synthesis at Rhodotorula glutinis
- Eschenbecher, F. (GDR): On the content of vitamins of respiratory deficient yeasts
- Elinov, N. P. (USSR): Extracellular polysaccharides produced by Rhodotorula spp.
- Šandula, J., Masler, L., Šikl, D. (CSSR): Immunochemical studies on yeast polysaccharides
- Kaliuzhny, M., Petrushko, G. (USSR): Polysaccharides of yeast-like organisms of Candida spp. producing lyophilic properties of the cultures and their thickening by flotation
- Balou, C. (USA): Species specificity of the structure of yeast cell wall mannans
- Šikl, D., Masler, L., Vojková-Lepšíková A., Šandula, J. (CSSR): On some characteristic cell wall polysaccharides of chosen yeast species
- Brucker, W. (GDR): Computer application to yeast biology
- Harman, H. H. (USA): How factor analysis can be used in yeast classification?
- Kocková-Kratochvílová, A., Ondrušová, D., Kuzmin, F. (CSSR): The grouping of species in the genus Candida Berhout
- Kocková-Kratochvílová, A., Blagodatskaja, V., Hronská, L. (CSSR,

- USSR): The grouping of species in the genus *Kluyveromyces van der Walt*
- Campbell, J. (England): Numerical taxonomy of serological groups of yeasts
- Phaff, H. J., Meyer, S. A. (USA): DNA base composition and DNA-DNA homology studies as tools in yeast systematics
- Bak, A. L., Christiansen, C., Christiansen, G., Meyer, S. A., Stenderup, A. (Denmark): Yeast DNAs
- Komagata, K. (Japan): DNA base composition in yeast
- Rodrigues de Miranda, L., Yarrow, D. (The Netherlands): The maintainance of stock cultures of yeasts
- Zsolt, J. (Hungary): About some curious yeasts
- Minárik, E., Nagyová, M., Šilhárová, Z. (CSSR): Contribution to the effect of some surface active substances on yeasts occurring on secondary habitats in wineries
- Kocková-Kratochvílová, A., Wegener, A. K., Ondrušová, D. (CSSR): Yeasts in North-East Mecklenburg
- Dorfwirth, K. (Austria): Taxonomic studies on yeasts isolated from mead
- Shavlovsky, G. (USSR): Some properties of *Candida guilliermondii* strains with blocked purine catabolism and the peculiarities of their maintainance
- Novák, E. K., Deák, T. (Hungary): General aspects of sugar transport in various taxonomic areas of yeasts
- Deák, T., Novák, E. K. (Hungary): Quantitative characteristics of sugar transport in various yeast species
- Pignal, M. C., Jacob, J. (France): Mutual action of yeasts and tannine
- Kostov, V. (Bulgaria): About the biosynthesis of lysine in *Candida tropicalis* and *Rhodotorula glutinis* under the action of certain substances
- Burjan, I. N. (USSR): The race of wine yeast for continuous fermentation under carbondioxide pressure
- Radler, F. (GFR): The formation of non volatile acids by strains of *Saccharomyces* during fermentation
- Kunkee, R. (USA): Attempt to control fusel oil production by metabolic inhibition
- Bezborodov, A. M., Rosenfeld, S. M., Disler, E. N. (USSR): Some characteristics of the metabolism of thiamine heterotrophic yeast *Candida lipolytica*
- Lozínov, A. B., Finogenova, T. K. (USSR): Organic acid biosynthesis during the growth of *Candida* spp. on alcane medium
- Biely, P., Krátký, Z., Bauer, Š. (CSSR): Metabolism of 2-deoxyglucose in yeast with different 2-deoxyglucose sensitivity
- Jakubowska, J. (Poland): Flocculent and non flocculent yeasts in top beer production. Some diagnostic features
- Bendová, O. (CSSR): Evaluation of the selection process of yeast strains in brewing industry
- Moštek, J., Šilhánková, L. (CSSR): Possibilities of the use of respiration deficient mutants of *Saccharomyces carlsbergensis* in brewing industry
- Koch, Y., Koch, H. A. (GDR): Morphological studies on cell nucleus of certain yeasts
- Holan, Z., Beran, K. (CSSR): Gas chromatographic determination

