

# PERIODICALS ROOM

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## Y E A S T

A Newsletter for Persons Interested in Yeast

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International Commission on Yeasts and Yeasts-like Microorganisms  
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June 1980

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Herman J. Phaff, Editor  
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Davis, California 95616

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

The strains listed below have been added to the ATCC since November 27, 1979. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

<u>Saccharomyces bailii</u> ATCC 38923 decomposition of malic acid during grape fermentation	S. Goto Research Inst. of Fermentation Yamanashi University Kofu, Japan
<u>Saccharomyces acidifaciens</u> ATCC 38924 decomposition of malic acid during fermentation of grape	"
<u>Kloeckera javanica</u> ATCC 38925 decomposition of malic acid	"
<u>Saccharomyces uvarum</u> ATCC 38926-38928 bottom-fermenting brewers yeast	A. D. Haukeli Als Ringnes Brewery Norway
<u>Saccharomyces cerevisiae</u> ATCC 38929 genetic studies	K. Wakabayashi University of Tokyo Tokyo, Japan
<u>Saccharomyces cerevisiae</u> ATCC 38976 killer toxin secretion	Kozo Ouchi Research Institute of Brewing Tokyo, Japan
<u>Saccharomyces bisporus</u> var. <u>bisporus</u> ATCC 38993 sherry production	D. F. Spiittstoesser Cornell University Geneva, New York
<u>Saccharomyces cerevisiae</u> ATCC 42015-42017 genetic studies	J. Bruenn State University of New York Buffalo, New York
<u>Saccharomyces cerevisiae</u> ATCC 42029 genetic studies	S. F. Cottrell Brooklyn College of New York Brooklyn, New York
<u>Saccharomyces cerevisiae</u> ATCC 42030-42034 genetic studies	H. Bussey McGill University Montreal, Quebec, Canada
<u>Candida guilliermondii</u> ATCC 42050 production of single cell protein from hydrocarbons	H. Hiss Industrial Biochim Lab. Sao Paulo, Brazil
<u>Saccharomyces cerevisiae</u> ATCC 42081 fermentation of "tesquino" beer	M. Ulloa University of Mexico Mexico 22, D.F.

Saccharomyces uvarum  
ATCC 42082  
fermentation of "tesquino" beer

M. Ulloa  
University of Mexico  
Mexico 22, D.F.

Saccharomyces cerevisiae  
ATCC 42130  
genetic studies

G. L. Petersen  
Carlsberg Lab  
Gamle Carlsberg Vej  
Valby, Denmark

Saccharomyces cerevisiae  
ATCC 42131  
genetic studies

R. Fahrig  
D-7800 Freiburg, Schoneck Str. 3  
Germany

Saccharomyces cerevisiae  
ATCC 42152-42153  
genetic studies

A. P. James  
Division of Biological Sciences  
Ottawa, Ontario, Canada

Filobasidiella neoformans  
ATCC 42161, 42163, 42168  
mating type  $\alpha$

K. Schmeding  
American Type Culture Collection  
Rockville, Maryland

ATCC 42162, 42165, 42169  
mating type  $\alpha$

"

ATCC 42165-42167  
self-fertile

"

Candida mucilagina  
ATCC 42171  
taxonomy

M. Miranda  
University of California  
Davis, California

Lodderomyces opuntiae  
ATCC 42172-42173  
taxonomy

"

Candida mucilagina  
ATCC 42174  
type culture

"

Yeast  
ATCC 42175-42176  
taxonomy

"

Candida utilis  
ATCC 42181  
physiology studies

H. Aiking  
University of Amsterdam  
The Netherlands

Saccharomyces cerevisiae  
ATCC 42182  
genetic studies

D. Wolf  
Biochem. Institut Universität  
Germany

Saccharomyces cerevisiae  
ATCC 42207-42209  
genetic studies

J. L. Bos  
Section for Molecular Biology  
Amsterdam  
The Netherlands

<u>Torulopsis taboadae</u> ATCC 42213 wine fermentation	M. Ulloa University of Mexico Mexico 22, D.F.
<u>Candida ravautii</u> ATCC 42214 onchomycosis	W. J. Crovier Wollongong Hospital Australia
<u>Saccharomyces cerevisiae</u> ATCC 42243-42244 genetic studies	A. Uchida Kobe University Kobe, Japan
<u>Saccharomyces cerevisiae</u> ATCC 42245 genetic studies	A. H. Hopper Milton S. Hershey Medical Center Hershey, Pennsylvania
<u>Cryptococcus laurentii</u> ATCC 42264 metabolizes 3-O-methyl-D-glucose	R. Brown Dalhousie University Canada
<u>Candida kefyr</u> ATCC 42265	D. Yarrow CBS The Netherlands
<u>Candida albicans</u> ATCC 42266	"
<u>Candida membranaefaciens</u> ATCC 42267	"
<u>Candida oregonensis</u> ATCC 42268	"
<u>Candida shehatae</u> ATCC 42269	"
<u>Candida silvae</u> ATCC 42270	"
<u>Candida hordei</u> ATCC 42271	"
<u>Cryptococcus ater</u> ATCC 42272-42273	"
<u>Cryptococcus lactativorus</u> ATCC 42244-42275	"
<u>Cryptococcus kuetzingii</u> ATCC 42276-42278	"
<u>Cryptococcus luteolus</u> ATCC 42279-42280	"

Candida pseudolipolytica  
ATCC 42281

D. Yarrow  
CBS  
The Netherlands

Saccharomyces cerevisiae  
ATCC 42295-42301  
genetic studies

R. B. Wickner  
NIH  
Bethesda, Maryland

Schizosaccharomyces pombe  
ATCC 42302-42305  
genetic studies

P. A. Fantes  
University of Edinburgh  
United Kingdom

Hansenula saturnus  
ATCC 42306  
production of l-amino  
cyclopropane -l-l

M. Honma  
Hokkaido University  
Sapporo, Japan

Pichia kluyveri  
ATCC 42328  
production of yeast killer toxin

E. J. Middelbeek  
University of Nijmegen  
Nijmegen  
The Netherlands

Saccharomyces cerevisiae  
ATCC 42329  
genetic studies

D. F. Callen  
Flinders University  
South Australia

Saccharomyces cerevisiae  
ATCC 42330-42334  
genetic studies

S. Doi  
Kinki University  
School of Medicine  
Osaka, Japan

Saccharomyces cerevisiae  
ATCC 42335-42337  
genetic studies

K. Suda  
Nara University  
Nara, Japan

Saccharomyces cerevisiae  
ATCC 42338-42340  
genetic studies

M. Wesolowski  
Institut Curie  
Orsay, France

Moniliella species  
ATCC 42342  
immunological studies

G. S. Bulmer  
University of Oklahoma  
Oklahoma City, Oklahoma

Filobasidiella neoformans  
ATCC 42343-42347  
immunological studies

"

Saccharomyces carlsbergensis  
ATCC 42367-42368  
biomedical studies

P. S. Lazo  
Universidad de Oviedo  
Spain

Trichosporon penicillatum  
ATCC 42397  
produces protopectin-  
solubilizing enzyme

Dr. Sakai  
University Osaka  
Osaka, Japan

Hansenula anomala  
ATCC 42398  
treatment of waste water  
from sake brewery

K. Yoshizawa  
Kito-kui  
Tokyo, Japan

Filobasidium capsuligenum  
ATCC 42399  
taxonomy

D. G. Ahearn  
Georgia State University  
Atlanta, Georgia

Candida utilis  
ATCC 42402  
production of extra-cellular invertase

T. Yamamoto  
Osaka University  
Osaka, Japan

Saccharomyces cerevisiae  
ATCC 42403-42405  
genetic studies

Dr. I. Takano  
The Central Research Institute  
Osaka 618, Japan

Saccharomyces cerevisiae  
ATCC 42407  
genetic studies

R. Serrano  
University Autonoma  
Madrid, Spain

Saccharomyces cerevisiae  
ATCC 42413-42414  
genetic studies

J. R. Pringle  
University of Michigan  
Ann Arbor, Michigan

Candida utilis  
ATCC 42416  
production of single cell  
protein from ethanol

P. Biely  
Slovak Academy  
Bratislava, Czechoslovakia

Saccharomyces ludwigii  
ATCC 42451-42456  
genetic studies

T. Yamazaki  
Yamanashi University  
Kofu, Japan

Saccharomyces bailii var. bailii  
ATCC 42476-42477  
production of sherry type wine

G. A. Farris  
Universita de Sassari  
Sassari, Italy

Saccharomyces bayanus  
ATCC 42478  
production of sherry type wine

"

Saccharomyces prostoserdovii  
ATCC 42479-42480  
production of sherry type wine

"

II. Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pushchino, Moscow Region 142292, USSR. Communicated by W. I. Golubev.

The Instability of Physiological and Morphological Characteristics in Metschnikowia lunata

The description of M. lunata (Golubev, W. I. 1977. ANTONIE VAN LEEUWENHOEK 43, 317) was based on the study of only one strain, CBS 5946. Recently, ascosporeulation was achieved in the strain CBS 7698 designated as Schizoblastosporion kobayashii Soneda (= Selenotila intestinalis Krassilnikov). On the basis

of mode of ascus formation, ascospore morphology, and physiological properties, it is also identified as M. lunata. In contrast to the type strain, the new one does not assimilate L-sorbose but assimilates raffinose weakly. Depending on the conditions of growing (the sources of carbon and nitrogen in the medium), the cultures of M. lunata can lose the ability to utilize ethanol, ribitol, mannitol, and glucitol. The ability to ferment glucose can become very weak and latent. In addition, changes can occur in size and shape of vegetative cells. These observations may explain the discrepancies between authors' descriptions of these strains and the results of the recent studies. Attention is paid to the fact that all cultures of M. lunata were isolated in maritime regions.

The following articles have been published recently:

Golubev, W. I., Tauson, E. L. 1979. Heterogeneity of the Yeast-like Fungus Cryptococcus terreus di Menna. MIKOLOGIA I PHYTOPATOLOGIA 13, No. 6, 462-467.

Schkidtschenko, A. N., Golubev, W. I. 1980. Continuous Cultivation of Mono- and Mixed Cultures of Yeasts Inhabiting Spring Fluxes of Birch. MIKROBIOLOGIA 49, No. 1, 44-48.

III. Istituto di Microbiologia Agraria e Tecnica, University of Perugia, 06100 Perugia, Italy. Communicated by A. Martini.

1. Dr. F. Federici from our Institute spent 8 months in the laboratory of Dr. M. W. Miller, Department of Food Science and Technology of the University of California at Davis, getting acquainted with procedures of yeast collection, maintenance, and working on a project on yeast ecology.
2. Dr. G. Rosini from this Institute is leaving next February to work for 10 months in the laboratory of Dr. Blachère at the Institute National de la Recherche Agronomique, Station de Génie Microbiologique, Dijon, France.
3. We received a grant from the Italian National Council for Research to work out the nuclear DNA/DNA relatedness relationship inside a relatively large group of Candida species in actual or potential use for SCP production.
4. The following papers have been submitted for publication, accepted or published:

"A Proposal for a New Approach to the Study of Yeast Ecology of Natural Substrates." A. Martini, F. Federici, and G. Rosini. Submitted to CANADIAN JOURNAL OF MICROBIOLOGY.

#### ABSTRACT

The yeast flora associated with the surface of various natural materials has been studied on the assumption that individual cells and micro-colonies are strongly retained by gummy and mucous secretions. Pre-isolation treatments based on vigorous shaking, percolation with excess of water, and the sonication of samples allowed the recovery of a much higher number of species when compared to traditional isolation procedures. Results confirmed that a sequence of aggressive and disruptive actions on samples, together with an enrichment culture, is necessary to obtain an exhaustive picture of the actual yeast flora.

\* \* \*

"Partial Purification and Characterization of a Yeast Extracellular Acid Protease." A. Martini and F. Federici. Submitted to JOURNAL OF DAIRY SCIENCE.

ABSTRACT

A yeast isolate, belonging to the species Cryptococcus albidus var. aerius and selected for its high milk coagulating activity in the course of a screening survey of 142 yeasts, is able to produce an extracellular protease in shake culture. A crude preparation was obtained from the cell-free broth by ammonium sulphate precipitation, showing an optimum pH for milk-clotting activity of 5.5-5.7 at 35°C, while maximum stability to pH occurred in the range 3.5 to 5.5. Its optimum temperature was 45°C. Inactivation by lead, iron, and mercury ions was observed.

