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YEAST

A Newsletter for Persons Interested in Yeast

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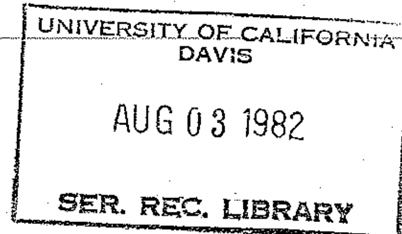

Herman J. Phaff, Editor
126 Cruess Hall
University of California
Davis, California 95616

Associate Editors

Anna Kocková-Kratochvílová
Slovak Academy of Sciences
Bratislava, Czechoslovakia

Susumu Nagai
Biological Laboratories
National Women's University
Nara 630, Japan

Richard Snow
Dept. of Genetics, Univ. of California
Davis, California 95616

Torsten O. Wiken
Runvistarvägen 53
Vallentuna, Sweden

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I regret to inform you that inflation has also caught up with the Yeast Newsletter; and, as a consequence, the annual subscription must be raised to $3 per year, an increase of one dollar. Even with this increase, we expect the Newsletter to operate at a deficit; and further increases in the not too distant future may be unavoidable.

Foreign Subscribers: It has come to our attention that mailing of the Yeast Newsletter by printed matter involves a 2-3 month delay in your receiving it. If you are not receiving the Yeast Newsletter by airmail (which takes approximately 2 weeks) and would like to, please let us know. An additional $4 per year is required to cover postage and handling for this service.

I wish all readers of the Yeast Newsletter a prosperous and scientifically rewarding New Year.

Herman J. Phaff
Editor

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

The strains listed below have been added to the ATCC since April 30, 1979. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

Strain	Source
Saccharomyces cerevisiae ATCC 38554 formation of ascospores with high heat resistance	H. M. C. Put Carl C. Conway Lab The Netherlands
Saccharomyces chevalieri ATCC 38555 formation of ascospores with high heat resistance	"
Pityrosporum ovale ATCC 38593 isolated from human scalp	G. Midgley St. Johns Hospital London, United Kingdom
Saccharomyces cerevisiae ATCC 38598 genetic studies	J. F. Lemantt Oak Ridge Lab Tennessee
Candida lambica ATCC 38617 spoilage of orange juice concentrate	B. J. Juven The Volcani Center Israel
Saccharomyces chevalieri ATCC 38618 spoilage of orange juice concentrate	"
Torulopsis magnoliae ATCC 38619 spoilage of orange juice concentrate	"
Candida fluviolilis ATCC 38621-38625 isolated from aquatic environment; 38621 is type culture	L. R. Hedrick Portland, Oregon
Saccharomyces cerevisiae ATCC 38626 genetic studies	G. Fink Cornell University Ithaca, New York
Saccharomyces cerevisiae ATCC 38636-38638 wine fermentation	R. Eschenbruck Ruakultura Agric. New Zealand
Saccharomyces species ATCC 38639 wine fermentation	"

Saccharomyces cerevisiae ATCC 38659-38662 genetic studies	N. Gunge Mitsubishi Chem. Ind. Japan
Candida albicans ATCC 38696 human isolate	M. O. Perrot Serv. Paracitologie France
Saccharomyces cerevisiae ATCC 38708-38712 genetic studies	C. Bizean C. R. A. M. Cedex, France
Candida rugosa ATCC 38772 isolated from artificial diet for silk worm	T. Nakase Central Research Labs Japan
Candida pararugosa ATCC 38774 isolated from artificial diet for silk worm, type culture	"
Candida tropicalis ATCC 38773 isolated from an Atlantic bottle-nosed dolphin	A. Widra University of Illinois Chicago, Illinois
Candida fusiformata ATCC 38777 isolated from cauliflower and cabbages	R. W. M. Buhagiar Food Research Institute United Kingdom
Candida species ATCC 38835 isolated from fuel tanks	M. E. May Naval Research Lab Washington, D.C.
Kluyveromyces fragilis ATCC 38866 Pectinolytic	M. Wilson Univ. of New South Wales Australia
Cryptococcus neoformans ATCC 38897 cryptococcal endothalmitis after corneal transplant	G. S. Kobayashi Washington University School of Medicine St. Louis, Missouri

II. United States Department of Agriculture, Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604. Communicated by C. P. Kurtzman.

The following paper has been accepted for publication:

Kurtzman, C. P., M. J. Smiley, C. J. Johnson, L. J. Wickerham, and G. B. Fuson. 1980. Two New and Closely Related Heterothallic Species: *Pichia amylophila* and *P. mississippiensis*, Characterization by Hybridization and DNA Reassociation. INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY (in press).

ABSTRACT

Two new species of the genus Pichia were isolated from frass obtained from loblolly pines growing in Mississippi. The new taxa, designated Pichia amylophila and P. mississippiensis, proved to be heterothallic. The species show interspecific mating, but ascospores from the crosses are infertile. The nuclear DNAs from the species differ by 2 mol% in guanine + cytosine content and exhibit low (25%) base sequence complementarity. Mating tests and DNA studies showed Candida obtusa var. arabinosa to be the imperfect form of P. mississippiensis. The new taxa showed no mating response with the phenotypically similar species P. rhodanensis and P. wickerhamii and only 4-8% DNA relatedness.

III. Department of Microbiology, Faculté de Pharmacie, Avenue Charles Flahault, 34060 Montpellier Cedex, France. Communicated by J. M. Bastide.

Below follow abstracts of two recent papers from this laboratory:

J. M. Bastide, E. Hadibi, and M. Bastide. Taxonomic Significance of Yeast Sphaeroplast Release after Enzymic Treatment of Intact Cells. JOURNAL OF GENERAL MICROBIOLOGY, 113, 147-153 (1979).

Treatment of whole yeast cells with a mixture of reducing agent and 1,3-β-glucanase isolated from Basidiomycete QM 806 led to the production of sphaeroplasts from ascomycetes, from some fungi imperfecti, but not from basidiomycetes. Association of 1,3-β-glucanase with a second enzyme, 1,4-α-glucanase, from Trichoderma viride was required for sphaeroplast release from some, but not all, basidiomycetes and fungi imperfecti. The ability of yeast cells to liberate sphaeroplasts following appropriate enzymic treatment is proposed as a taxonomic criterion for differentiating basidiomycetous from ascomycetous yeasts and for classifying fungi imperfecti yeasts.

* * *

M. Bastide, E. Hadibi, D. Scheiber, M. Miegeville, C. Vermeil, and J. M. Bastide. Release of Saccharomyces cerevisiae Protoplasts: Scanning Electron Microscope Study. ANN MICROBIOL., (Institute Pasteur), 130A, 419-433, 1979.

In a previous study, we described the minimal methodology used to obtain protoplasts from ascomycetous yeasts. Using a reducing agent associated with 1,3-β-glucanase at 26°C, protoplasts were invariably obtained. In the present study, we localized the disruption spots of the cell wall using the two same reagents. The observations were made with the scanning electron microscope. The disruption site was always in the subterminal region; and this very simple structure (proteins with disulphide bridges and 1,3-β-glucans) was opposite the birth scar.

The dissociation of the two reagents used for preparing protoplasts showed that a small part of the yeast population was able to release protoplasts with only glucanase. We believe these very sensitive yeasts (2 to 10% of the population) to be very young cells.

These disruption sites seemed very different from budding sites. They might be identical with elongation sites or with the opening in the ascus wall during germinating ascospore release.

IV. Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-110007, P.O. Box No. 2101, India. Communicated by H. S. Randhawa.

1. Two theses submitted in 1978 by research students of the department, particulars of which are given below, were approved by the University of Delhi for the award of Ph.D. in Medical Mycology/Microbiology.

   Studies on Cryptococcus neoformans and allied yeast-like fungi with special reference to ecology and reappraisal of laboratory diagnostic procedures. By D. K. Paliwal.

   Studies on yeasts and yeast-like fungi with special reference to their association with foodstuffs. By V. C. Misra.

2. A list of recently published papers follows:

Paliwal, D. K., and Randhawa, H. S. (1978). A rapid pigmentation test for identification of Cryptococcus neoformans. ANTONIE VAN LEEUWENHOEK 44, 243-246.

Paliwal, D. K., and Randhawa, H. S. (1978). Evaluation of a simplified Guizotia abyssinica seed medium for differentiation of Cryptococcus neoformans. JOURNAL OF CLINICAL MICROBIOLOGY 7, 346-348.

Paliwal, D. K., Randhawa, H. S., and Kowshik, T. (1979). A modified method for testing inositol assimilation by Cryptococcus species. CANADIAN JOURNAL OF MICROBIOLOGY 25, 1188-1190.

V. University of Lyon, Section Levures, Bât. 405, 45 Bd., 11 Nov. 1918, 69622 Villeurbanne Cedex, France. Communicated by M. C. Pignal.

The following articles have been accepted and will soon appear in MYCOPATHOLOGIA:

F. H. Jacob, M. Fauré-Reynaud, and M. C. Berton

Torulopsis pilati nov. sp., une nouvelle espèce de levure isolée de la litière du sapin Abies alba Mill.

Description is given of Torulopsis pilati, a new species of anascosporogenous yeast, isolated from Abies alba Mill. litter. A comparison is made with allied species.

* * *

M. C. Pignal and D. LaChaise

Les levures des Drosophiles de savane d'Afrique intertropicale (savanes de Lamto, Côte d'Ivoire).

Yeasts from crops of Drosophilidae (154 flies belonging to 22 species) of Lamto savannas (Ivory Coast) were studied. One hundred fifty yeast strains (belonging to 19 species) were isolated and studied. The most

frequently isolated yeasts were Hanseniaspora and Pichia membranaefaciens s.l.; these yeasts ferment and assimilate few of the carbon compounds. Some crops contained up to 100,000 yeast cells. In 25% of the flies, these yeasts belonged to several different species.

* * *

J. B. Fiol and S. Poncet

Systématique de genre Kluyveromyces: hybridations ADN-ADN.

Deoxyribonucleic acids of eight species of Kluyveromyces and one round-spored species of Pichia have been compared with P^{32} labelled DNA of K. marxianus in view of a systematic study by a method of hybridization in liquid media.

