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

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****

David Grounds, Rockville, Maryland 53
H. J. Phaff, Davis, California 56
W. I. Golubev, Moscow, Russia 57
D. C. Vacek, Tucson, Arizona 58
A. N. Hagler, Rio de Janeiro, Brazil 58
M. C. Pignal, Villeurbanne, France 60
Donald G. Ahearn, Atlanta, Georgia 60
J. D. Buck, Noank, Connecticut 61
N. J. W. Kreger-van Rij, Groningen, The Netherlands 61
J. Friis, Odense, Denmark 62
Wolfgang Dunze, Bochum, West Germany 62
J. Schwencke, Gif Sur Yvette, France 63
L. Ferenczy, Szeged, Hungary 64
G. D. Clark-Walker, Canberra City, Australia 66
Norio Gunge, Yokohama, Japan 66
M. J. Leibowitz, Piscataway, New Jersey 67
Tamotsu Morita, Shizuoka, Japan 67
S. R. Snow, Davis, California 68
Pencho Venkov, Solfia, Bulgaria 70
F. K. Zimmermann, Darmstadt, West Germany 71
Graeme M. Walker, Edinburgh Scotland 74
T. W. James, Los Angeles, California 75
K. Poralla, Tübingen, F. R. Germany 76
R. K. Poole, London, England 77
N. van Uden, Oeiras, Portugal 79
C. W. Price, Atlanta, Georgia 81
L. Julia Douglas, Glasgow, Scotland 81
Kenji Soda, Kyoto-Fu, Japan 82
A. Kimura, Kyoto, Japan 83
Harlyn O. Halvorson, Waltham, Massachusetts 85
A. E. Beezer, London, England 85
R. Hütter, Zürich, Switzerland 86
H. Suomalainen, Helsinki, Finland 87
K. Watson, Townsville, Australia 90
Steve Oliver, Kent, England 91
Roy J. Thornton, Palmerston North, New Zealand 93
P. Ribereau-Gayon, Talence, France 93
E. Minarik, Bratislava, Czechoslovakia 95

G. G. Stewart, London, Canada 95
S. Windisch, Berlin, West Germany 97
Amin S. El-Nawawy, Tripoli, Libya 97
Nancy J. Moon, Experiment, Georgia 99
National and International Meetings on Yeasts 100
Brief News Items 109

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

I. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852. Communicated by David Grounds.

The strains listed have been added to the ATCC since April 10, 1978. Complete information on these strains may be obtained upon request from the Mycology Department of ATCC.

Torulopsis wickerhamii ATCC 36540, Type Culture	D. Yarrow CBS
Trichosporon capitatum ATCC 36553, Human pathogen	K. Grunder Abt. für Dermat. der MHL West Germany
Cryptococcis neoformans ATCC 36555-36556 Virulence factor studies	T. R. Kozel Univ. of Nevada at Reno Reno, Nevada
Rhodotorula glutinis ATCC 36575, Biochemical properties of NAD(P)H: Nitrates	S. Windisch Forchunginst. Mikrob. West Germany
Cryptococcus neoformans ATCC 36582, Human pathogen	N. G. Miller Univ. of Nebraska Omaha, Nebraska
Torulopsis candida ATCC 36584, Type Culture, Taxonomic studies	D. Yarrow CBS
Torulopsis nitratophila ATCC 36585, Type Culture, Taxonomic studies	"
Torulopsis norvegica ATCC 36586, Type Culture, Taxonomic studies	"
Torulopsis domercqii ATCC 36587, Type Culture, Taxonomic studies	"
Torulopsis cantarellii ATCC 36588, Type Culture, Taxonomic studies	"
Torulopsis anatomiae ATCC 36589, Type Culture, Taxonomic studies	"
Torulopsis mogii ATCC 36590, Type Culture, Taxonomic studies	"

Torulopsis tannotolerans
ATCC 36591, Type Culture,
Taxonomic studies

D. Yarrow
CBS

Torulopsis pignaliae
ATCC 36592, Type Culture
Taxonomic studies

"

Torulopsis nemodendra
ATCC 36593, Type Culture,
Taxonomic studies

"

Hansenula alni
ATCC 36594-36596
Taxonomic studies

M. Miranda
Univ. of California
Davis, California

Torulopsis etchellsii
ATCC 36631, Taxonomic studies

D. Yarrow
CBS

Torulopsis halonitratophila
ATCC 36632, Taxonomic studies

"

Torulopsis mannitofaciens
ATCC 36633, Taxonomic studies

"

Torulopsis pampelonensis
ATCC 36642, Type Culture,
Beech Forest soil and leaves

C. Ramirez
Inst. "Jaime Ferrand"
Spain

Cryptococcus vishniacii
ATCC 36649, Type Culture

H. S. Vishniac
Univ. of Rochester
New York

Hansenula polymorpha
ATCC 36669, Mutant of wild type
strain ATCC 26012

S. Sanchez
Inst. Invest. Biomed.
Mexico

Candida tropicalis
ATCC 36729, Isolated from
shrimp larva

Jorge Leong
Natl. Ocean. Atmoph.
Galveston, Texas

Rhodotorula marina
ATCC 36764, Isolated from
Pacific Ocean

K. Yamasato
Univ. Tokyo
Japan

Cryptococcus albidus var. *albidus*
ATCC 36765, Isolated from
Pacific Ocean

"

Rhodotorula rubra
ATCC 36766, Isolated from
Pacific Ocean

"

Culture	Depositor
Debaryomyces hansenii ATCC 36767, Isolated from Pacific Ocean	K. Yamasato Univ. Tokyo Japan
Candida krissii ATCC 36768, Type Culture, Isolated from Pacific Ocean	"
Cryptococcus infirmo-miniatus ATCC 36769, Isolated from Pacific OCean	"
Candida diddensii ATCC 36780, Isolated from Pacific Ocean	"
Candida albicans ATCC 36801, serotype A	K. J. Kwon-Chung NIH Bethesda, Maryland
Candida albicans ATCC 36802, 36803, serotype B	"
Cryptococcus laurentii ATCC 36832-36833, L-α-Amino-E-caprolactamase producer	T. Fukumura Osaka City University Osaka, Japan
Pichia opuntiae var. thermotolerans ATCC 36834, Mating type of 36835	M. Miranda Univ. of California Davis, California
Pichia opuntiae var. thermotolerans ATCC 36835, Mating type of 36834	"
Pichia opuntiae var. opuntiae ATCC 36836, Mating type of 36837	"
Pichia opuntiae var. opuntiae ATCC 36837, Mating type of 36836	"
Torulopsis azyma ATCC 36850, Ascomycetous affinity, Type Culture	D. Yarrow CBS
Torulopsis azyma ATCC 36851, Ascomycetous affinity, Isolated from soil and rupicolous lichens in South Africa	"
Torulopsis geocharis ATCC 36852, Ascomycetous affinity, Type Culture	"

Saccharomyces cerevisiae ATCC 36858-36859, Mutant strain lacking hexokinase activity	Z. Lobo Tata Inst. Fund. Res. India

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

1. The following three papers will appear in the January 1979 issue of INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY:

   M. A. Lachance and H. J. Phaff. A Comparative Study of Molecular Size and Structure of Exo-β-glucanases from Kluyveromyces and Other Yeast Genera: Evolutionary and Taxonomic Implications.

   G. B. Fuson, C. W. Price, and H. J. Phaff. Deoxyribonucleic Acid Sequence Relatedness Among Some Members of the Yeast Genus Hansenula.

   H. J. Phaff, M. W. Miller, and M. Miranda. Hansenula alni, a New Heterothallic Species of Yeast from Exudates of Alder Trees.

2. Eric A. Johnson and Herman J. Phaff. Rhodotorula fujisanensis, A New Taxonomic Combination. CURRENT MICROBIOLOGY 1, (No. 4), 1978 (in press).

ABSTRACT

The demonstration of carotenoid pigments in the type strain and one additional strain of Torulopsis fujisanensis Soneda necessitates the transfer of this species to the genus Rhodotorula as Rhodotorula fujisanensis (Soneda) Johnson et Phaff comb. nov.

3. W. T. Starmer, H. J. Phaff, Mary Miranda, M. W. Miller, and J. S. F. Barker. Pichia opuntiae, A New Heterothallic Species of Yeast Found in the Decaying Cladodes of Opuntia inermis and in Necrotic Tissue of Cereoid Cacti. (Submitted to INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY.)

ABSTRACT

A new heterothallic species of Pichia has been recovered 25 times from widely separated cactus substrates. The organism has been named Pichia opuntiae because the sexually most compatible strains were isolated from Opuntia inermis in Australia. Two varieties are designated based on differences in physiology, habitat, and geographic distribution. P. opuntiae var. opuntiae has a maximum temperature for growth of 30-33°C, assimilates citric acid strongly, but assimilation of cellobiose is latent, weak, or negative. P. opuntiae var. thermotolerans grows well at 37°C but not at 39°C; it assimilates cellobiose strongly but does not assimilate citric acid. Ecologically, P. opuntiae var. opuntiae is associated with Opuntia inermis (Tribe Opuntiaeae, subtribe Opuntiinae) in Australia; P. opuntiae var. thermotolerans is associated with species of the cactus tribe Pachycereae, subtribe Pachycereinae, from various

locations in the North American Sonoran Desert. A discussion of the physiologic and host-plant shifts for these two varieties and three similar cactophilic yeasts is presented. The base composition of the nuclear deoxyribonucleic acid of P. opuntiae var. opuntiae (average of 4 strains) is 33.64 ± 0.25 mol% guanine plus cytosine, and that of P. opuntiae var. thermotolerans (average of 3 strains) is 33.13 ± 0.23 mol% G + C. The type strain of P. opuntiae and of the type variety P. opuntiae var. opuntiae is UCD-FS&T 77-40 (= ATCC 36836 = CBS 7010). The type strain of P. opuntiae var. thermotolerans is UCD-FS&T 76-211 (= ATCC 36834 = CBS 7012).

4. The second, revised and enlarged edition of THE LIFE OF YEASTS by H. J. Phaff, M. W. Miller, and E. M. Mrak was published November 1978. (Harvard University Press, 79 Garden Str., Cambridge, Mass. 02138.)

III. Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pushchino, Moscow Region 142 292, USSR. Communicated by W. I. Golubev.

The following studies are being carried out in our laboratory:

The nucleotide composition of DNA was analyzed in several strains of Cryptococcus terreus. It has been found that its GC-content is distributed into two groups of values. There is correlation between the mol% G+C and morphology of vegetative cells, giant colonies, and assimilatory characteristics. On the basis of these findings, we propose to divide Cr. terreus di Menna 1954 into two species: Cr. terreus (di Menna) Golubev and Cr. elinovii Golubev nov. sp. The first species is characterized by globose and subglobose cells, inability to assimilate melezitose, and exhibits a GC-content of 59-61 mol%. The latter species has a GC-content of 57-58%, subglobose and oval cells, and assimilates melezitose. Cr. himalayensis Goto et Sugiyama 1970 is identical to Cr. terreus (di Menna) Golubev by morphological and physiological properties, but base composition of the type strain is 55.7 mol%.

Working with Cr. magnus, Mr. A. R. Manukayan has found that D-mannose and peptone are the best sources of carbon and nitrogen for capsule synthesis. Optimal concentrations of these substances are 3% and 0.2%, respectively. An increase in the sugar content and particularly that of nitrogen in the medium inhibits capsule formation. Addition of yeast extract produces larger capsules than a supply of only biotin and thiamine. Comparison of these data with those on Cr. neoformans shows that there are no significant differences in nutritional requirements for capsule production between saprophytic and pathogenic yeasts.

Mrs. I. V. Golubeva studies dimorphism in Metschnikowia lunata. The cellular form of this yeasts may be either lunate or ellipsoidal in shape. Development of cell form is affected by carbon and nitrogen sources, age of culture, and temperature of incubation. Cell populations almost exclusively of lunate or ellipsoidal forms can be obtained in chemically defined media.

The following articles have been published:

Golubev, W. I., and L. M. Vagabova. 1977. Nucleotide composition of DNA in some yeast species. Izvestia USSR Acad. Sci., SER. BIOL., N 6, pages 933-936.

Golubev, W. I., I. P. Bab'eva, and S. N. Novik. 1977. Yeast succession in birch fluxes. EKOLOGIA, N 5, pages 21-26.

IV. Department of Ecology and Evolutionary Biology, The University of Arizona, Tucson, Arizona 85721. Communicated by D. C. Vacek.

Vacek, Don C., William T. Starmer, and William B. Heed. 1978. The relevance of the ecology of Citrus yeasts to the diet of *Drosophila*. Microbial Ecology 5 (No. 1). In press.

A study of the yeast flora of necrotic oranges and associated *Drosophila* yielded a total of 221 isolates composed of *Kloeckera apiculata* (75), *Pichia fermentans* (75), *Pichia kluyveri* (50), *Torulopsis stellata* (17), *Hanseniaspora uvarum* (2), *P. membranaefaciens* and *Candida vini* (1). The yeast species of all samples of oranges and adult *Drosophila* were very similar. However, the species of *Drosophila* contained a higher proportion of *P. fermentans* and a lower proportion of *K. apiculata* than was found in the rotting oranges. *P. fermentans* was subsequently found more frequently on the surface of the necrotic tissue, where the flies feed, than was found internally. Since *P. fermentans* characteristically produces a pellicle and pseudomycelium and *K. apiculata* does not, it is concluded that the growth characteristics of the yeasts are an important factor determining adult *Drosophila* diets.

V. Universidade Federal do Rio de Janeiro. Instituto de Microbiologia, Rio de Janeiro, Brazil. Communicated by A. N. Hagler.

Below follows the summary of my doctoral dissertation which was completed in May 1978 (Text Portuguese):

THE ECOLOGY AND TAXONOMY OF YEASTS IN A POLLUTED ESTUARY AND MARINE ENVIRONMENTS OF RIO DE JANEIRO, BRAZIL

A review of the literature on yeasts in aquatic environments, including freshwater, estuary, marine, sewage, and sediments, makes up the bulk of the Introduction. Tables in the Introduction and Discussion indicate the prevalent yeast genera and species in aquatic environments. Some notes on coliform counts as pollution indicators and on yeast taxonomy are included.

This study was focused on six sites along a polluted estuary. Three of these were in an inner channel with pH and salinity well below that of seawater. They were more heavily polluted than three other sites along an outer channel which had pH and salinity near that of seawater. Yeast counts from the inner channel were typically

within a 10 to 50 CFU/ml range, and those of the outer channel were typically within a 0.5 to 10 CFU/ml range. Yeast counts in cleaner marine waters of recreational beaches and unpopulated areas were rarely above one per ml.

The 549 strains of yeasts and yeast-like organisms isolated were classified into 67 species. Most of these were identified with existing species, although some variation from standard descriptions was common. Characteristics most typical of the isolates were fermentation of glucose, growth at 37°C but not at 40°C, requirement of one or more vitamins for optimum growth, failure to use nitrate as the sole nitrogen source, and lack of sexual spores. The species most frequently isolated from the polluted estuary included some commonly isolated from marine sources and some commonly isolated from fecal material and sewage, which are potential pathogens. The 20 prevalent yeasts of the polluted estuary water which accounted for 86% of the total yeast counts were, in order of frequency: *Rhodotorula rubra*, *Candida krusei*, *C. sorbosa*, *Trichosporon penicillatum*, *Kloeckera apiculata* (+ *Hanseniaspora uvarum*), *Torulopsis holmii* (+ *Saccharomyces exiguus*), *T. candida* (+ *Debaryomyces hansenii*), *C. tropicalis*, *Pichia terricola*, *C. sorboxylosa*, *Hansenula anomala*, *Aureobasidium pullulans*, *C. guilliermondii*, *C. intermedia*, *C. lipolytica*, *C. species* A, *Tr. cutaneum*, *C. brumptii*?, *C. parapsilosis*, and *P. membranaefaciens*.

The yeast population of the intertidal sediment is usually restricted to the top few centimeters and is generally higher than that in the water. Yeasts of the intertidal sediments appeared to be independent from the yeasts of water in the polluted estuary. *Candida krusei*, *Pichia membranaefaciens*, and similar species were prevalent among the sediment yeast; and some species, such as *Torulopsis glabrata*, *Prototheca zopfii*, and an unidentified *Candida*, a species similar to *C. ingens*, were mostly restricted to the sediments.

Low level of utilizable carbon sources was the principal nutritional factor limiting growth of yeasts in polluted estuary water, but nitrogen sources and phosphate were also less than optimal, and low vitamin levels should influence growth of some strains. The sodium chloride concentration of oceans and estuaries may prevent or inhibit growth of some yeasts, but most yeasts had sodium chloride tolerances of twice the concentration in seawater or more. The most frequently needed vitamin was biotin, followed by thiamine and pyridoxine. Requirements for other vitamins were unusual. Phosphate requirements of prevalent yeasts were about the concentration of phosphate in the polluted estuary water (5×10^{-6} to 1×10^{-7} molar) or higher, thus confirming its importance as a limiting nutrient. The alkaline pH of marine and estuary environment was also indicated as an important factor influencing which yeasts can grow there. Some prevalent species, such as *C. krusei*, *P. membranaefaciens*, and *Kl. apiculata*, cannot grow under high pH or low phosphate conditions and should be good indicators of microbial contamination in the marine and estuary environments. Other species, such as *C. tropicalis*, *D. hansenii*, and *R. rubra*, can grow in these conditions; and high counts of these should indicate pollution by chemicals, which can

serve as nutrients in addition to indicating microbial contamination. Some factors in autoclaved clean seawater and polluted estuary water favored survival of seven representative yeasts relative to a saline control. Survival in these three situations was species dependent; but, in general, toxicity toward yeasts was low or not apparent.