\* \* \*

"Variations of Cellular Nitrogen Components of Different Yeasts During Growth." A. Martini, Ann E. Martini, and M. W. Miller. Accepted for publication in the JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE.

ABSTRACT

The amino acid composition of whole cells of eight yeast species as well as their protein and RNA contents were determined at three selected points of the growth curve. Total nitrogen and crude protein contents showed a decrease during the growth cycle, while true protein concentrations appeared to be more stable. RNA contents significantly decreased in the late stationary phase. Variability was negligible for overall amino acid patterns from different phases of growth.

\* \* \*

"Amino Acid Composition of Whole Cells of Different Yeasts." Ann E. Vaughan Martini, M. W. Miller, and A. Martini. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 27:982-984 (1979).

IV. Universidade Federal do Rio de Janeiro, Instituto de Microbiologia, Ilha do Fundão, Rio de Janeiro, RJ, Brasil. Communicated by Allen N. Hagler.

Below follows the English summary of the recently completed masters thesis of Renilson Batista de Oliveira (Text Portuguese):

The Yeasts of Cashew and Mango Fruits

SUMMARY

This is a quantitative and taxonomic study of yeasts, removed by washing off, associated with the skin of, or inside of cashew and mango fruits and of cashew flowers. The method developed to wash yeasts from the surface of fruits was to shake them at 120 RPM for 30 minutes in steril plastic bags containing 100 ml steril 0.85% NaCl and 0.05% tween 40 in distilled water for each fruit; this suspension was blended aseptically for one minute before plating. Rose bengal at 2.5 and 3 mg% concentrations caused 12 to 69% reduction in yeast counts on Sabouraud agar.

Ninety-three percent of the yeasts of cashew fruits collected in the field were removed by washing, but less than 50% were washed from the surface of cashews collected from markets of Rio de Janeiro. Ninety-eight percent of the yeasts of both sword and pink mangos were removed by washing.

Yeast counts on cashew fruits ranged from  $4.3 \times 10^3/g$  for those collected in the field to more than  $2 \times 10^7/g$  for some collected in the market. Total yeasts counts on mangos were  $2.5 \times 10^5/g$  for the pink variety and  $4.1 \times 10^3/g$  for the sword variety. Yeasts are probably important in the spoilage of cashew but not of mango fruits.

The 204 strains of yeasts and yeast-like organisms isolated were grouped into 49 species. Of these, only 13 species could be identified; Candida diddensii, C. guilliermondii, C. krusei, C. lusitaniae, C. sorbosa, C. tropicalis, Cryptococcus albidus var. diffluens, Kloeckera apiculata, Pichia membranaefaciens, P. ohmeri, P. terricola, Rhodotorula pilimanae, and Torulopsis candida. The yeasts isolated were predominantly fermentative which may reflect the high level of sugars in their environment. A large proportion of the organisms studied were yeasts which could not be identified and are probably new species.

The prevalent yeast of cashew fruits was Kloeckera apiculata which made up 78% of the population on the surface and 90% of the population inside the fruits. The prevalent species on rose mangos were Candida krusei, Kloeckera apiculata, and Candida lusitaniae in decreasing order of incidence. The predominant yeast on sword mangos was Candida krusei which made up about 50% of the total yeast population.

V. Microbiology Department, Indian Drugs Research Laboratory (IDRA), 561/B Shivajinagar, Poona 411 005, India. Communicated by V. B. Rale.

1. V. B. Rale and J. R. Vakil. Microbial ecology of Achras sapota L. (Chikoo). Presented at the 19th Annual Conference, 1978, of the Association of Microbiologists of India, Baroda).

A microbial survey of the yeasts of Achras sapota L. in an orchard over two consecutive years revealed a regular pattern of microflora and indicated (i) a dominant and (ii) transient microbial population on Chikoos. Although the yeast counts made in 1974 were different from those of 1975, the total picture and their occurrence pattern did not change appreciably. It seemed that the microbial ecosystem in the orchard consisted of three zones: terrestrial, plant, and aerial. There was some overlapping of each zone. This was unavoidable since certain vectors, e.g. insects, are likely to have affected the zone microflora qualitatively and quantitatively. Thus, Hansenula sydowiorum, an insect-borne species, was found throughout the development stages of Chikoos. The occurrence of different groups of microorganisms at various stages of development of the Chikoo can be used to construct a biological calendar.

2. V. B. Rale and J. R. Vakil. The yeasts of Achras sapota L. (Chikoo). Presented at the 19th Annual Conference, 1978, Association of Microbiologists of India, Baroda, and to appear in BIOVIGYANAM, 1980.

Systematic studies on the microflora of fruits have been few, except those concerned with the microbial spoilage of soft fruit both before and after harvest. In particular, their yeast flora have been largely ignored;

where they have been studied, only total counts have been made. Apart from the detailed studies of yeasts on strawberries and other soft fruits by Buhagiar and his associates, little attention has been given to the yeast flora of soft fruits. A systematic program of isolation of yeasts from A. sapota L. fruits was undertaken by us over two consecutive years (1974-75) based on the isolation methods modified and developed in our laboratory.

The major yeasts isolated and identified were Hansenula, Rhodotorula, Saccharomyces, and Sporobolomyces species. Among the minor flora, Pullularia pullulans (= Aureobasidium pullulans) was the most dominant.

The paper describes and emphasizes the utility of newer isolation and identification techniques for yeasts from fruits that are applicable to other ecosystems.

VI. Department of Plant Sciences, University of Western Ontario, London, Canada N6A 5B7. Communicated by M. A. Lachance.

To be presented at the Vth International Symposium on Yeasts, London, Canada, July 1980.

#### Yeasts Associated with Black Knot Disease of Trees

Various substrates in the southern Ontario peninsula were screened for the presence of sexually reproducing yeasts to establish a source of organisms suitable for natural speciation studies. Black Knot, a tree gall caused by the fungus Dibotryon morbosum (= Apiosporina morbosa), was found to be an exceptionally reliable source of ascogenous yeasts. Due to the ubiquity of Aureobasidium and Cryptococcus species, a selective technique favoring fermentative yeasts were used, although several strictly oxidative ascosporegenous yeasts were often recovered.

The genera Kluyveromyces and Saccharomyces predominated, while less abundant isolates included species of Hanseniaspora, Hansenula, and Pichia. A large number of Candida species, often the anamorphic counterparts of the above genera, were also recovered.

The survey was extended to several host trees, including Prunus virginiana, P. avium, P. pensylvanica, P. serotina, P. pumila, and Malus species, from localities in the "Deciduous" and "Great Lakes-St. Lawrence" Canadian forest regions. These variables, as well as the habitats of the trees, the age of the galls, their colonization by insects, season, and climate were factors found to have a profound influence, qualitatively and quantitatively, on the yeast crop.

\* \* \*

#### A Simple Method for the Determination of Deoxyribonucleic Acid Relatedness by Thermal Elution in Hydroxyapatite Micro-columns

Marc-André Lachance (1980)

INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY 30(2):433-436

## ABSTRACT

A convenient method has been devised to chromatograph simultaneously numerous samples of reassociated deoxyribonucleic acid (DNA) under thermo-regulated conditions. Small amounts of hydroxyapatite (0.2 ml bed) are used, and the effluent volumes for separating single-stranded DNA from DNA duplexes are 1.5 ml each. The method is comparable in reliability and reproducibility to other well-established techniques; but it combines improvements in ease of operation, in rapidity, and in simplicity, which are desirable in large taxonomic studies.

VII. Laboratoire de Biologie et Cytophysiologie Végétales, U.E.R. des Sciences de la Nature, Université de Nantes, Le Petit Port, 44300 Nantes, France. Communicated by Liliana Simon.

Below follow abstracts of two studies conducted in our laboratory:

L. Simon et A. Poulard (1979) Occurrence of Aureobasidium pullulans (de Bary) Arnaud in the Vineyard of Nantes (France). A Microbiological and Ecological Study. BULL. SOC. SC. NAT. OUEST DE LA FRANCE 1, 57-68.

### SUMMARY

Aureobasidium pullulans strains have been isolated from the fermentative oxydative microflora of the regional vineyard of Nantes (soil, inflorescences, grape juices, musts, and wine). This species is widely distributed among the oxydative group forms.

Microbiological characteristics of some strains have been determined from solid or liquid aseptic cultures; yeast-like cells and hyphae present a very rapid growth. Four or 5 days after inoculation, colonies of some strains develop a brown pigmentation. Fermentative capacities of such strains are weak or lacking.

The ecological distribution of this species during two years (1976 and 1977) is discussed. Climatic variations are very important in the distribution.

\* \* \*

A. Poulard et L. Simon (1979) Rare Yeasts of the Nantes Vineyard (France): Some Ecological and Metabolic Properties. BULL. SOC. SC. NAT. OUEST DE LA FRANCE 1, 185-196.

### SUMMARY

Seventeen oxydative species of yeasts and yeast-like organisms have been isolated from soil, inflorescences, black and white juices, and musts of the Nantes regional vineyard (Aureobasidium pullulans, Candida krusei, C. sake, C. sorbosa, C. zeylanoides, Endomycopsis vini, Exophiala jeanselmei, Pichia rhodanensis, P. vini, Rhodotorula pilimanae, Torulopsis colliculosa, T. gro-pengiesseri, T. lactis-condensi, Trichosporon capitatum, Tr. cutaneum, Tr. fermentans, Tr. penicillatum); some of them, Exophiala jeanselmei, Trichosporon (=Geotrichum) penicillatum, Candida sorbosa, are unknown or little known in viticultural ecosystems. Aureobasidium pullulans is the most dominant. The fermentative activity of these species has been determined for some sugars; among them, glucose, galactose, and sucrose. The ecological distribution of this minor oxydative flora is discussed for two

years, 1976-1977, years characterized by strong climatic variations. The results of these studies are compared with that obtained in other french vineyards.

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

Below follows an abstract published in SOCIETY FOR GENERAL MICROBIOLOGY QUARTERLY, Vol. 7, page 85 (1980). The paper was presented orally by Dr. Salmon at the Easter meeting of the Society at Cambridge.

Activities of Some Mitochondrial Enzymes in the  
Cell Cycle of Sterigmatomyces halophilus

I. Salmon and R. K. Poole

ABSTRACT

Sterigmatomyces halophilus is an unusual budding yeast in which daughter cells are formed remote from the mother cells on fine projections called sterigma<sup>1</sup>. The cell cycle of this yeast was studied by isopycnic fractionation of exponentially-growing cultures into various size classes using linear urografin gradients (24-44% w/v) in a BXIV zonal rotor. Successive fractions from such separations correspond to different stages in the cell cycle.

Protein, RNA, and the activities of various mitochondrial enzymes were assayed in extracts of each fraction. During the cell cycle, these components showed an overall doubling on a per cell basis; however, while protein and RNA increased continuously, the enzymes showed oscillations. If the cell cycle is expressed as a linear scale between 0 and 1, and it is assumed that cell age is directly proportional to cell size, then succinate dehydrogenase showed a single maximum at  $0.25 \pm 0.05$  [standard deviation of 5 experiments]. Two other enzymes each exhibited two peaks per cycle, though at different timings, NADH cytochrome c oxidoreductase at  $0.41 \pm 0.08$  [5] and  $0.75 \pm 0.09$  [5] and cytochrome c oxidase at  $0.25 \pm 0.06$  [8] and  $0.66 \pm 0.06$  [8]. The cytochrome a + a<sub>3</sub> complex (the structural counterpart of cytochrome c oxidase activity) was measured in low-temperature difference spectra<sup>2</sup> in several independent experiments and found to vary in phase with cytochrome c oxidase activity. This indicates that, at least for this enzyme, the variation in activity actually reflects a variation in enzyme amount. In contrast, Cottrell et al.<sup>3</sup> observed a step-wise increase in oxidase activity but a continuous increase in spectrophotometrically-detectable oxidase in induced synchronous cultures of Saccharomyces cerevisiae.

<sup>1</sup>Salmon, I., and Poole, R. K. 1978. BULLETIN OF THE BRITISH MYCOLOGICAL SOCIETY 12, 121.

<sup>2</sup>Salmon, I., and Poole, R. K. 1978. SOCIETY FOR GENERAL MICROBIOLOGY QUARTERLY 6, 34.