The species related to K. marxianus by currently employed systematic characters show generally a good nucleotide sequence homology (>70%), except K. wickerhamii.

On the contrary, K. africanus, K. phaffii, and P. abadiae show a very low percentage of hybridization with K. marxianus.

This molecular approach yields useful information to test the value of usual criteria of yeast systematics.

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

The following are recent reports from our laboratory:

Crow, S. A., S. L. Bell, and D. G. Ahearn. The Uptake of Aromatic and Branched Chain Hydrocarbons by Yeast. BOTANICA MARINA 22 (in press).

ABSTRACT

Studies of the hydrocarbon utilizing yeasts Candida maltosa and C. lipolytica have shown that both were capable of reducing recoverable amounts of branched chain and aromatic hydrocarbons in a mixture of naphthalene, tetradecane, hexadecane, and pristane (tetramethylpentadecane). Cells of C. lipolytica grown on either glucose or tetradecane were capable of binding (rendering unextractable) nearly 50% of the hydrocarbon mixture within three hours. In contrast cells of C. maltosa bound hydrocarbons only after growth on a hydrocarbon medium. Uptake of selected paraffinic hydrocarbons was not altered by the presence of naphthalene. Pristane uptake was concentration dependent for C. maltosa but not for C. lipolytica. Uptake, transport, and metabolism of hydrocarbons in C. maltosa and C. lipolytica differ.

* * *

Ahearn, D. G., and S. A. Crow. Yeasts from the North Sea and Amoco Cadiz Oil. BOTANICA MARINA 22 (in press).

ABSTRACT

The species and densities of yeasts isolated from North Sea waters before and after the production of oil were compared. Debaryomyces hansenii

was the predominant species in both sets of samples; but after oil production, Candida guilliermondii, a hydrocarbonoclastic yeast, was more commonly isolated, and the frequency of Aureobasidium pullulans decreased. Relatively few fungi were isolated from Amoco Cadiz oil collected 12 days after the start of the spill. The highest densities were obtained from the oil samples when they were first emulsified in a Tween 80-seawater solution. It is suggested that the high concentrations of aromatics in the unweathered Iranian crude, which constituted about half the spilled oil, were inhibitory to the yeast flora.

* * *

Nurudeen, Taofeeq A., and Donald G. Ahearn, 1979. The Regulation of Melanin Production by Cryptococcus neoformans. JOURNAL OF CLINICAL MICROBIOLOGY (in press).

ABSTRACT

Species of Filobasidiella, the agents of cryptococcosis, produced melanin-like pigments within 4-48 hours with diphenol, aminophenol, and diaminobenzene compounds as substrates. The rate of phenyloxidase activity was found to be regulated by glucose and nitrogen catabolite repression. Increased glucose concentration reduced pigmentation of all serotypes of Filobasidiella, whereas repression by nitrogen sources varied with the strain. Glutamine repressed the phenyloxidases of all isolates except those of serotype B, and $(NH_4)_2SO_4$ repressed the phenyloxidase of all isolates except that of serotype A. Tyrosine and glycine appeared to be near optimal for the phenyloxidase activity but not necessarily for growth of all strains examined. Representatives of serotype C were unique in that their phenyloxidase system was adaptive in contrast to the constitutive system found in the other serotypes.

VII. Department of Biology, Guru Nanak Dev University, Amritsar-143005, Punjab, India. Communicated by D. K. Sandhu.

Currently, we are working on the yeasts associated with pollinating bees, nectary of flowers, and fermented foods. We are specially interested in the symbiotic relationship, if any, between pollinating bees and yeasts.

Below follows an abstract of a paper which was recently presented at the 20th Conference of the Association of Microbiologists of India held from November 1-3 at Hissar, India. The paper has been submitted to the INDIAN JOURNAL OF MEDICAL RESEARCH for publication.

D. K. Sandhu, M. K. Warraich, and Sukhdev Singh. Sensitivity of yeasts isolated from clinical and saprophytic sources to aqueous extracts of garlic.

Sensitivity of 61 yeasts and yeast-like fungi was tested against aqueous garlic extract. Thirty-nine strains were isolated from vagina, cervix, and buccal swabs of vaginitis patients and included 26 strains of Candida albicans. The remaining 22 strains were isolated from pollinating bees. All except 2 strains of C. albicans proved sensitive and showed zones of inhibition up to 45 mm by the paper disc method. By the tube dilution method, also all the strains were inhibited and killed. The minimum inhibitory concentration (MIC) varied from 1:128 to 1:1024 while minimum lethal concentration (MLC) varied from 1:32 to 1:512 dilution of aqueous garlic extract.

VIII. Microbiology Department, Indian Drugs Research Laboratory (I D R A), 561/B Shivajinagar, Poona 411 005, India. Communicated by V. B. Rale.

The following two papers were read at the 1978 Annual Conference of Association of Microbiologists of India held at Baroda:

1. V. B. Rale and J. R. Vakil. Microbial Ecology of *Achras sapota* L. (Chikoo).

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

2. V. B. Rale and J. R. Vakil. The Yeasts of *Achras sapota* L. (Chikoo).

Systematic studies on the microflora of fruits have been few, except those concerned with the microbial spoilage of soft fruit both before and after harvest. In particular, their yeast flora have been largely ignored; where they have been studied, only total counts have been made. Apart from the detailed studies of yeasts on strawberries and other soft fruits by Buhagiar and his associates, little attention has been given to the yeast flora of soft fruits. A systematic program of isolation of yeasts from *A. sapota* L. fruits was undertaken by us over two consecutive years (1974-75) based on the isolation methods modified and developed in our Laboratory. The major yeasts isolated and identified were *Hansenula*, *Rhodotorula*, *Saccharomyces*, and *Sporobolomyces* species. Among the minor flora *Pullularia pullulans* (*Aureobasidium pullulans*) was the most dominant. The paper describes and emphasizes the utility of newer isolation and identification techniques for yeasts from fruits applicable to other ecosystems.

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

1. Professor Yuzo Yamada from Shizuoka University, Japan, will spend a sabbatical year, beginning January 1980, in my laboratory to participate in our research on the molecular taxonomy of yeasts.

2. The following are abstracts of publications in press:

Eric A. Johnson, Tomás G. Villa, Michael J. Lewis and Herman J. Phaff

Lysis of the Cell Wall of the Yeast *Phaffia rhodozyma* by a Lytic Enzyme Complex for *Bacillus circulans* WL-12

JOURNAL OF APPLIED BIOCHEMISTRY 1, (1979) in press

An enzyme complex that lysed whole cells and cell walls of the yeast *Phaffia rhodozyma* was obtained from the culture fluid of *Bacillus circulans* WL-12 grown on a medium containing cell walls of *P. rhodozyma*. A number of carbohydrates were tested for their ability to induce lytic enzymes, but only the cell wall-induced enzyme complex caused complete lysis. When the lytic enzyme complex was incubated for 12 hours with log phase yeast cells, spheroplasts of *P. rhodozyma* were produced. Hydrolytic enzyme activities detected in the enzyme mixture included β-(1→3)-glucanase, β-(1→6)-glucanase, α-(1→3)-glucanase, xylanase, chitinase. To specify those enzyme activities essential for cell wall hydrolysis, the lytic system was fractionated by ion-exchange and gel permeation chromatography and by affinity methods. It was concluded that β-(1→6)- and β-(1→3)-glucanases were most important in cell wall lysis and that α-(1→3)-glucanase played a minor role.

* * *

Gayle B. Fuson, C. W. Price, and H. J. Phaff

Deoxyribonucleic Acid Base Sequence Relatedness Among Strains of *Pichia ohmeri* that Produce Dimorphic Ascospores

INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY
January issue 1980

Strains of *Pichia ohmeri* that form either spherical or hat-shaped ascospores comprise a well-defined assemblage, distinct in deoxyribonucleic acid (DNA) base sequence and composition from other phenotypically similar yeasts. Thus, spore morphology, usually a stable systematic character, is not invariably reliable as a major taxonomic criterion among yeasts.

* * *

T. G. Villa and H. J. Phaff

Recovery of Invertase and Laminarinases from Industrial Waste Broths of Baker's Yeast

EUROPEAN JOURNAL OF APPLIED MICROBIOLOGY (in press)

SUMMARY

A procedure is described for the recovery of invertase and three laminarinases from commercial waste broths of baker's yeast. The procedure includes adsorption steps on DEAE-Sephadex A50, on concanavalin A-Sepharose 4B, and an additional gel exclusion chromatography step on Sephacryl S-200. The isolated laminarinases were: laminarinase I (exo-β-D-glucanase), laminarinase II [endo-β-(1-3)-D-glucanase], and laminarinase III (exo-β-D-glucanase). The efficiency of the main concentration step (DEAE-Sephadex A50) depended on the pre-dialysis of the crude broth. Thus, the recovery in invertase and laminarinase from nondialyzed samples was 7% and 16%, respectively. These values were increased to 62% and 100%, respectively, when the samples were previously dialyzed.

* * *

William T. Starmer (Biology Department, Syracuse University, Syracuse, New York 13210); Henry W. Kircher (Department of Nutrition and Food Science, University of Arizona, Tucson, Arizona 85721); and Herman J. Phaff (Department of Food Science and Technology, University of California, Davis, California 95616).

Evolution and Speciation of Host Plant Specific Yeasts

EVOLUTION (in press)

SUMMARY

In summary, three stages in the formation of species are discernible in cactus inhabiting yeasts. *Pichia heedii* may represent the initial stage in host race formation and exhibits a single gene change accompanying the shift of host plants within the same subtribe. *Pichia amethionina* could represent the final stage of host race formation and the beginning of the speciation stage. This species shows changes at two independent loci when it shifts between two related subtribes of cactus and exhibits post-mating breakdown for hybrids of the host varieties. The varieties differ by their resistance to triterpene glycosides found in one subtribe of columnar cacti. The difference is shown to be controlled by a single temperature sensitive gene and this genetic difference may be singularly responsible for the divergence of the two varieties. The two varieties of *Pichia opuntiae* may illustrate the final stage of the speciation process. This species shows considerable metabolic change when it shifts between hosts of different cactus tribes and demonstrates pre-zygotic barriers to gene exchange.