- of the content of N-acetylglucosamine and its polymers in the cell wall of yeasts
- Semushina, T., Monakhova, N. (USSR): Yeast-like fungi: main procedures of protein yield on hydrolyzate media
- Kozlov, K. (USSR): On air oxygen consumption in fodder yeast cultivation on hydrolyzate media
- Kalačević, I., Pejin, D., Marinković, R. (Yugoslavia): Studies on the changes of physiological properties on baker's yeast in aerobic growth on alcohol as the only carbon source
- Nawawy, E. El. (UAR): Studies on the total carbohydrates, their fraction in baker's yeast of UAR
- Rozmanova, N. (USSR): Baker's yeast cultivation in technological cycle as a model of synchronous cell propagation
- Gusarova, L. (USSR): On the action of furfural on fodder yeast metabolism
- Vraná, D., Fencl, Z. (CSSR): Dependence of the size of cells, their substance and the content of nucleic acids on the dilution rate in two-stage continuous cultivation of Candida utilis
- Lieblová, J., Beran, K. (CSSR): Characterization of cell groups of a given age in a continuous culture of Saccharomyces cerevisiae

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Specialized Symposium on Yeasts. 1972.

Place of meeting: Kyoto, Japan

Period: March 19 through 24, 1972, in connection with the IVth International Fermentation Symposium

Proposed focal topics:

- (1) Taxonomy and Ecology of Yeasts
- (2) Subcellular Structure and Function of Yeasts
- (3) Sexuality, Gene Action and Breeding of Yeasts

Some other sessions for general subjects will be arranged depending on the applications for presentation. An additional meeting on the pathogenicity and immunology is now under planning. Detailed information circular will be sent to interested investigators upon request to:

Dr. Y. Oshima, Yeast Organizing Group
 Department of Fermentation Technology
 Faculty of Engineering, Osaka University
 Suita near Osaka 565, Japan

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The Third International Symposium on Yeast Protoplasts will be held October 2-5, 1972. The Meeting will be held at the Faculty of Sciences of the University of Salamanca. The official language of the Symposium will be English. Papers may be presented in any European language. No facilities for simultaneous translation will be available.

Scientific Programme:

- A. Plenary Lecture
- B. The following topics have been selected for the Symposium
 - Session I. Yeast cell wall lytic enzymes.
Organized by C. Hardisson.
 - Session II. Structure and Morphogenesis.
Organized by F. Uruburu.

- Session III. Physiology and biochemistry of yeast protoplasts.
Organized by S. Gascón.
- Session IV. Protoplasts of moulds.
Organized by Isabel García-Acha.
- Session V. Protoplasts of higher plants.
Organized by R. Sentandreu.

C. Free communications

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Third International Symposium on Yeast Protoplasts
Departamento de Microbiología
Facultad de Ciencias
Universidad de Salamanca
Salamanca, Spain
Cable: YEASTSYMPOSIUM (Salamanca)

XXXI Brief News Items

1. Professor F. M. Clark of the University of Illinois and an Associate Editor for many years, has retired. His contributions to the Yeast News Letter are greatly appreciated and we wish him the very best in good health in the years ahead.

Professor Torsten D. Wiken, Chairman of the Commission on Yeasts and Yeast-like Organisms (IAMS), has accepted appointment as Associate Editor to succeed Professor Clark.

2. Dr. and Mrs. L. J. Wickerham are in the process of establishing their new home at the following address: 5540 West Bar X Street, Tucson, Arizona 85713. They love the Arizona deserts and mountains and are taking a course in desert biology at a local college. In August the Wickerhams will combine a music workshop and back packing in Colorado.

Dr. Wickerham would greatly appreciate receiving reprints from all contributors of the Yeast News Letter.

3. Instituto Nacional de Investigaciones Agronómicas, Avda. Puerta de Hierro, Madrid 3, Spain. Prof. J. Santa María reports that the following recent publications are currently in press:

J. Santa María and Concepción García Aser: "Debaryomyces yarrowii nov. spec.". Anales Instituto Nacional de Investigaciones Agronomicas, 1971. In press. Description of a new Debaryomyces species isolated from frass.

J. Santa María: "Candida ergatensis nov. spec.". Anales Instituto Nacional de Investigaciones Agronomicas, 1971. In press. Description of a new Candida species, able to form true mycelium, isolated from larvae.

4. Dr. L. R. Batra, United States Department of Agriculture,

Agricultural Research Service, Plant Science Research Division, Beltsville, Maryland 20705, reports: I have just completed a monograph on the hemiascomycetes which predominantly live in mycelial form. A comparative account of the morphology and developmental stages of all species of the genera Ascoidea, Dipodascus, Eremascus, Eremothecium, Nematospora, Endomyces and the monotypic genera Ashbya, Cephaloascus, Endomycopsella, Helicogonium, and Spermophthora are discussed. The doubtful genera for which no material was available (Coccidiascus, Conidiascus, Fragosia, and Oscarbrefeldia etc.) are also treated. A key to the genera and species are provided and almost all species are illustrated. Although some formalities have not yet been completed, the monograph has been accepted for publication by J. Cramer, Lehre, Germany.