<sup>3</sup>Cottrell, S. F., Rabinowitz, M., and Getz, G. S. 1975. JOURNAL OF BIOLOGICAL CHEMISTRY 250, 4087.

IX. Laboratorium voor Microbiologie, R. U. Groningen, Kerklaan 30, Haren (GR.), Holland. Communicated by K. B. Zwart.

The following papers have been accepted for publication:

Development of Amine Oxidase-Containing Peroxisomes in Yeasts during growth on Glucose in the Presence of Methylamine as the Sole Source of Nitrogen

K. Zwart, M. Veenhuis, J. P. van Dijken, and W. Harder

ARCHIVES OF MICROBIOLOGY, in press

#### ABSTRACT

The metabolism of methylamine as the nitrogen source for growth of the non-methylotrophic yeast Candida utilis and the methylotrophic yeast Hansenula polymorpha was investigated. Growth of both organisms in media with glucose and methylamine was associated with the presence of an amine oxidase in these cells. The enzyme catalyses the oxidation of methylamine by molecular oxygen into ammonia, formaldehyde, and hydrogen peroxide; and it is considered to be the key enzyme in methylamine metabolism in the organisms studied.

In addition to synthesis of amine oxidase, derepression of catalase, formaldehyde and formate dehydrogenases was also observed upon transfer of cells of the two organisms from media containing ammonium ions into media containing methylamine as the nitrogen source.

The synthesis of enzymes was paralleled by the development of a number of large microbodies in the cells. Cytochemical staining experiments indicated that the amine oxidase activity was located in the microbodies in both organisms. Catalase activity was also demonstrated in these organelles, which can, therefore, be considered as peroxisomes. The present contribution is the first description of a peroxisomal amine oxidase.

\* \* \*

An Electron Microscopical Study of the Development of Peroxisomes during Formation and Germination of Ascospores in the Methylotrophic Yeast Hansenula polymorpha

M. Veenhuis, I. Keizer-Gunnink, and W. Harder

ANTONIE VAN LEEUWENHOEK, accepted for publication

#### ABSTRACT

Ascospore formation was studied in liquid cultures of the yeast Hansenula polymorpha, previously grown under conditions in which the synthesis of alcohol oxidase was repressed (glucose as growth substrate) or de-repressed (methanol, glycerol, and dihydroxyacetone as growth substrates and after growth on malt agar plates). In ascospores obtained from repressed cells, generally one small peroxisome was present. The organelle probably originated from the small peroxisome, originally present in the vegetative cells. They had no crystalline inclusions and cytochemical experiments indicated the presence of catalase, urate oxidase, and amino acid oxidase activities in these organelles. In ascospores obtained from de-repressed cells, generally

1-3 crystalline peroxisomes were observed. These organelles also originated from the peroxisomes originally present in the vegetative cells by means of fragmentation or division. They contained, in addition to the enzymes characteristic for peroxisomes in spores from repressed cells, also alcohol oxidase. The latter enzyme is probably responsible for the crystalline substructure of these peroxisomes.

Peroxisomes had no apparent physiological function in the process of ascospore germination. A glyoxysomal function of the organelles during germination of the ascospores was also not observed. Germination of mature ascospores in media containing different carbon- and nitrogen sources showed that the function of the peroxisomes present in these ascospores of Hansenula polymorpha is probably identical to that in vegetative haploid cells. They are involved in the oxidative metabolism of different carbon- and nitrogen sources. Their enzyme profile is a reflection of that of peroxisomes of vegetative cells, and their presence may enable the formation of cells which are optimally adapted to environmental conditions extant during spore germination.

\* \* \*

Cytochemical Localization of Glucose Oxidase  
in Peroxisomes of Aspergillus niger

J. P. van Dijken and M. Veenhuis

EUROPEAN JOURNAL OF APPLIED MICROBIOLOGY, in press

ABSTRACT

The subcellular localization of glucose oxidase (E.C. 1.1.2.4.) in mycelia of Aspergillus niger has been investigated using cytochemical staining techniques. Mycelia from fermenter cultures, which produced gluconic acid from glucose, contained elevated levels of glucose oxidase and catalase. Both enzymes were located in microbodies. In addition, when the organism was grown on glucose with methylamine as a nitrogen source, amine oxidase activity was detected in the microbodies. These organelles can, therefore, be designated as peroxisomes.

The following paper has recently been published in ARCHIVES OF MICROBIOLOGY:

Th. Egli<sup>1</sup>, J. P. van Dijken<sup>2</sup>, M. Veenhuis<sup>2</sup>, W. Harder<sup>2</sup>, and A. Fiechter<sup>1, 3</sup>.  
"Methanol Metabolism in Yeasts: Regulation of the synthesis of catabolic enzymes".

X. Division of Biological Sciences, National Research Council, Ottawa, Canada K1A 0R6. Communicated by H. Schneider.

The following is an abstract of a paper recently accepted by BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS:

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<sup>1</sup>Chair of Microbiology, Swiss Federal Institute of Technology, ETH-Hönggerberg, CH-8093, Zürich, Switzerland.

<sup>2</sup>Department of Microbiology, University of Groningen, Kerklaan 30, 9751 NN Haren, the Netherlands.

<sup>3</sup>To whom requests for offprints should be sent.

Patrick Y. Wang, Charles Shopsis, and Henry Schneider

Molecular Genetics Group, Division of Biological Sciences  
National Research Council of Canada, Ottawa, Canada K1A 0R6

### Fermentation of a Pentose by Yeasts

#### SUMMARY

Several yeasts have been found to be able to ferment D-xylulose, a catabolite of D-xylose and to produce ethanol thereby. The fermentation is carried out by several species which can utilize D-xylose oxidatively as well as by several which cannot do so. Xylose itself, and the other aldopentoses, are not utilized anaerobically by yeasts. Fermentation of D-xylulose by D-xylose-oxidising species indicates that a control operates under conditions of low oxygen tension which prevents the catabolism of D-xylose to D-xylulose. The results are pertinent in efforts to obtain yeasts which can ferment biomass pentoses, a problem of interest in attempts to obtain a liquid fuel from a renewable resource.

XI. Department of Microbiology, University of Glasgow, Alexander Stone Building, Garscube Estate, Bearsden, Glasgow, Scotland. Communicated by L. Julia Douglas.

#### Inhibition and Activation of Mannan Synthesis in Saccharomyces cerevisiae Spheroplast Lysates

Charles R. Harrington and L. Julia Douglas

JOURNAL OF BACTERIOLOGY (in press, June 1980 issue)

#### ABSTRACT

Mannan synthetase activity in spheroplast lysates prepared from Saccharomyces cerevisiae was measured by following the incorporation of [ $^{14}\text{C}$ ] mannose from GDP-[ $^{14}\text{C}$ ] mannose into material precipitable with cold 0.3M perchloric acid. When enzyme activity was assayed at high concentrations of spheroplast lysate protein (10 mg/ml), in the presence of 7.5 mM  $\text{MnCl}_2$ , a severe inhibition was observed. This inhibition could be relieved by pre-incubation of the spheroplast lysate at 4°C for 16-32 hours before assay, by repeated freezing and thawing of the spheroplast lysate, or by the omission of  $\text{MnCl}_2$  from assay mixtures. The addition of EDTA or monovalent cations removed inhibition in the presence of  $\text{Mn}^{2+}$ . No similar inhibition was observed when a washed membrane fraction was substituted for spheroplast lysate as the source of mannan synthetase. The supernatant fluid obtained by centrifuging spheroplast lysate at 100,000 x g, when added to assay mixtures containing either spheroplast lysate pre-incubated at 4°C or washed membrane fraction, also caused inhibition of enzyme activity. This inhibition required 7.5 mM  $\text{MnCl}_2$  and was destroyed by heating the supernatant fluid at 60°C for 10 minutes or by trypsin treatment at 30°C. These results indicate the existence of a protein inhibitor of mannan synthesis whose inhibitory activity in spheroplast lysates may be modulated by pre-incubation at low temperature or by varying the available  $\text{Mn}^{2+}$  concentration.

XII. Faculty of Science of Living, Osaka City University, Sugimotocho, Sumiyoshi-ku, Osaka 558, Japan. Communicated by Akira Misaki.

Below follow abstracts of two recent papers:

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

Mariko Kakuta, Yoshiaki Sone  
Tomiyo Umeda and Akira Misaki

AGRICULTURAL AND BIOLOGICAL CHEMISTRY 43(8), 1659-1668, 1979

Acidic heteropolysaccharides, D-glucurono-D-xylo-D-Mannans were isolated from the water and alkaline extracts of the fruit body of Tremella fuciformis Berk. Similar polysaccharides were isolated from the growing culture of the haploid cells of two strains (T-19 and T-7) of T. fuciformis, when they were cultured in sucrose or glucose-yeast extract medium. The extracellular polysaccharides contain, D-glucuronic acid, D-xylose and D-mannose [molar ratios, 1.3:1.0:3.5 (T-7) and 0.8:1.0:2.1 (T-19)] and, in addition, small proportions of L-fucose and O-acetyl groups. Methylation and Smith degradation studies indicated that both fruit body and extracellular polysaccharides are built up of  $\alpha$ -(1 $\rightarrow$ 3)-linked D-mannan backbone chain to which  $\beta$ -linked D-glucuronic acid and single or short chains of  $\beta$ -(1 $\rightarrow$ 2)-linked D-xylose residues are attached at the C-2 position. L-fucose residues in the extracellular polysaccharides may form single branches. The structural features of these polysaccharides are discussed in comparison with the similar polysaccharides from other fungi.

\* \* \*

Antigenicities of Several Cell Wall Mannan Preparations and Cell Envelope Preparations from Baker's Yeast

Youichi Tamai<sup>1</sup>, Takahiro Nakashima<sup>1</sup>  
Masayoshi Takakuwa<sup>1</sup>, and Akira Misaki

AGRICULTURAL AND BIOLOGICAL CHEMISTRY 44(1), 49-53, 1980

Antigenicity of mannan preparation obtained by the usual method so far published and that prepared by our method was investigated with the rabbit antiserum against the intact yeast cell. The antigenicity of the former was a little less than the latter, but both mannan preparations were much less in the antigenicity as compared with the intact yeast cell. Thus, the preparation of an intact surface antigen was attempted through treating the yeast whole cells successively with acetic acid, pepsin, acetone, and ether. The cell envelope obtained by the procedure was almost intact microscopically and maintained the antigenic activity similar to that of the intact cells. The dry weight of the cell envelope was one half of the whole cell, and its composition was sugar 60%, crude protein 32%, and lipid 5%.

<sup>1</sup>Department of Agricultural Chemistry, Faculty of Agriculture, Ehime University, Matsuyama 790, Japan.

XIII. Departamento Interfacultativo de Bioquímica, Facultades de Ciencias y Medicina, Universidad de Oviedo, Oviedo, Spain. Communicated by F. Moreno.

The following items are abstracts of papers recently published or accepted for publication from our laboratory:

1. Moreno, F., Herrero, P., Parra, F., and Gascón, S. "Invertase and  $\alpha$ -Galactosidase Synthesis by Yeast." CELLULAR AND MOLECULAR BIOLOGY 25, 1-6, 1979.

ABSTRACT

The effects of 2-deoxy-D-glucose and D-xylose on the synthesis of yeast invertase and  $\alpha$ -galactosidase have been studied. The presence of 2-deoxy-D-glucose in the culture medium produces an increase of invertase synthesis when galactose is used as carbon source. Conversely,  $\alpha$ -galactosidase synthesis is inhibited by the deoxysugar. When glucose is the carbon source, invertase is not superproduced if 2-deoxy-D-glucose is present in the culture medium. D-xylose increases invertase synthesis both when glucose and galactose are used as carbon sources.

A hypothesis by which these effects are related to the catabolite repression is discussed.

2. Herrero, P., Moreno, F., and Gascón, S. "Role of Vesicles on the Transport and Secretion of Exocellular Enzymes by Yeast." CELLULAR AND MOLECULAR BIOLOGY, in press.

ABSTRACT

We have studied the role of yeast vesicles on the secretion of the exocellular enzymes invertase and  $\alpha$ -galactosidase.

By means of gentle osmotic lysis of protoplasts, we have managed to release the vesicles which were purified by floating in Ficoll gradient. In the Ficoll layer which contained the vesicles, two different populations could be seen, some bright and refracting which appeared empty inside, and others dark and containing intravesicle corpuscles.