X. Department of Biology, Faculty of Medicine, J. E. Purkyně, University, 66243 Brno, Czechoslovakia. Communicated by Marie Kopecká.

The following papers have been published recently:

1. Nečas, O., Janisch, R.: Plastic deformation and surface shells of latex spheres after freeze fracturing. In: Viklický, V., and Ludvik, J. (eds.); Proc. of the Czech. Conf. E. Micr., Prague 1977, Vol A., p. 227-228, 1977.

2. Nečas, O., Gabriel, M.: Viability of spores after repeated freezing and thawing shocks. FOLIA MICROBIOLOGICA 23, 126-132, 1978.

3. Nečas, O., Gabriel, M.: The relationship between ultrastructure and survival of spores of *Rhizopus nigricans* damaged by freezing and thawing shocks. FOLIA MICROBIOLOGICA (in press).

4. Kopecká, M., Gabriel, M.: Staining cell nuclei in cells and protoplasts of yeasts, moulds, and green algae with the antibiotic lomofungin. ARCHIVES OF MICROBIOLOGY 119, 305-311, 1978.

5. Svoboda, A.: Fusion of yeast protoplasts induced by polyethylene glycol. JOURNAL OF GENERAL MICROBIOLOGY 109, 169-175, 1978.

6. Kreger, D. E., Kopecká, M.: The nature of the nets produced by protoplasts of *Schizosaccharomyces pombe* during the first stage of wall regeneration in liquid media. JOURNAL OF GENERAL MICROBIOLOGY 108, 269, 1978.

7. Kopecká, M., Farkaš, V.: RNA-synthesis and the formation of cell wall. Effect of lomofungin on regenerating protoplasts of Saccharomyces. JOURNAL OF GENERAL MICROBIOLOGY 110, 453-462, 1979.

8. Kopecká, M., Gabriel, M.: Staining cell nuclei in cells and protoplasts of yeasts, moulds, and green algae with the antibiotic lomofungin. ARCHIVES OF MICROBIOLOGY 119, 305-311, 1978.

9. Kreger, D. R., Kopecká, M.: On the molecular organization of chitin in regenerated and normal walls of Saccharomyces cerevisiae. Program and Abstracts of Vth Internat. Protoplast. Symp. Szeged, p. 145, 1979.

XI. Department of Microbiology, School of Dentistry, Kagoshima University, Ushuku-machi 1208, Kagoshima 890, Japan. Communicated by Michiko Tokunaga.

Our group will move into a new place (as indicated above) after April 1980.

Below follows abstracts of work conducted in our laboratory:

1. M. Niimi, K. Niimi, J. Tokunaga, and H. Nakayama[1]. Changes in the Levels of Cyclic Nucleotides and Dimorphic Transition in Candida albicans. (Submitted for publication in the JOURNAL OF BACTERIOLOGY.)

[1]Kyushu University

SUMMARY

The relationship between the levels of cyclic nucleotides and dimorphic transition in Candida albicans was examined. The results show that cells of this pathogenic fungus contain cyclic adenosine 3', 5'-monophosphate (cAMP) and cyclic guanosine 3', 5'-monophosphate (cGMP), the concentration of the latter being about one-tenth of that of the former in stationary phase cells of the yeast form. Our results further indicated that germ tube formation induced by incubation at 40°C followed a rise in cAMP concentration in the cell with no accompanying change in cGMP content. Cysteine, that suppressed germination, also reversed the increase in intracellular cAMP concentration. Dibutyryl cAMP (1mM) significantly promoted germination in proline medium at 32°C to 34°C. These results suggest that cAMP is related causally to the morphological transition in Candida albicans.

2. M. Tokunaga and J. Tokunaga. Immuno-Electron Microscopic Study of Localization of Polysaccharide Antigen in Regeneration of Candida albicans Protoplasts. (In preparation.)

3. M. Tokunaga and J. Tokunaga. Ultrastructural Host-Fungus Relationships: Fungi Pathogenic for Warm Blooded Hosts. (Presented at 11-ICMS München.)

Send correspondence to: M. Tokunaga

Present Address: Department of Bacteriology
Kyushu Dental College
Kokura, Kitakyushu 803, Japan
(January-March 1980)

New Address: Department of Microbiology
School of Dentistry
Kagoshima University
Ushuku-machi 1208
Kagoshima 890, Japan
(after April 1980)

XII. Carlsberg Foundation Biological Institute, DK-2200 Copenhagen, N., 16, Tagensvej, Denmark. Communicated by Graeme M. Walker.

The following are abstracts of two recent papers from our laboratory*. Part of the work for the second paper was carried out at The Biological Institute of the Carlsberg Foundation, Copenhagen, Denmark, and we thank its Director, Professor Erik Zeuthen, for helpful discussion.

1. "An Investigation into the Potential Use of Chelating Agents and Antibiotics as Synchrony Inducers in the Fission Yeast, *Schizosaccharomyces pombe*." JOURNAL OF GENERAL MICROBIOLOGY 114, 391-400 (1979).

ABSTRACT

Following the discoveries that the divalent cation ionophore A23187 and the divalent cation chelating agent EDTA can be used to synchronize yeast cell division, a study has been undertaken of the possible use of other chelating agents and antibiotics which interact with divalent cations in controlling cell division in the fission yeast *Schizosaccharomyces pombe*. All of the agents studied (five chelators and two antibiotics) arrested cell division in growing cultures of this yeast, but only sodium pyrophosphate and citrate induced synchrony of cell division. Novobiocin produced a transient inhibition of cell division, treated cells exhibiting "endogenous recovery" in the continued presence of the antibiotic. The results obtained are discussed in relation to the hypothesis that the concentration of intracellular Mg^{++} regulates cell division.

* * *

2. "Magnesium Ions and the Control of the Cell Cycle in Yeast." JOURNAL OF CELL SCIENCE, in press.

ABSTRACT

A study has been made of the role of Mg ions in cell division cycle control in the fission yeast, *Schizosaccharomyces pombe*, and the budding yeast, *Kluyveromyces fragilis*. Synchronization of cell division in these organisms can be induced by restoring Mg to Mg-exhausted cultures. In *S. pombe*, a correlation exists between the time taken for cells to enter the first synchronous division and the period of Mg-exhaustion. During short term incubation in Mg-deficient media, *S. pombe* cells are observed to continue growth in length, but they fail to make a cell plate and divide; long term Mg-deficiency results in the production of aberrant cell forms and a reduction in viability. Analysis of total cell Mg in cultures

*N.B. Both papers by: Graeme M. Walker[+] and John H. Duffus, Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh, Scotland.

[+]Present address: The Biological Institute of the Carlsberg Foundation, 16 Tagensvej, Copenhagen, N., DK 2200, Denmark.

of both S. pombe and K. fragilis, synchronized by various induction and selection procedures, revealed that there is a fairly steady fall in Mg concentration as cells grow, terminating in a rapid influx of Mg just

before cell division. This leads to the hypothesis that falling Mg concentration may act as a transducer of cell size, eventually triggering spindle formation and a membrane change which permits rapid uptake of Mg to a concentration which brings about spindle breakdown. The hypothesis was tested directly using the divalent cation ionophore, A23187, in the absence of Ca ions; the results obtained showed that a short pulse of A23187, very late in the cell cycle, accelerated cells into division and shortened the subsequent cycle. The hypothesis is discussed in relation to current models of cell cycle regulation.

XIII. Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan. Communicated by B. A. Siddiqi.

Below follows a summary of the paper that was presented at the International Symposium on New Researches in Biology and Genetics held at Islamabad, Pakistan, from December 8 to 13, 1979:

Testing of Some Food Colours and Flavours for the Induction of Mitotic Gene Conversion in Saccharomyces cerevisiae

B. A. Siddiqi and S. R. Chughtai

SUMMARY

Colouring and flavouring agents comprise two very important groups of food additives, but very little information is available about their genotoxicity. We have been investigating a number of food colours and flavours for the induction of mitotic gene conversion in the diploid strain D4 of Saccharomyces cerevisiae. Altogether, 26 food colours and 5 flavours, widely used in Pakistan and other parts of the world, have been tested. The strain D4 is heteroallelic at two unlinked loci, ade2 and trp5. The two alleles in the ade2 locus (ade2-2/ade2-1) and in the trp5 locus (trp5-12/trp5-27) do not complement. Thus, D4 requires both adenine and trytophan for growth. Gene conversion creates cells no longer requiring either adenine or tryptophan or, very rarely, cells requiring neither adenine nor tryptophan.

Food colours tested in the present investigation include Amaranth, Tartrazine, Brilliant Blue FCF, Sunset Yellow FCF, Indigotine, Ponceau 4R, Ponceau 6R, Violet Acid 5B (Benzyl violet 4B), New Blue VN (Blue VRS), Diazolrein Blue 6B (Chlorazol Sky Blud FF), Patent Blue, Metanil Yellow, Lemon Yellow, Carminic Acid, Bixine, Orange H. K., Mocca Brown, Madiera Colour, Syregul, Laksefarge, Seelachfarbe, Dunkel Blue, Kirsebaerrod, Raspberry Red, and Apple Green; and the flavours include Vanilla, Mango, Elachi, Kewra, and Afza.

The treatments were carried out at three different pH levels; 5.91, 6.96, and 8.05. Ethyl methanesulphonate (EMS) was used as a positive control. The results of this study indicate that the tested colour and flavour additives, without any further metabolic activation are non-mutagenic in diploid yeast. They failed to increase the frequency of mitotic gene conversion in the strain D4 of S. cerevisiae both at the ade2

and trp5 loci. Moreover, no significant cell killing was observed with any of the tested additives. EMS, which was used as a positive control, exhibited mutagenic (recombinogenic) activity. This is evidence for the reliability of the test system.

This work is in further progress, and many more food additives are being investigated for their recombinogenic effects.

XIV. Albert-Ludwigs Universität, Biochemisches Institut, D-7800 Freiburg I. Br., West Germany. Communicated by Dieter H. Wolf.

Recent research in our group is concerned with elucidation of the physiological function of the intracellularly occurring proteinases and proteinase inhibitors.