VI. Section Levures - Biologie Végétale 2°cycle Bld. 405, University Claude Bernard, 43, Blvd. du 11 Novembre 1918, 69 Villeurbanne, France. Communicated by M. C. Pignal.

Below follows some news items from our laboratory.

The following papers have been published:

1. J. B. FIOL & G. BILLON-GRAND. Osidases, nitrite et nitrate reductases dans les genres Hanseniaspora et Kloeckera. Mycopathololologia 63: 47-51, 1978.

2. M. FAURE-REYNAUD & F. H. JACOB. Microflore de la litière du sapin Abies alba Mill.: bactéries et levures. Bull. mens. Soc. Linn. Lyon 47: 392-404, 1978.

   Mise en évidence des bactéries et des levures colonisant les feuilles de la litière. Evolution de cette microflore dans les couches L et F de la litière. Etude des bactéries et des levures isolées, recherche de quelques caractères de ces microorganismes.

3. R. MONTROCHER. Serological studies in the genus Candida. VIth Intern. Spec. Symp. Yeasts, Montpellier July 1978, Abstr. S III: 9-10.

The following papers are in press:

1. F. H. JACOB & S. PONCET. Production de protéines à partir de cellules-levures: Tendances actuelles pour l'utilisation des résidus industriells. Bull. Mens. Soc. Linn. Lyon.

2. M. OHJA & S. PONCET. Ribosomal RNA genes in Kluyveromyces marxianus. Accepted for publication in J. Gen Microbiol.

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

The following are recently published papers:

Ahearn, D. G. 1978. Medically important yeasts. ANN. REV. MICROBIOL. 32:59-68.

Ahearn, D. G., and D. L. Holzschu. 1978. The white yeasts as disease agents: Historical perspective. In Proc. 4th Internat. Cong. Mycoses, Pan Am. Health Org., Washington, D.C., PAHO SCI. PUBL. NO. 356:119-123.

Ahearn, D. G., J. Sangvi, G. J. Haller, and L. A. Wilson. 1978. Mascara contamination: In-use and laboratory studies. J. SOC. COSMETIC CHEMISTS 29:127-131.

Bowman, P. I., and D. G. Ahearn. 1978. Ecology and physiology of *Cryptococcus neoformans* in Georgia. *In* Proc. 4th Internat. Cong. Mycoses, Pan Am. Health Org., Washington, D.C., PAHO SCI. PUBL. NO. 356:258-268.

Ogletree, F. A., A. T. H. Abdelal, and D. G. Ahearn. 1978. Germ-tube formation by atypical *Candida albicans*. ANTONIE VAN LEEUWENHOEK 44:15-24.

VIII. The University of Connecticut, Marine Sciences Institute, Marine Research Laboratory, P.O. Box 278, Noank, Connecticut 06340. Communicated by J. D. Buck.

Below follow some recent publications from our laboratory:

Buck, J. D., P. M. Bubucis, and T. J. Combs. 1977. Occurrence of human-associated yeasts in bivalve shellfish from Long Island Sound. APPL. ENVIRON. MICROBIOL. 33:370-378.

Buck, J. D. 1977. *Candida albicans*. In: Bacterial indicators/Health hazards associated with water. (A. W. Hoadley and B. J. Dutka, eds.) Pages 139-147. ASTM-STP 635, AMERICAN SOCIETY FOR TESTING & MATERIALS, PHILADELPHIA.

Buck, J. D. and P. M. Bubucis. 1978. Membrane filter procedure for enumeration of *Candida albicans* in natural waters. APPL. ENVIRON. MICROBIOL. 35:237-242.

Buck, J. D. 1978. Comparison of *in situ* and *in vitro* survival of *Candida albicans* in seawater. MICROBIOL. ECOL. (in press).

Our current research is concerned with associations of human-associated yeasts with marine mammals and the effects of various sewage treatment processes on the survival of potentially pathogenic yeasts. I would appreciate greatly hearing from any of the Yeast Newsletter readers with information on these topics.

IX. Laboratory for Medical Microbiology R.U., Oostersingel 59, Groningen, The Netherlands. Communicated by N. J. W. Kreger-van Rij.

Below follows a list of publications from this laboratory:

1. N. J. W. Kreger-van Rij, Ultrastructure of *Hanseniaspora* ascospores. ANTONIE VAN LEEUWENHOEK, 43: 225-232, 1977.

2. N. J. W. Kreger-van Rij, Electron microscopy of germinating ascospores of *Saccharomyces cerevisiae*. ARCH. MICROBIOL. 117: 73-77, 1978.

3. J. Lodder and N. J. W. Kreger-van Rij, Proposal (446) for the conservation of the generic name Debaryomyces Lodder et Kreger-van Rij against Debaryomyces Klöcker, Taxon, 27: 306-307, 1978.

4. N. J. W. Kreger-van Rij, Ultrastructure of the ascospores of the new yeast genus Sporopachydermia Rodrigues de Miranda. ANTONIE VAN LEEUWENHOEK, 44: 1978, in press.

X. Dept. of Molecular Biology, Odense University, Niels Bohrs Alle, DK-5230 Odense, M, Denmark. Communicated by J. Friis.

Below follows the summary of a recently published paper.

Kay B. Gulløv and Jørgen Friis

Isolation of Chromatin from Saccharomyces cerevisiae: The Metabolic State in Relation to the Protein Content of Chromatin and Its Susceptibility to Micrococcal Nuclease

EXPERIMENTAL MYCOLOGY 2, 161-172 (1978)

The structure and composition of yeast chromatin were analyzed in cells actively engaged in RNA synthesis and in cells in which the RNA synthesis was abolished. Isopycnic banding of hydrated chromatin from both cell types indicate that yeast chromatin is homogeneous with respect to the protein content along the DNA strand. The genome from transcribing cells is isolated as a membrane-bound structure, while the genome from nontranscribing cells might be isolated as a freely sedimenting structure. The DNA of yeast chromatin is susceptible to a steady micrococcal nuclease degradation. The absence of a limit digest plateau is evident and independent of the physiological state of the cell. During codigestion of chromatin from transcribing and nontranscribing cells, isopycnic banding reveals that the protein content decreases continuously and in an identical manner. A relatively stable element, with a protein content less than half of what is normally registered in core particles at limit digest, is eventually formed.

XI. Ruhr Universität Bochum, Institut Für Physiologische Chemie, D 4630 Bochum 1, Postfach 10 21 48, West Germany. Communicated by Wolfgang Dunze.

Purification and Characterization of a-Factor from S. cerevisiae

Dr. Richard Betz in our laboratory has succeeded in purifying to homogeneity the specific mating-hormone of a-mating type cells (a-factor) and is currently trying to elucidate the structure of this hormone. The following is a summary of a manuscript which is in preparation and will be submitted for publication.

SUMMARY

Cells of Saccharomyces cerevisiae exhibiting the a mating type secrete into the culture medium a mating-type specific hormone

activity, (a-factor) which specifically causes a transient arrest of DNA replication and cell division in cells of the opposite mating type α. Three peptides exhibiting a-factor activity have been found in culture filtrates of a cells. The most active compound has been purified more than 10^5 fold and appears to be homogeneous on the basis of thin layer chromatography and thin layer electrophoresis in different systems. We propose that this compound represents pure a-factor. a-Factor has been characterized as a linear undecapeptide with the following amino acid composition:

Asx_1, Pro_1, Gly_1, Val_1, Ala_1, Ile_2, Tyr_1, Phe_1, Lys_1, Trp_1

Amino acid analysis and gel filtration indicate a molecular weight in the range of 1300-1400 daltons. The purified compound exhibits in α-cells all biological activities that have been attributed to a-factor, such as G1-arrest of the cell cycle, induction of the "shmoo" morphology, and induction of sexual agglutinability.

Although the biological effects of a-factor are remarkably similar to those produced by α-factor in a-cells, there appears to be little similarity in the chemical structure of the two hormones, except for the fact that both are hydrophobic linear oligopeptides.

XII. Laboratoire D'Enzymologie, C.N.R.S., 91190 Gif Sur Yvette, France. Communicated by J. Schwencke.

Below follows a method for the preparation of yeast protoplasts of *Schizosaccharomyces* cells, recently described by us in METHODS IN CELL BIOLOGY (D. M. Prescott, editor), Vol. XX, 101-105 (1978), Academic Press, New York.

Our previously described method (Housset et al., 1975) has been subsequently modified as follows:

1. *Schizosaccharomyces pombe* cells, wild-type strain 972h-, are grown at 30°C in a complete medium containing in 1 liter: 5 g. of yeast extract, 5 g. of Difco peptone, and 30 g. of glucose. The addition of sulfur amino acids (Svihla et al., 1961) has been found to be unnecessary. Protoplasts are prepared from exponentially growing cells (up to 8 X 10^6 cells/ml), harvested by centrifugation (5000 g. for 1 min.), and washed once with distilled water.

2. The yeast pellet (10^9 cells, about 350 mg wet weight) is resuspended in 5 ml of a freshly prepared solution containing 100 mM Tris, 5mM EDTA, and 5 mM dithiothreitol brought to pH 8 with HCl. After 15 minutes at 30°C, the cells are separated by centrifugation (5000 g. for 1 minute) and washed once with 0.6 M sorbitol containing 20 mM (N-morpholino) ethanesulfonic acid (MES) brought to pH 6 with KOH.

3. The cells are then incubated (10^9 cells/ml) with gentle shaking at 30°C in a fresh mixture of 0.6 M KCl, 5 mM dithiothreitol, and 40 mg/ml lyophylized snail enzymes (Industrie Biologique Française), to which is added 5 mg/ml of α-1, 3-glucanase (3 units/mg) and 5 mg/ml of β-1, 3-glucanase (10 units/mg). (The last two enzymes were

obtained from E. T. Reese, Food Microbiology Group, U.S. Army, Natick Laboratories, Natick, Mass.) This enzyme mixture is previously clarified by centrifugation at 12,000 g. for 15 minutes. Complete conversion of cells to protoplasts is obtained after about 60 min. of incubation for cells grown in complete medium without sulfur amino acids, and after about 80 minutes for those grown in their presence.

4. Protoplasts may be purified from debris by sedimentation at 2000 g. for 5 minutes through a layer of 0.8 M sucrose containing 0.2 M KCl. The pelleted protoplasts are resuspended and stored in 0.6 M sorbitol containing 0.2 M KCl and 20 mM MES (pH 5, KOH).

REMARKS

Both α-1, 3 and β-1, 3-glucanase are indispensable for good results. Protoplasts obtained and stored under the conditions described above maintain good microscopic and metabolic characteristics as indicated by the study of their guanine uptake (Housset et al., 1975). Early stationary cells and cells grown in mineral media also have been converted to protoplasts using a stronger pretreatment medium (Schwencke et al., 1977). The method described by Kopecka (1975) should also be useful. A limitation common to both methods is the lack of a commercial source of α-1, 3-glucanase. A mixture of β-1, 3-glucanases is available in gram quantities under the trade name Zymolase from Kirin Brewery Co., Ltd., Takasaki, Gumma Prefecture, Japan. However, as expected, complete conversion of cells into protoplasts with this enzyme preparation alone has not been obtained.

XIII. Department of Microbiology, Attila József University, H-6701 Szeged, P. O. Box 428, Hungary. Communicated by L. Ferenczy.

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

Sipiczki, M., and L. Ferenczy. Protoplast fusion in *Schizosaccharomyces pombe* auxotrophic mutants of identical mating type. MOLEC. GEN. GENET., 151, 77-81 (1977).

Ferenczy, L., and A. Maráz. Transfer of mitochondria by protoplast fusion in *Saccharomyces cerevisiae*. NATURE, 268, 524-525 (1977).

Sipiczki, M., and L. Ferenczy. Fusion of Rhodosporidium (Rhodotorula) protoplasts. FEMS LETTERS, 2, 203-205 (1977).

Novák, E. K., E. Heszler, A. Pólay, A. Maráz, I. Rojik, L. Sztankov, and L. Ferenczy. India-ink immuno reactions with anti *Candida albicans*-protoplast serum. PROC. 5TH INTERN. SPEC. SYMP. YEAST, Keszthely, Hungary, p. 29-30 (1977).

Vallin, C., and L. Ferenczy. Diploid formation of *Candida tropicalis* via protoplast fusion. ACTA MICROBIOL. ACAD. SCI. HUNG., in press (1978).

Maráz, A., M. Kiss, and L. Ferenczy. Protoplast fusion in Saccharomyces cerevisiae strains of identical and opposite mating types. FEMS LETTERS, in press (1978).

Sipiczki, M., and V. Farkas. Morphogenic effects of 2-Deoxy-D-Glucose in Rhodosporidium toruloides. ARCH. MICROBIOL., in press (1978).

Protoplast fusion in *Saccharomyces cerevisiae* strains of identical and opposite mating types

A. Maráz, M. Kiss, and L. Ferenczy

FEMS LETTERS (1978)

Protoplasts of *S. cerevisiae* are able to fuse in the presence of polyethylene glycol as inducer. When auxotrophic mutants are used, the fusion products can be selectively regenerated in consequence of nutritional complementation. The frequency of complementation in the case of identical (*a* and *a* or *α* and *α*) or different (*a* and *α*) mating types proved to be similar indicating that the mechanism of fusion and stability of fused protoplasts are not affected by the mating characters.

The fusion products are diploids. In biochemical and genetic properties the [*a* + *α*] fusion products resemble the diploid cells produced by sexual processes; e.g., they can be induced to sporulate, and the parental types can be recovered by meiotic segregation. Sporulation, however, cannot be induced in [*a* + *a*] and [*α* + *α*] diploids, but by induced haploidization, cells of parental biochemical characters and recombinants can be recovered by mitotic segregation.

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Morphogenic Effects of 2-Deoxy-D-Glucose in *Rhodosporidium toruloides*

M. Sipiczki and V. Farkas

ARCH. MICROBIOL. (1978)

The presence of 2-deoxy-D-glucose (DG) in the growth medium caused marked morphological changes in the cells of *Rhodosporidium toruloides*. The originally elongated ellipsoidal cells grew spherically in the presence of DG, displayed differences in cell division and separation, and were larger than the control cells. After exhaustion of glucose from the medium, the cells died, although practically no lysis was observed. The morphological changes were accompanied by significant alterations in the carbohydrate composition of the cell wall. The wall of *Rh. toruloides* grown in the presence of DG was thinner and contained higher proportions of chitin and glucan, while the relative contents of mannose and galactose polymers decreased drastically in comparison to normal cells.

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Diploid formation of Candida tropicalis
via protoplast fusion

C. Vallin and L. Ferenczy

ACTA MICROBIOL. ACAD. SCI. HUNG., in press (1978)

Haploid auxotrophic mutants were produced from Candida tropicalis, and protoplast fusion was induced by polyethylene glycol. The resulting nutritional complementation was due to heterokaryon formation and, at a much lower frequency, to spontaneous diploidization. During cultivation, heterokaryotic clones regularly gave rise to heterozygous diploids from which, in turn, haploids could be isolated.

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

The following is a summary of an article to appear in the November issue of GENETICS.

K. M. Oakley & G. D. Clark-Walker

Abnormal Mitochondrial Genomes in Yeast Restored to Respiratory Competence

ABSTRACT

When crosses are performed between newly-arisen spontaneous petite mutants of Saccharomyces cerevisiae, respiratory competent (restored) colonies can form. Some of the restored colonies are highly sectored and produce large numbers of petite mutants. The high frequency petite formation trait is inherited in a non-Mendelian manner and elimination of mitochondrial DNA from these strains results in the loss of the trait. These results indicate that abnormal mitochondrial genomes are sometimes formed during restoration of respiratory competence. It is hypothesized that these abnormalities result either from recombination between mitochondrial DNA fragments to produce molecules having partial duplications contained on inverted or transposed sequences or else recombinational "hot spots" have been expanded.

XV. Central Research Laboratories, Mitsubishi Chemical Industries Limited, 1,000 Kamoshida-Cho, Midori-Ku, Yokohama-Shi, Kanagawa-Ken, Japan. Communicated by Norio Gunge.

The following is a summary of our recent paper on the fusion of mitochondria with protoplasts in Saccharomyces cerevisiae, which is to appear in MOLECULAR & GENERAL GENETICS. Part of this work was presented at the Rochester Conference on Yeast Genetics and Molecular Biology, 1978.

Protoplasts prepared from a neutral petite haploid B060AF-1 (a ade2 arg4 leu2 trp C° E° O° ω° ρ°), S. cerevisiae, were mixed with mitochondria isolated from an oligomycin resistant respiring haploid

$AN^{R}OR12D$ (a his4 leu2 thr4 C^{S} E^{S} O^{R} ω^{+} ρ^{+}) and treated with 30% polyethylene glycol and $CaCl_2$. When the treated mixture was spread and incubated on selective agar plates, respiration-sufficient colonies appeared with low frequency. All of these colonies carried the mitochondrial genotype of C^{S} E^{S} O^{R} ω^{+} ρ^{+} and showed the same mating type and nutritional requirements as did B060AF-1, evidencing the mitochondrial transfer into protoplasts. Recombination and transmission of the mitochondrial drug resistance markers were studied, using the strains issued from the mitochondria accepted protoplasts.