5. Miami University, Department of Microbiology, Oxford, Ohio 45056. Dr. J. K. Battacharjee reports the publication of the following papers:

Sinha, A. K. and J. K. Battacharjee. 1970. Control of a lysine-biosynthetic step by two unlinked genes of Saccharomyces. Biochem. Biophys. Res. Commun., 39:1205-1210.

Bhattacharjee, J. K. 1970. Leaky mutation and coordinate regulation of the accumulation of lysine-precursors in Saccharomyces. Can. J. Genet. Cytol., 12:785-789.

Bhattacharjee, J. K. 1970. Microorganisms as potential source of food. Adv. Appl. Microbiol. 13:139-161.

Bhattacharjee, J. K. 1971. Population explosion and microorganisms as potential source of food. The Biologist. 53: 22-28.

6. Peter Austwick, formerly at the Central Veterinary Laboratory, Weybridge, Surrey England, informed the editor that he is presently at Nuffield Institute of Comparative Medicine, Zoological Society of London, Regents Park, London, NW1 4RY. He writes: My field of work is in the morphology of fungi in tissue and its relationship to the reaction of the body to invasion. I hope to include a number of yeasts in this study and would be pleased to hear of anyone working in the field who could supply or loan to me fixed tissue, blocks or sections known to be infected by the following fungi:

Candida tropicalis
Candida guilliermondii
Trichosporon cutaneum
Geotrichum candidum

We have just spent a most enjoyable year in New Zealand working on bovine mycotic abortion mostly caused by Mortierella wolfii, at the Ruakura Agricultural Research Centre, Hamilton.

7. Dr. S. Fogel, Department of Genetics, University of California, Berkeley, Ca. 94720 writes: Dr. Robert Mortimer and I have been funded by the National Science Foundation to establish a yeast culture collection. We are prepared to supply only those stocks of which we will present a listing to be distributed to the yeast

genetics community. We plan to make available stocks which are useful for mapping purposes and for identifications against the known mutants. We would welcome those unique additions to the collection which have utility. Most emphatically, we do not plan to serve as a repository for the preservation of miscellaneous stocks of limited interest.

8. Dr. K. Kodama points out the following errors in Chapter 5 (Sake yeast) of *The Yeasts*, Vol. III (A. H. Rose and J. S. Harrison eds.), Academic Press, London.

ERRATA

Page	Line	For	Read
228	Table 1-10	glucoside -	glucoside +
"	31	sakē process	sakē brewing process
231	Fig 2-2	yeast on potato agar	yeast, <u>Kyokai</u> No. 7 strain on potato agar
234	14	differed	differs
236	31	caused by	due to
238	15	mashes from	mashes of
"	21	breweries in	mashes of breweries in
241	14	fraction of <u>Koji</u>	fraction of <u>Koji</u> mould
246	25	1962c	1962d
247	8	the nitrogen	cellular nitrogen
"	13	is normally accom- panied	is normally carried out accompanied
"	21	1962d	1962e
"	23	up to 40-45%	up to 30-35%
"	30	p 263	p 265
249	5	5-9	5-7
251	10	p 266	p 270
253	19	slight graded	slight and graded
258	16	p 227	p 229
259	19	changed	changes
"	33	a precursor	a special precursor
262	33	sufficient pure active yeast	sufficient crop of pure and active yeast
265	26	If, however, the growth	If the growth
266	20	the mash	the alcohol-added mash
272	13	12-16%	15-25%
273	3-4	putrefaction	spoilage

December 1971

Dear readers,

As you know the financial operation of the Yeast News Letter has been left up till now to voluntary contributions by the readers. Some of you have faithfully paid \$1.00 per year, others have been more generous and still others contribute hardly ever. Last year mailing and reproduction (exclusive of secretarial time) cost \$871.00 and the income was \$226.00 from 15% of the subscribers. The deficit was contributed by the Department of Food Science and Technology, University of California. Considering the financial problems of the University we can no longer absorb such a large deficit. Added to this is the fact that recently mailing costs have gone up sharply.

As a first step to overcome this problem it has become necessary to institute a billing system and to increase the subscription price to \$1.50 for the two issues per year. Those who already paid for 1972 or beyond will receive credit for their contributions. Many countries with foreign currency restrictions are nevertheless permitted to send Unesco Coupons or International Postal Exchange Coupons. Where none of these alternatives are available interested readers may still continue to receive the News Letter. If you are no longer interested in the News Letter please notify us promptly.

H. J. Phaff
Editor

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