From these preparations, it has been possible to establish that invertase and  $\alpha$ -galactosidase are contained in these vesicles at a high specific activity. By means of filtering the lysed vesicles preparations through Sephadex G-200, we have studied the molecular forms of these enzymes.

The vesicles contain, in all cases, heavy invertase ( $V_e/V_o = 1.05$ ) and intermediate molecular forms of the enzyme ( $V_e/V_o = 1.05-1.60$ ).  $\alpha$ -Galactosidase elutes in only one peak of activity; but by treating these vesicles with Triton X-100 and EDTA, it is possible to observe at least two forms of  $\alpha$ -galactosidase, one of which was identified with the exocellular enzyme and the other with a lower molecular weight form.

3. Iglesias, C. F., Moreno, F., and Gascón, S. "Light and Intermediate Molecular Forms of Yeast Invertase as Precursor of the Heavy Enzyme." FEBS. LETTERS, in press.

## INTRODUCTION

Yeast invertase has been found to exist in several molecular forms which differ not only in molecular weight but in cell localization. The heavy enzyme is a glycoprotein containing 50% mannan and 3% glucosamine. The light one contains no carbohydrate, and the intermediate molecular forms might represent different degrees of glycosylation of the enzymatic protein.

The cellular content of the different invertase molecular forms and their distribution in yeasts have been shown to be dependent on the glucose concentration of the culture medium, which is the main factor in controlling the synthesis of the enzyme. In repressed cells, the concentrations of the heavy and light forms of invertase are similar; and most of the enzyme is intracellular. On the other hand, the heavy invertase represents 95% of the total enzyme in de-repressed cells; and this molecular form is located mainly outside the cytoplasmic membrane.

In the present paper, we report our studies on the relationship between the metabolic state of the yeast cells and the distribution of the different molecular forms of invertase in the yeast strain *Saccharomyces* 303-67. We introduce the intermediate molecular forms in the preexisting scheme, giving them a role as intermediates in the process of secretion of the heavy enzyme and as precursors of the exocellular invertase. [References omitted; ed.].

4. Fernández, M. P., Suárez Rendueles, M. P., and Gascón, S. "Molecular Forms of Yeast Acid Phosphatase--Cellular Localization". CELLULAR AND MOLECULAR BIOLOGY, in press.

## ABSTRACT

Two molecular forms of acid phosphatase have been proven to be present in cell-free extract from *Candida utilis*. The two enzymes differ in their molecular weight as shown by gel filtration on Sephadex G-200. They have a different elution volume void volume ratio, being 1.1 for the heavy form and 1.5 for the light. DEAE-Sephadex chromatography has shown that the two isozymes are equally adsorbed by this gel eluting at the same NaCl concentration. The Michaelis constants are different, the  $K_m$  values being 3 mM for the heavy form and 0.8 mM for the light, using p-nitrophenyl phosphate as substrate. Optimum pH is 4.5 for both isozymes; but while the heavy form has no activity in the alkaline pH range, the light one has an appreciable activity in the alkaline pH range. pH stability is different for both isozymes, the light acid phosphatase having a broader range of pH stability. Heat inactivation is slightly different as well. The localization of the two isozymes has been studied; and we have found that both are released to the supernatant during preparation of protoplasts, proving their external localization.

- XIV. Laboratory of Industrial Biochemistry, Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Yosida, Kyoto, Japan. Communicated by Tejiro Kamihara.

Below follow abstracts of two recent papers from our laboratory:

Effects of Thiamine and Pyridoxine on Respiratory Activity in *Saccharomyces carlsbergensis* Strain 4228

Ichiro Nakamura, Naohiko Isobe, Tejiro Kamihara, and Saburo Fukui

ARCHIVES OF MICROBIOLOGY 1980 (in press)

## ABSTRACT

Studies were made to elucidate the relationship among the thiamine-induced growth inhibition and decrease in cellular vitamin B<sub>6</sub> content and respiratory deficiency in Saccharomyces carlsbergensis strain 4228 [Nakamura et al., BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS 59, 771-776 (1974)]. Addition of pyridoxine to the thiamine-added culture at the beginning or in the course of cultivation brought about appearance of cytochrome spectra and an increase in the activity of heme-containing enzymes and in respiratory activity (Q<sub>o2</sub>). The effects of pyridoxine occurred prior to the restoration of growth. Pyridoxine was effective even in the presence of high levels of glucose in the growth medium (not less than 3%). On the basis of these results, the mechanism of the effects of thiamine and pyridoxine is discussed.

\* \* \*

Morphological Change in Candida tropicalis pK 233  
Caused by Ethanol and its Prevention by Myo-inositol

Yoshio Tani, Yukiko Yamada<sup>1</sup>, and Tejiro Kamihara

(<sup>1</sup>Seibo Women's Junior College, Osaka, Japan)

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS  
Vol. 91, No. 1, 1979, pages 351-355

### SUMMARY

The cells of Candida tropicalis pK 233 grew in filamentous form when cultivated in a synthetic medium supplemented with ethanol. The ethanol-grown cells excreted significant amounts of polysaccharides into the culture medium. Myo-inositol added simultaneously with ethanol prevented both the morphological change and the extracellular production of polysaccharides.

XV. Department of Genetics, Research School of Biological Sciences, The Australian National University, P.O. Box 475, Canberra City, A.C.T. 2601, Australia. Communicated by G. D. Clark-Walker.

Below follow summaries of three manuscripts which have recently appeared or are in press:

The Size of Yeast Mitochondrial Ribosomal RNAs

K. S. Sriprakash and G. D. ClarkWalker

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS  
93:186-193 (1980)

### SUMMARY

The sizes of mitochondrial rRNAs of Saccharomyces cerevisiae and Torulopsis glabrata have been investigated by agarose gel electrophoresis after glyoxal treatment. The large and small mt rRNAs from S. cerevisiae were found to be 3100 and 1460 bases respectively, values which are considerably smaller than previous estimates (1). The corresponding lengths for T. glabrata mt rRNAs are 2700 and 1400 bases, indicating that the size of mitochondrial rRNAs in yeasts is not conserved.

\* \* \*

Hybridizable Sequences between Cytoplasmic  
Ribosomal RNAs and 3 Micron Circular DNAs of  
Saccharomyces cerevisiae and Torulopsis glabrata

G. D. Clark-Walker, Department of Genetics  
and A. A. Azad, Molecular Biology Unit

In: NUCLEIC ACIDS RESEARCH (1980) 8:1009

ABSTRACT

In the present report we show that 2.8 and 3.1  $\mu$ m circular DNA molecules previously reported to be present in Saccharomyces cerevisiae and Torulopsis glabrata, respectively, contain sequences hybridizing to cytoplasmic ribosomal RNAs. In S. cerevisiae, the 2.8  $\mu$ m circular DNA appears to be identical to the rDNA repeating unit from nuclear DNA, both in length (approximately 9000 base pairs), and in the location of the 25, 18, and 5.8S rRNA sequences on the large HindIII fragment (6500 bp), and the presence of the 5S rRNA sequence on the small HindIII fragment. The 3.1  $\mu$ m molecule from T. glabrata is approximately 2000 base pairs longer than the S. cerevisiae molecule; and, in addition, one of the HindIII sites lies within the region hybridizing to 25, 18, and 5.85 rRNAs. In S. cerevisiae, the 4-5 copies of the 2.8  $\mu$ m circular DNA molecules per cell, which have an extranuclear location, do not appear to be essential for cell viability as in one strain they were undetectable.

\* \* \*

Mapping of Mitochondrial DNA  
from Torulopsis glabrata:  
Location of Ribosomal and Transfer RNA Genes

G. D. Clark-Walker, K. S. Sriprakash, and C. R. McArthur  
Department of Genetics

and  
A. A. Azad  
Molecular Biology Unit

CURRENT GENETICS (1980) 1, in press

SUMMARY

Large and small rRNAs have been isolated from mitochondria of the yeast Torulopsis glabrata and have been shown to have lengths of 2700 bases and 1400 bases, respectively. Construction of a restriction endonuclease site map of mitochondrial DNA has enabled us to position these rRNAs by hybridization of labelled RNA and DNA fragments transferred to nitrocellulose. The large and small mt rRNA genes are separated by a minimum of 1820 bp and a maximum of 2765 bp on the 18,870 bp mitochondrial genome. tRNA genes map within this separating sequence, but they are also located distal to both rRNA genes. The implication of these results to the structural relationships of mitochondrial DNAs from yeasts is discussed.

XVI. Biochemistry Laboratory, University of Sussex, Brighton, Sussex  
BN1 9QG, England. Communicated by Sydney Shall.

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

# Isolation of Chromosomal Origins of Replication in Yeast

David Beach, Melanie Piper, and Sydney Shall

NATURE Vol. 284, No. 5752, pages 185-187, March 13, 1980

Origins of replication have been identified in the DNA of viruses, mitochondria, bacterial plasmids, and the bacterial chromosome. However, origins of replication of eukaryote chromosomes have remained elusive because of the large size and sequence complexity of chromosomes and in particular for want of a suitable assay for their detection. Recent development of techniques for genetic transformation of yeast by autonomously replicating cytoplasmic plasmids now makes it possible to search for eukaryote origins in a manner analogous to that used for bacteria. Here we describe the construction and properties of a plasmid which contains no effective eukaryote replication origin and whose efficiency of replication in yeast is greatly enhanced by insertion of certain fragments of yeast chromosomal DNA. We believe these to contain replication origins, since yeast transformants are shown to contain copies of the transforming plasmids.

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

In our work on the genetics of the mating-type system of Schizosaccharomyces pombe, we presently study (1) spontaneous lethal mutations which map in mat2 and (2) mutations that reduce the frequency of switching between plus and minus activity in homothallic strains.

Most of our work on the mating-type system was performed with the S. pombe strains originally introduced by U. Leupold into genetic research. We are now interested to include S. pombe strains of different geographical origins in our studies. We would very much appreciate if yeast researchers who have isolated any S. pombe strains would supply us with subcultures.

XVIII. Department of Physiology, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark. Communicated by Morten C. Kielland-Brandt.

The following papers have been published:

1. Jørn Dalgaard Mikkelsen, Poul Sigsgaard  
Arne Olsen, Kenneth Erdal, Morten C. Kielland-Brandt  
and Jens G. Litske Petersen

Thiaisoleucine Resistant Mutants in Saccharomyces carlsbergensis Increase the Content of D-amyl Alcohol in Beer

CARLSBERG RESEARCH COMMUNICATIONS 44, 219-223 (1979)

Four presumably dominant thiaisoleucine resistant mutants of a brewing strain of Saccharomyces carlsbergensis were previously shown to produce more D-amyl alcohol than the parent strain when grown in synthetic minimal medium with aeration. In the present work, an increased production of D-amyl alcohol was also found in fermenting wort under brewing conditions resulting in a beer with an altered ratio between D-amyl alcohol and isoamyl alcohol. The

synthesis of 2, 3-pentanedione plus its precursor,  $\alpha$ -aceto- $\alpha$ -hydroxybutyrate, was generally higher in the mutants. The mutants showed a somewhat lower rate of increase in cell concentration during fermentation than the parent strain. With respect to other analytical data and taste, the three mutants isolated after moderate mutagenesis did not show deviations outside the range of the parent. One mutant obtained from a more heavily mutagenized culture deviated in several respects.

\* \* \*

2. Bjørn Eggert Christensen

Somatic Hybridization in Saccharomyces cerevisiae: Analysis of Products of Protoplast Fusion

CARLSBERG RESEARCH COMMUNICATIONS 44, 225-233 (1979)

Yeast strains of identical mating type were converted into protoplasts with snail gut enzymes. Fusion induced with polyethylene glycol gave products in which nutritional requirements had complemented each other. The somatic hybrids were invariably maters and non-sporulators. When the strains differed in non-Mendelian markers not used in the selection, these markers were found in the expected parental and recombinant combinations in the fusion products. Fusion products of two strains of mating type  $\alpha$  were mated with fusion products of two strains of mating type  $a$ . Two cycles of sporulation and tetrad analysis of the presumed tetraploid gave a regular distribution of all markers showing that the somatic hybrids were indeed diploids homozygous for mating type. A single exception to the expected segregation was found not to be due to an abnormal chromosome number but is attributed to a gene conversion or a mitotic crossing over.