XVI. Rutgers Medical School, Department of Microbiology, University Heights, Piscataway, New Jersey 08854. Communicated by M. J. Leibowitz.

Below is the abstract of a recent paper from this laboratory.

M. J. Leibowitz and R. B. Wickner, "PET18: A chromosomal gene required for cell growth and for the maintenance of mitochondrial DNA and the killer plasmid of yeast". MOLECULAR AND GENERAL GENETICS, in press, 1978.

Mutations in the pet18 gene of Saccharomyces cerevisiae (formerly denoted pets) confer three phenotypes on mutant strains: (i) inability to respire (petite), (ii) inability to maintain the double-stranded RNA killer plasmid (sensitive), and (iii) temperature sensitivity for growth. We find that pet18 mutants lack mitochondrial DNA. However, despite their inability to maintain the killer RNA plasmid and mitochondrial DNA, pet18 mutants still can carry the other yeast plasmids, [URE3-1], [PSI], and 2-micron DNA. The temperature sensitivity of the pet18 mutants is not expressed as a selective defect in total DNA, RNA, or protein synthesis.

XVII. Department of Microbiology, Shizuoka College of Pharmacy, 2-2-1 Oshika, Shizuoka, 422 Japan. Communicated by Tamotsu Morita.

1. The following paper previously listed as submitted to Mutation Research has now been published:

   Tamotsu Morita and K. Lemone Yielding: Photolytic binding of the monoazido analog of ethidium to yeast mitochondrial DNA-Competition by ethidium. MUTATION RESEARCH 54, 27-32, 1978.

   SUMMARY

   In Yeast Newsletter, Volume XXVI, number 2, page 21.

2. Below is a summary of a recently published paper: Masahito Fukunaga, Tamotsu Morita, and Ichiji Mifuchi: Loss and retention of mitochondrial drug resistance in rho^{-} mutants of yeast induced by 4-nitroquinoline 1-oxide and ethidium bromide. J. GEN. APPL. MICROBIOL. (Tokyo) 24, 215-222, 1978.

SUMMARY

Loss and retention of mitochondrially coded drug reisistance markers were studied in cytoplasmic respiration-deficient (rho$^-$) mutants of yeast, Saccharomyces cerevisiae, induced by 4-nitroquinoline 1-oxide (4-NQO) and ethidium bromide. When the yeast cells resistant to chloramphenicol, erythromycin and oligomycin were treated with ethidium bromide, this drug resistance was lost progressively with increasing time of treatment. In the case of 4-NQO, the retention rate of drug resistance was very high and did not change by treatment for 1, 5, and 24 hours.

Crossing experiments between drug-sensitive rho$^-$ mutants and resistant cells indicated that the loss of drug resistance was caused not by mutation of drug-resistance genes but by deletion of genes on mitochondrial DNA. Interpretations concerning the deletion of mitochondrial drug-resistance genes by 4-NQO and ethidium bromide are given, and the difference in their actions is discussed.

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

1. Abstract of the Ph.D. Dissertation of Terry L. Strike, Department of Genetics, U.C. Davis. CHARACTERIZATION OF MUTANTS OF YEAST SENSITIVE TO X-RAYS.

ABSTRACT

This study deals with the characterization of mutants at the rad50 to rad57 loci selected on the basis of their sensitivity to X-rays. They were also examined for sensitivity to UV and MMS and for characteristics of mutation induction, heteroallelic reversion (gene conversion), liquid holding recovery from X-rays, and sporulation. All the mutants were slightly to moderately sensitive to UV, though they did not show the extreme sensitivity of the rad1 to rad22 mutations; and all demonstrated cross sensitivity to both X-rays and MMS. If a mutant was very sensitive to X-rays, it was usually very sensitive to MMS also.

Rad50 is very sensitive to X-rays and MMS and slightly sensitive to UV. It has negative liquid holding recovery, reduced mutation induction at low doses of mutagenic agents, reduced heteroallelic reversion, slightly reduced sporulation ability, and almost zero spore viability.

Rad51 is moderately sensitive to X-rays and MMS and slightly sensitive to UV. It has normal liquid holding recovery, normal mutation induction except for some depression at low doses of X-rays, slightly lowered heteroallelic reversion, and extremely poor sporulation; but those spores that are produced are usually viable.

Rad52 is moderately to very sensitive to X-rays and MMS and slightly sensitive to UV. It has greater than normal liquid holding recovery, almost normal induction except for a slight elevation at

high doses of mutagenic agents, almost normal heteroallelic reversion, and very poor sporulation and spore viability. It is known to be deficient in the repair of double strand breaks in DNA and could have a meiotic defect in chromosome pairing or recombination.

Rad53 is very sensitive to X-rays and MMS and moderately sensitive to UV. It has normal liquid holding recovery, normal mutation induction except for an elevation at high doses of mutagenic agents, slightly less than normal heteroallelic reversion, and almost normal sporulation but poor spore viability.

Rad54 is very sensitive to X-rays and MMS and slightly sensitive to UV. It has normal liquid holding recovery, normal mutation induction with most mutagenic agents except for depressed X-ray mutation induction, reduced heteroallelic reversion, wild-type sporulation, and good spore viability.

Rad55 is very sensitive to X-rays and MMS and slightly sensitive to UV. It has elevated liquid holding recovery, slightly lower than normal mutation induction (except for UV), elevated heteroallelic reversion, and very poor sporulation and spore viability.

Rad56 is slightly sensitive to X-rays, MMS, and UV. It has lower than normal X-ray mutation induction but has almost normal UV and MMS mutation induction, normal heteroallelic reversion, liquid holding recovery, sporulation, and spore viability.

Rad57 is very sensitive to X-rays and MMS and slightly sensitive to UV. It has normal liquid holding recovery, depressed mutation induction at low doses of mutagenic agents, elevated heteroallelic reversion, slightly reduced sporulation, and poor spore viability.

The rad50 to rad57 series of genes has been called a minor UV pathway by several researchers, but a more suitable name would be the X-ray and MMS repair pathway. All of these genes have been reported to be epistatic and thus should control various steps in a single pathway. However, as it is very difficult to group the various rad alleles into discrete types and to discern a probable order, it is possible that these mutants might affect more than one pathway. It is probably too simple an idea to think of the rad50 series as a linear pathway; rather it may be a highly branched pathway with still unknown genes acting in concert. Instead of a number of enzymes working independently, all or most of the enzymes might be aggregated into an enzyme complex which carries out various repair functions as needed. It is evident that some of the gene products have other cellular functions, especially during cell division and meiotic recombination.

2. The following papers have recently been published:

Snow, R. 1978. Absence of suppressible alleles at the his1 locus of yeast. MOLEC. GEN. GENET. 164:341-342.

Scott, K. E., and R. Snow. 1978. A rapid method for making glass micromanipulator needles for use with microbial cells. JOUR. GEN. APPL. MICROBIOL. 24.

3. The following two papers are in press:

Snow, R. Maximum likelihood estimation of linkage and interference from tetrad data. (GENETICS, in press.)

ABSTRACT

Maximum likelihood equations have been derived for estimation of map distance and interference from two-point and ranked tetrad data. The estimators have been applied to data from Saccharomyces cerevisiae and Schizosaccharomyces pombe. S. cerevisiae consistently shows quite strong interference over the mapped genome. In striking contrast, S. pombe consistently shows much weaker interference, and many crosses exhibit negative interference. In neither species was there a conspicuous tendency for intervals spanning a centromere to show less interference than those which did not. Since the amount of recombination per microgram of DNA in the two species is similar, the difference in interference characteristics seems to be a reflection of some fundamental difference in the recombination process of the two species.

Snow, R. Toward genetic improvement of wine yeasts. (AMERICAN JOURNAL OF ENOLOGY AND VITICULTURE, in press.)

ABSTRACT

Planned genetic improvement programs for wine yeasts should be successful, as wine strains contain a great deal of variability. The genetic groundwork which has been undertaken indicates that the methods in current use in yeast genetics and molecular biology research can be readily applied to them. Most wine strains appear to be homothallic and to contain a great deal of heterozygosity for lethal genes or genes affecting growth rate. Some may be polyploid or aneuploid. A number of auxotrophic mutants have been induced in the Montrachet strain and certain of these have been used to produce tetraploid strains through cell mating or protoplast fusion techniques. There is a high degree of genetic homology between Montrachet and laboratory strains. Although in many cases it is easy to specify desirable genetic improvements in wine yeasts, it is likely that one of the major problems which will be encountered in attempts to improve flavor and aroma characteristics will be identification of those biochemical changes which would result in better wine.

XIX. Institute of Molecular Biology, Department of Molecular Genetics, Bulgarian Academy of Sciences, Solfia 1113, Bulgaria. Communicated by Pencho Venkov.

Below follow summaries of our recent studies concerning the further characterization of the osmotic sensitive Saccharomyces cerevisiae mutants isolated in our laboratory several years ago. These investigations have been done in collaboration with our colleagues from Leningrad Nuclear Physics Institute, USSR (Dr. T. Kozhina) and Department of Biology, Friedrich-Schiller University, Jena, DDR (Drs. G. Reuter, U. Märkisch, V. Hering).

1. GENETIC ANALYSIS OF AN OSMOTIC-SENSITIVE SACCHAROMYCES CEREVISIAE MUTANT. T. Kozhina, L. Stateva, and P. Venkov, submitted for publication in MOLEC. GEN. GENETICS.

The genetic analysis of VY1160 sorbitol-dependent, osmotic-sensitive yeast mutant led to the identification of three different nuclear, recessive mutations. Two of them, designated sorb$^-$ and ts1, are closely linked to one another and could be separated only by genetic analysis of hybrids obtained by second set of crosses. The mutation sorb$^-$ determines the lysis, while the mutation ts1 increases the ability for lysis of the sorbitol-dependent cells. The third mutation, ts2, segregates independently from the other two mutations and is localized apart from them. Studies on the rifampicin sensitivity of strains having different genotypes supplied evidence that ts2 mutation is involved in the susceptibility of VY1160 mutant towards this antibiotic.

2. CELL WALL STUDIES ON THE OSMOTIC SENSITIVE SACCHAROMYCES CEREVISIAE MUTANT VY1160 AND ITS PARENTAL STRAIN S288C. G. Reuter, U. Märkisch, V. Hering, and P. Venkov, submitted for publication in DIE ZEITSCHRIFT FÜR ALLGEMEINE MICROBIOLOGIE.

Determination of the polysaccharide contents and structural studies on the mannan by acetolysis and permethylation analysis have shown a changed polysaccharide biosynthesis in the osmotic sensitive VY1160 mutant. The mutant contains more glucan, less mannan, and less alkali soluble glycogen. Its mannan has more short side chains and less long side chains. Its main chain is 1→6 linked, but its side chains consist of more 1→3 than 1→2 linked mannose units.

XX. Institut für Mikrobiologie, Technische Hochschule Darmstadt, Schnittspahnstr. 10, 6100 Darmstadt, Federal Republic of Germany. Communicated by F. K. Zimmermann.

M. Ciriacy and I. Breitenbach: The genetics and physiology of glycolytic pathway mutants in Saccharomyces cerevisiae.

Our work on yeast glycolysis mutants was continued. Forty-five EMS-induced mutants either unable to ferment glucose and maltose or with reduced glucose catabolism were selected for a further analysis. The following mutant types could be identified:

1. pgi - mutants had no phosphoglucose isomerase activity. On glucose medium, mutant cells accumulated a 10-20 times more glucose-6-phosphate than wild type. Glucose strongly inhibited growth, whereas fructose supported growth.

2. One mutant lacked phosphofructokinase (pfk). Growth on and fermentation of glucose were only slightly reduced, suggesting in vivo leakiness of the mutant. Glucose-grown mutant cells contained abnormally high concentration of glucose-6-phosphate and fructose-6-phosphate (20 times the wild-type level), whereas fructose-1, 6-bisphosphate was hardly detectable.

3. A single mutant was found with a defect in triosephosphate isomerase activity (tpi). It grew extremely slow both on YEP-glycerol-ethanol and on YEP-glucose. Dihydroxyacetone phosphate was accumulated both on glycerol and on glucose. Glucose was not fermented.

4. Mutants lacking phosphoglycerate kinase (pgk) showed high levels of glyceraldehyde-3-phosphate, dihydroxyacetone phosphate, and of fructose-1, 6-bisphosphate. Glucose did not stimulate growth and was not fermented.

5. Phosphoglyceromutase mutants (pgm) had a similar phenotype as pgk mutants. Additionally, 3-phospho-glycerate accumulated on glucose.

6. Eight mutants lacked pyruvate kinase activity (pyk). They were allelic to a pyk - mutant isolated by Clifton et al., and showed abnormal accumulation of PEP, 2-Phosphoglycerate, and of 3-phosphoglycerate, whereas hexosephosphate levels were low on glucose.

Glycolysis mutants showed a rapid depletion of ATP when glucose was added to ethanol grown cells. Even in the pfk - (which grew nearly normal on glucose), ATP could hardly be detected. It has been argued by several authors that the inhibitory effect of glucose in pgi-mutants is caused by toxic accumulation of hexosephosphates. This seems to be unlikely because the pfk - mutant grew nearly normal, although it contained up to 100 nmoles glucose-6-phosphate + fructose-6-phosphate; pgi-mutant: 80-100 nmoles glucose-6-phosphate/mg d. wt.; wild type: 4-6 nmoles/mg d. wt.

Catabolite repression of mitochondrial and gluconeogenetic enzymes were tested in the pfk - mutant. It showed normal repression, indicating that the marked increase of fructose-1, 6-bisphosphate level in glucose-grown wild-type cells has no clear-cut effect on catabolite repression.

F. K. Zimmermann: Recombination events during yeast meiosis. In continuation of previous work (L. W. Olson and F. K. Zimmermann: Meiotic recombination and synaptonemal complexes in Saccharomyces cerevisiae. MOLEC. GEN. GENET., in press, 1978).

We have used strain:

$$\text{D10:}\; \frac{\text{ade5} \;\; + \;\; \text{aro2} \;\; + \;\; \text{cyh2} \;\; + \;\; \text{leu1}}{+ \;\; \text{lys5} \;\; + \;\; \text{met13} \;\; + \;\; \text{trp5} \;\; +} 0 \;\; \frac{\text{his4}}{+} 0 \frac{\text{a}}{\text{alpha}} \;\; 0 \frac{\text{ade2}}{\text{ade2}}$$

which is red and adenine requiring because of the condition ade2/ade2 but does not express any of the other recessive markers. This strain was incubated in a sporulation medium, and progress of meiotic recombination followed by plating samples on a rich growth medium supplemented with cycloheximide. Generation of recombinants is signaled by the appearance of cycloheximide resistant colonies. Interruption of meiosis before meiotic anaphase I can be established by the low incidence of colonies expressing centromere marker leu1. The diploid state is demonstrated by the lack of mating ability.

The number of resistant colonies started to increase at 4 hours in sporulation medium and was followed up to 11 hours when the majority of the resistant colonies expressed mating type indicating haploidization. An isolated crossing-over event between the centromere and cyh2 will generate a white colony expressing aro2 and ade5 distal to the selected marker cyh2. Any additional single crossing-over event distal to aro2 will lead to a red colony. Consequently, white cyh2, aro2, ade5 colonies signal one crossing-over, red cyh2, aro2 colonies two events. Gene conversion without additional crossing-over generates red colonies expressing only cyh2. Additional single crossing-over events beyond aro2 will lead at a 25% probability to a white colony expressing only cyh2. Since the marker arrangements allow to determine interruption of meiosis before meiotic anaphase I, we conclude that all recombinational events are meiotic but followed by a mitotic segregation of chromosomes. The results shown in the table suggest that recombination in meiosis starts with low coincidences with the typical coincidence values reached only late before meiotic chromosome segregation.

h in sporul. medium	4	5	6	7	8	9	10	11
total X-over cyh2 and aro2	7	37	116	141	129	127	78	20
red cyh2 and aro2 % of total	14.3	5.4	11.2	6.4	7.8	21.3	38.5	65.0
total conversion cyh2	3	17	48	88	75	70	69	21
white cyh2 % of total	none	none	10.4	5.7	6.7	12.9	14.5	23.8

M. K. Grossmann: New aspects of invertase genetics.

There are five known SUC-genes in Saccharomyces cerevisiae, any one of which is sufficient for fermentation of raffinose or sucrose. Heat inactivation of invertases from strains carrying only one of the five SUC-genes showed that SUC1-invertase was rapidly inactivated at 55°C. The others were much more heat resistant and indistinguishable among themselves. Electrophoresis of the invertases revealed a different behaviour of the internal isoenzymes. Those formed in strains with genes SUC2 to SUC5 were migrating close to the front but not equally fast. The internal invertase of SUC1 moved distinctly slower in the polyacrylamid gel. Diploids of SUC1 with each one of the other genes were constructed, and electrophoresis of these was carried out. All diploids, except the cross SUC1-SUC2, showed three bands in the region of the internal isoenzyme: 1) a slow band, corresponding to the SUC1-invertase, 2) a fast band, determined by the other parent, and 3) an intermediate band. The intermediate band could be a hybrid of one SUC1 and one SUC4 monomer, for example. Trimble and Maley have shown the purified internal enzyme consisted

of two identical subunits. These findings support our interpretation of the heterozygote behaviour. SUC1-SUC2 diploids only gave the slow band of the SUC1-invertase. This suggested that SUC1 could have a regulatory component epistatic over SUC2.