In addition to the published material, two review articles are in press:

1. D. H. Wolf and H. Holzer, in: Transport and Utilization of Amino Acids, Peptides, and Proteins by Microorganisms, (J. W. Payne, ed.) John Wiley and Sons, Chichester, in press "Proteolysis in Yeast".

2. D. H. Wolf, in: Advances in Microbial Physiology, (A. H. Rose and J. G. Morris, eds.) Vol. 21, Academic Press, London-New York (1980), in press "Control of Metabolism in Yeast and Other Lower Eucaryotes through Action of Proteinases".

Recently published:

3. D. H. Wolf and Claudia Ehmann (1979). Studies on a Proteinase B mutant of Yeast. EUROPEAN JOURNAL OF BIOCHEMISTRY 98:375-384.

4. Ingrid Beck, Gerald R. Fink[1], and Dieter H. Wolf. The Intracellular Proteinases and Their Inhibitors in Yeast: A Mutant with Altered Regulation of Proteinase A--Inhibitor Activity. JOURNAL OF BIOLOGICAL CHEMISTRY (submitted).

[1]Division of Biological Sciences
Cornell University
Ithaca, New York 14853

SUMMARY

Our genetic and biochemical studies on the biological function of the known intracellular proteinases and proteinase-inhibitors in yeast led to detection of a temperature and pH-sensitive mutant with altered regulation of proteinase A-inhibitor activity. Conditions, which in wild type yeast bring about a simultaneous de-repression of proteinases A and B and their respective specific inhibitors, lead to a 70% reduction in proteinase A-inhibitor activity in mutant cells without a change in the activities of proteinase A and B. The mutant shows also an early growth stop. The altered physiological and biochemical phenotypes of the mutant segregate together 2:2 in meiotic tetrads. Results obtained with this mutant indicate that proteinase A and its specific inhibitor are two independently synthesized polypeptide chains rather than one polypeptide chain, an inactive proteinase A-zymogen, which is then cleaved into proteinase A and its

inhibitory activity. In addition, the mutant indicates that regulation of proteinase A and proteinase A-inhibitor activity differs at least in one regulatory element, leaving the cell the possibility to probably regulate these proteins independently from each other. The mutant studies lead to the same conclusions concerning the regulation of proteinase A-inhibitor activity and proteinase B-inhibitor activity: At least one element seems to be different in their regulatory device. Whereas a surplus of proteinase A-inhibitor over proteinase A can be calculated for wild type cells, mutant cells exhibit an about 2.5 fold excess of proteinase A over proteinase A-inhibitor. Overall protein degradation is not altered in the mutant. However, tryptophan synthetase and proteinase B-inhibitor,--proteins sensitive to proteinase A attack _in vitro_--, show an enhanced loss of activity in the mutant. Immunological studies uncovered a two-fold accelerated degradation of proteinase B-inhibitor in mutant cells.

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

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

A. Technical publications:

1. P. Subrahmanyam, B. S. Rao, N. M. S. Reddy, M. S. S. Murthy, and U. Madhvanath. Modification of high LET radiation-induced damage and its repair in yeast by hypoxia. INTERNATIONAL JOURNAL OF RADIATION BIOLOGY (in press).

2. M. S. S. Murthy, V. V. Deorukhakar, and B. S. Rao. Hyperthermic inactivation of diploid yeast and interaction of damage caused by hyperthermia and ionising rAdiation. INTERNATIONAL JOURNAL OF RADIATION BIOLOGY 35, 333-341 (1979).

3. V. V. Deorukhakar, B. S. Rao, and U. Madhvanath. Interaction between hyperthermia and gamma radiation in diploid yeast. AMPI MEDICAL PHYSICS BULLETIN 4, 175-179 (1979).

4. N. M. S. Reddy, M. S. S. Murthy, and U. Madhvanath. Effect of sucrose solution on stationary and log phase diploid yeast: Shock excretion of UV absorbing cell constituents and modification of radiation sensitivity. INDIAN JOURNAL OF EXPERIMENTAL BIOLOGY 17, 549-552 (1979).

5. N. Sankaranarayan and M. S. S. Murthy. Testing of some permitted food colours for the induction of gene conversion in diploid yeast. MUTATION RESEARCH 78, 309-314 (1979).

6. B. S. Rao, N. M. S. Reddy, and U. Madhvanath. Genetic control of radiation sensitivity in diploid yeast. INEDIAN JOURNAL OF EXPERIMENTAL BIOLOGY (in press).

7. B. S. Rao, V. V. Deorukhakar, and U. Madhvanath. Effect of temperature and duration of hyperthermic treatment on the gamma radiation response of yeast cells. IBID (in press).

8. P. Subrahmanyam, B. S. Rao, and U. Madhvanath. Relative biological effectiveness of fast neutrons for induction of genetic damage in diploid yeast. IBID (in press).

B. Presentation of Reports at Conferences:

1. N. M. S. Reddy, B. S. Rao, M. S. S. Murthy, and U. Madhvanath. Genetic control of cellular recovery from radiation damage in diploid yeast Saccharomyces cerevisiae. 6th International Congress of Radiation Research, Tokyo, May 1979.

2. B. S. Rao, V. V. Deorukhakar, and U. Madhvanath. Role of cellular repair processes in the interaction between hyperthermia and radiation. 9th Annual Meeting of the Indian Biophysical Society, Gorakhpur, March 1979.

3. N. M. S. Reddy, M. S. S. Murthy, U. Madhvanath. Radiation response of diploid yeast Saccharomyces cerevisiae strains D4 and D7: Survival, mitotic gene conversion, and mitotic crossing over. IBID.

4. B. S. Rao and U. Madhvanath. Role on RAD 52 locus in budding cell resistance and liquid holding recovery in yeast cells exposed to gamma radiation. 20th Annual Conference of Association of Microbiologists of India, Hissar, November 1979.

5. V. V. Deorukhakar, B. S. Rao, and U. Madhvanath. Interaction between hyperthermia and radiation in repair deficient mutants of diploid yeast. IBID.

C. Summeries of recently completed research projects:

1. Factors influencing the induction and expression of mitotic gene conversion in diploid yeast by the promutagen cyclophosphamide.

Cyclophosphamide (CP), an alkylating agent, is used in the treatment of certain malignancies. Induction of mitotic gene conversion leading to arginine prototrophy in yeast by this drug has been studied. Cells in stationary and log phase were incubated with CP in phosphate buffer at different temperatures. The effect of metabolic activation by rat liver microsomes was also studied. The convertants were scored on synthetic medium without arginine (Arg^-) and with 0.8 mg (Arg^+) of arginine per litre of medium. When the treatment was in buffer, induced convertants could be observed only on Arg^+ medium but not on Arg^- medium. Induction of convertants at 37C was higher than at 30C. Under similar conditions of treatment, induction of prototrophs in log phase cells was higher than in stationary phase cells. When microsomes were added to the incubation mixture, the conversion rate was very high compared to buffer treatment. These results suggest that 1) trace amounts of arginine supplementation in the medium is required for the better expression of gene conversion; 2) selection of suitable temperature, medium, and cell stage is necessary for observing the maximum convertogenic activity of CP; and 3) CP requires one or more metabolic steps for activation. Yeast cells, by themselves, can bring about these reactions only to a limited extent when compared to rat liver microsomes.

2. Radiobiological aspects of radiation-sensitive mutants of diploid yeast exposed to gamma radiation: a) survival; b) liquid holding recovery (LHR) from the damage induced under euoxic and anoxic conditions; c) budding cell resistance; and d) oxygen enhancement ratio (OER).

Wild type diploid yeast strains 211 and X2180 and their mutants rad 2, 9, 6, 18, 50, 51, 52, and 57 were exposed to cobalt-60 gamma radiation in order to study their radiation sensitivity, oxygen enhancement ratio (OER), and recovery from potentially lethal damage during post irradiation liquid holding (LHR). Compared to wild type, the mutants showed increased sensitivity to radiation lethality as judged by D_o and D_q values of survival curves. Shoulder was absent in rad 51 and was reduced in the other mutants. OER was also reduced in rad 2, 9, 18, 50, 51, and 57 mutants liquid holding recovery (LHR) from the damage induced under euoxic and hypoxic conditions was present in wild type and most of the mutants, but was absent in rad 50, 51, and 52 strains. Logphase resistance was absent in rad 50, 51, and 52 mutants. These observations suggest that the expression of RAD 50, 51, and 52 loci play an important role in the post irradiation cellular recovery processes.

3. Hyperthermia.

Interaction between hyperthermia and gamma radiation has been investigated in wild type and radiation sensitive diploid yeast strains rad 9, rad 51, and rad 52. Hyperthermic inactivation curves of rad mutants indicate that there is no correlation between hyperthermic and radiation sensitivity. On exposure to nonlethal hyperthermic treatment (51°C for 30 minutes), both the wild type and sensitive strains showed sensitization to subsequent gamma exposure. Degree of sensitization as judged by DMF values at 10% survival level was around 1.6 for wild type strain and around 1.2-1.4 for repair-deficient strains.

Effect of pre- and post-irradiation hyperthermic treatment (nonlethal) on liquid holding recovery (LHR) has been investigated using the diploid yeast Saccharomyces cerevisiae BZ 34. Pre-irradiation hyperthermia (51°C for 20 minutes) did not interfere with LHR. Post-irradiation hyperthermia at 49°C for 1 hour slightly reduces the extent of LHR while treatment at 51°C for 20 minutes completely inhibited LHR.

XVI. Department of Biology, York University, Faculty of Science, 4700 Keele Street, Downsview, Ontario M3J 1P3, Canada. Communicated by Robert H. Haynes.

Below follows the abstracts of two recent papers from our laboratory:

Bernard A. Kunz, Mohammed A. Hannan[1], and R. H. Haynes. The Effect of Tumor Promoters on UV Induced Mutation and Mitotic Recombination in Saccharomyces cerevisiae. CANCER RESEARCH (in press).