\* \* \*

3. Steen Holmberg, Jens G. Litske Petersen  
Torsten Nilsson-Tillgren and Morten C. Kielland-Brandt

Molecular Characterization of  
A Saccharomyces Plasmid  
Containing the HIS4 Gene

CARLSBERG RESEARCH COMMUNICATIONS 44, 269-282 (1979)

In a previous study, genetic transformation in yeast was carried out with a mixture of the yeast plasmid 2-micron DNA and total yeast DNA, which was treated with restriction endonuclease PstI and DNA ligase. Strains were derived that contain a plasmid which carries the HIS4 gene. In the present study, new plasmids have been constructed by combining the HIS4 carrying yeast plasmid, as well as parts of it, with bacterial plasmids. The hybrid plasmids were propagated in bacteria and analysed. From the data obtained, the original plasmid was inferred to be a 2-micron DNA circle with a 9.4 kb insertion carrying the HIS4 gene. The structure was confirmed by restriction endonuclease analysis of plasmid DNA from the original transformant, using molecular hybridization to detect fragments which contain sequences of the 9.4 kb insert. The insert which is bordered by two PstI sites, was mapped with restriction endonucleases EcoRI, HindIII, and Sall. No BamHI site is present in the insert.

\* \* \*

4. Steen Holmberg, Morten C. Kielland-Brandt  
Torsten Nilsson-Tillgren and Jens G. Litske Petersen

Molecular Characterization of  
Three his4 Deletion Mutants  
in Saccharomyces cerevisiae

CARLSBERG RESEARCH COMMUNICATION 44, 283-288 (1979)

DNA was isolated from haploid strains of Saccharomyces cerevisiae carrying three different deletions in the his4 gene, his4-15, his4-24, and his4-29, as well as his4 point mutant and a strain carrying the HIS4 wild type gene. Samples of the DNA preparations were treated with restriction endonucleases BamHI, EcoRI, HindIII, PstI, and Sali; and the DNA fragments were separated according to size by agarose gel electrophoresis. A bacterial plasmid containing the yeast DNA sequences of a region including the HIS4 gene allowed the detection by molecular hybridization of the fragments in the electropherograms that contained these sequences. From the sizes of these fragments, the mutations his4-15 and his4-24 were both determined to be deletions of about 0.5 kb, while his4-29 was found to be a deletion of approximately 0.9 kb. The data allowed an extension of our previously constructed cleavage map of the region, as well as approximate location of the deletions on the map. Comparisons with available data on the his4 gene and its product allowed an approximate positioning of the three functional regions of the his4 gene on the map and indicated that no large intervening sequences are present within the his4 AB region.

\* \* \*

5. Jens G. Litske Petersen

In Vivo Labelling of Proteins Associated  
with Folded Chromosomes of Yeast

CARLSBERG RESEARCH COMMUNICATIONS 44, 395-402 (1979)

Proteins associated with the pre-replicative ( $g_1$ ) and post-replicative ( $g_2$ ) folded chromosomes of Saccharomyces cerevisiae can be labelled in vivo by growing cells in acetate vegetative medium containing [ $^{35}$ S] methionine. In both sporulating (MAT $\alpha$ /MAT $\alpha$ ) and non-sporulating (MAT $\alpha$ /MAT $\alpha$ , MAT $\alpha$ /MAT $\alpha$ ) diploids, proteins associated with the resting stage genome ( $g_0$ ) can be labelled with [ $^{35}$ S] methionine during nitrogen starvation and in sporulation medium. In addition, in MAT $\alpha$ /MAT $\alpha$  diploids proteins associated with the meiotic replication form (r) can also be labelled. SDS-polyacrylamide gel electrophoresis and autoradiography of the labelled proteins from the various folded genome forms showed that the  $g_1$  and  $g_2$  patterns are, with the exception of one polypeptide band, essentially identical. Several differences distinguish the r and  $g_0$  patterns. No significant differences were observed between the  $g_0$  proteins of sporulating and non-sporulating diploids.

\* \* \*

6. Torsten Nilsson-Tillgren  
Jens G. Litske Petersen, Steen Holmberg  
and Morten C. Kielland-Brandt

Transfer of Chromosome III during *karl*  
Mediated Cytoduction in Yeast

CARLSBERG RESEARCH COMMUNICATIONS 45, 113-117 (1980)

We describe the transfer, at low frequency, of a limited number of nuclear markers during *karl* mediated cytoduction of the  $RHO^+$  factor. By selection for a chromosome III marker in *KARI HIS4* [ $RHO^+$ ] x *karl his4* [ $rho^-$ ] crosses, strains disomic for chromosome III were isolated. Markers carried on five other chromosomes in the *HIS4* donating strain could be shown to be absent from the disomic strains. When these disomic strains were force-mated to haploid tester strains, the rare prototrophic products were sporulators with good spore viability. These observations suggest that one or possibly a few chromosomes were transferred to the recipient strain during cytoduction of the  $RHO^+$  factor.

\* \* \*

7. Morten C. Kielland-Brandt, Barbara Wilken  
Steen Holmberg, Jens G. Litske Petersen  
and Torsten Nilsson-Tillgren

Genetic Evidence for Nuclear  
Location of 2-Micron DNA in Yeast

CARLSBERG RESEARCH COMMUNICATIONS 45, 119-124 (1980)

Progeny from *karl* x *KARI* crosses in *Saccharomyces cerevisiae* were isolated by selection for a mitochondrial marker from each parent. Haploid progeny (heteroplasmons, cytoductants) were obtained at high frequency because of the *kar* mutation and were identified by replication of progeny colonies to media differentiating on the basis of chromosomal markers. In some of the crosses, nuclei of both parental types were found among the progeny, although the *KARI* nuclei were in majority.

In crosses where only one parent contained the plasmid 2-micron DNA, all progeny inheriting the nucleus from that parent also had the plasmid, whereas only about 25-50% of the progeny with the other nucleus had the plasmid. In crosses where the size of 2-micron DNA of the parents was different, the haploid progeny had a plasmid size identical to that of the parent from which it had received its nucleus.

On the basis of these observations, we suggest a nuclear location of 2-micron DNA. We propose that one or a few copies of the plasmid are frequently transferred from one nucleus to another in *karl* mediated transient heterokaryons.

- XIX. Abteilung Genetik, Fachbereich Biologie, Technische Hochschule Darmstadt, D-6100 Darmstadt, Federal Republic of Germany. Communicated by F. K. Zimmermann.

Genetics of Carbon Metabolism  
in *Saccharomyces cerevisiae*

1. The regulatory roles of glucose-6-phosphate and phosphoglucoseisomerase in controlling the glycolytic flux, glycolytic enzymes, and carbon catabolite inactivation: In his doctoral thesis, H. RASENBERGER (*Physiologische Eigenschaften von Glykolysemutanten von Saccharomyces cerevisiae*, Technische Hochschule, Darmstadt, 1979) reported on some unexpected properties of a mutant with strongly reduced phosphoglucoseisomerase (about 6% wild type activity): *pgi*. Even though phosphoglucoseisomerase is not required for fermentation of fructose, the *pgi* mutant fermented fructose at only 50% of the wild type rate, accumulated up to 80 nmoles/mg dry weight of fructose-1, 6-bisphosphate (wild type 10-15), and formed pyruvate kinase almost constitutively, whereas the increase in pyruvate decarboxylase, usually observed when yeast cells are transferred from a nonsugar to a sugar medium, was strongly reduced. Three more *pgi* mutants were isolated in our group, M. CIRIACY and I. BREITENBACH (*JOURNAL OF BACTERIOLOGY* 139, 152-160, 1979) and investigated by C. STUEWER. She confirmed this extraordinary behavior for the additional mutants. The problem of accumulation of fructose-1, 6-bisphosphate in *pgi* mutants was studied in more detail. Normally, yeast cells growing on ethanol as a carbon source form 300 mU of aldolase. Upon addition of a fermentable sugar, this activity increases to 600-700 mU. Heat inactivation revealed that there is only one heat sensitive fraction of aldolase in nonfermenting cells. However, fermenting cells have a more heat labile aldolase that accounts for about 100 mU. All *pgi* mutants formed this labile isozyme when incubated on glucose but not on fructose media. An attractive hypothesis would be to consider the minority aldolase as the proper glycolytic enzyme which is formed only when glycolysis can operate. A possible signal stimulating this postulated glycolytic isozyme (either de novo synthesis of a new isozyme or modification of the majority isozyme) could be glucose-6-phosphate since on fructose media in wild type the intracellular levels are around 5 nmole/mg dry weight, whereas in *pgi* mutants levels remain low between 0.3-1.0 nmole. All *pgi* mutants on glucose accumulate very high levels of glucose-6-phosphate (up to 70 nmoles). Catabolite inactivation has been postulated to be triggered by an interaction of hexokinases with their substrates and/or their products, ENTIAN (*MOLECULAR GENERAL GENETICS* 158, 201-210, 1977). A detailed study of the catabolite inactivation of fructose-1, 6--bisphosphatase revealed indeed that *pgi*-mutants inactivate this enzyme faster than wild type when glucose is the inactivating carbon source; however, fructose causes a delayed inactivation (residual activity after 60 minutes: wild type 40%, *pgi* mutants 83%). The tentative conclusion is that glucose-6-phosphate is the metabolic signal opening up the upper glycolytic pathway and also is the low molecular effector which in combination with hexokinases triggers catabolite inactivation of fructose-1, 6-bisphosphatase. An additional source for the control of glycolytic activity requires the activity of phosphoglucoseisomerase, especially the formation of pyruvate kinase and pyruvate decarboxylase.
2. Phosphofructokinase: I. BREITENBACH has isolated nine mutants lacking all phosphofructokinase activity. They are all recessive and fall into two genes *pfk1* and *pfk2*. Normal yeast cells usually accumulate fructose-1, 6-bisphosphate up to 10-15 nmole/mg dry weight after exposure to fermentable sugars. If glucose or fructose are the only carbon sources for *pfk* mutants, no such accumulation is observed. This suggested that the mutants are not leaky in vivo. It was, therefore, quite surprising to find that all *pfk* mutants readily fermented hexoses and produced ethanol, even though this ethanol production occurred only after a lag of 90-180 minutes after sugar addition to the medium. In wild type,

there was no such lag. A double mutant pfk pgi has a block in fructose utilization for straight glycolysis and also for its degradation via the pentose phosphate shunt. Even such a double mutant can produce ethanol from sugars. The only pathway for a fermentative fructose degradation requires a third branch from fructose-6-phosphate to the triose phosphate level, and this may be "induced" in the presence of sugars.

3. Pyruvate decarboxylase: H. D. SCHMITT has isolated a number of recessive mutants with strongly reduced pyruvate decarboxylase activity. They also fall into two genes pdcl and pdcl2. Growth on glucose is reduced and completely inhibited when respiration is blocked with antimycin. On glucose media alone, the mutants excrete pyruvate at rates of up to 4  $\mu$ moles/mg dry weight. h.
4. Genetics of invertase: M. K. GROSSMANN has isolated inulin-utilizing mutants in a strain carrying SUC4. Inulin utilization was due to two mutations. One was the activation of a new SUC gene called SUC7 which coded for an exceedingly temperature sensitive invertase and a second gene called INU1. Both mutant alleles were dominant. Invertases were purified from strains carrying one of the following relevant genes: SUC1-SUC5, SUC7, and SUC7 INU1. The  $k_m$  values for sucrose were between 25-29 mM and for raffinose between 145<sup>m</sup>-165 mM for the first five genes. SUC7 and SUC7 INU1 had  $k_m$  values for raffinose of 71 and 50 mM respectively. For inulin hydrolysis, rates were 13-39 mU for SUC1-SUC5, 680 for SUC7, and 1140 for SUC7 INU1. The carbohydrate/protein ratios were 7:1 for SUC4, 6:1 for SUC7, and 1:1 for SUC7 INU1. It is assumed that the ready inulin utilization of SUC7 INU1 strains has to do with a reduced glycosylation of the protein moiety and better accessibility of the enzyme to inulin which cannot be taken up by the cell.
5. Environmental mutagenesis: F. K. ZIMMERMANN and I. SCHEEL have developed a plate incorporation test for the detection of chemically induced mitotic gene conversion in strain D7. This allows to detect in a 90 mm diameter Petri dish the genetic activity of 1.6 nl diepoxybutane, 24  $\mu$ g canavanine, 26.7  $\mu$ g carofur (a nitrofurane derivative), 36  $\mu$ g p-fluorophenylalanine, and 0.4  $\mu$ l EMS. Only 5-fluorouracil was found inactive. A microscopic examination of the plates allows one to readily detect cell killing and growth inhibition. Using classical liquid suspension tests and liver S9 activation, the following indirect mutagens turned out to be good positive controls: hexamethylphosphoramide, 4-acetylaminofluorene, 2-naphtylamine, and benzo(a)pyrene, provided enough solvent can be tolerated by the yeast cells.