Tetrad analysis of a SUC1-SUC4 heterozygote revealed the typical segregation pattern of two unlinked genes for raffinose fermentation. Electrophoresis of the tetrads showed the expected distribution of the internal isoenzymes. One recombinant spore in the tetratype and two spores in the nonparental ditype tetrad had the three band pattern.

A nonfermenting mutant of SUC3, previously isolated by F. K. Zimmermann, was crossed with SUC1. The resulting diploid showed two bands of internal invertase: a slow band corresponding to the pure SUC1 enzyme and a second band with intermediate mobility. This hybrid presumably consisted of an active SUC1 and an inactive SUC3-monomer. The pure SUC3 band was missing. Again, this isoenzyme pattern was found in tetrad analysis in tetratypes and nonparental ditypes. The lack of fermentation in the SUC3-mutant strain was, therefore, caused by the formation of an inactive enzyme. SUC3, SUC4, and possibly SUC5 seem to be real structural genes, whereas SUC1 may comprise an additional regulatory component epistatic over SUC2.

XXI. Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh, Scotland. Communicated by Graeme M. Walker*.

Abstract of Ph.D. Thesis. September, 1978.

METAL IONS AND THE CONTROL OF THE CELL CYCLE IN FISSION AND BUDDING YEASTS

A study has been made of the role of calcium and magnesium ions in the regulation of the cell cycle in a fission yeast, *Schizosaccharomyces pombe*, and a budding yeast, *Kluyveromyces fragilis* (formerly, *Saccharomyces fragilis*).

The cell cycle of these yeasts may be controlled by limiting the availability of magnesium but not calcium. This was demonstrated using various chelating agents and metal ion-deficient growth media. Such studies revealed that cell division could be inhibited by lowering intracellular magnesium concentrations and that exponentially growing cultures could be synchronized by induction, either by using short pulses of chelators or by restoring magnesium to magnesium-exhausted cells. Analysis of total cell calcium and magnesium in cultures synchronized by various induction and selection procedures was accomplished routinely using flameless (electrothermal) atomic absorption spectrophotometry. This revealed that a close temporal relationship exists between magnesium ion uptake and cell

*Present Address: The Biological Institute of the Carlsberg Foundation, Tagensvej 16, 2200-Copenhagen N, Denmark.

division. This is discussed in the context of studies on metal ion compartmentalization and the distinction between free and bound ions. In S. pombe, as well as controlling cell division, magnesium is clearly shown to regulate aspects of cell morphology, including the induction of filamentation and inhibition of cell plate formation. Similar phenomena have been observed in temperature sensitive cell cycle mutants of the same yeast by other workers, and it is conceivable that such strains possess an altered capacity to accumulate magnesium ions.

It is concluded from results in the first part of this Thesis that in both fission and budding yeasts changes in the level of free intracellular magnesium ions play an important role in the regulation of cell division. This concept is discussed in relation to current models on the control of cell cycle, and a hypothesis has been formulated implicating magnesium with the in vivo regulation of spindle microtubule assembly and breakdown. This hypothesis receives indirect experimental support from studies on magnesium limitation and from studies with the specific antimicrotubular drug, Nocodazole.

The second part of this Thesis was devoted to a study of the control of glutamine metabolism during the fission and budding yeast cell cycle, with special reference to the role of calcium and magnesium ions. The activity of glutamine synthetase was monitored in synchronized cultures and was shown to fluctuate in a way which suggested a complex control system, perhaps involving an unstable enzyme modulated by magnesium ions.

XXII. Biology Department, University of California, Los Angeles, California 90024. Communicated by T. W. James.

The following is an abstract of:

Selection and Evolution of Yeast Cells in a Chemostat. T. W. James. In Cell Reproduction: Honoring Daniel Mazia (E. Dirksen, D. Prescott, and C. F. Fox, Eds.), Vol. 12, 127-138, Academic Press, New York, 1978.

ABSTRACT

Selection based on the energetic cost of gene maintenance has important implications in cell evolution (1). Yeast is one eucaryotic system in which selection can be measured readily with a chemostat. Two methods were used in which respiratory deficient cytoplasmic mutants, petites, were examined relative to parental wild type cells in anaerobic chemostats in which glucose was used as the energy-limiting substrate. The first method involves the comparison of growth rate vs. substrate concentration plots. Under these conditions, linear Lineweaver-Burk plots are obtained and Michaelis-Menton kinetics apply. Consequently, a specific strain or mutant can be characterized by a growth rate maximum (V_{max}) and a glucose affinity constant (K_m). These characteristics were obtained by setting the chemostat to a series of growth rates (dilution rates) and measuring the glucose concentration at each with a glucose electrode. When these characteristics are compared for wild type and petite cells by

superimposing their Michaelis-Menton plots, it can be seen that at lower substrate concentrations the petite mutant has the selection advantage. A plot of the numerical difference between any two Michaelis-Menton curves against the substrate concentration allows a pairwise comparison of strains and gives regions of substrate concentration where growth rate differences go through a maximum. This difference function indicates that for a specific pair selection is in favor of the mutant in regions of low concentration or growth rate, while at higher concentrations the wild type ought to have the selective advantage. This suggests that there is a substrate concentration at which selection is neutral for the two strains. Since the function obtained by the method can be determined by another method, a direct test was undertaken. It consists of measuring the change in ratio of wild type to mutant cells which occur with time in a mixed culture chemostat (2, 3, 4). The initial slope of the logarithm of this ratio against time yields a value which is the difference in growth rates of the two strains and is theoretically identical to the differences obtained by the previous method. When this procedure is applied to mixed wild type and petite cells maintained at growth rates of 0.037/hr (18.5 hr generation time), 0.082/hr (8.5 hrs), and 0.165/hr (4.2 hrs), selection is in favor of the petite mutants. These occur at steady state concentrations of glucose of 0.17, 0.40, and 0.93 mM, respectively, and are at the onset of selection. The differences in the growth rate of the mutants and wild type cells determined by this method are in agreement with those found by the growth rate/substrate concentration plots on single cell types, indicating that the two methods are compatible and the interactions between cell types in mixed systems do not appear to affect the selection relationships. Selection for the wild type over the petite mutant predicted by the kinetic data to occur at glucose concentrations above 2.25 mM has not been tested and has not been ruled out. This work is in good agreement with what has been found in procaryote cells (3, 4). It also indicates that eucaryotic mitochondria are subject to energetic selection pressures similar to those acting on nuclear systems.

1) James, T. W. (1969). In _The Cell Cycle_ (G. Padilla and I. Cameron, Eds.), Vol. 1, Acad. Press, N.Y., p. 3.

2. Moser, H. (1958). Carnegie Institution of Washington, Publication #614.

3) Zamenhof, S. and H. Eichhorn (1967). _Nature_, 216:456.

4) Dykhuizen, D. E. (1971). Ph.D. Dissertation, University of Chicago.

XXIII. _Institute for Biology II, Microbiology I, University of Tübingen, D-74 Tübingen, FRG. Communicated by K. Poralla._

Some years ago we have found that the antibiotic borrelidin is a very specific and potent inhibitor of threonyl-tRNA-synthetase in pro- and eucaryotes (K. Poralla, 1975, In J. W. Corcoran and E. F. Hahn [ed.], ANTIBIOTICS, Vol. III, Springer Verlag, Heidelberg).

1. G. Nass and K. Poralla (1976). Genetics of Borrelidin Resistant Mutants of Saccharomyces cerevisiae and Properties of their Threonyl-tRNA-Synthetase. MOL. GEN. GENETICS 147, 39-43 (1976).

SUMMARY

Twenty-two borrelidin resistant mutants of Saccharomyces cerevisiae were isolated, studied genetically and their threonyl-tRNA-synthetase was investigated. The borrelidin resistant mutants are classified into four groups. In the first group, borrelidin resistance is coupled to the gene HOM3 coding for aspartokinase, in the second group, to the gene LEU1. The borrelidin resistance in group three and four is not coupled to anyone of the genetic markers tested. Borrelidin resistance exhibited dominant behavior in all mutants, except in the mutant of group four. The properties of the ThrRS of the mutants of group one, two, and four were found to be like the ones of the wild types. However, the mutants of group three exhibit a structurally altered ThrRS, which is no longer inhibited by borrelidin.

2. K. Poralla, M. Seibold, and K. Nill. Borrelidin resistant Mutants of Saccharomyces cerevisiae with a Threonine Insensitive Aspartokinase and elevated Pools of Threonine and Homoserine. In preparation.

SUMMARY

The group of resistant mutants coupled to the gene HOM3 (aspartokinase) was biochemically characterized. The aspartokinase of these mutants is not inhibited by threonine. As a consequence, the pool of threonine is 22 times and of homoserine 14 times elevated. By feeding aspartate to these mutants, the pools of aspartate and threonine are unaltered, whereas the pool of homoserine is drastically elevated over that of threonine.

XXIV. Queen Elizabeth College, (University of London), Campden Hill Road, W8 7AH, London, England. Communicated by R. K. Poole.

The following is an abstract to be published in the SOCIETY FOR GENERAL MICROBIOLOGY QUARTERLY (including Proceedings). It should appear in the November issue; the paper will be presented orally at Cardiff in January at the S.G.M. meeting.

THE COMPLEXITY OF CYTOCHROMES IN A BUDDING YEAST, STERIGMATOMYCES HALOPHILUS

By I. Salmon and R. K. Poole (Department of Microbiology).

Intact S. halophilus cells from exponentially growing cultures contain a-, b-, and c-type cytochromes spectrally similar to those of other yeasts. The use of appropriate inhibitors of electron transport identified the absorption maxima in reduced-minus-oxidized difference spectra (at 77°K) to be at 440-447 nm and 600 nm for cytochrome $a + a_3$, at 431, 520, and 560 nm for cytochrome(s) b, and

at 422, 508, and 548 nm for c-type cytochromes. Spectra of isolated mitochondrial fractions were similar, except that extensive loss of cytochrome c_{548} from such particles revealed a further shoulder of absorption at 552 nm.

Finite difference analysis was used to explore the possiblity that the broad absorption maxima observed were due to multiple overlapping peaks, such as have been described in Escherichia coli (1) and in Prototheca zopfii (2). Absorption was recorded at 0.2 nm intervals in reduced-minus-oxidized difference spectra of isolated mitochondrial fractions, and the fourth-order finite difference spectra computed using the algorithm of Butler & Hopkins (2). In the γ region (390-460 nm) at least six peaks (404, 411, 420, 430, 441, and 450 nm) were resolved. In the β region (505-540 nm) a further six peaks (510, 513, 517, 521, 529, and 536 nm) were revealed. Analysis of the broad α peak observed between 540 and 570 nm in conventional difference spectra revealed several peaks (542, 545, 548, 552, 558, and 561 nm) attributable to b and c type cytochromes. Similar computations failed to resolve the oxidase peak at about 600 nm into multiple components. Many of these resolved peaks are clearly those observed at similar wavelengths in conventional difference spectra. We have not yet determined whether the additional peaks represent the presence of (a) many functionally or structurally discrete cytochromes, or (b) fewer cytochromes with split absorption bands.

(Ian Salmon is the holder of an S.R.C. Research Studentship.)

(1) Shipp, W. S. (1972) ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 150, 459-472.

(2) Butler, W. L., Hopkins, B. W. (1970) PHOTOCHEMISTRY AND PHOTOBIOLOGY 12, 439-450.

The following short communication was just published.

"Which cells are selected from exponential cultures by continuous-flow centrifugation? The selection of small cells from cultures of E. coli and Schizosaccharomyces pombe that exhibit minimal density fluctuations during their cell cycles."

R. K. Poole and A. M. Pickett. J. GEN. MICROBIOL. 107, 399-402 (1978).

ABSTRACT

"Synchronous cultures of many cell types can be produced by continuous-flow centrifugation. An exponential culture is allowed to flow through the rotor so that about 90% of the cells are retained in the rotor; the remaining minority in the effluent medium constitute a synchronous culture, being a homogeneous age class. It was previously suggested that size is not the only, perhaps not the primary, factor in determining the age of cells selected since the cells selected from exponentially grown E. coli or Schizosaccharomyces pombe were

those of the lowest density rather than of smallest size, (Poole, R. K., FEMS MICROBIOL LETT. 1, 305, 1977). In the present paper this hypothesis was tested by applying the continuous flow method to cultures that were found to exhibit only insignificant density changes during the cell cycle. These were: (a) S. pombe 972 h$^-$ grown at suboptimal temperature (17°C) and (b) E. coli grown in a chemostat, in which the entering concentration of growth-limiting nutrient fluctuated between 0.02% and 0.04% at 2.5 h periods. Densities of cells from such cultures were determined by centrifugation in dextran (S. pombe) or Ludox/polyvinylpyrrolidone (S. pombe). Continuous flow centrifugation selected the smallest cells from each type of culture, suggesting that the age of cells selected in previous experiments was influenced by density fluctuations occurring during the cell cycle."

XXV. Laboratory of Microbiology, Gulbenkian Institute of Science, Oeiras, Portugal. Communicated by N. van Uden.

1. The following papers were published recently:

I. Spencer-Martins and N. van Uden. Yields of yeast growth on starch. EUROP. J. APPL. MICROBIOL. BIOTECHNOL. 4, 29-35, 1977.

B. Simões-Mendes, A. Madeira-Lopes, and N. van Uden. Kinetics of petite formation and thermal death in Saccharomyces cerevisiae growing at superoptimal temperatures. Z. ALLG. MIKROBIOL. 18, 275-279, 1978.

M. Vidal-Leiria. Distribution of myo-inositol oxidizing enzymes among yeasts. Z. ALLG. MIKROBIOL. 18, 719-721, 1978.

2. The following are abstracts of papers accepted for publication:

I. Spencer-Martins and N. van Uden. Extracellular amylolytic system of the yeast Lipomyces kononenkoae. EUROP. J. APPL. MICROBIOL. BIOTECHNOL.

A strain of the yeast Lipomyces kononenkoae, which converted starch into SCP with a high yield, produced three extracellular amylases which were purified from the culture fluid by Ficoll concentration, dialysis, isopropanol precipitation, and DE-cellulose chromatography: an α-amylase, a glucoamylase, and a debranching transferase. The latter transferred α-1, 6-glucosyl units from panose to glucose forming maltose and appeared to have some debranching activity on amylopectin. The α-amylase had the following properties: MW 38000 daltons; no effect of added calcium ions on activity; optimum temperature and pH for activity around 40°C and pH 5.5; $\Delta H\neq$ and $\Delta S\neq$ of heat inactivation 24360 cal mole^{-1} and 29.2 cal deg^{-1} mole^{-1}; range of pH stability pH 4-6.5; final low molecular

weight products of starch hydrolysis, maltose, and glucose; K_m (40°C, pH 5.5) for starch 2.7 gl^{-1}, for maltotriose 109 gl^{-1}; uncompetitive inhibition by maltose with K_i (40°C, pH 5.5) 29.5 gl^{-1}. The glucoamylase had the following properties: MW 81500 daltons; optimum temperature and pH for activity around 50°C and pH 4.5; $\Delta H \neq$ and $\Delta S \neq$ of heat inactivation 20400 cal $mole^{-1}$ and 17.7 cal deg^{-1} $mole^{-1}$; range of pH stability pH 4-6.5; K_m (30°C, pH 4.5) for soluble starch 16.2 gl^{-1}, for maltose 0.36 gl^{-1}, for p-nitrophenyl-α-D-glucoside 0.35 gl^{-1}; competitive inhibition by glucose with K_i (30°C, pH 4.5) 4.7 gl^{-1}.

A. Madeira-Lopes and N. van Uden. Thermal association and dissociation in thermosensitive mutants of *Saccharomyces cerevisiae*. Z. ALLG. MIKROBIOL.

A thermosensitive mutant of *S. cerevisiae* defective in energy metabolism displayed thermal dissociation similar with the thermal profiles of psychrophilic yeasts, while three thermosensitive mutants defective in protein synthesis had associative thermal profiles, such as are characteristic of mesophilic yeasts. The results are considered to support the theory that the thermal death sites are identical or have a thermosensitive component in common with the T_{max} sites in the case of mesophilic yeasts while they are wholly separate entities in psychrophilic yeasts.

***

M. Vidal-Leiria, H. Buckley, and N. van Uden. Distribution of the maximum temperature for growth among yeasts. MYCOLOGIA.

The maximum temperature for growth (T_{max}) was determined in 594 yeast strains belonging to 112 species of the genera *Candida*, *Torulopsis*, *Hansenula*, *Pichia*, *Metschnikowia*, and *Leucosporidium*. Less than 2% of the strain population was psychrophilic having T_{max} values below 24 C. More than 98% consisted of mesophilic strains with T_{max} values ranging from 26-48 C, the highest frequency being in the 34-38 C range. No thermophilic yeasts were encountered.

The intraspecific variation of T_{max} in 41 species of which more than 5 strains each were studied did not exceed a range of 5 degrees C in 76% of the species, the highest frequency pertaining to the range of 3 degrees C. Analysis of the T_{max} variation in the 7 species of which more than 15 strains were available brought further evidence that in most yeasts T_{max} is fixed on the species level within a narrow range of temperatures.

3. Current research is concerned with an extracellular yeast pullulanase (Isabel Spencer-Martins), the symbiotic bioconversion of starch by yeasts (Benilde Simões-Mendes), extracellular β-glucosidases from yeasts (Manuela Vidal-Leiria), cellulase of *Fusarium graminearum* (Mané Dias), the bioconversion of eucalyptus wood (Manuel Beja da Costa), bacterial xylanases (Ana Vieira and Margarida Guerreiro), and the genetic improvement of an amylolytic yeast (Amândio Madeira Lopes and Cecília Silva).