[1]Environmental Mutagenesis-Carcinogenesis
Ephraim McDowell Cancer Network
University of Kentucky
Lexington, Kentucky 40506

ABSTRACT

Recently it has been suggested that mitotic recombination is involved in tumor promotion. On this basis, one might expect tumor promoters to be recombinagenic. D7 is a diploid strain of yeast in which both mutation and mitotic recombination can be measured. We have used this strain to assay the known tumor promoters iodoacetate, anthralin and TPA, and the cocarcinogen catechol for mutagenicity, recombinagenicity, and the ability to potentiate UV-induced genetic events. In the absence of pre-irradiation with UV, iodoacetate was found to be recombinagenic whereas catechol was mutagenic; however, in both cases the effects were small. Iodoacetate, anthralin, and catechol enhanced UV-induced mitotic crossing-over, colored colony formation, and mutation, while catechol also increased UV-induced gene conversion. We were unable to detect any genetic effect of TPA either in whole cells or in spheroplasts. Our results do not indicate any consistent correlation between tumor-promoting activity and the ability of an agent to induce mitotic recombination in yeast. However, the ability to potentiate UV-induced mutation and mitotic recombination may relect the cocarcinogenic activity of certain promoters.

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Friederike Eckardt, Soo-Jeet Teh, and R. H. Haynes. Heteroduplex Repair as an Intermediate Step of UV Mutagenesis in Yeast. GENETICS (in press).

ABSTRACT

We have measured ultraviolet light (UV) induced mutation frequencies in yeast in a forward nonselective assay system by scoring white adex ade2 double auxotrophs among parental red pigmented ade2 clones. The frequencies of sectored and pure mutant clones were determined separately. In excision, defective strains carrying the genes rad1-1, rad3-2, and rad4-4, as well as in the double mutants rad1-1rad3-2 and rad1-1rad4-4, considerably more sectored than pure clones, are induced in the low dose range; in repair competent strains, pure mutant clones substantially outnumber the sectored clones. These results can be explained on the basis of known differences in the timing of error-prone repair during the cell division cycle: that is, we assume that error-prone repair occurs primarily before replication in RAD wild type strains but after replication in excision-deficient mutants. It has been suggested that excision deficiency has a pleiotropic effect on heteroduplex repair and nucleotide excision repair; however, the high percentage (36.6%) of half sectored clones found in the rad1-1 strain is hard to reconcile with this hypothesis. We propose that heteroduplex repair occurs subsequent to error-prone repair in both excision-proficient and excision-deficient strains.

XVII. Institut National Agronomique, Chaire de Génétique, 16, Rue Claude-Bernard, Paris-5, France. Communicated by H. Heslot.

Below follows the abstract of a paper recently published in COMPTES RENDUES ACADÈMIE SCIENCE (Paris), série D (1979):

Cloned β-galactosidase gene of *Escherichia coli* is expressed in the yeast *Saccharomyces cerevisiae*

J. J. Panthier[1], P. Fournier[2]
H. Heslot[2], and A. Rambach[1]

[1]Institut Pasteur, and
[2]Laboratoire de Génétique, Institut National Agronomique, Paris, France

Early DNA cloning experiments were done with a prokaryote, the bacterium Escherichia coli. Recently the eukaryote, Saccharomyces cerevisiae, has emerged as a possible host vector for heterologous DNA cloning. It is, therefore, important to know if foreign structural genes can be expressed as their protein product in this eukaryotic host.

We have chosen to study the expression of the lac Z gene of E. coli into S. cerevisiae. This model gene was introduced in a chimeric plasmid. The resulting transformant yeast expresses a β-galactosidase. The specific activity is 2.5 international units in crude extracts.

The coli-in-yeast enzyme appears identical, by size and immunological criteria, to the E. coli enzyme. This means that the yeast expresses the bacterial gene and makes the correct protein product. It also means that other foreign DNA fragments inserted into the E. coli lac Z gene of this plasmid should be expressed in S. cerevisiae.

XVIII. Suntory Institute for Biomedical Research, 1-1-1 Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618, Japan. Communicated by Isamu Takano.

A. The following papers have been published recently or are in press. These papers are related to the technique of protoplast fusion or application of the technique to genetic analyses of the homothallic genes in Saccharomyces yeasts.

1. Isamu Takano and Kenji Arima, Evidence of the insensitivity of the α-inc allele to the function of the homothallic genes in Saccharomyces yeasts. GENETICS 91:245-254 (1979).

2. Kenji Arima and Isamu Takano, Multiple fusion of protoplasts in Saccharomyces yeasts. MOLECULAR AND GENERAL GENETICS 173:271-277 (1979).

3. Kenji Arima and Isamu Takano, Protoplast fusion in microorganisms. A review, HAKKO KOGAKU KAISHI 57:380-395 (1979), in Japanese.

4. Kenji Arima and Isamu Takano, Evidence for co-dominance of the homothallic genes, HMα/hmα and HMa/hma, in Saccharomyces yeasts. GENETICS in press (September issue, 1979).

In paper Number 4, we have proposed the revised nomenclature for the homothallic genes. The correspondence between the old and revised genetic symbols for the homothallic genes is as follows:

Old Symbol	Revised Symbol
HO	HO
ho	ho
HMα	HMRa
hmα	HMRα
HMa	HMLα
hma	HMLa

Small letters, hmr and hml, can be assigned for recessive or defective alleles of the HMR and HML loci, respectively.

B. The following papers by Takehiro Oshima and Isamu Takano have been submitted to GENETICS for publication:

1. Mutants showing heterothallism from a homothallic strain of *Saccharomyces cerevisiae*.

2. Duplicated genes producing transposable controlling elements for the mating-type differentiation in *Saccharomyces cerevisiae*.

3. Mating-type differentiation by transposition of controlling elements in *Saccharomyces cerevisiae*.

ABSTRACTS

1. Mutants showing heterothallism were isolated from EMS-treated ascospores of a perfect homothallic strain having the HO HMRa HMLα genotype of *Saccharomyces cerevisiae*, and they were divided into seven different classes of mutation: I is an HO to ho mutation, II is a mutation at a new gene which is unlinked to the HO gene and controls the expression of the HO allele (designated csm mutation), III and IV are mutations at the HMRa and HMLα loci, respectively, V and VI are mutations at the a and α mating-type alleles, respectively, and VII is a mutation blocking specifically the expression of a or α mating type (tentatively designated mex mutation). One of the temperature-sensitive mutants characterized was involved in the VII class of mutation.

2. Mutation of HMLα was classified into two groups: one is a mutation from the original functional allele to the alternate functional allele (functional mutation); i.e., phenotypically HMLα to HMLa mutation, and the other is a mutation from the functional allele to the nonfunctional allele (nonfunctional mutation); i.e., HMLα to hmlα mutation. All mutants tested of HMRa gene were due to a functional mutation; i.e., phenotypically HMRa to HMRα mutation. It was strongly suggested that the functional mutation is caused by transposition of a genic product (a controlling element) from an HM locus into the other HM locus.

3. Nonfunctional mutants of HMLα were divided into two types: one is a leaky mutation defective in producing a normal element for α mating-type information, and the other is a mutation giving a sterile α mating-type (α-ste) information. In the latter type of mutation, switches of mating types, a to α-ste and α-ste to a switches, were observed in the manner similar to that of a wild-type homothallic cell.

These observations strongly suggested that transposable controlling elements participate in the differentiation of the mating-type alleles by the homothallic genes in Saccharomyces yeasts.

C. Below are abstracts of our recent works which are in preparation for publication:

1. We have investigated 2 μm-size of DNA plasmids in various Saccharomyces brewing yeasts and found four types of plasmids having mutually different cleavage sites by restriction enzymes. One of them was a new type of 2 μm-size plasmid which had one EcoRI site, three Hind III sites, two HpaI sites, and one PstI site. The other three had the physical map similar to either one of SCP1, SCP2, or SCP3, reported by Cameron, Philippsen and Davis (NUCLEIC ACID RESEARCH 4:1429, 1977). 2 μm-size plasmids were found in whisky, wine, beer, and baker's yeasts, and in genetic strains but not observed in 22 sake yeasts tested except one isolated from sake-mash. All the plasmids observed consisted of two forms.

2. We have studied mutants bearing a recessive mutation which blocks the expression of the homothallism gene, HO, and identified three mutant alleles, csm1, csm2, and csm3, all of which were unlinked to the HO allele and to each other. These csm mutations also seemed to be involved in meiotic processes. In an a/α diploid strain, homozygous configuration for csm1 allele remarkable diminished sporulation ability of the strain while homozygous configuation for csm2 and csm3 alleles promoted the sporulation processes and produced asci containing more than four spores. Diploid strains heterozygous for these csm alleles showed normal sporulation ability. These observation suggested that excision/recombination of DNA is involved in the homothallism gene system of Saccharomyces yeasts.

XIX. The University of Sussex, Biology Building, Falmer, Brighton, BN1 9QG, England. Communicated by Andrew J. Morgan.

Below follow abstracts of two papers that have been submitted to MOLECULAR AND GENERAL GENETICS:

Andrew J. Morgan and Peter A. Whittaker

Protoplast Fusion in a petite-Negative Yeast
Kluyveromyces lactis I
Protoplast Formation, Regeneration, and Fusion

SUMMARY

Stable "hybrids" of Kluyveromyces lactis have been constructed by protoplast fusion between heterothallic strains of identical mating type. The fact that these "hybrids" are incapable of sporulating suggests that the mating-type alleles of this yeast affect not only conjugation but also the process of sporulation. In this respect, K. lactis resembles several other species of yeast.

Protoplast fusion has also been found to be effective for the isolation of stable "hybrids" from crosses between wild-type (ρ^+) cells and _petite_ (ρ^-) cells of like mating type.

* * *

Andrew J. Morgan, Aurora Brunner, and Peter A. Whittaker

Protoplast Fusion in a _petite_-Negative Yeast
Kluyveromyces lactis II
Biochemical, Cytological, and Genetical
Characterization of Fusion Products

SUMMARY

In _Kluyveromyces lactis_, protoplast fusion between heterothallic strains of like mating-type results in the formation of stable hybrids that are incapable of sporulation. The majority of these sporulation-deficient hybrids have been shown to be of diploid size and to carry the diploid DNA content. Nuclear staining reveals the presence of only one nucleus per hybrid cell. Karyogamy has been further demonstrated by the recovery of parental and recombinant segregants through haploidisation. Protoplast fusion also leads to the formation of a small proportion of haploid recombinants.