XX. Institute of Biochemistry and Biophysics, 02-532 Warszawa ul., Rakowiecka 36, Warsaw Poland. Communicated by Tomasz Biliński.

We have undertaken systematic studies on the genetic aspects of hemoprotein formation in Saccharomyces cerevisiae. In the following papers, we present data on the isolation and characterization of mutants affected in the heme and hemoprotein formation:

J. Pachecka, J. Litwińska, and T. Biliński. Hemoprotein Formation in Yeast. I. Isolation of catalase and cytochrome-deficient mutants. MOLECULAR AND GENERAL GENETICS 134:299-305 (1974).

J. Rytka, A. Sledziwski, J. Litwińska, and T. Biliński. Hemoprotein Formation in Yeast. II. Isolation of catalase regulatory mutants. MOLECULAR AND GENERAL GENETICS 145:37-42 (1976).

J. Rytka, A. Sledziewski, J. Lukaszewicz, and T. Biliński. Hemoprotein Formation in Yeast. III. The role of carbon catabolite repression in the regulation of catalase A and T formation. MOLECULAR AND GENERAL GENETICS 160:51-57 (1978).

T. Biliński, J. Lukaszewicz, and A. Sledziewski. Demonstration of Anaerobic Catalase Synthesis in the cz1 Mutant of *Saccharomyces cerevisiae*. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS 83:1225-1233 (1978).

J. Lukaszewicz and T. Biliński. Effect of Anaerobiosis and Glucose on the Content of Haem and its Precursors in Intact Yeast Cells. ACTA BIOCHIMICA POLONICA 26:161-169 (1979).

Below follow abstracts of two recent papers from this laboratory:

T. Biliński, A. Sledziewski, and J. Rytka. Hemoprotein Formation in Yeast. VI. Mutants with changed levels of catalase and other haem enzymes under conditions of glucose repression. ACTA MICROBIOLOGICA POLONICA (1980, in press).

Three mutants with altered response of the hemoprotein level to glucose repression and anoxia were characterized biochemically. Two mutants show complete resistance of catalase activity to glucose repression and a lower degree of resistance of cytochrome c oxidase and of some other enzymes. Liquid nitrogen spectra of these mutants grown in high glucose are typical for those of glucose de-repressed spectra of wild-type cells.

The third mutant is hypersensitive to glucose repression and shows only traces of catalase T activity when grown in high-glucose media. Under these conditions, its spectrum is almost devoid of typical peaks of cytochromes. When grown on ethanol or raffinose, it forms catalase and shows a typical de-repressed spectrum of cytochromes. The regulatory mechanism impaired in the mutants is not known. It seems likely that the regulation of the heme pathway in the mutants does not differ from that of the wild-type.

T. Biliński, J. Litwińska, A. Sledziewski, and J. Rytka. Genetic Analysis of Pleiotropic Mutants affected in the Response to Glucose Repression and Anoxia. ACTA MICROBIOLOGICA POLONICA (1980, in press).

Genetic analysis of three pleiotropic mutants with changed regulation of hemoprotein level was performed. It was proved that single recessive mutations *cgr4*, *cas1*, and *cgh1* are responsible for simultaneous changes of catalase T, cytochrome c oxidase, and of some other enzyme levels, under conditions of glucose repression. Two mutations *cgr4* and *cas1*, responsible for glucose resistance of hemoprotein formation in aerobic conditions, enable also anaerobic synthesis of catalase T. Mutation *cgh1* causing hypersensitivity of several respiratory enzymes to glucose repression is partly epistatic to *cas1* and *cgr4* mutations.

XXI. Division of Radiological Protection, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India. Communicated by B. S. Rao.

Following is a list of publications and abstracts of recently completed projects in the areas of radiobiology, chemical mutagenesis, and hyperthermia:

## Technical Publications

1. B. S. Rao, N. M. S. Reddy, and U. Madhvanath. Gamma Radiation Response and Recovery in Radiation Sensitive Mutants of Diploid Yeast. INTERNATIONAL JOURNAL OF RADIATION BIOLOGY (in press).
2. M. S. S. Murthy and K. B. Anjaria. Deactivation of Furyl Furamide (AF-2) by Rat Liver Microsomes and its Implication to the Short-Term Tests for Mutagenicity/Carcinogenicity. MUTATION RESEARCH 77, 127-134 (1980).
3. N. M. S. Reddy and B. S. Rao. On the Nature of the Target Molecule involved in Hyperthermic Inactivation: Studies on cell stage sensitivity of repair deficient mutants of diploid yeast. AMPI MEDICAL PHYSICS BULLETIN 5, 56-58 (1980).
4. B. S. Rao, K. B. Anjaria, and V. V. Deorukhakar. Influence of Hyperthermic Treatment on the Repair of Potentially Lethal Damage induced by Gamma Radiation in Diploid Yeast. IBID 5, 54-56 (1980).
5. V. V. Deorukhakar and B. S. Rao. Evidence for the Existence of Different Targets for Hyperthermic and Gamma Radiation Killing in Diploid Yeast. IBID 5, 79-81 (1980).

## Summaries of recently completed research projects

1. Interaction between Mutagenic Action of Radiation and Chemicals in Low Dose Range.

Interaction between mutagenic action of radiation and chemicals was studied using a diploid yeast strain Saccharomyces cerevisiae BZ 34, with gene conversion as the end point. EMS, MMS, and 4,NQO were used in combination with gamma radiation. It was observed that 4,NQO treatment when administered before or after gamma irradiation exhibited antagonistic effect. The two alkylating agents, EMS and MMS, exhibited additive effect when the chemical treatment was given following irradiation. On the other hand, when EMS or MMS treatment was given prior to gamma irradiation, synergistic response was observed.

2. Testing of Some Permitted Food Colors for Genotoxicity in Diploid Yeast with Microsomal Activation.

Permitted food colors Amaranth, Indigo Carmine, carmoisine, Ponceau-4R, Fast Red E, Scarlet F, Fast Green FCF, Wool Green BS, Tartrazine, Erythrosine, and Sunset Yellow FCF were screened using mitotic gene conversion in Saccharomyces cerevisiae BZ 34 with microsomal activation. At a concentration of 5 mg/ml, none of the dyes caused any significant increase in the frequency of arginine convertants, under the experimental conditions employed.

XXII. Chaire de Génétique et Microbiologie, E.N.S.A.M.-I.N.R.A., 34060 Montpellier Cedex, France. Communicated by P. Galzy.

The following publications have been published recently or are in press:

K. Oteng-Gyang, G. Moulin, and P. Galzy. Properties and Conditions for Excretion of an Amylase and two Glucoamylases of Schwanniomyces castellii. Meeting on extracellular products of microorganisms. FEMS, Dublin, September 18-21, 1979.

K. Oteng-Gyang, G. Moulin, and P. Galzy. Effect of Medium Composition on Excretion and Biosynthesis of the Amylases of Schwanniomyces castellii. EUROPEAN JOURNAL OF APPLIED MICROBIOLOGY AND BIOTECHNOLOGY 9, 129-132, 1980.

G. Moulin, Maguy Guillaume, and P. Galzy. Alcohol Production by Yeast in Whey Ultrafiltrate. BIOTECHNOLOGY AND BIOENGINEERING 22, No. 7, 1980, in press.

J. F. Arthaud, C. Bizeau, and P. Galzy. Development of Yeast Population in Mixed Cultures. ACTA MICROBIOLOGICA ACAD. SC. HUNGARIAE, in press.

C. Bizeau, J. F. Arthaud, P. Galzy, C. L'Homme. Design of a Growth Model for Yeast. FOLIA MICROBIOLOGICA, No. 4, 1980, in press.

XXIII. Department of Genetics, Haryana Agricultural University, Hissar 125004, India. Communicated by R. K. Vashishat.

Below follow a summary of work conducted recently in our laboratory:

R. K. Vashishat, Manjula Vasudeva, and S. N. Kakar

Genetic Effects of Agricultural/Industrial  
Chemicals on Saccharomyces cerevisiae

#### SUMMARY

Three systemic fungicides, carboxin, thiabendazole and hinosan, were tested for the induction of mitotic gene conversion and reverse mutation in diploid cells of D7 strain of Saccharomyces cerevisiae. Treatment of stationary phase cells with carboxin and thiabendazole at concentrations of 25, 50, 100, and 200  $\mu\text{g/ml}$  and with hinosan at concentrations of 25, 50, 100, and 200  $\mu\text{l/ml}$  for 30, 60, 90, and 120 minutes did not cause any increase in mitotic gene conversion and reverse mutation over the control. No significant cell killing was observed when the cells were treated with carboxin and thiabendazole, but hinosan treatment significantly reduced the survival. The absence of lethal and genetic effects in case of carboxin and thiabendazole may be due to lack of penetration of these compounds into the cells. Hinosan treatment killed cells but failed to induce any genetic effects. Since the treatment of yeast cells with these fungicides did not increase the frequencies of convertants and revertants over the controls, it is, therefore, concluded that these fungicides are not convertogenic and mutagenic for yeast.

Epichlorhydrin (1-chloro-2:3-epoxy-propane) was also tested for the induction of reverse mutation, mitotic crossing-over, and gene conversion in Saccharomyces cerevisiae. Treatment of stationary phase cells of D<sub>7</sub> strain of yeast with 0.065M and 0.13M epichlorhydrin, inactivated yeast cells. The decrease in percentage of survivors was dependent on the concentration used and time of exposure. The decrease in cell viability was accompanied by increase in the percentage of mitotic cross-overs and total aberrant colonies as well as in the frequencies of revertants and convertants. The percentage of induced mitotic cross-overs and total aberrant colonies as well as the frequencies of revertants and convertants were dependent on the concentration of epichlorhydrin used and the time of exposure.

The following papers have been published:

1. Vashishat, R. K., and S. N. Kakar. 1979. UV-sensitive mutants of Saccharomyces cerevisiae. I. Isolation and characterization. INDIAN JOURNAL OF EXPERIMENTAL BIOLOGY 17:28-32.
  2. . 1979. UV-sensitive mutants of Saccharomyces cerevisiae. II. UV-mutagenesis. INDIAN JOURNAL OF EXPERIMENTAL BIOLOGY 17:33-35.
- XXIV. Research Institute for Viticulture and Enology, Matúškova 25, 886 15 Bratislava, Czechoslovakia. Communicated by E. Minárik.

The following are summaries of papers accepted for publication:

Influence of Herbicides on the Yeast  
Flora of Spontaneously Fermenting Musts

E. Minárik and P. Rágala

WEIN-WISSENSCHAFT (GFR) 35, 1980, No. 4

The application of triazin and mixed triazin herbicides (Herbex, Caragard, Combi, Semparol) in viticultural practice does not cause any changes in the usual composition of the yeast flora of spontaneously fermenting grape juice. Uncovered Prefix G (chlorthiamide) and urea-containing triazin herbicides (e.g., Ustinex Spezial) caused a slight inhibition of sporogenous yeast species. The results are in good accordance with previous investigations on the neutral behaviour of most herbicides, applied in viticulture for weed control, towards yeasts and yeast-like microorganisms.

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Influence of Insecticides and Acaricides  
on the Yeast Flora of Grapes and Musts

E. Minárik and P. Rágala

KVASNY PRUMYSL (PRAGUE) 26, 1980, No. 4

Insecticides and acaricides applied in vine protection against insects and mites usually display lower secondary actions on yeasts and yeast-like microorganisms of fermenting musts, as compared to treatments with fungicides. Toxic actions could be registered when pesticides were applied in higher concentrations than usual in practice and/or when short waiting periods were necessary in vine treatment. Arafosfotion and Dicarban showed the strongest inhibition of yeast activity among all insecticides and acaricides tested.

- XXV. Microbiology and Fermentation Technology Section, Department of Brewing Research and Development, Adolph Coors Company, Golden, Colorado 80401. Communicated by J. J. Ruocco.