XXVI. Department of Biology, Georgia State University, Atlanta, Georgia 30303. Communicated by C. W. Price.

Below follows the abstract of a recently published paper.

Purification and Properties of the Arginine-specific Carbamoyl-phosphate Synthase from *Saccharomyces cerevisiae*

By C. W. Price, Judy H. Holwell, and Ahmed T. H. Abdelal

JOURNAL OF GENERAL MICROBIOLOGY (1978), 106, 145-151

The arginine-specific carbamoyl-phosphate synthase of yeast was stabilized sufficiently to allow partial purification of the enzyme (30- to 40-fold). The synthase (mol. wt 115000) comprised two unequal subunits: a heavy subunit (mol. wt 80000) capable of catalysing synthesis of carbamoyl phosphate with ammonia as a nitrogen donor and a light subunit conferring upon the holoenzyme the ability to utilize glutamine. The enzyme had unusually high affinity for ATP (K_m = 0·2 mM) and atypical negative cooperativity for glutamine binding ($[S]_{0\cdot5}$ = 0·25 mM). Glutamine activity was not modulated by possible effectors such as arginine, ornithine, or N-acetylglutamate. Thus, although the yeast arginine enzyme physically and functionally resembles the single enteric synthase, the systems differ substantially both in kinetic properties and in regulation of activity.

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

EFFECTS OF PHOSPHATE LIMITATION OF GROWTH ON THE CELL-WALL AND LIPID COMPOSITION OF *SACCHAROMYCES CEREVISIAE*

A. M. Ramsay and L. Julia Douglas

J. GEN. MICROBIOL. (In Press)

SUMMARY

The phosphorus content of phosphate-limited *Saccharomyces cerevisiae* was only 71% of that of non-limited yeast. Walls prepared from phosphate-limited cells contained slightly less phosphorus than control walls. No evidence was obtained for the presence in these walls of uronic acid or succinyl residues. The carbohydrate content of walls of limited yeast was less than that of non-limited walls, and this was reflected in a decreased glucan content. There was only a slight decrease in glucosamine content while that of protein increased. The major change in the lipid composition of phosphate-limited yeast was a decrease in both sterol esters and triacylglycerols. These was a decrease in total lipid content but increased production of phosphatidylethanolamine and phosphatidylcholine. The phosphatidylserine content was decreased. These results suggest a diminished population of intracellular low-density vesicles in phosphate-limited yeast.

(This work was presented, in part, at the VIth International Specialized Symposium on Yeasts held in Montpellier, France, July 1978.)

XXVIII. *Laboratory of Microbial Biochemistry, Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan. Communicated by Kenji Soda.*

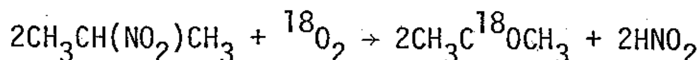
Research has continued in our laboratory on the enzymological aspects, particularly the reaction mechanism, of 2-nitropropane dioxygenase of *Hansenula mrakii*. The following are the summaries of our papers published recently.

1. 2-Nitropropane Dioxygenase from *Hansenula mrakii*: Generation and Participation of Superoxide Anion in the Reaction.

Kenji Soda and Toshiko Kido (The laboratory of Microbial Biochemistry, Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan) and Kozi Asada (The Research Institute for Food Science, Kyoto University, Uji, Kyoto-Fu 611, Japan).

In: Biochemical and Medical Aspects of Active Oxygen. Ed. by O. Hayaishi and K. Asade, University of Tokyo, p. 119-133 (1977).

2-Nitropropane dioxygenase from a yeast, *Hansenula mrakii*, is a nonheme iron flavoprotein which catalyzes the following reaction:



$$2CH_3CH(NO_2)CH_3 + {}^{18}O_2 \rightarrow 2CH_3C^{18}OCH_3 + 2HNO_2$$

The enzyme, which has been purified to homogeneity, is unique in that it incorporates 2 atoms of oxygen molecule into two molecules of the same acceptor. The enzyme has a molecular weight of approximately 62,000 and consists of two subunits nonidentical in molecular weight (39,000 and 25,000). The enzyme exhibits absorption maxima at 274, 370, 415, and 440 nm and a shoulder at 470 nm and contains 1g-atom of nonheme iron and 1 mol of FAD/mol of enzyme protein as prosthetic groups. The enzyme-bound FAD is reduced by 2-nitropropane under anaerobic conditions, but the enzyme-bound Fe^{3+} is not affected.

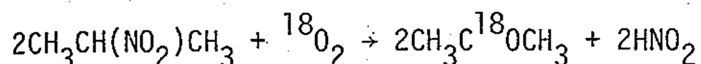
The enzyme activity is significantly inhibited by superoxide dismutase and various scavengers of O_2^-, such as cytochrome *c*, epinephrine, NADH, tiron, and thiols. The reduction of cytochrome *c* and the oxidation of epinephrine and NADH do not occur in the presence of superoxide dismutase or in the absence of 2-nitropropane and oxygen. The enzyme catalyzes the formation of nitrite from 2-nitropropane by KO_2 even under anaerobic conditions. These findings indicate the generation of O_2^- and its participation in the oxygenation of 2-nitropropane. One mole of NADH is bound to 1 mol of the enzyme and the *pro-R* hydrogen of bound NADH is mainly transferred to O_2^- formed enzymatically or given exogenously. Thus, the diastereotopic hydrogen of NADH is discriminated by the enzyme, though not completely.

2. Generation and Participation of O_2^- in 2-Nitropropane Dioxygenase Reaction.

Toshiko Kido, Katsumi Hashizume, and Kenji Soda (The laboratory of Microbial Biochemistry, address as above) and Kozi Asade (The Research Institute for Food Science, address as above).

PHYTOCHEMISTRY AND PHOTOBIOLOGY, 29, 729-732 (1978).

2-Nitropropane dioxygenase (EC class 1. 13. 11) of a yeast Hansenula mrakii catalyzes the oxygenative denitrification of 2-nitropropane as follows:



$$2CH_3CH(NO_2)CH_3 + {}^{18}O_2 \rightarrow 2CH_3C^{18}OCH_3 + 2HNO_2$$

The enzyme is significantly inhibited by superoxide dismutase and various scavengers for superoxide, such as cytochrome c, epinephrine, thiols, and polyhydric phenols. The scavengers added to the reaction mixture were oxidized or reduced. The addition of superoxide dismutase and the omission of 2-nitropropane or oxygen prevented the oxidation and the reduction of the scavengers. The enzyme catalyzes the formation of nitrite from 2-nitropropane by KO_2 added anaerobically.

One mol of NADH is bound per mol of the enzyme, and predominantly the pro-R hydrogen of bound NADH is transferred to superoxide formed enzymatically or provided externally. The enzyme shows incomplete stereospecificity for hydrogen transfer from NADH.

XXIX. The Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan. Communicated by A. Kimura.

Below follow abstracts of two of our recent papers, also recently presented on the same subject:

(1) M. Asada, K. Nakanishi, R. Matsuno, Y. Kariya, A. Kimura, and T. Kamikubo, "Continuous ATP Regeneration Utilizing Glycolysis and Kinase Systems of Yeast". AGR. BIOL. CHEM., 42, 1533-1538 (1978).

SUMMARY

During the course of phosphorylation of adenosine and AMP by acetone dried cells of yeast, enzymes related to glycolysis and adenosine kinase as well as adenylate kinase were found to be released into the supernatant of the reaction mixture. The supernatant effectively phosphorylated adenosine and AMP at high phosphate concentrations utilizing glucose as an energy source, and the ATP accumulated was maintained at a high level still after 24 hours of incubation. A continuous reaction apparatus for regeneration of ATP equipped with a semipermeable membrane was designed and constructed taking into account the specific characteristic of the present enzyme reaction systems. The released enzymes were applied to the apparatus equipped with semipermeable membranes, and continuous phosphorylation of adenosine to ATP was examined under different operational conditions. When the residence time was 18 hours, 80% of the initial adenosine was continuously phosphorylated to ATP by the apparatus, in which the collodion membrane was used as a semipermeable membrane.

(2) A. Kimura, Y. Tatsutomi, N. Mizushima, A. Tanaka, R. Matsuno, and H. Fukuda, "Immobilization of Glycolysis System of Yeasts and Production of Cytidine Diphosphate Choline". EUR. J. APPL. MICROBIOL. BIOTECHNOL., 5, 13-16 (1978).

SUMMARY

Glycolysis system of yeast was successfully immobilized into a derivative of polyethylene glycol hydroxyethylacrylate. The immobilized system could produce ATP and then phosphorylate nucleotides (CMP). The CTP thus formed was effectively converted to CDP-choline in the same system.

This system is a kind of bioreactor, consisting of energy (ATP) generating and transformation systems of various substances.

However, this paper should be read with the following erratum.

On page 13 the structural formula should have been labeled Figure 1.

The legend should have read: According to the number of n, various polymerized compounds with different average molecular weight (M.W.) were prepared; for example, average M.W. 1000, 2000, 4000, and 6000, but only M.W. 1000 was good for entrapping the whole enzyme system.

Figure 1 on page 14 should have been Figure 2.

The second sentence in the last paragraph on page 14 should have read: Only the smallest one (average M.W. = 1000) effectively entrapped whole enzymes as shown in Figure 3.

The figure at the top of page 15 should have been Figure 3.

The legend should have read: The reaction was carried out by the cells immobilized in ENT of M.W. 1000. After 3, 6, and 9 h in the first reaction, the immobilized cells were separated by centrifugation, washed 3 times with deionized water and reused in the fresh reaction mixture containing 2 mM ATP, and 2 mM NAD in addition to the usual components (although ATP was not always necessary).

The figure at the bottom of page 15 should have been Figure 4.

The legend should have read: The immobilized cells were kept at 4°C for the days indicated (2, 7, and 14 days), then the cells were used as described in the legend for Figure 3. The cells were reused after 3 h reaction.

(3) A. Kimura, M. Okuda, T. Tatsutomi, Y. Miyao, and H. Fukuda, "Control Mechanism of Fermentative Production of Nucleotides by Yeasts".

This paper was presented at the Third International Symposium on Genetics of Industrial Microorganisms, held on June 4-9 at the University of Wisconsin, Madision, Wisconsin.

SUMMARY

When mitochondria of yeast cells of Hansenula jadinii developed, glucose was not metabolized because one of three hexokinase isoenzymes, which is resistant to high concentration of phosphate, was not biosynthesized. Suppression of mitochondrial development by acriflavine recovered the biosynthesis of the phosphate-resistant isozyme of hexokinase. The various properties of each hexokinase isozyme were also shown.

These results will be published in the near future.

XXX. Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02154. Communicated by Harlyn O. Halvorson.

Perlman, D., and Hopper, J. E., "Constitutive Synthesis of the GAL4 Protein, a Galactose Pathway Regulator in Saccharomyces cerevisiae".

The following is a summary from a paper appearing in the January 1979 issue of CELL.

We have demonstrated that induction of synthesis of the mRNAs encoding the galactose catabolizing enzymes galactokinase (EC 2.7.1.6) and transferase (EC 2.7.7.12) occurs in Saccharomyces cerevisiae following the addition of α-D-galactose during continuous exposure to 100 μg cycloheximide per ml. The prompt induction and sustained accumulation of galactose message activity (detected in the wheat germ in vitro translation system) following the cessation of all detectable protein synthesis in vivo indicates that the GAL4 regulatory protein, and any yet undefined positive effectors of transcription of the galactose genes, exist prior to induction and are, therefore, synthesized constitutively. We have carried out synchronous matings in which inducible gal1 cells lacking galactokinase function were mated with uninducible gal4 cells containing either the normal GAL80 encoded galactose sensitive repressor or a dominant $GAL80^S$ encoded galactose-insensitive repressor. All cells were cultured in the continuous presence of galactose to allow for the possibility of inducible synthesis of the GAL4 specified protein. Galactokinase was expressed in the GAL80/GAL80 zygote (the control experiment) and not in the $GAL80^S$/GAL80 zygote indicating that a galactose-insensitive repressor can neutralize already existing GAL4 protein activity.

These results, particularly the cycloheximide finding, necessitate a revision of a central element of the Douglas and Hawthorne model for galactose regulation (1966), in which it was postulated that the GAL80 gene product acts at a GAL4 operator to repress GAL4 transcription. Our observations indicate that the repressor must control GAL4 regulatory activity at a post-translational level.

XXXI. Chelsea College, Manresa Road, London, SW3 6LX, England. Communicated by A. E. Beezer.

The following papers relating to yeasts have been accepted for publication:

R. F. Cosgrove, A. E. Beezer, and R. J. Miles, In vitro Studies of Amphotericin B in Combination with the Imidazole Antifungal Compounds Clotrimazole and Miconazole. J. INFECTIOUS DISEASES, 1978.

A. E. Beezer, R. D. Newell, and H. J. V. Tyrrell, Characterization and Metabolic Studies of Saccharomyces cerevisiae and Kluyveromyces fragilis by Flow Microcalorimetry. ANTONIE VAN LEEWENHOEK, 1978.

R. F. Cosgrove, A. E. Beezer, and R. J. Miles, The Application of Cryobiology to the Microbiological Assay of Nystatin. J. PHARM. PHARMACOL., 1978.

A. E. Beezer and P. B. Sharma, Bioassay of Nystatin; Measurement of Mg^{2+} by Atomic Absorption Spectroscopy. TALANTA, 1978.

R. F. Cosgrove, A. E. Beezer, and R. J. Miles, A Comparative Study of the Microbiological Assays Currently Available for Nystatin Raw Material. J. PHARM. PHARMACOL., 1978.

R. F. Cosgrove and A. E. Beezer, A Rubidium Ion Selective Electrode Suitable for the Assay of Polyene Antibiotics. ANALYT. CHIM. ACTA, 1978.

A. E. Beezer, R. D. Newell, and H. J. V. Tyrrell, Flow Microcalorimetric Investigation of Yeast Growth on a Complex Medium. MICROBIOS, 1978.

XXXII. Eidgenössische Technische Hochschule Zürich, ETH-Zentrum, Universitätstr. 2, CH-8092 Zürich, Switzerland, Mikrobiologisches Institut. Communicated by R. Hütter.

Below follow the titles of two recent publications from our yeast group:

1. Miozzari, G., P. Niederberger, and R. Hütter (1977). Action of tryptophan analogues in Saccharomyces cerevisiae. ARCH. MICROBIOL. 115: 307-316.

2. Miozzari, G., P. Niederberger, and R. Hütter (1978). Tryptophan biosynthesis in Saccharomyces cerevisiae: Control of the flux through the pathway. J. BACTERIOL. 132: 48-59.

A third publication has been accepted by ANALYT. BIOCHEMISTRY. Title and abstract are presented below:

3. Miozzari, G., P. Niederberger, and R. Hütter (1978). Permeabilization of microorganisms by Triton X-100.

Abstract: A simple permeabilization procedure has been developed which allows the reliable determination of enzyme activities in situ in a variety of different microorganisms. Permeabilization is obtained by freezing cell suspensions in the presence of a low concentration of the anionic detergent Triton-X-100. After

thawing, the cells can be used directly in the enzyme assays. The procedure has been optimized using the yeast *Saccharomyces cerevisiae*. Yeast cells are completely permeabilized by Triton-X-100 concentrations of 0.05% (v/v), and permeabilization is independent of cell age and cell concentration. The treatment makes the cells freely diffusible for macromolecules up to molecular weights around 70,000. Cytoplasmic and mitochondrial amino acid biosynthetic enzymes, as well as aminoacyl-tRNA synthetases, could be readily measured in treated cells. The method as been successfully applied to the determination of enzyme activities in other fungi, as well as in gram-positive and gram-negative bacteria.

XXXIII. ALKO, Box 350, SF-00101, Helsinki 10, Finland. Communicated by H. Suomalainen.

Below follow abstracts of our work published since June 1978.

1. ESTERASES OF BAKER'S YEAST.

Elke Parkkineñ, Erkki Oura, and Heikki Suomalainen.

Abstract of paper presented at the Sixth International Specialized Symposium on Yeast, July 2-8, 1978, Montpellier, France.

We recently reported that esterases of baker's yeast are located on both sides of the plasma membrane and are easily transferred into the supernatant from disintegrated yeast cells by centrifugation (Parkkineñ et al., 1978, J. INST. BREW. 84, 4-7).

Further investigation has now revealed the following. The ammonium sulphate precipitates of the yeast sphaeroplast lysates as well as those of the cell wall digests fractionated by Sephadex G-100 showed four esterase-active peaks, designated E_1 - E_4. The ratio of E_1:E_2:E_3:E_4 did not change much from extraction to extraction, and 55% ammonium sulphate precipitates was about 10 : 68 : 10 : 12 and in 55% -75% ammonium sulphate precipitates about 16 : 58 : 0 : 26. The molecular weights, the pH optima, temperature optima, and heat stabilities of the esterases were determined.

On the basis of substrate specificities using p-nitrophenyl esters with increasing length of carbon number of the acids (C_2-C_{12}) and on the basis of sensitivity to different esterase inhibitors, the esterases are believed to be catalytically different enzymes.

2. THE EFFECT OF CULTURE CONDITIONS ON THE REQUIREMENTS OF BIOTIN BY BAKER'S YEAST.

Erkki Oura.