For genetic analysis, sporulation-competent hybrids have been constructed by protoplast-fusion between homothallic strains. These "homothallic hybrids" can be distinguished from sexually produced diploids by their low levels of sporulation (<2%). Tetrad analysis shows a 2:2 segregation of nuclear markers.

The evidence suggests that hybrids isolated by protoplast fusion are predominantly diploid.

XX. _Institut für Mikrobiologie u. Biochemie, Lehrstuhl für Biochemie, der Universität Erlangen-Nürnberg, Egerlandstraße 7, 852 Erlangen, West Germany. Communicated by E. Schweizer._

The following article has been submitted for publication:

Fatty Acid-Requiring Mutant of _Saccharomyces cerevisiae_

Defective in Acetyl-CoA Carboxylase

Rainer Roggenkamp, Sosaku Numa[1], and Eckhart Schweizer

[1]Department of Medical Chemistry
Kyoto University Faculty of Medicine
Kyoto 606, Japan

ABSTRACT

The isolation and biochemical properties of a _Saccharomyces cerevisiae_ mutant (_acc 1-167_) defective in acetyl-CoA carboxylase activity is described [acetyl-CoA:carbondioxide ligase (ADP forming) EC 6.4.1.2]. The mutant is deficient in _de novo_ long chain fatty acid biosynthesis and specifically requires a saturated fatty acid of 14-16 C-atoms chain length for growth.

While fatty acid synthetase levels were normal, the acetyl-CoA carboxylase specific activity of the purified enzyme was reduced to approximately 5 percent compared to wild type yeast. Upon sodium dodecyl sufate polyacrylamide gel electrophoresis, the purified mutant enzyme migrated as a single band and was essentially indistinguishable from the wild type enzyme. The study of acetyl-CoA carboxylase partial activities revealed that the biotin binding capacity and the transcarboxylase partial activity were unaffected while the biotin carboxylase component enzyme exhibited less than 10 percent of wild type specific activity. This biotin carboxylase mutational deficiency could be ascribed to a more than 10-fold reduction of V_{max} and to a comparable increase in the K_M-value for ATP which was accompanied by an increased requirement for Mg^{2+}. It is concluded that acc 1-167 contains a structural gene mutation in the biotin carboxylase domain of acetyl-CoA carboxylase.

XXI. Division of Biological Sciences, National Research Council, Ottawa, Canada K1A 0R6. Communicated by G. B. Calleja.

The following are abstracts of recent papers from our laboratory:

Calleja, G. B., Zuker, M., and Johnson, B. F. (1979). Estimation of meiosis and sporulation efficiencies in the fission yeast by ascus analysis. GENET. RES. CAMB., 33, 109-119.

Populations of linear asci are classified according to the number of spores in an ascus. The resultant five numerical classes are further classified into ten spatial classes according to the arrangement of the spores in an ascus and, by inference, into ten historical classes according to the number and origins of failures during the developmental process. An analysis of the observed frequencies of numerical classes allows derivation of the efficiencies of the first meiotic division, the second meiotic division and sporulation in a fission yeast. The analytical method may be useful in locating the site of action of sporulation inhibitors and in identifying meiosis mutants from sporulation mutants.

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Calleja, G. B., Johnson, B. F., Zuker, M., and James, A. P. (1979). The mating system of a homothallic fission yeast. MOLECULAR GENERAL GENETICS, 172, 1-6.

Exposed to iodine vapors, colonies of a homothallic strain of Schizosaccharomyces pombe were of two classes: P, with many black streaks; and d, with scarcely any. Contiguous P and d colonies, but not contiguous P colonies nor contiguous d colonies, gave the iodine junction reaction, a black line along the common boundary of two colonies. Neither class could be purified. On replating, a P colony gave rise to a P plate, which contained mostly P but also d colonies; a d colony gave rise to a d plate, which contained mostly d but also P colonies. The P/d colony ratio of a fresh isolate (if isolated as a P colony) was very high or (if isolated as a d colony) very low. It fell, if initially high, or rose, if initially low, on subsequent replatings of the same isolate. Maintained for many generations, an isolate attained a fairly constant P/d colony ratio that was less than unity. Tetrad analysis showed 2:2 segregation of the classes. We conclude that a homothallic clone is a mixture of two types of cells:

P, which gives rise to a P colony, and d, to a d colony. The two types are sexually complementary and interconvertible. The rate of interconversion of P to d exceeds that of d to P by a factor of about 2.

* * *

Johnson, B. F., Calleja, G. B., Boisclair, I., and Yoo, B. Y. (1979). Cell division in yeasts, III. The biased, asymmetric location of the septum in the fission yeast cell Schizosaccharomyces pombe. EXPERIMENTAL CELL RESEARCH (in press).

Living, dividing, log-phase fission yeast cells (178 pairs) were photographed by fluorescence microscopy of their fluorochromed walls. Analysis of the lengths, volumes, and fission scar distributions of these cells led to the following conclusions: the new septum is sited asymmetrically at division by length parameters, and the asymmetric site is biased toward the newer end (that end generated by the previous cell division) of the dividing cells. The volumes of the resultant sibs, however, are equal. Some rather simple models for siting of the septum are presumed untenable on the basis of the evidence.

* * *

Calleja, G. B., and Johnson, B. F. (1979). Temperature sensitivity of flocculation induction, conjugation, and sporulation in fission yeast. ANTONIE VAN LEEUWENHOEK, JOURNAL OF MICROBIOLOGY AND SEROLOGY (in press).

Homothallic cultures of Schizosaccharomyces pombe, anaerobically grown to stationary phase in broth at 32°C, were induced by aeration to flocculate. Flocculation was followed by copulation, conjugation, zygote formation, meiosis, and sporulation. Cultures grown to stationary phase at 32°C and then aerated at 37°C did not sporulate. Grown to stationary phase at 37°C, cultures were not immediately inducible when aerated at 32°C. To identify which events in the developmental sequence were thermosensitive, we grew and induced cultures at 32°C and then shifted them at various times to 37°C. We observed the following events to be thermosensitive: development of respiratory sufficiency, readiness (inducibility of a culture within 1 hour), flocculation induction, copulation, conjugation, and early sporulation (including meiosis). Respiration, flocculation, and spore maturation were thermoresistant. Conjugation-induced lysis and post-developmental deflocculation were enhanced at 37°C.

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

The following papers have been published:

1. Jens G. Litske Petersen, Lauritz W. Olson, and Denise Zickler. Synchronous Sporulation of Saccharomyces cerevisiae at High Cell Concentrations. CARLSBERG RESEARCH COMMUNICATIONS 43. 241-253 (1978).

A procedure for the sporulation of yeast is described which combines good synchrony and high cell concentration, important features in biochemical studies of meiosis. Meiosis and ascospore formation were followed by a modified Feulgen staining procedure, which allowed the detection of chromosomes during meiotic prophase. The sporulation process was also characterized with respect to nucleic acid synthesis, commitment to meiosis, and the segregation of the mutant and wild type alleles at the ade2 locus.

2. Steen Holmberg. Isolation and Characterization of a Polypeptide Absent from Non-flocculent Mutants of Saccharomyces cerevisiae. CARLSBERG RES. COMMUN. 43, 401-413 (1978).

Alkaline cell extracts obtained from while cells of a flocculent strain of Saccharomyces cerevisiae, containing the dominant gene for flocculence FLO4, and a non-flocculent mutant (FLO4, fsu1) were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The mutant lacked a low molecular weight (13,000) polypeptide present in the extract from the parent strain. This polypeptide difference was also observed when five other independently isolated non-flocculent mutants of the parent strain were analyzed. One of these five mutants was characterized genetically and also in this case was non-flocculence shown to be due to an unlinked suppressor mutation of FLO4. This suppressor gene is designated fsu2. Analysis of petites with different flocculation phenotypes further extended the correlation between non-flocculence and the absence of the polypeptide.

The polypeptide was isolated by gel filtration in the presence of sodium dodecyl sulfate followed by ion-exchange chromatography on DEAE-Cellulose. By electrophoretic analysis, the purity of the preparation was estimated to be 95%. From amino acid analysis, the polypeptide was calculated to consist of 121 residues with a molecular weight of 12,900.

Differential extraction of proteins iodinated in situ by lactoperoxidase suggested an external location of the polypeptide.

3. Jens G. Litske Petersen and William F. Sheridan. High Mobility Group Proteins in Yeast. CARLSBERG RES. COMMUN. 43, 415-422 (1978).

A class of low molecular weight proteins was extracted from isolated yeast nuclei by 5% (w/v) perchloric acid followed by fractional acetone precipitation. Their solubility properties, electrophoretic mobility, and the amino acid composition were similar to those of the high mobility group (HMG) chromosomal proteins of higher eukaryotes.

4. Morten C. Kielland-Brandt, Jens G. Litske Petersen, and Jørn Dalgaard Mikkelsen. Mutants in the Biosynthesis of Isoleucine in a Non-Mating, Non-Sporulating Brewing Strain of Saccharomyces carlsbergensis. CARLSBERG RES. COMMUN. 44, 27-36 (1979).

Mutants resistant to 2-amino-3-(methylthio)butanoic acid (thiaisoleucine) were selected from a non-mating, non-sporulatiing brewing strain of Saccharomyces carlsbergensis after treatment with N-methyl-N'-nitro-N-nitrosoguanidine. One closer studied mutant, C77-T70 had a threonine deaminase activity which was less sensitive to L-isoleucine than that of the parent strain. The inhibition with varying concentrations of L-isoleucine showed that part of the activity was as sensitive as that of the parent strain, suggesting that the mutant is heterozygous for a dominant change in the structural gene for threonine deaminase. Of twelve mutants selected on 25 mM-thiaisoleucine, all are believed to have undergone a change similar to that of C77-T70, since their threonine deaminase activity was partly resistant to 1 mM-L-isoleucine like that of C77-T70. Four such mutants were compared to the parent strain for the production of fusel alcohols during aerobic growth in minimal medium. The synthesis of 2-methyl-1-butanol (D-amyl alcohol) was 2-5 times higher in the mutants than in the parent strain, while the

syntheses in the mutants of 3-methyl-1-butanol (isoamyl alcohol) and 2-methyl-1-propanol (isobutanol) were similar or slightly reduced compared to the level found in the parent strain.