The following paper was presented at the annual meeting of the Master Brewers Association of America, September, 1979:

J. J. Ruocco, R. W. Coe, and C. W. Hahn. Computer Assisted Exotherm Measurement in Full Scale Brewery Fermentations. MASTER BREWERS TECHNICAL QUARTERLY, April, 1980.

### ABSTRACT

The use of small, dedicated computers and sensitive thermistors to measure the rate of heat evolution during fermentation (exotherm) was demonstrated with full scale brewing fermentors. By recording the temperature change with time when cooling is off in a fermentor, a thermogram may be constructed which approximates the yeast growth curve and fermentation rate. Experiments with different temperatures, yeast inocula, and different fermentation substrates showed that exotherm or the instantaneous rate of heat generation varies under different environmental and physico-chemical conditions and reflects the rate of fermentation. The use of the exotherm as a means of controlling the rate of fermentation in a closed loop, computer system in sub-ambient fermentation is currently under investigation.

The following paper was presented at the annual meeting of the American Society of Brewing Chemists, May, 1980:

L. M. King, D. O. Schisler, and J. J. Ruocco. An Epifluorescent Method for Detection of Nonviable Yeast.

### ABSTRACT

Several fluorescent dyes were evaluated for their effectiveness in accurately staining both viable and nonviable yeast cells. A simple, accurate epifluorescent method was developed for the detection of nonviable yeast using a fluorochrome, the magnesium salt of 1-anilino-8-naphthalene sulfonic acid (Mg-ANS). This procedure is comparable in accuracy to the methylene blue method for detection of nonviable yeast at high viabilities. At low yeast viabilities, the MG-ANS procedure is more accurate than methylene blue because it reduces the interpretative inaccuracy inherent in such conventional brightfield dye techniques as methylene blue.

XXVI. Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. Communicated by Eric A. Johnson.

Below follow abstracts of two recent papers based on work performed at the University of California, Davis, California 95616:

#### Pigmentation of Egg Yolks with Astaxanthin from the Yeast *Phaffia rhodozyma*

Eric A. Johnson, Michael J. Lewis, and C. R. Grau\*

(\*Department of Avian Sciences)

POULTRY SCIENCE, in press

### ABSTRACT

The red yeast *Phaffia rhodozyma* was tested as a dietary pigment source for egg yolks of laying hens and Japanese quail. It was found that astaxanthin from broken yeast or prepared yeast oil but not from intact yeast cells were deposited in egg yolks. The efficiency of carotenoid deposition was approximately 4%, and it was only slightly dependent on the astaxanthin concentration in the diet. Astaxanthin was probably deposited without metabolic alteration in egg yolks. When *P. rhodozyma* was fed to laying hens at several concentrations and in combination with marigold flower pigments or yellow corn, a wide

range of colors was achieved; depending on the yeast concentration in the feed, the dominant wavelength of chicken egg yolks ranged from 571 nm to 598 nm.

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Phaffia rhodozyma as an Astaxanthin Source in Salmonid Diets

E. A. Johnson, T. G. Villa, and M. J. Lewis

AQUACULTURE, in press

ABSTRACT

The red yeast Phaffia rhodozyma has possible application as a component of diets for use in aquaculture. Its primary value lies in its content of astaxanthin, which is much higher (5-50 times) than that found in crustacean meals. When fed to rainbow trout, the deposition of astaxanthin in the fish flesh was dependent on the proper preparation of yeast cells before their inclusion into the feed. No astaxanthin was nutritionally available from intact yeast. If P. rhodozyma was mechanically ruptured, its pigments were transferred to the flesh of rainbow trout coloring it salmon pink. The most efficient deposition of astaxanthin in trout occurred when the cell wall of P. rhodozyma was partially removed by enzymatic digestion.

The proximate composition, amino acid content, fatty acid profile, and astaxanthin content were determined for P. rhodozyma. When grown under the conditions used in this study, P. rhodozyma has a low protein content (~25%) and a high total lipid content (~17%) compared to most other microorganisms. Its amino acid profile is well balanced but is deficient in methionine. The predominant fatty acids present in the yeast are oleic, linoleic, and palmitic acids.

XXVII. Laboratory of Industrial Biochemistry, Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Yoshida, Kyoto, Japan. Communicated by Saburo Fukui.

The following is a publication list of papers on yeast and related topics (1979) from the Laboratory of Industrial Biochemistry, Department of Industrial Chemistry, Faculty of Engineering, Kyoto University:

Professor Dr. Saburo Fukui  
Associate Professor Dr. Tejiro Kamihara  
Assistant Professor Dr. Atsuo Tanaka  
Assistant Professor Dr. Tetsuo Toraya

S. Ikeda and S. Fukui. Immobilization of Vitamin B<sub>6</sub> Coenzyme (Pyridoxal 5'-phosphate: PLP) and PLP-dependent Enzymes on Sepharose. METHODS IN ENZYMOLOGY 62, 517-527 (1979).

T. Nihira, T. Toraya, and S. Fukui. Pyridoxal 5'-phosphate Sensitized Photo-inactivation of Tryptophanase and Evidence for Essential Histidyl Residue in the Active Site. EUROPEAN JOURNAL OF BIOCHEMISTRY 101, 341-347 (1979).

✓ Y. Tani, Y. Yamada, and T. Kamihara. Morphological Change in Candida tropicalis pK 233 caused by Ethanol and its Prevention by Myo-inositol. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS 91, 351-355 (1979).

S. Kawamoto, T. Yamada, A. Tanaka, and S. Fukui. Distinct Subcellular Localization of NAD-linked and FAD-linked Glycerol-3-phosphate Dehydrogenases in Alkane-grown Candida tropicalis. FEBS LETTERS 97, 253-256 (1979).

S. Fukui and A. Tanaka. Yeast Peroxisomes--A Review. TRENDS IN BIOCHEMISTRY SCIENCE 4, 246-249 (1979).

S. Fukui and A. Tanaka. Peroxisomes of Alkane- and Methanol-grown Yeasts--Metabolic Functions and Practical Applications--A Review. JOURNAL OF APPLIED BIOCHEMISTRY 1, 171-201 (1979).

S. Fukui and A. Tanaka. Microbial Products from Alkane Media--A Review. DEHEMA-MONOGRAPHS Vol. 83, "Microbiology Applied to Biotechnology," pages 181-196 (1979).

T. Omata, A. Tanaka, T. Yamane, and S. Fukui. Immobilization of Microbial Cells and Enzymes with Hydrophobic Photo-cross-linkable Resin Prepolymers. EUROPEAN JOURNAL OF APPLIED MICROBIOLOGY AND BIOTECHNOLOGY 6, 207-215 (1979).

K. Sonomoto, A. Tanaka, T. Omata, T. Yamane, and S. Fukui. Application of Photo-cross-linkable Resin Prepolymers to Entrap Microbial Cells--Effects of Increased Cell-Entrapping Gel Hydrophobicity on the Hydrocortisone  $\Delta^1$ -Dehydrogenation. EUROPEAN JOURNAL OF APPLIED MICROBIOLOGY AND BIOTECHNOLOGY 6, 325-334 (1979).

A. Tanaka, I. N. Jin, S. Kawamoto, and S. Fukui. Entrapment of Microbial Cells and Organelles with Hydrophilic Urethane Prepolymers. EUROPEAN JOURNAL OF APPLIED MICROBIOLOGY AND BIOTECHNOLOGY 7, 351-354 (1979).

N. Itoh, N. Hagi, T. Iida, A. Tanaka, and S. Fukui. Photo-cross-linkable Resin Prepolymer Method for Immobilization of Biocatalysts: Preparation of Immobilized Glutamate Decarboxylase Tubes and their Application to Automated Analysis of L-Glutamate. JOURNAL OF APPLIED BIOCHEMISTRY 1, in press.

M. Asada, K. Morimoto, K. Nakanishi, R. Matsuno, A. Tanaka, A. Kimura, and T. Kamikubo. Continuous ATP Regeneration using Immobilized Yeast Cells. AGRICULTURAL AND BIOLOGICAL CHEMISTRY 43, 1773-1774 (1979).

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

The following is a recent report from our laboratory:

R. Bhaduria and D. G. Ahearn. Loss of Effectiveness of Preservative Systems of Mascaras with Age. APPLIED AND ENVIRONMENTAL MICROBIOLOGY Vol. 39, No. 3, March 1980, pages 665-667.

#### ABSTRACT

The preservative systems of unused, anhydrous mascaras were challenged periodically with microorganisms, using a modified membrane procedure. Shelf life of preservative activity against Pseudomonas aeruginosa varied for different brands from as little as 1 month to over 36 months. Generally, P. aeruginosa grew in mascaras after the mascaras were challenged first with Staphylococcus epidermidis or Candida albicans.

XXIX. Meetings.

1. International Yeast Commission, I.A.M.S.  
VIIIth International Specialized Symposium on Yeast  
Cell Surface  
September 24-26, 1981  
University of Valencia, Spain

In accordance with the resolution taken by the International Yeast Commission in the meeting held in Montpellier, France, during the VIth International Specialized Symposium on Yeast in July 1978, the VIIth International Specialized Symposium on Yeast (Cell Surface) will be held in Valencia, Spain, September 24-26, 1981.

The official language of the Congress will be English; but papers may be presented in other languages, if necessary. Abstracts must be submitted in English. The Secretariat<sup>1</sup> of the VIIth International specialized Symposium on yeast will be happy to send a personal invitation to the Meeting to any scientist who requests it. It should be understood that such an invitation is only to give assistance in raising travel funds and is not a commitment on the part of the organizers to provide any financial support.

SCIENTIFIC PROGRAMME

- a) Plenary Lectures
- b) Free Communications (which will be presented orally or a poster)

PROPOSED TOPICS

- a) Plasma Membrane
  - 1) Structural Components
  - 2) Biosynthesis and Turnover
  - 3) Catalytic Activities
- b) Cell Wall
  - 1) Macromolecular Components
  - 2) Biosynthesis and Assembly
  - 3) Exocellular Enzymes
- c) The Secretory Process

The poster sessions will constitute the usual free communications of recent research data. This type of presentation proved, at previous Meetings, to be superior to the traditional oral presentations in stimulating discussions and in allowing personal contacts.

2. I would like to bring to the attention of readers of the Newsletter that the VIIIth Congress of the International Society for Human and Animal Mycology

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<sup>1</sup>VIIIth I.S.S.I. Secretariat, Departamento de Microbiologia, Facultad de Farmacia, Avenida Blasco Ibanez 13, Valencia-10, Spain.

will be held at this University, February 8-12, 1982. Among the sessions planned are Ecology, Clinical, Therapy, Immunology and Serology, Taxonomy, Opportunism, Pathology, and Fungal Toxins, are likely to be of interest to yeast specialists.

Chairman of the Organising Committee is Dr. M. Baxter of the Department of Veterinary Pathology, Massey University; and all details of the Congress can be obtained from the Secretariat, P.O. Box 63, Palmerston North, New Zealand.

3. The Tenth International Conference of Yeast Genetics and Molecular Biology will be held at the Campus of Louvain-la-Neuve, Belgium, from September 8-12, 1980. For further information, write to the 10th International Conference on Yeast Genetics and Molecular Biology, Laboratoire d'Enzymologie, Place Croix du Sud, 1, Box 8, 1348 Louvain-la-Neuve, Belgium (telephone 10/41 81 81).