Abstract of paper presented at the Sixth International Specialized Symposium on Yeast, July 2-8, 1978, Montpellier, France.

During growth in normal media biotin auxotrophic baker's yeast needs the biotin-containing pyruvate carboxylase and acetyl-CoA carboxylase to function. When the common nitrogen source ammonium

salt is replaced by urea, an active urea amidolyase enzyme, which contains biotin as the prosthetic group, is induced. Since the need for the function of biotin-containing enzymes in yeast varies with the growth conditions, it has been assumed that the biotin requirements of baker's yeast can vary similarly. Where all three biotin enzymes are necessary, such as during anaerobic growth on glucose with urea as the nitrogen source, the requirements for biotin are maximal. During anaerobic growth based on glucose and ammonium salt, the function of only two biotin enzymes, pyruvate carboxylase and acetyl-CoA carboxylase, are essential; and the biotin requirements of yeast should be lower. When oxaloacetate is formed besides by the pyruvate carboxylase reaction also by the function of the glyoxylate cycle as is the case during aerobic growth on glucose, the requirements should be again lower; and lower still when only acetyl-CoA carboxylase is needed, as is in the case of yeast growing on ethanol.

This hypothesis was tested by continuous cultivation of baker's yeast under the various conditions using a synthetic medium with varying amounts of biotin (0.5 - 100 ug/1000 ml). According to these results, the requirements of baker's yeast for biotin is affected by the growth conditions and in the manner proposed. When biotin is used at the higher concentrations, the effect, although clear, is not as pronounced as with the lower amount concentration. By comparing the results obtained with the lower biotin concentration, the following remarks can be made. The need for biotin in urea amidolyase is very great, as can be seen from the fact that when urea is used as the nitrogen source the yield was less than half of that in yeasts cultured with ammonium salt. With this low biotin concentration, a completely aerobic metabolism of glucose could not be achieved, nor in the cases where a low dilution rate ($0.02\ h^{-1}$) was used. The reason for this could be the biotin-limited growth in which glucose tends to accumulate in the medium and to be fermented.

At any rate increased aerobicity decreased the need of yeast for biotin; it being about 20% lower in aerobic than under anaerobic cultivations. The highest value being obtained for ethanol-grown yeast confirms the view that the function of pyruvate carboxylase is then very small or negligible.

3. THE USE OF CONTINUOUS CULTIVATION IN ESTIMATING THE FLUX OF THE SUBSTRATE AT DIFFERENT STEPS OF METABOLISM DURING MICROBIAL GROWTH.

Erkki Oura.

Abstract of paper presented at the Seventh International Symposium on Continuous Cultivation of Microorganisms, July 10-14, 1978, Prague.

During anaerobic continuous cultivation of yeast on glucose, almost all the acetaldehyde formed from pyruvate is metabolized to ethanol. Thus, the specific ethanol formation rate reflects the flow of acetaldehyde through the reaction catalyzed by alcohol dehydrogenase. On the other hand, all the sugar consumed will be transformed into glucose phosphate, and the specific rate of substrate

consumption gives a value for the amount of glucose metabolized in the hexokinase reaction. A part of the product of this reaction, glucose phosphate, is used in the formation of cellular carbohydrates, nucleotides, and some amino acids. Knowing the detailed composition of the yeast, this proportion can be calculated. The remainder is further metabolized in the phosphoglucose isomerase and phosphofructokinase reactions. When the use of all components of the intermediary metabolism for biosyntheses had been estimated, the flux of substrates at different steps could be assessed for yeasts grown anaerobically on glucose and aerobically on glucose, ethanol, or a mixed glucose-ethanol substrate.

The measured *in vitro* activities of several enzymes were compared with the values for the flux of substrate through the corresponding enzymatic reactions. Most of the *in vitro* activities greatly exceeded the values for the substrate turnover during growth, indicating a large excess of the enzymes in the cells; but for some of the reactions the two values were in close agreement. Using this kind of examination, some candidates for pacemaker enzymes and for enzymes having an important regulatory role in cellular metabolism have been found.

4. ETHANOL AS SUBSTRATE FOR BAKER'S YEAST.

Heikki Suomalainen and Erkki Oura.

Abstract of paper presented at the 12th International Congress on Microbiology, September 3-8, 1978, Munich.

In addition to sugars, ethanol is a normal substrate for baker's yeast, as is apparent from its acting as substrate during the second logarithmic growth phase of aerated batch cultivations. The enzymes required for oxidative metabolism are not the only ones induced in the cells during the lag phase between the first and second logarithmic phases; also induced are the specific enzymes that yeast needs during growth on ethanol.

Yeast is able to grow with ethanol as the sole carbon source. The specific gluconeogenic enzymes (isocitrate lyase, phosphoenolpyruvate carboxykinase, and fructose-1, 6-bisphosphatase) have especially high activities in yeast grown under these conditions. The yield of cell material from yeast grown on ethanol as the sole substrate has been reported to be 50-70 g yeast (d.m.) per 100 g ethanol consumed. During the last, strongly aerobic growth stage in the industrial production of baker's yeast, it is possible to feed the yeast with ethanol as well as molasses. This has been done at Alko ever since 1946, and it has been found that 100 g ethanol corresponds to about 140 g sugar. In a laboratory cultivation in an ethanol/glucose (15/85) medium, the yield obtained from ethanol has been estimated to be 81 g yeast (d.m.) per 100 g ethanol. Here ethanol is used only for the synthetic reactions associated with the TCA cycle and as energy source.

5. The following publication has appeared since the last communication. The abstract of the report has been given in the Yeast Newsletter 26 (1977):2, 30.

Sampsa Haarasilta, Enzymatic determination of biotin. ANALYTICAL BIOCHEMISTRY 87 (1978), 306-315.

XXXIV. Department of Chemistry and Biochemistry, James Cook University of North Queensland, Townsville 4811, Australia. Communicated by K. Watson.

K. Watson, H. Morton, H. Arthur, and M. Streamer. Membrane lipid composition: A determinant of anaerobic growth and petite formation in psychrophilic and thermotolerant yeasts. BIOCHEM. SOC. TRANS. 6:380-382, 1978. A summary of this paper was presented in Yeast Newsletter XXVII, p. 28.

K. Watson. Thermal adaptation in yeasts: Correlation of substrate transport with membrane lipid composition in psychrophilic and thermotolerant yeasts. BIOCHEM. SOC. TRANS. 6:293-296, 1978.

SUMMARY

The uptake of ^{14}C-leucine and ^{14}C-glucose as a function of temperature was studied in psychrophilic, mesophilic, and thermotolerant yeasts. Substrate transport was high at low temperatures (2°C) in the psychrophilic yeast, *Leucosporidium frigidum*. By contrast, uptake was negligible at 40°C. Transport in the mesophilic yeast, *Candida lipolytica*, was most active at 25°C. There was minimal net uptake at 2 and 40°C. A completely different pattern of substrate transport was observed in the thermotolerant yeasts, *Torulopsis bovina* and *Saccharomyces telluris*. Uptake of labelled substrate was rapid at 40°C, in contrast with no uptake at 2°C. The transport studies correlated well with membrane lipid composition data which showed that the fatty acid unsaturation index increased dramatically from psychrophile to mesophile to thermotolerant yeast.

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K. Watson, H. Arthur, and H. Morton. Thermal adaptation in yeast: obligate psychrophiles are obligate aerobes and obligate thermophiles are facultative anaerobes. J. BACTERIOL., Nov. 1978. In press.

SUMMARY

Obligate psychrophilic yeasts are defined as yeasts with an upper temperature limit for growth of 20°C. We propose that it is unnecessary to restrict the definition to encompass only those yeasts which grow well (usually defined as visible growth after 1 to 2 weeks) at 0°C. Accordingly, a yeast which can grow in the temperature range of, for example, 5 to 18°C would be classified as a psychrophilic yeast. The term thermophile as applied to yeasts is much more nebulous since there are no yeasts which can grow at temperatures above 46°C. This temperature is generally the lower limit for growth of procaryotic microorganisms classified as thermophilic. There are a number of yeasts which are known to grow in the restrictive temperature range of between 20 and 46°C, and these have been referred to in the literature as thermophilic or psychrophobic. The present studies examined the ability of psychrophilic and thermophilic yeasts to grow under strictly anaerobic conditions.

The results illustrated that the obligate psychrophilic yeasts were obligate aerobes whereas the obligate thermophilic yeasts were facultative anaerobes. In summary, a further differentiating property, apart from their temperature limits of growth, of the psychrophilic and thermophilic yeasts examined in this report is the ability, in the case of the thermophiles, and inability, in the case of the psychrophiles, to grow under strictly anaerobic conditions. At this point in time, it is not possible to conclude whether the properties of anaerobic growth, synthesis of polyunsaturated fatty acids (Arthur & Watson, J. BACTERIOL. 128:56-68, 1976), and formation of respiratory-deficient mutants (Watson et al., Abstr. AUST. BIOCHEM. SOC. 11:96, 1978) in psychrophilic and thermophilic yeasts are the results of growth temperature or if these factors control the growth temperature range of these yeasts. Nevertheless, these properties are open to experimental test and should provide new insights into the mechanisms of microbial adaptation to extreme temperatures.

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H. Morton, K. Watson, and M. Streamer. Thermal adaptation in yeast: Temperature characteristics of ^{14}C-glucose uptake in psychrophilic, mesophilic, thermophilic *Torulopsis* yeasts. FEMS LETT. In Press.

SUMMARY

In this communication the temperature characteristics of glucose uptake in psychrophilic, mesophilic, and thermophilic yeasts from the same genus, namely *Torulopsis*, is described. It was clear from the results that *T. psychrophila*, a psychrophilic yeasts, was the only species which was capable of glucose transport at a high rate at low temperatures (2°C). Conversely, in the case of the thermophile, *T. bovina*, no uptake was observed at 2°C while there was a rapid and high uptake at 37°C. The mesophilic yeasts, *T. candida*, was intermediate in its transport properties having low activity at 2°C but high activity at 25 and 37°C, with maximum uptake at 25°C.

Our interpretation of these results is that at low temperatures, the only yeast whose membranes were in a semi-fluid, functional state was the psychrophilic species. On the other hand, at high temperatures, the membranes of the psychrophile were so fluid as to be nonfunctional. By contrast, the thermophilic yeasts membranes were fully functional at high temperatures but were in a nonfunctional gel state at low temperatures. Membranes of the mesophilic yeast were intermediate in their properties, with optimum fluidity at 25°C. These interpretations were supported by analysis of cell lipids and determination of the degree of fatty acid unsaturation. The psychrophilic yeast, *T. psychrophila*, had the highest unsaturation index, followed by *T. candida* and *T. bovina*, in that order.

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

The following two abstracts will be presented by members of our laboratory at forthcoming scientific meetings:

S. G. Oliver and J. J. Clare, "The effect of starvation on the synthesis and maintenance of P1 dsRNA in *Saccharomyces cerevisiae*".

(Was presented at the 188th meeting of the Genetical Society, U.C., London, November 17, 1978, and published in HEREDITY.)

The yeast virus-like particle (VLP) neither lyses nor infects its host cell and, therefore, can only be propagated through cellular growth and division. This constraint requires that VLP replication is coordinated with host cell metabolism. We have investigated the effect of amino acid and nitrogen starvation on the synthesis of P1 double-stranded ribonucleic acid (P1 dsRNA), the VLP genome.

When protein synthesis is prevented by starvation for a required amino acid or by addition of cycloheximide, the rate of P1 dsRNA synthesis is reduced markedly. During nitrogen starvation, the synthesis of P1 dsRNA continues but is accompanied by the degradation of preexisting molecules. This degradation appears to require the induction of new enzymes, and it is likely that the breakdown products are used to enable the cell to complete its division cycle. However, all of the copies of the VLP genome are not degraded in this process; some are conserved and can replenish the amount of P1 dsRNA on return to growth condition. Hence, the pattern of VLP replication is well-adapted to its dependence on the survival of the host cell.

S. W. Brown, D. E. F. Harrison, and S. G. Oliver, "Difference in the ethanol tolerance of aerobic growth and fermentation in the yeast Saccharomyces cerevisiae". (To be presented at the 84th meeting of the Society for General Microbiology, Cardiff, January 3, 1979, and published in the S. G. M. QUARTERLY.)

YEAST 12-78

The effects of ethanol on aerobic growth and fermentation have been studied in two strains of Saccharomyces cerevisiae. One strain, NCYC 479, is a commercial sake yeast whilst the other, 5D-cyc, is a laboratory haploid strain. The growth rate of these two strains was followed colorimetrically, and fermentation was measured as the production of carbon dioxide using a Gilson respirometer.

The effect of ethanol on the growth of the two yeast strains was very similar. Ethanol concentrations of 2% (v/v) and below had no observable effect on growth rate. At concentrations above 2% (v/v), ethanol caused an increasingly severe inhibition of growth; this inhibition being complete at a concentration of ca. 10% (v/v). At concentrations up to 5% (v/v), the effect of ethanol on fermentation paralleled that on growth. However, at the higher concentrations the fermentation rate was less inhibited by ethanol than was the growth rate. Complete inhibition of fermentation was not observed until ethanol concentrations of ca. 30% (v/v). Thus, above 5% (v/v) the responses of growth and fermentation to ethanol were markedly noncoordinate. Possible explanations for the biphasic response of fermentation to ethanol inhibition will be discussed.

XXXVI. Department of Microbiology and Genetics, Massey University, Palmerston North, New Zealand. Communicated by Roy J. Thornton.

HYBRIDISATION AS A MEANS OF IMPROVING PURE-CULTURE WINE YEASTS

Undesirable properties have been revealed in certain pure-culture wine yeasts; e.g., excess sulphide and sulphite formation and foaming. Although most of the pure-culture wine yeasts in the Ruakura A.R.C. collection, New Zealand, are homothallic, it has been shown that hybridisation techniques may be successfully employed in the elimination of undesirable properties. Two dominant genes FRO 1 and FRO 2 are responsible for the foaming phenotype in wine yeasts; they are allelic to the foaming genes isolated from sake yeasts. Either gene alone can cause foaming, but it is not clear whether there is a gene dosage effect. FRO 1 and FRO 2 are linked together 21 centimorgans apart, and FRO 1 is linked distally to the ade 3 locus on chromosome VII at a distance of 15 centimorgans. Two hybrid strains developed in the course of these investigations have been assessed in small scale wine making trials and compare quite favorably with some commercial pure-culture wine yeasts and are currently being evaluated in commercial scale fermentations. A general conclusion that may be drawn is that undesirable properties of pure-culture wine yeasts can be eliminated by hybridisation providing they have a simple genetic basis. The incorporation of desirable properties may depend upon a more complete understanding of the various factors involved but is the subject of current investigations.

Publications:

Homothallism in Wine Yeasts. R. J. Thornton and R. Eschenbruch. 1976. ANT. VAN LEEUWENHOEK 42, pp. 503-509.

Investigations on the Genetics of Foaming in Wine Yeasts. R. J. Thornton. 1978. EUROPEAN J. APPL. MICROBIOL. BIOTECHNOL. 5, pp. 103-107.

The Improvement of Pure-Culture Wine Yeasts by Hybridisation. R. J. Thornton and R. Eschenbruch. 1978. Proceedings of the IVth International Specialized Symposium on Yeast, Montpellier, France.

The Mapping of Two Dominant Genes for Foaming in Wine Yeasts. R. J. Thornton. 1978. FEMS MICROBIOLOGY LETTERS (in press).

XXXVII. Université De Bordeaux II, Institute D'Oenologie 351, cours de la Liberation, 33405 Talence, France. Communicated by P. Ribereau-Gayon.

The following papers have been published:

"The survival factors of yeasts and their role in the fermentation of grape must." This is the subject of the doctoral dissertation of

Françoise Larue under the direction of Professeur P. Ribéreau-Gayon and S. Lafon-Lafourcade. (BORDEAUX II, 1978.)

ABSTRACT

A new concept is introduced by this work: just as there are growth factors which increase growth rates and yields, there are "survival factors" which increase the percentage of the cells which remain alive when the multiplication ceases. Certain biological molecules which do not promote growth are able to maintain a high level of viability of non-proliferating cells. This phenomenon is particularly significant in the case of yeasts in certain media such as grape must which allow a limited growth, but which contains a high concentration of sugar which must be fermented by non-proliferating cells.

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S. Lafon-Lafourcade, F. Larue, P. Ribéreau-Gayon. "The survival factors of the yeasts in fermenting grape must." VIth International Specialized Symposium on yeasts - Montpellier 1978.

ABSTRACT

Three classes of substances have been identified capable of maintaining a more constant rate of viability and fermenting activity in yeast populations in their death-phase: sterols, oleanolic acid, oxytocin.

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S. Lafon-Lafourcade et P. Ribéreau-Gayon. Origines de l'acidité volatile des grands vins liquoreux. C. R. ACAD. AGRIC. 1977-63 (9) 551-558.

ABSTRACT

In the highly sugared grape musts which are parasitized by *Botrytis cinerea*, an important formation of acetic acid is observed. It has hitherto been thought that this excretion resulted from bacterial growth. Certain analytical tests establish that the level of acetic acid is due to a variation in the yeast metabolism induced by both the high sugar concentration of the grape musts and the substances secreted by *Botrytis cinerea*.

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The following papers have been submitted for publication:

S. Lafon-Lafourcade, F. Larue et P. Ribéreau-Gayon. Evidence for the existence of "survival factors" as an explanation for some peculiarities of yeast growth especially in grape must of high sugar concentration.