5. Morten C. Kielland-Brandt, Torsten Nilsson-Tillgren, Steen Holmberg, Jens G. Litske Petersen, and Bo A. Svenningsen. Transformation of Yeast without the use of Foreign DNA. CARLSBERG RES. COMMUN. 44, 77-87 (1979).

Isolated circular molecules of the yeast plasmid 2-micron DNA were converted to linear molecules with restriction endonuclease PstI and ligated with T4 DNA ligase to PstI restriction fragments of total yeast DNA. A haploid strain of Saccharomyces cerevisiae carrying a deletion in the his4 locus was transformed with the ligated DNA mixture to histidine prototrophy. One unstable histidine prototrophic transformant was obtained. Grown in the absence of histidine, 70-75% of the cells were auxotrophic; and this number increased in nonselective medium. The histidine auxotrophic variants carried a deletion in the his4 locus which had patterns of complementation and UV-induced mitotic recombination identical to the original his4 deletion. When the transformant was crossed to a his4 strain and sporulated, the unstable histidine prototrophy segregated in a non-Mendelian way: All five possible segregations from 0:4 to 4:0 were observed. When strains carrying the transformed character were crossed to a his4 karl strain, a low frequency of cytoduction of the unstable histidine prototrophy was observed. Nucleic acid from the transformant was able to transform a strain which carried another deletion in the his4 locus. Treatment of the transformant with ethidium bromide caused an extensive induction of petites without any observable change in the frequency of histidine prototrophic cells.

It is concluded that the HIS4 gene function in the transformant is not stably associated with any chromosome. We take the instability as indication that only one or a few copies of the gene conferring the prototrophy are present in the prototrophic cells. The data are consistent with the assumption that the transformant contains a 2-micron DNA in which is inserted a chromosomal DNA region containing the HIS4 gene. A derivative of the transformant with increased stability has been isolated.

6. Jens G. Litske Petersen, Morten C. Kielland-Brandt, and Torsten Nilsson-Tillgren. Protein Patterns of Yeast during Sporulation. CARLSBERG RES. COMMUN. 44, 149-162 (1979).

High resolution two-dimensional gel electrophoresis was used to study protein synthesis during synchronous meiosis and ascospore formation of Saccharomyces cerevisiae. The stained protein patterns of samples harvested at any stage between meiotic prophase and the four-spore state in two sporulating strains showed the same approximately 250 polypeptides. Of these, only a few seemed to increase or decrease in concentration during sporulation. The characteristic pattern of sporulating yeast was identical to the pattern of glucose-grown stationary yeast cells adapted to respiration. The latter type of cells readily initiates meiosis when transferred to sporulation medium. This pattern differed from the protein patterns of exponentially growing cells in glucose or acetate presporulation medium. Five major proteins in stationary and sporulation yeast cells were not detected in either type of exponential culture. Two-dimensional autoradiograms of [^{35}S]methionine-labelled yeast proteins revealed that some proteins were preferentially labelled early during sporulation, while other proteins were labelled at later stages. These

patterns differed from the autoradiograms of exponentially growing yeast cells in glucose presporulation medium in a number of spots. No differences were observed when stained gels or autoradiograms of sporulating cultures and non-sporulating strains in sporulation medium were compared.

XXIII. Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. Communicated by Arnold L. Demain.

Below follows the abstract of a dissertation submitted to the Department of Nutrition and Food Science on June 27, 1979, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Emmanuel Owei Denenu

Genetic De-regulation of the Aromatic Amino Acid Pathway in a Methanol-Utilizing Yeast *Hansenula polymorpha*, DL-1

The elimination or circumvention of control mechanisms through the isolation of de-regulated mutants is a useful approach in the utilization of microorganisms for the production of microbial metabolites. This strategy was applied in this work to the aromatic amino acid biosynthetic pathway in *Hansenula polymorpha*. The overall pathway leads to tryptophan, phenylalanine, and tyrosine; in this work, the emphasis was placed on the tryptophan branch.

Different classes of mutants, de-regulated to enhance the flow of aromatic intermediates through the tryptophan biosynthetic branch, were obtained. Of special interest was the behavior of the de-regulated mutants with respect to the accumulation of tryptophan and/or tryptophan metabolites in the extracellular fermentation broth.

Key regulatory enzymes of aromatic amino acid biosynthesis were studied in *Hansenula polymorpha*, DL-1. It was found that the activity of DAHP synthetase (EC 4.1.2.15) is subject to an additive type of feedback inhibition by phenylalanine and tyrosine but not by tryptophan. Exogenous aromatic amino acids, singly or in combinations, did not repress the synthesis of this enzyme. It was also found that anthranilate synthetase (EC 4.1.3.27) activity is inhibited by tryptophan.

Using a sequential resistant development program, three mutants resistant to an antimetabolite of tryptophan (5-fluoro-tryptophan) were isolated. Wild type and 5-fluorotryptophan-resistant mutants showed greater sensitivity to growth inhibition by the analogue in media containing methanol rather than glucose as the sole carbon-energy source. Each 5-fluorotryptophan-resistant mutant produced a higher amount of total indole derivatives than its predecessor. In the first two mutants, the overproduction of tryptophan and its metabolites was due primarily to a partial desensitization of anthranilate synthetase to feedback inhibition by tryptophan. On the other hand, the third mutant differed from its parent by an apparent de-repression of anthranilate synthetase. The specific and volumetric productivities of the three mutants were 15-60 and 10-20 times higher, respectively, than those of the wild type.

Two other classes of mutants were also isolated: (i) a mutant resistant to anthranilate (an inhibitory intermediate in the tryptophan

branch), and (II) a phenylalanine plus tyrosine bradytroph. Each of these mutant types produced higher extracellular titer of total indole derivaties than its immediate parent.

Yeast extract (250 mg/l) significantly stimulated the production of total indole derivatives by Hansenula polymorpha strains. When genetic de-regulation was coupled with media manipulations to maximize phenotypic expression, total production of indole derivatives showed 70 to 80-fold increases in the latter de-regulated strains when compared to the original production levels of the wild type.

Three tryptophan catabolites (tryptophol, indoleacetic acid, and indoleacetaldehyde) were identified in the extracellular broths of the mutants but not in that of the wild type. These same three compounds were produced by resting cells from added tryptophan or tryptamine. The mutants also produced two to three additional non-indolic compounds which were not produced by the wild type. For the mutants, tryptophan is only a minor product, with tryptophol being the major broth metabolite. Among the de-regulated strains, there were not only differences in the relative proportions but also in the spectrum of the compounds produced.

Thesis Supervisor: Arnold L. Demain, Professor of Industrial Microbiology.

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

Ian Salmon and Robert K. Poole. The Mitochondrial Cytochromes of an Unusual Budding Yeast Sterigmatomyces halophilus: Spectral Characterization Exploiting Fourth Order Finite Difference Analysis. JOURNAL OF GENERAL MICROBIOLOGY (accepted for publication).

ABSTRACT

Low-temperature difference spectra of gradient-purified mitochondria from Sterigmatomyces halophilus revealed the presence of a, b, and c-type cytochromes, spectrally similar to those of other yeasts. Fourth-order finite difference analysis resolved the broad α-band of b and c-type cytochromes into eight peaks. Absorption maxima at about 559,543.5 and 547.5 nm were attributed to one or perhaps two cytochromes c that are loosely bound to the mitochondrial membrane. Cytochrome c_1 was identified at 550.5-551.5 nm. Maxima at about 554, 556, 559, and 562 nm were attributed to three or four distinct b type cytochromes on the basis of their differential reduction by NADH, dithionite, or ascorbate plus NNN'N'-tetramethyl-p-phenylenediamine in the absence or presence of antimycin. Difference spectra in the presence of CO or cyanide indicated the presence of cytochrome a (600 nm) and a_3 (608 nm). Finite difference analysis of the cytochrome oxidase peak centered at 600 nm revealed two components; the major component at 600 nm was identified as ctyochrome a and the minor component at 605 nm as cytochrome a_3. A further CO-binding haemoprotein was present, tentatively identified as cytochrome o.

XXV. Laboratoire d'Enzymologie, Centre National de la Recherche Scientifique, 91190 Gif sur Yvette, France. Communicated by J. Schwencke.

Below follows the abstract of a paper recently published in ARCHIVES OF MICROBIOLOGY 121 (1979) 169-175:

This paper is the result of work done in collaboration with the group of Dr. Wiemken at Zürich, Switzerland.

Sequestration of Arginine by Polyphosphate in Vacuoles of Yeast (*Saccharomyces cerevisiae*)

M. Durr[1], K. Urech[1], Th. Boller[1], A. Wiemken[1]
J. Schwencke[2], and M. Nagy[2]

[1]Institut für Allgemeine Botanik der ETH, Labor für Pflanzenphysiologie, Sonneggstr. 5, CH-8092 Zürich, Switzerland

[2]Laboratoire d'Enzymologie, CNRS, F-91190 Gif-sur-Yvette, France

ABSTRACT

Isolated and purified vacuoles from yeast protoplasts contain the bulk of the cellular pool of arginine. The arginine is firmly retained in the isolated vacuoles despite the presence of a permease which mediates arginine diffusion through the vacuolar membrane (Boller et al., 1975). It is shown, mainly by equilibrium dialysis on vacuolar extracts, that the retention of arginine in the vacuoles is due to binding by polyphosphate. The polyphosphate appears to be located exclusively in the vacuoles. Enzymes hydrolysing polyphosphate are also located in the vacuoles. Isolated vacuoles from arginine grown cells contain about three times as much polyphosphate as vacuoles from ammonium grown cells; the vacuolar pool of arginine is correspondingly greater. Thus, there seems to be a close correlation between the storage of arginine and polyphosphate. This confirms the observation that under conditions provoking "polyphosphate overcompensation" (Liss and Langen, 1962) the accumulation of enormous quantities of polyphosphate is associated with that of corresponding quantitites of arginine, provided this amino acid is supplied in the medium. Yet, under certain growth conditions, the cells are able to store, and to mobilize, both arginine and polyphosphate independently.