4. XIIth Annual Conference on Yeasts held in Smolenice castle (CSSR), February 13-15, 1980. Communicated by A. Kocková-Kratochvilová

#### CYTOLOGY OF YEASTS (Chairmen: Dr. Nečas and Dr. Streiblová)

Streiblova, E.: Yeast-like models for the study of cell cycle

Svoboda, A.: The international protoplasts symposium held in Szeged 1979

Voríšek, J., Sajdl, P.: Cytology of ascomycetes

#### POSTERS:

Havelková, M., Křipalová, J.: A new model for the study of cell cycle

Havelková, M.: The use of ruthenium red for demonstration of regenerating protoplast cell wall in yeasts

Svoboda, A., Pesti, M.: Ultrastructural changes of plasma-membrane of Candida albicans caused by nystatin

#### EPIDEMIOLOGY, SEROLOGY, GENETICS, AND BIOCHEMISTRY (Chairmen: Dr. Koch and Dr. Augustin)

Koch, Y.: Study of the epidemiology of yeast mycoses

Drga, J.: Possibilities of using immuno-precipitation for serological diagnostics of candidoses

Vraná, D., Votruba, J.: The life cycle of yeasts influenced by external conditions

Šubik, J., Takaczová, G.: Mucidin resistant yeast mutants and mozaic organization of structural gene for cytochrom b

Gbeliská, Y.: The influence of bongkreking acid on the metabolism of macromolecules in respiration-deficient yeasts

Augustin, J.: Study of the degradation of yeast nucleic acids after the activation of intracellular nucleases

Valášková, M., Masler, L.: Characteristics of saccharidic fragments obtained by alkaline degradation of cryptococcal mannans

NATURAL AND INDUCED VARIABILITY IN YEASTS (Chairmen: A. Kocková-Kratochvilová and J. Zemek)

Kocková-Kratochvilová, A.: Natural variability of yeasts

Zemek, J.: Enzyme variability of yeasts

POSTERS:

Kocková-Kratochvilová, A., Markovič, O., Sláviková, E., Hronská, L.: The variability of tryptophan and tyrosine concentration in yeasts

Kocková-Kratochvilová, A., Sláviková, E.: The variability of N-compounds among yeast genera

Kocková-Kratochvilová, A., Sláviková, E., Hronská, L.: Extracellular RNA-ases and DNA-ases

Sláviková, E., Kocková-Kratochvilová, A.: The variability in the genus Debaryomyces

Sláviková, E., Kocková-Kratochvilová, A., Čerňáková, M.: Models for the selection of productive yeast strains

Farkaš, V., Kollárova, N., Kocková-Kratochvilová, A., Bauer, Š.: Alcoholic fermentation of cellobiose

Lieblová, J., Beran, K.: Biochemical differences in the cell cycle of S. cerevisiae

Bendová, O.: The variability of brewing yeasts

Šilhánková, L., Veleminský, J., Gichner, T.: The mutagenic effect of azide and its metabolite from barley on S. cerevisiae

Šilhánková, L.: The interference of cavitation ultrasound with the course of excision repair in S. cerevisiae

ENZYMOLGY AND BIOCHEMISTRY (Chairman: Dr. Farkaš)

Farkaš, V., Lehotský, J., Svoboda, A.: The biosynthesis of chitin in regenerating protoplasts of S. cerevisiae and its relation to the localization of chitin-synthetase

Biely, P., Krátky, Z., Vršanská, M.:  $\beta$ -xylosidase in the system of enzymes degrading xylans in Cryptococcus albidus

Krátký, Z., Biely, P.: The changes in transport system for  $\beta$ -xylosides during the induction of extracellular  $\beta$ -xylanase in Cryptococcus albidus

Vršanska, M., Krátky, Z., Biely, P.: The kind of effect of extracellular endo-1, 4- $\beta$ -xylanase of Cryptococcus albidus

POSTERS:

Augustin, J.: The enzyme equipment in the genus Saccharomycopsis

Zemek, J., Augustin, J.: The production of glucose-isomerase in yeasts

Kotyk, A., Michaljaničová, D., Knotková A., Vacata, V.: The energization of the yeast cell and transport of compounds

Mislovičová, D., Gemeiner, P., Kuniak, L., Zemek, J.: The affinity chromatography of some yeast dehydrogenases on antrachinonetriazine derivatives of pearl-cellulose

Zemek, J., Joniak, D., Košíková, B.: Enzymatic degradation of lignin-cellulose complex

Zemek, J., Kuniak, L., Šallaiová, Z.: Characteristics of glucamylase of yeast origin

Zámocký, J., Zemek, J., Gemeiner, P., Kučár, Š., Kuniak, L.: Specific sorbents for proteases, esterases, and phosphatases

TECHNOLOGY (Chairman: Dr. Bendová)

Běhalová, B., Pichová, A., Beran, K.: The proteolytic fraction of autolyzing des-integrated S. cerevisiae cells

Rychtera, M., Pichová, A., Grégr, V.: The relation between the composition of cultivation media and activity of baker's yeasts

Páca, J.: The effect of the kind of O<sub>2</sub>- addition on the character of growth of C. utilis in mostestep tower fermentor

Králíčková, E.: Morphological changes of C. utilis cells during continuous cultivation

Páca, J., Ruml, T.: The changes in catabolic activity of resting cells of C. utilis caused by pH of the medium

Čepička, J., Moštěk, J., Šabatová, O.: The influence of productive strains of bottom brewing yeasts on the amino acid content in beer

Minárik, E., Rágala, P., Navara, A.: The influence of insecticides and acaricides on yeast flora of grapes and ciders

Navara, A., Minárik, E.: Yeast strains suitable for the production of natural and dessert red wines

Malik, F., Černoch, I.: The technology of the propagation of wine yeasts

Šmogrovičová-Vasil'ová, D., Augustin, J., Garaj, J.: The effect of N-monosubstituted and N-disubstituted derivatives of dithio-carbamoic acids against yeasts

RECENT STATE OF BIOMASS PRODUCTION (Chairmen: Dr. Volfová, Dr. Fencí, and Dr. Hal'ama)

Longauerová, D., Hal'ama, D.: The study of inhibiting effect of acid hydrolyzates on microorganisms

Hermonová, M., Šandula, J., Ebringerová, A.: The growth of yeasts on saccharidic wastes from alkaline hydrolysis of cellulose

Vojtková-Lepšíková, A.: The characteristics of yeast isolated from industrial wastes

Kysliková, E., Volfová, O.: The preparation of yeast fodder proteins from acidic hydrolyzates of cellulose wastes

Krumphanzl, V.: The recent state and perspectives in the utilization of nontraditional wastes for the preparation of microbial biomass

Barta, J., Verner, M.: New trends in the utilization of sulfite liquor for microbial biomass

Rosa, M., Štross, F.: Recent possibilities for utilizing ethanol for the production of biomass

Volfová, O.: Recent state and perspectives in the utilization of methanol for the preparation of microbial proteins

5. The Alfred Benzon Symposium No. 16 "Molecular Genetics in Yeast" was held in Copenhagen, Denmark, from June 15-19, 1980. Attendance to the Symposium was limited to 37 invited speakers. The proceedings of the Symposium will be published at an early date by Munksgaard Publishing Company. The editors are D. von Wettstein, J. Friis, M. Kielland-Brandt, and A. Stenderup. Address for correspondence: Professor D. von Wettstein, Carlsberg Laboratory, 10 Gamle Carlsberg Vej, 2500 Copenhagen, Denmark.

XXX. Brief News Items.

1. We deeply regret to inform the readers of the Yeast Newsletter that Professor Erik Zeuthen passed away on January 10, 1980, at the age of 65 years.

The Biological Institute of the  
Carlsberg Foundation, Denmark

2. With deep sorrow, I inform you of the death of Dr. Mikio Amaha, Director of our Laboratories.

He died on June 13, 1980, of illness after three months of treatment in the hospital. He was fifty-six years of age and left Mrs. Amaha as well as two daughters.

We shall continue to work for the progress of brewing science to which Dr. Amaha had dedicated himself throughout his whole academic life.

Yoshihiro Tsumura  
Succeeding Director  
Central Research Laboratories  
Asahi Breweries, Ltd.  
13-1 Ohmori-kita 2 chome, Ohta-ku  
Tokyo 143, Japan

3. I have moved from Yamagata University to the Biological Institute, Faculty of Science, Yamaguchi University, Yamaguchi 753, Japan, effective April 1980. My interests and projects of the staff in my laboratory will be focussed mainly on the aging and sporulation mechanisms in yeast in collaboration with a group in Yamagata University.

Nobundo Sando

4. Edith Gollub has moved from Mount Sinai Medical Center, New York, to the Department of Biological Sciences, Barnard College, 120th and Broadway, New York City, New York 10027. She is continuing work of the heme mutants of *S. cerevisiae*. The most recent publication on these mutants resulted from a collaboration with Dr. Diana Beattie, Mt. Sinai School of Medicine: Synthesis of the apoprotein of cytochrome b in heme-deficient yeast cells. L. Clejan, D. S. Beattie, E. B. Gollub, K. P. Lin, and D. B. Sprinson. JOURNAL OF BIOLOGICAL CHEMISTRY 255:1312-1316, 1980.

5. Dr. J. M. Bastide gave a lecture before the "Société Française de Mycologie Médicale; April 29-30, 1980" on the following subject:

"Cell-wall structure of yeasts" (BULLETIN DE LA SOCIÉTÉ, DE MYCOLOGIE MÉDICALE, in press). Another paper is in press in the BULLETIN DE LA SOCIÉTÉ DE MYCOLOGIE MÉDICALE: "Etude taxonomique de *Candida curvata* et de *Candida marina*" by E. Gueho, S. Jouvert, and M. Bastide. We consider these two species as belonging to the genus *Cryptococcus* according to the G+C evaluation, the assimilation of inositol, and the presence of capsular material shown in scanning electron microscopy.

M. Bastide  
Département de Microbiologie  
Laboratoire d'immunologie,  
virologie et parasitologie et  
Laboratoire de Microbiologie  
Industrielle  
Université de Montpellier 1, France

6. The following papers have been accepted for publication:

Allan, R. A., and Miller, J. J. Influence of S-adenosylmethionine on DAPI-induced fluorescence of polyphosphate in the yeast vacuole. CANADIAN JOURNAL OF MICROBIOLOGY.

Bilinski, C. A., and Miller, J. J. Induction of normal ascosporeogenesis in two-spored *Saccharomyces cerevisiae* by glucose, acetate, and zinc. JOURNAL OF BACTERIOLOGY.

J. J. Miller  
Department of Biology  
McMaster University  
1280 Main Street West  
Hamilton, Ontario L8S 4K1  
Canada

7. Don C. Vacek, who in December completed a Ph.D. in Ecology and Evolutionary Biology under the supervision of William B. Heed at the University of Arizona, Tucson, Arizona, is spending two years, beginning February 1980, in my laboratory to participate in our research on the mechanisms maintaining

enzyme polymorphisms in Drosophila. He has been awarded a Junior Research Fellowship by the Australian Research Grants Committee to study yeast-Drosophila interactions and selection in natural populations of the cactophilic Drosophila, D. buzzatii and D. aldrichi that breed in rots in the introduced Opuntia of Australia.

Professor J. S. F. Barker  
Department of Animal Science  
University of New England  
Armidale, New South Wales  
Australia, 2351

8. The following publications have appeared since the last communications. The abstracts of reports have been given in Yeast Newsletter XXVII (1978):2, 89 and XXVIII (1979):2, 78.

Heikki Suomalainen and Erkki Oura. Ethanol as substrate for baker's yeast. Proceedings of the 12th International Congress of Microbiology, Munchen 1978, DECHEMA (Deut. Ges. Chem. Apparatewesen) MONOGR. 83 (1979):1704-1723, 43-51.

Anssi Saura, Juhani Lokki, Erkki Oura, and Heikki Suomalainen. Qualitative yeast enzyme analysis by electrophoresis. EUROPEAN JOURNAL OF APPLIED MICROBIOLOGY AND BIOTECHNOLOGY 7 (1979), 355-364.

Dr. Heikki Suomalainen, Director  
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Alko, Box 350  
SF-00101 Helsinki 10, Finland

9. The mention by Duran in the last issue of the Yeast Newsletter (XXVIII, No. 2, page 76) of two articles (Duran and Cabib, JOURNAL OF BIOLOGICAL CHEMISTRY 253:4419-4425, 1978; and Duran, Cabib and Bowers, SCIENCE 203:363-365, 1979) may have given the impression that the work reported in these papers originated in the Department of Microbiology of the University of Salamanca, Spain. Actually, the papers were submitted from our laboratory; and the work was carried out in the Bethesda Laboratory during Dr. A. Duran's stay in my laboratory as a "Guest Worker".

Enrico Cabib  
National Institutes of Health  
Bethesda, Maryland 20014

10. On Tuesday, June 10, 1980, the National collection of Yeast Cultures will be moved to the Agricultural Research Council's Food Research Institute.

All the services currently provided by the N.C.Y.C. will be continued. It is not anticipated that there will be any appreciable interruption to services as a result of the move, and any delays will be of a temporary nature.

The new address of the N.C.Y.C. is:

National Collection of Yeast Cultures  
Food Research Institute  
Colney Lane, Norwich  
Norfolk NR4 7UA  
Telephone No.: Norwich (0603) 56122