F. Larue, S. Lafon-Lafourcade et P. Ribéreau-Gayon. Relationship between the sterol content of yeast cells and their fermentation activity in grape must.

XXXVIII. Research Institute for Viticulture and Enology, 886 15 Bratislava, Matuskova 25, Czechoslovakia. Communicated by E. Minarik.

E. Minarik: Annual meeting of technical subcommissions of the Office International de la Vigne et du Vin (in Slovak). KVASNY PRUMYSL 24, 212-213 (1978).

The 14th Annual Meeting of the Subcommission "Microbiology of Wine" of the O.I.V. was held in Paris on May 24, 1978. Delegates from 20 countries attended the meeting. The following papers and communications were presented:

1. E. Minarik (Czechoslovakia): Practical methods of examination of enological properties of yeasts.

2. F. Radler (GFR): Microbiological studies on active dry yeasts (evaluation of public inquiry).

3. C. Llaguno (Spain): Enzymatic equipment of yeasts: distinction of yeasts used in enology.

4. V. Kovac (Jugoslavia): Investigation of grape oxydase inactivation by chemical methods.

5. J. V. Gomes (Portugal): Differentiation of living and dead lactic bacteria by coloration and diffraction.

The following publication will appear soon:

E. Minarik, V. Svec, and O. Jungova: Estimation of glucophilic and fructophilic wine yeasts of various genera (in German). MITTEILUNGEN KLOSTERNEUBURG 1978 (in press).

The fermentation behavior of different yeast species occurring on grapes and in fermenting grape juice towards glucose and fructose of the invert sugar of the must was examined. Most yeast species are more or less markedly glucophilic. *S. bailii* var. *bailii* and *T. stellata* belong to fructophilic yeast species and are detrimental for sweet table wines. Some important properties of glucophilic and fructophilic yeasts are given, and possibilities of utilizing preferential glucose and fructose fermentation in yeast taxonomy are discussed.

XXXIX. Labatt Breweries of Canada, Ltd., London, Ontario, Canada N6A 4M3. Communicated by G. G. Stewart.

GENETIC CONTROL OF YEAST FLOCCULATION

Previous reports (1, 2, 3) have indicated the presence of three independent dominant genes in *Saccharomyces sp.* coding for flocculation (*Flo 1*, *Flo 2*, and *Flo 4*). Further study, however, has indicated that all three are allelic. Consequently, it is proposed that they be consolidated as a single gene locus and be known in future as

Flo 4. Flo 4 is located on the right arm of chromosome I (4), 39 cM from the centromere (5). At least one further dominant gene for flocculation has been identified (Flo 5) which is nonallelic with Flo 4. Currently, the chromosome location of Flo 5 is being studied.

References:

1. Lewis, C. W., J. R. Johnston, and P. A. Martin. J. INST. BREWING (1976) 82, 158.

2. Lewis, C. W., and J. R. Johnston. SOC. GEN. MICROBIOL. PROC. (1974) 1, 73.

3. Stewart, G. G., I. F. Garrison, T. E. Goring, M. Meleg, P. P. Pipasts, and I. Russell. KEMIA-KEMI (1976) 3, 465.

4. Stewart, G. G., and I. Russell. CAN. J. MICROBIOL. (1977) 23, 441.

5. Sherman, F., and P. Helms, 10TH INTERNATIONAL CONGRESS OF YEAST GENETICS AND MOLECULAR BIOLOGY, Rochester, N.Y., June 1978.

I. Russell and G. G. Stewart
Labatt Breweries of Canada, Ltd.
London, Ontario
Canada

P. A. Martin
Allied Breweries, Ltd.
Burton-on-Trent
England

J. R. Johnston and H. P. Reader
Strathclyde University
Glasgow
Scotland

The following is the abstract of a paper that will shortly appear in the J. INST. BREWING, coauthored by I. Russell and G. G. Stewart.

SPHEROPLAST FUSION OF BREWER'S YEAST STRAINS

Spheroplasts of brewing polyploid yeast strains have been successfully fused with spheroplasts of haploid yeast strains. After regeneration of the cell wall, stable fusion recombinants were isolated. Genetic analysis of these recombinants revealed that they contained the genotype of both parents, sporulated well with each ascus containing four spores, and were indeed diploid. Spheroplast fusion thus affords a means to genetically analyze brewing yeast strains, such an analysis having been difficult if not impossible by conventional hybridization techniques.

The following is the abstract of a paper that was presented at the VITH INTERNATIONAL SPECIALIZED SYMPOSIUM ON YEASTS held in Montpellier, France, July 1978, coauthored by G. G. Stewart, J. Erratt, I. F. Garrison, and I. Hancock.

Normally, brewing yeast cultures take up wort sugars in a distinct but overlapping order: sucrose, glucose, fructose, maltose, and maltotriose. Before sucrose is taken into the cell, it is hydrolyzed into glucose and fructose by invertase. Invertase is only produced when the yeast cell possesses the correct genetic complement. Glucose is taken into the cell at a faster rate than

fructose, a fact of little consequence in traditional worts; however, with the advent of syrups, predominant in one sugar (e.g., high fructose syrups), such basic information is of importance to the brewer. Maltose is the wort sugar present in greatest concentration. In contradistinction to glucose, maltose uptake into the yeast cell requires the expenditure of metabolic energy; consequently, the cell will take up glucose in preference to maltose. Maltotriose uptake also has an energy demand and once inside the cell shares a common hydrolytic system (α-glucosidase) with maltose; however, each sugar possesses its own permease. In traditional beers the wort's dextrin is unfermented. One method of reducing dextrins is to genetically manipulate the yeast so that it will produce an extracellular amyloglucosidase thereby hydrolyzing the dextrin during fermentation, and thus lowering the caloric content of the beer.

XL. Technische Universität Berlin, Fachbereich 13, Lebensmitteltechnologie und Biotechnologie, Lehrstuhl für Mikrobiologie, Seestr. 13, 1000 Berlin 65, West Germany. Communicated by S. Windisch.

The following represents recent work from our laboratory.

S. Windisch, S. Kowalski, and I. Zander: Dough-raising tests with hybrid yeasts. EUROPEAN J. APPL. MICROBIOL. 3, 213-221, 1976.

Summary: The raising capacity of hybrid yeast strains was determined in hard biscuit, shortcake, and flour dough by measuring the CO_2 production at 30°C with an Epsom fermentometer. Of the 90 strains tested, 26 hybrids were able to ferment in all three types of dough very well, reaching CO_2 levels of approx. 300, 150, and 220 ml CO_2/g dry weight of yeast after 135 min in hard biscuit, shortcake, and flour dough, respectively. The pattern of CO_2 production shown by the hybrid yeasts in the different doughs is compared and discussed.

S. Windisch, S. Kowalski, and I. Zander: Demonstration of osmotolerant yeasts in almonds. ZUCKER- UND SÜSZWARENWIRTSCHAFT 31 (5) 177-180, 1978.

Summary: The same osmotolerant (ot) yeasts which spoil marzipan were found among those ot yeasts isolated from unblanched almonds (e.g., Saccharomyces rouxii). These yeasts seem to be attached to the seed coat, which explains why ot yeasts were not found previously in samples of blanched almonds. The origin of contamination by the ot yeasts in marzipan can now be traced directly to the almonds. The presence of ot yeasts can be demonstrated by testing the osmotolerance of fermentation in a 75% fructose (w/v) solution following enrichment cultures in nutrient medium with 40% glucose.

XLI. Department of Food Science, Faculty of Agriculture, Alfateh University, Tripoli, Libya. Communicated by Amin S. El-Nawawy.

XII International Congress for Microbiology (ICM)

Munich, September 3-8, 1978

Comparative Studies on Some Yeasts and Their Efficiency in Utilizing Rub el Tamr Wastes for Production of Food Yeasts

Amin S. El-Nawawy

Rub el Tamr (date syrup) is produced in Libya from some varieties of dry dates. The waste, after syrup extraction, contains 10% sugars, giving a possibility for being a rich raw material for some fermentation processes.

After extraction of the remaining sugars from the ground waste by hot water, the two parts were used separately; i.e., sugar extract (SE) and dried ground waste (DGW) containing mainly the cellulose fraction of the dates.

(SE) proved to be a good carbon source but not a nitrogen source for the yeasts: (1) C. utilis, (2) C. utilis var. thermophila, (3) C. pelliculosa, (4) Hansenula anomala, (5) C. tropicalis, (6) Endomycopsis fibuligera. However, addition of $(NH_4)_2SO_4$ (0.5%) and KH_2PO_4 (0.2%) under moderate aeration conditions (rotary shaking at 500 r.p.m.) caused utilization of 80-90% of the sugars, giving the following yields, compared to the utilized sugars by each strain: (1) 40, (2) 38, (3) 35, (4) 36, (5) 40, (6) 34%. Protein percent was 55, 52, 56, 54, 52, 40, respectively.

Trichoderma viride was used either alone or mixed with one of the strains: 1, 2, 3, 4, 5; and propagated on (DGW) 1% medium provided with N&P salts. The cellulose was partially hydrolysed by T. viride, resulting in good yeast growth. Best results were determined with T. viride + H. anomala followed by T. viride + C. tropicalis. The remaining part of DGW + microbial growth contained (on the dry weight basis) 18 and 16% protein, respectively.

Production of Microbial Protein from Rice Hulls in Egypt

S. A. Z. Mahmoud, W. A. Mashhoor, A. S. El-Nawawy and E. M. Ibrahim

In Egypt, rice hulls are one of the major agricultural wastes where about 250 thousand tons remain each year. In a previous investigation (Mahmoud et al., 1974) various treatments were applied to find the optimal method for rice-hull hydrolysis. H_2SO_4 hydrolysate contained reducing sugars equal to 15% of hulls weight with relatively low concentration of furfural. Five strains of Candida were found to grow successfully on that hydrolysate after being neutralized and supplemented with nitrogen and phosphate salts.

The present work was conducted to optimize the environmental conditions of C. pelliculosa growing on rice-hulls hydrolysate medium for the highest yield of biomass and protein. Therefore, type and concentration of added salts, pH, size of inoculum, temperature, and perod of incubation were investigated.

Results showed that supplementation of the hydrolysate with 0.03% nitrogen as ammonium sulfate and 0.08% of KH_2PO_4 gave the highest yield of yeast protein. The use of 8% inoculum of a two days culture was the best inoculum size. In addition, it was found that a pH value of 5 and incubation at 28°C for 72 hours had been shown superior among either treatment.

The protein of C. pelliculosa grown on rice-hulls hydrolysate medium contained all the essential amino acids in sufficient concentration. However, as in most microbial protein, the concentration of sulfur amino acids was found to be low.

XLII. Department of Food Science, The University of Georgia, College of Agriculture, Experiment, Georgia 30212. Communicated by Nancy J. Moon.

Conversion of cheese whey to yeast lipid and single cell protein. 1977. Nancy J. Moon, Ph.D. dissertation at Iowa State University in Food Technology under the direction of Earl G. Hammond.

Title: Utilization of Dairy Processing Wastes by Trichosporon cutaneum and Candida curvata to Produce Cells Rich in Triglycerides. Nancy J. Moon.

A process was developed for fermenting cheese whey and ultrafiltered whey permeate by yeast that produced large amounts (56% dry wt) of lipids. Fungi known to be fat producers (Rhodotorula sp and Lipomyces sp), as well as many unidentified isolates, were examined for their ability to grow on whey, use lactose, and produce fat. Four organisms isolated from dairy plants were selected for study: 2 strains each of Candida curvata and Trichosporon cutaneum.

Optimum conditions for submerged fermentation were determined for logarithmic growth and stationary phase. Nutritional optimization of growth and fat production was also determined in fermentation studies. Minimal additions were necessary (1.28 g nitrogen/10L) to achieve maximum cell numbers and growth. Sixty hours complete fermentations of 10L of whey by these organisms reduced the organic content a maximum of 85%, produced 89 g fat, and left most of the whey protein unused. Fermentation of whey permeate for 72 hours reduced the organic content 95% to 3000 mg/L and produced (depending on the organism) from 39 to 149 g fat, 196 to 268 g cells, and 22 to 32 g single cell protein. The two strains of C. curvata were the most efficient.

The fat was composed of oleic (∿50%), palmitic (∿30%), stearic (∿15%), and linoleic (∿8%) acids. The composition changed with the age of the culture; the older cells being higher in oleic and lower in linoleic acids. At lower temperatures (15C) more oleic (∿60%) and less stearic acids were produced.

XLIII. National and International Meetings on Yeasts.

1. Vth INTERNATIONAL SYMPOSIUM ON YEAST - LONDON, CANADA

July 20-26, 1980

The International Commission on Yeast and Yeast-Like Microorganisms met during the VIth International Specialized Symposium on Yeasts that was held in Montpellier, France, in July 1978. It was decided, because of the withdrawal of the U.S.S.R., to hold the Vth International Symposium on Yeasts (ISY) in conjunction with the VIth International Fermentation Symposium (IFS) on the campus of the University of Western Ontario, London, Canada, from July 20-26, 1980. The two Symposia will have a common adminstration, registration fee, and social programs but separate scientific programs.

The Scientific Program Committee consists of B. Johnson (National Research Council of Canada, Ottawa), C. F. Robinow (University of Western Ontario), E. R. Tustanoff (University of Western Ontario), and G. G. Stewart, Chairman (Labatt Breweries of Canada, Ltd.). It has been decided that the symposium will consist of the following:

1. Plenary Lectures
2. Symposia
3. Free Communications
4. Round Table Discussions
5. Poster Sessions

PLENARY LECTURES

There will be a morning of four plenary lectures on Monday, July 21, 1980. The following people have accepted invitiations to speak:

A. H. Rose	University of Bath, U.K.
H. J. Phaff	University of California, Davis
F. Sherman	University of Rochester, N.Y.
C. F. Robinow	University of Western Ontario, London

SYMPOSIA

There will be symposia on the following topics:

a) Industrial and Agricultural Uses of Yeast
b) Genetics
c) Sporulation and Conjugation
d) Biochemistry
e) Taxonomy and Ecology
f) Cell Cycle

Suggestions, comments, etc. with regard to any aspect of the organization of the Vth ISY are solicited and should be addressed to:

G. G. Stewart
Chairman, Vth ISY
Labatt Breweries of Canada, Ltd.
150 Simcoe Street
London, Ontario, Canada N6A 4M3

2. VIth INTERNATIONAL FERMENTATION SYMPOSIUM - LONDON, CANADA

July 20-26, 1980

The VIth IFS will be held on the campus of the University of Western Ontario, London, Canada, in conjunction with the Vth ISY, July 20-26, 1980. In line with tradition, the scientific program of the VIth IFS will cover a wide range of topics, including both basic and applied aspects of fermentation. The Symposium will be divided into three general sections:

(i). Basic Aspects of Microbiology and Biochemistry

(e.g., Genetics, Biosynthesis, Biotransformations, Continuous Culture, Growth Kinetics, etc.)

(ii). Basic Aspects of Bioengineering and Biotechnology

(e.g., Process Design, Scale-up Procedures, Instrumentation, Process Economics, Medium Formulations, etc.)

(iii). Applications in Processes and Products

(e.g., Waste Treatment, Hydrocarbon Fermentations, Mineral Ore Leaching, SCP, Tissue Culture, Antibiotics, Vaccines, Enzymes, etc.)

Suggestions, comments, etc. with regard to any aspect of the organization of the VIth IFS are solicited and should be addressed to:

J. E. Zajic
Chairman, VIth IFS
Faculty of Engineering Science
The University of Western Ontario
London, Ontario, Canada N6A 5B9

3. Society for Applied Bacteriology Symposium on Yeasts, The University, Newcastle-upon-Tyne, England. Approximately 16 papers (45 minutes) by invitation: topics include habitats, classification procedures, systematics, sugar utilization, physiology, genetics,

pathogenic yeasts, aspects of spoilage of foods including resistance, workshop on selective and differential media. Four simultaneous paper-reading sessions, Wednesday a.m. (papers 15 minutes each). Contact:

Dr. T. A. Roberts
Agricultural Research Council
Meat Research Institute
Langford, Bristol BS18 7DY, England

Program and reservation forms available approximately March 1979. Total cost (includes accommodation, Monday p.m. to Friday a.m., all meals, conference registration fee) estimated $60. Open to nonmembers of SAB.

4. INTERNATIONAL MYCOLOGICAL ASSOCIATION
THIRD INTERNATIONAL MYCOLOGICAL CONGRESS

The Executive Committee of the International Mycological Association has accepted the invitation of the Mycological Society of Japan to hold the Third International Mycological Congress (IMC3) in Japan in 1983. The Congress will be based at either Kyoto or Tokyo, most probably in late August.

Further information will be circulated by the Organizing Committee of the Congress when detailed arrangements have been finalized.

D. L. Hawksworth
Secretary, IMA

CMI, KEW, England
September 1978

5. 25th ANNIVERSARY OF I.S.H.A.M.

VII CONGRESS OF THE INTERNATIONAL SOCIETY
FOR HUMAN AND ANIMAL MYCOLOGY

Jerusalem, Israel, March 11-16, 1979

Please address correspondence to:

The Secretariat
VII Congress of the ISHAM
P.O.B. 16271
Tel Aviv, Israel

A "package tour" for the Israel meeting of ISHAM is available for $998. Contact either Dr. S. Shadomy, Box 85, MCV Station, Richmond, Virginia 23298 or Crown International Travel, 655 South Hope Street, Suite 107, Los Angeles, California 90017 for details.

6. During the 6th International Specialized Symposium on Yeasts in Montpellier, a meeting of the members present of the International Commission on Yeasts (ICY) was held, and the following resolution was adopted:

(i). The members present of the ICY like to thank Professor Galzy and his coworkers most sincerely for the excellently organized 6th ISSY.

(ii). As new members of the International Commission of Yeast were nominated:

France	Dr. Jean Bastide, Laboratoire d'immunologie, Faculté de Pharmacie, Avenue Charles Flahault F-34000 Montpellier
United Kingdom	Dr. A. H. Rose, Zymology Laboratory, School of Biological Sciences, Bath University, Bath, England
	Dr. Ian Dawes, Microbiology Department, West Mains Road, Edinburgh EH8 3JG, Scotland
Spain	Dr. Juana Gancedo, Instituto de Enzimologia, CSIC, Facultad de Medicina Autonoma, Arzobispo Morcillo, Madrid 34
	Dr. Rafael Santandreu, Department of Microbiology, Facultad de Ciencias, Salamanca

(iii). Next Symposia:

1980	5th General Symposium July 20-26, London, Ontario, Canada
1981	7th Specialized Symposium, Spain Topic: Yeast Cell Surface
1982	8th Specialized Symposium, Great Britain Topic: Yeast Physiology
1983	9th Specialized Symposium, Czechoslovakia Topic: Ecology and Taxonomy
1984	10th Specialized Symposium, Bulgaria Topic: Molecular Biology and Genetics
1985	6th General Symposium, Montpellier, France

(iv). Members of the ICY (August 1978):

Australia: B. C. Rankine; Austria: H. Klaushofer, U. Sleytr; Belgium: H. Verachtert; Brazil: A. Panek; BRD: S. Windisch; Bulgaria: P. Venkov; Canada: C. Robinow, G. Stewart; Czechoslovakia: A. Kockova-Kratochvilova, A. Kotyk, E. Minarik; Denmark: A. Stenderup; DDR: H. Koch, P. Lietz, W. Nordheim;

Egypt: A. S. ElNawawy; England: A. H. Rose, J. Spencer; Finland: H. Suomalainen; France: P. Galzy, J. Bastide, H. Heslot; Hungary: E. Novak; Ireland: R. B. Gilliland; Israel: C. Shalitin; Japan: Y. Fukazawa, K. Iwata, S. Nagai; Netherlands: J. C. Hoogerheide, L. Rodrigues de Miranda; Poland: J. Jakubowska, B. Bachman (observer); Portugal: L. do Carmo-Sousa; Scotland: T. Dawes, E. O. Morris; South Africa: J. P. van der Walt; Spain: J. Galzedo, J. Santa Maria, R. Santandreu; Sweden: K. Jarl; Switzerland: A. Fiechter, P. Matile; USA: H. J. Phaff; USSR: N. Elinov, M. Meissel, G. Shavlovsky, I. Babjeva; Yugoslavia: V. Johanides; Vice Chairman: T. Wikén (Sweden).

D. Klaushofer
Gregor Mendel Strasse 33
Vienna A-1180, Austria

7. THE ELEVENTH ANNUAL MEETING OF YEAST GENETICS CONFERENCE - JAPAN

The Eleventh Annual Meeting of Yeast Genetics Conference - Japan was held from October 11 to 13, 1978, at Ohmori Plant of Asahi Breweries Ltd., Tokyo, Japan. The following topics were presented and discussed.

Session 1 : Radiation and Mutation (Chairpersons, K. Hieda and T. Ito)

A. Ito and T. Ito (Inst. Phys., Col. Gen. Edu., Univ. Tokyo, Komaba, Meguro-ku, Tokyo 153). The development of the technique for the vacuum ultra-violet irradiation of yeast cells in a wet state.

S. Nakai and I. Machida (Div. Genet., Natl. Inst. Sci., Anakawa-cho, Chiba 280). Induction of mutation intra- and intergenic recombination by UV-light during meiosis of *Saccharomyces*.

Y. Nakatomi (Res. Lab., Oriental Yeast Co. Ltd., Azukizawa, Itabishi-ku, Tokyo 174). The use of color differentiation medium for the detection of mutants.

K. Hieda (Biophys. Lab., Fac. Sci., Rikkyo Univ., Nishi-ikebukura, Teshima-ku, Tokyo 171). Isolation and characterization of dry-sensitive mutants of *Saccharomyces cerevisiae*.

A. Fukui (Biophys. Lab., Dept. Phys., Rikkyo Univ.). Number of photo-reactivating enzymes in yeast cells.

Session 2 : Gene Regulation (Chairperson, N. Gunge)

Y. Adachi, K. Matsumoto*, and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., Yamadakami, Suita 565 and *Dept. Indust. Chem., Fac. Eng., Tottori Univ.). The meiotic mapping of the *GAL81* and *gal4* mutants in the galactose regulation system.

K. Matsumoto and Y. Oshima* (Dept. Indust. Chem., Fac. Eng., Tottori Univ., Koyama-cho, Tottori 680 and *Dept. Ferment. Technol., Osaka Univ.). Isolation and characterization of catabolite repression-resistant mutants in Saccharomyces cerevisiae.

T. Akamatsu and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). One of two complementary suppressors for phoS, phoW, may be involved in inactive transport of inorganic phosphate.

Y. Kaneko and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). The structural gene for nonspecific alkaline phosphatase in the phoH gene.

Session 3 : Mating and Life Cycle (Chairpersons, I. Takano, S. Harashima, M. Tsuboi, and K. Tanaka)

H. Tohyama (Biol. Inst., Fac Sci., Ehime Univ., Maysuyama 790), A. Aoyagi (Dept. Chem., Fac. Sci., Kyushu Univ.), A. Sakurai (Inst. Phys. Chem. Res.), and N. Yanagishima (Biol. Inst., Fac. Sci., Nagoya Univ.). The agglutinability-inducing action of synthetic peptides on inducible and physiologically repressed a cells.

K. Nishi and N. Yanagishima (Biol. Inst., Fac. Sci., Nagoya Univ., Chigusaku, Nagoya 464). Temperature sensitivity of induction by α peptidyl sex factor of sexual agglutinability in inducible a mating-type strain.

Y. Nakagawa and N. Yanagishima (Biol. Inst., Fac. Sci., Nagoya Univ.). Gene regulation of inducibility of sexual agglutinability.

I. Takano and K. Arima (Ctr. Res. Inst., Suntory Ltd., Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618). Evidence of co-dominant function in the homothallism gene system by means of protoplast fusion.

T. Oshima and I. Takano (Ctr. Res. Inst., Suntory Ltd.). Mutation of homothallism genes HMα and HMa, in Saccharomyces yeast.

K. Ohashi, M. Tsuboi, and M. Hayashibe (Dept. Biol., Fac. Sci., Osaka City Univ., Sugimoto-cho, Sumiyoshi-ku, Osaka 558). Sexual process in the fission yeast Schizosaccharomyces japonicus.

H. Inoue and C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.). Trehalase activity in germinating spores of Schizosaccharomyces pombe.

M. Miyata (Gifu Col Pharm., Mitabora, Gifu 502). Action of aculeacin A, a new antifungal antibiotic, on the cell wall of the fission yeasts.

E. Tsuchida, S. Fukui (Inst. Appl. Microb., Univ. Tokyo, Yayoi, Bunkyo-ku, Tokyo 113), Y. Sakagami (Dept. Agr. Chem.,

Univ. Tokyo), and M. Fujino (Ctr. Res. Div., Takeda Chem. Indust. Ltd.). Biochemical response of Tremella mesenterica 9313 to tremerogen A-10, a novel fungal sex hormone.

K. Abe and S. Fukui (Inst. Appl. Microb., Univ. Tokyo). Cell division cycle and the transition stage to sexual differentiation in the heterobasidomycetous yeast Rhodosporidium toruloides.

Session 4 : Recombination, Fine Structure, and Mapping (Chairperson, M. Mori)

T. Takahashi (Ctr. Res. Lab., Asahi Brew. Ltd., Ohmori-kita, Ohta-ku, Tokyo 143). Mitomycin C induced mitotic recombination rate and map distance. ——————, Location of β-phenylethyl-alcohol sensitive gene pea2.

S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Mapping functions for dihybrid-tetraploid segregation in yeasts.

M. Tsuboi (Dept. Biol., Fac. Sci., Osaka City Univ.). Genetical studies on a Saccharomyces cerevisiae strain which sporulates in a nitrogen-rich medium.

Session 5 : Cytoplasmic Inheritance and Drug Resistance (Chairpersons, K. Suda and T. Kamihara)

S. Murakami (Cancer Res. Inst., Kanazawa Univ., Takara-cho, Kanazawa 920). Effects of demecolcin on the growth of Saccharomyces cerevisiae and identification of tubulin protein of Saccharomyces cerevisiae.

F. Miyamoto (Dept. Biol., Fac. Edu., Wakayama Univ., Masago-cho, Wakayama 640). Induction of RD mutants in yeast under anaerobic condition.

N. Gunge, A. Tamaru, and K. Sakaguchi* (Ctr. Res. Lab., Mitsubishi Chem. Indust., Kamoshida, Midori-ku, Yokohama 227 and *Mitsubishi-Kasei Inst. Life Sci.). Fusion of mitochondria with protoplasts in Saccharomyces cerevisiae.

S. Nagai, S. Ochi, and K. Nishimura (Dept. Biol., Fac. Sci., Nara Women's Univ., Nara 630). Production of rho$^-$ mutants by some acid dyes.

M. Sugii and Y. Arakatsu (Dept. Biol., Fac. Sci., Konan Univ., Okamoto, Kobe 658). Saccharomyces mutants resistant to β-phenethylalcohol.

Session 6 : Metabolism and Biochemistry (Chairperson, T. Segawa)

H. Yamakatsu and M. Miyazaki (Inst. Mol. Biol., Fac. Sci., Nagoya Univ.). Some problems in isolation of highly active ribosomes from yeasts.

T. Kamihara, I. Nakamura, and S. Fukui (Lab. Indust. Biochem., Dept. Indust. Chem., Fac. Eng., Kyoto Univ., Yoshiakami, Sakyo-ku, Kyoto 606). Effects of thiamine and pyridoxine on alcoholic fermentation in Saccharomyces carlsbergensis 4228. ✓

11th Annual Yeast Genetics Conference Tokyo '78

H. Fukuda and A. Kimura (Res. Inst., Food Sci., Kyoto Univ., Uji, Kyoto 611). Intracellular localization of yeast hexokinase.

Session 7 : Structure and Function of Cell Organelles (Chairpersons, A. Kimura and M. Osumi)

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

K. Tanaka (Inst. Appl. Microb., Univ. Tokyo). Fine structure of mitosis in yeasts.

N. Kawakami, H. Mondo, H. Tanaka, and H. Kawakami* (Fac. Eng., Hiroshima Univ., Senda-cho, Hiroshima 730 and *Dept. Nutrition, Suzugamine Women's Col.). Thylakoid membrane incorporation in yeast protoplasts.

K. Arima and I. Takano (Ctr. Res. Inst., Suntory Ltd.). Multiple protoplast fusion in Saccharomyces yeast.

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

Y. Ikeda (Honorary Professor, Univ. Tokyo). Progress of genetics in microorganisms during the past 25 years.

B. Ono (Dept. Pharm. Technol., Fac. Pharm. Sci., Okayama Univ., Tsushimanaka, Okayama 700). Yeast suppressors.

The next annual meeting of the yeast genetics conference - Japan will be held in Tokyo or Nagoya in the fall of 1979.

Isamu Takano
Central Research Institute
Suntory Ltd.
Wakayamadai, Shimamoto-cho
Mishima-gun, Osaka 618
Japan

8. Natl. Res Inst. Brewing, Japan, Kita-ku, Tokyo 114, Japan. Communicated by H. Akiyama.

THE THIRD GENERAL YEAST SYMPOSIUM - JAPAN

The Third General Yeast Symposium - Japan, organized by Seishu Kobo Kenkyukai (Saké Yeast Conference, Japan) was held on May 25 and 26, 1978, at Ienohikari Kaikan Hall, Iidabashi, Tokyo. Around 240 yeast researchers met, and the following subjects were presented and discussed. The proceedings of this meeting (in Japanese) will be edited by the executive committee of Seishu Kobo Kenkyukai (Dr. Hiroichi Akiyama) and published through Japan Scientific Societies Press (Mukogaoka, Tokyo 113) next February.

Scientific program:

1. M. Takakuwa (Fac. Agr. Ehime Univ., Matsuyama 790): *In vivo* degradation of ribosomes during storage of pressed baker's yeast and its significance in preservability of pressed yeast.

2. H. Mori (Noda Inst. Sci. Res., Noda-shi, Chiba 278): Life cycle and breeding of a haploid yeast, *Saccharomyces rouxii*.

3. I. Takano (Ctr. Res. Inst., Suntory Ltd., Mishimagun, Osaka 618): Breeding of polyploid yeast.

4. H. Tamaki (Doshisha Women's College, Kyoto 602): Genetic studies and utilization of ability to ferment starch in *Saccharomyces*.

5. K. Yoshizawa, K. Kodama* (Natl. Res. Inst. Brewing, Tokyo 114, *Kodama Gomei Co., Akita 018-15): Treatment of waste water from a saké brewery using yeast.

6. S. Goto (Res. Inst. Ferment., Yamanashi Univ., Kofu 400): Yeast flora in grapes, musts, and wines and selection of wine yeasts.

7. T. Inoue (Res. Lab. Kirin Brewery Co. Ltd., Takasaki 370-12): Selection of yeast for brewing beer without diacetyl odor.

8. T. Ishikawa, K. Yoshizawa (Natl. Res. Inst. Brewing, Tokyo 114): Effects of cellular fatty acids on flavor esters formation by yeast and its contribution to saké brewing.

9. I. Takagahara, Y. Suzuki, T. Fujita (Oriental Yeast Co. Ltd., Osaka 564): Systematic methods for purification of enzymes in yeast cells.

10. T. Obata (Natl. Res. Inst. Brewing, Tokyo 114): Proteolytic enzyme from *Oerskovia* sp. CK lysing viable yeast cells.

11. N. Kawakami (Fac. Engineer. Hiroshima Univ., Hiroshima 730): Fission of yeast protoplasts.

12. M. Doi, S. Akiyama, Y. Nakao (Ctr. Res. Division, Takeda Chemical Ind. Ltd. Osaka 532): A ribonuclease deficient mutant of *Candida lipolytica*.

13. Y. Uzuka (Fac. Engineer. Yamanashi Univ., Kofu 400): Yeasts as potential resources of edible oil.

14. T. Hirata, T. Ishitani (Natl. Food Res. Inst., Tokyo 135): Microbiological identification of SCP yeast by PMR spectrum of cell-wall mannan.

15. N. Kato, S. Shimizu, Y. Tani, H. Yamada (Dept. Agr. Chem., Kyoto Univ., Kyoto 606): Metabolism and utilization of methanol by yeasts.

16. H. Kawaharada, K. Kagotani, Y. Shimada, K. Watanabe (Kanegafuchi Chemical Ind. Co. Ltd., Takasago 676), K. Ishii, T. Kaneko (Inst. Phys. Chem. Res., Saitama 351): Production of yeast cells from n-paraffin.

17. K. Iwata (Fac. Med. Univ. Tokyo, Tokyo 113): The pathogenicity and toxigenicity of yeasts with special reference to the production of single cell protein.

XLIV. Brief News Items.

RESEARCH ASSOCIATESHIPS

1. The National Research Council of Canada at Ottawa offers Research Associateships in open competition annually. One of the areas in which this appointment is available is in the Molecular Genetics Group of the Division of Biological Sciences. The interests of this Group are focussed on yeast genetics and encompass a variety of different areas. Applications are available through the NRC Research Associateship Office, National Research Council, Ottawa, Canada K1A OR6. Those interested in appointments to be taken up in 1979 should submit their qualifications by January 15, 1979. Initial appointments are for one year and may be renewed subject to each Associate's performance and to the requirements of the Division. Renewals are considered annually. The term as a Research Associate may vary but will not exceed five years. The 1979 Ph.D. recruiting rate without experience will be approximately $18,000 per annum (Canadian). Additional information regarding the work of the Group may be obtained by writing to any of the following: A. P. James, Group Leader, R. Brousseau, B. F. Johnson, C. V. Lusena, S. A. Narang, A. Nasim, H. Schneider, V. Seligy, L. Visentin, and M. Yaguchi.

Division of Biological Sciences
National Research Council of Canada
Ottawa, Ont., Canada K1A OR6

2. Dennis M. Dixon, Ph.D., has completed his research on the occurrence of dematiacious pathogenic fungi ("Black Yeast") in Virginia and has accepted an appointment as Assistant Professor, Department of Biology, Loyola College, Baltimore, Maryland.

Dr. H. Jean Shadomy has been promoted to the rank of Professor of Microbiology, Virginia Commonwealth University, Richmond, Virginia, effective July 1, 1978.

Dr. S. Shadomy presented a Workshop on Susceptibility Testing for Fungi in Rio de Janeiro in conjunction with the Xth Triennial World Congress of Pathology, September 26, 1978.

An opening exists as a Postdoctoral Fellow in the Research Training in Infectious Diseases program conducted by the Department of Microbiology, Virginia Commonwealth University. It is hoped that this opening will be filled by someone with an interest in medical mycology.

Smith Shadomy, Professor of Medicine