XXVI. Instituto de Enzimologia del C.S.I.C., Facultad de Medicina de la Universidad Autónoma, Arzobispo Morcillo s/n., Madrid-34, Spain. Communicated by Carlos Gancedo.

As a result of our search for control mechanisms for the degradation of protein in yeast, the following papers have been completed recently:

Juana M. Gancedo and Carlos Gancedo. "Inactivation of Gluconeogenic Enzymes in Glycolytic Mutants of *Saccharomyces cerevisiae*." EUROPEAN JOURNAL OF BIOCHEMISTRY, in press (1979).

SUMMARY

Yeast mutants blocked at different steps of the glycolytic pathway have been used to study the inactivation of several gluconeogenic enzymes upon addition of sugars. While phosphorylation of the sugars appears a requisite for the inactivation of fructose 1,6-bisphosphatase and phosphoenolpyruvate carboxykinase, malate dehydrogenase is inactivated by fructose in mutants lacking hexokinase. The normal inactivation elicited

by glucose in a mutant lacking phosphofructokinase indicates that the process does not require metabolism of the sugar beyond hexose monophosphates. A possible role for ATP in the inactivation process is suggested.

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Shigehiro Funayama, Juana M. Gancedo, and Carlos Gancedo. "Turnover of Yeast Fructose 1,6-bisphosphatase in Different Metabolic Conditions". In preparation.

SUMMARY

Earlier work from our laboratory demonstrated that addition of glucose to yeast growing on noncarbohydrate carbon sources sharply reduces the levels of fructose 1,6-bisphosphatase (FbPase). Recent work indicates that the decrease in the levels of FbPase is accompanied by a parallel decrease of cross-reacting material to specific FbPase antibody. Use of the specific antibody shows that the loss of activity is irreversible and that its reappearance requires de novo synthesis of protein. It has also been shown that the protein is highly stable during active growth on ethanol.

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Juana M. Gancedo, Soledad López, and Francisco Ballestero. "Calculation of half lives of proteins in vivo--Heterogeneity in the rate of degradation of yeast proteins." In preparation.

SUMMARY

A method is given for the calculation of half lives of proteins in vivo from the measurement of the decrease of radioactivity in pulse-labelled proteins along time. This method could be particularly useful for the study of the degradation of proteins in liver or brain.

The method applied to growing yeast indicates that there are two major classes of protein. The class with low turnover constitutes the bulk of yeast protein and has a half life of 150 hours in a medium with glucose or galactose and of 50 hours in a medium with ethanol. The class of proteins with high turnover (half life around 1 hour) represents from 1% of total protein in yeast growing on glucose to 7% in yeast growing on ethanol.

It is shown that some proteins which are de-repressed during growth on ethanol or induced during growth on galactose are particularly susceptible to degradation in a medium which contains glucose.

It is proposed that protein degradation is regulated by a coarse control at the level of protease activity and a fine control on the conformation of some particular proteins.

* * * YEAST 12-79

In continuation of her previous work on yeast energetics, Rosario Lagunas has published the following paper: "Energetic Irrelevance of Aerobiosis for S. cerevisiae Growing on Sugars." MOLECULAR AND CELLULAR BIOCHEMISTRY (1979) 27, 139-146.

SUMMARY

The net benefit that Saccharomyces cerevisiae obtains from aerobiosis as compared to anaerobiosis has been studied. For this purpose, yeasts with different respiratory capacities have been obtained by growing them in batch cultures on different substrates. Even with sugars with low catabolite repression effect, as is the case of galactose, aerobiosis increased the growth rate and the growth yield by less than two-fold. These variations, which are much lower than the expected considering the actual oxygen utilization, indicate that either the amount of ATP produced in respiration is much lower than the theoretically expected or a much greater expenditure of ATP occurs in aerobic than in anaerobic growth. The results show that S. cerevisiae obtains only a slight benefit from aerobiosis when growing on sugars at the relatively high concentration prevailing in its natural habitats.

The inhibition of sugar consumption rate by aerobiosis (Pasteur effect) has also been studied. Pasteur effect was almost unnoticeable during growth on any tested sugar and very low during ammonia starvation. These results contrast with the general belief that Pasteur effect is a quantitatively important phenomenon in yeast. It is concluded that the relevant observation of Louis Pasteur has little relationship with the phenomenon that bears his name.

XXVII. Lvov Branch of A. V. Palladin Institute of Biochemistry, Ukrainian SSR Academy of Sciences, 290005 L V O V, Drahomanov Street, 14/16, USSR. Communicated by George M. Shavlovsky.

Below follow the abstracts of some articles on yeast which have been published recently:

1. Shavlovsky, G. M., Zharova, V. P., Shchelokova, I. F., Trach, V. M., Sibirny, A. A., and Ksheminskaya, H. P. Flavinogenic activity of natural strains of the yeast Pichia guilliermondii. APPLIED BIOCHEMISTRY AND MICROBIOLOGY (Moscow) 14:184-189 (1978).

SUMMARY

Flavinogenic activity of 144 natural and 3 collection strains of the yeast Pichia guilliermondii was studied during their growth on media containing sucrose as carbon source and either optimal or lowered iron concentration. This activity varied on the media of both types. Out of a total of 147 strains, 146 were capable to synthesize and to excrete great amounts of riboflavin in the iron deficient medium. The lack of capacity of the only strain, 1453, for the riboflavin super-synthesis was controlled by a recessive allele. All strains showed a relationship between flavinogenic activity and activity of riboflavin synthase. Some strains synthesized increased quantities of riboflavin, when parafin oils were used as carbon source in the presence of optimal amounts of iron in the medium.

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2. Shavlovsky, G. M., Koltun, L. V., and Kashchenko, V. E. Regulation of activity of GTP-cyclohydrolase, the enzyme of the first step of flavinogenesis in yeasts. BIOKHIMIYA (Moscow) 43:2074-2081 (1978).

SUMMARY

The kinetic properties and regulation of activity of GTP-cyclohydrolase, the enzyme of the first step of flavinogenesis in the Pichia guilliermondii yeast, partially purified by gel-filtration were studied. It was found that the curve of the dependence of reaction rate on substrate concentration is nonhyperbolic. FAD inhibited the enzyme activity, while riboflavin and FMN had no such effect. In addition to FAD, 5'-AMP, 3',5'-AMP, ADP, ATP, NAD, and NADP inhibited the enzyme activity. Under combined action of FAD and AMP on GTP-cyclohydrolase, no synergistic or antagonistic effects of the inhibitors on the enzyme activity were observed. The enzyme appreciably lost its sensitivity to FAD and AMP after thermal treatment. The data obtained suggest that GTP-cyclohydrolase from P. guilliermondii is an allosteric enzyme, which is inhibited by the end product of flavinogenesis FAD, as well as by other 5'-AMP-containing nucleotides.

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3. Kashchenko, V. E., Shavlovsky, G. M., Babyak, L. Ya., and Zhilenko, L. N. Investigation of substrate and inhibitory specificity of riboflavin kinase from Pichia guilliermondii yeast. BIOKHIMIYA (Moscow) 43:2201-2210 (1978).

SUMMARY

The interaction of purified riboflavin kinase (EC 2.7.1.26) from Pichia guilliermondii yeast with 44 structural vitamin B_2 analogues was studied. The presence of D-ribityl lateral chain in an analogue structure was found to be necessary for the substrate activity. The substitution of CH_3 groups in the 7 and 8 positions of isoalloxazine ring in the riboflavin molecule for CF_3, Cl, H, NH_2, and $N(CH_3)_2$ resulted in the decrease of the analogue affinity to riboflavin kinase as compared with the natural substrate, vitamin B_2. The most efficient enzyme inhibitors of analogues without substrate properties turned out to be trifluoromethylisoalloxazines, containing 2'-hydroxyethyl group at N_{10}. The elongation of D-ribityl lateral chain, the elimination or change of CH_3 groups in the 7 and 8 positions for CF_3-, Cl-, COOH-substitutors resulted in the decrease of the inhibitory effect of flavines. Modifications in the structure of isoalloxazine ring, etherification of OH-groups in the lateral D-ribityl chain, and the introduction of volume substitutors (N-piperidyl, D-ribitylamine, hydroxyethylamine) prevented the interaction of the analogue with riboflavin kinase. Flavin nucleotides (FMN and FAD) did not affect the rate of vitamin B_2 phosphorylation.

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4. Logvinenko, E. M., Shavlovsky, G. M., and Zakalsky, A. E. On the product formed at the second step of riboflavin biosynthesis by Pichia guilliermondii. MIKROBIOLOGIYA (Moscow) 48:756-758 (1979).

SUMMARY

Dialyzed extracts of the mutants of Pichia guilliermondii RG80 (rib3, his^-) and RA39 (rib3, ade^-) in which the third step of flavinogenesis was genetically blocked converted GTP into 2,5-diamino-6-hydroxy-4-ribitylaminopyrimidine phosphate and 2,5-diamino-6-hydroxy-4-ribitylamino-

pyrimidine in the presence of $NADPH_2$. Apparently, 2,5-diamino-6-hydroxy-4-ribitylaminopyrimidine phosphate is a true precursor of riboflavin, whereas 2,5-diamino-6-hydroxy-4-ribitylaminopyrimidine is formed upon its hydrolysis.

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5. Shavlovsky, G. M., Sibirny, A. A., Kshanovskaya, B. V., Koltun, L. V., and Logvinenko, E. M. Genetic classification of riboflavinless mutants of Pichia guilliermondii yeast. GENETIKA (Moscow) 15:1561-1568 (1979).

SUMMARY

One hundred-fourteen riboflavinless mutants were selected from the genetic line of Pichia guilliermondii. By means of accumulation test, the mutants were divided into five biochemical groups. In genetic experiments, seven complementation classes were found among 106 mutants. The strains of the I biochemical group, accumulating no specific products, corresponded to complementation class rib1; II group, accumulating 2,4,5-triaminopyrimidine--to the class rib2; III group, accumulating 2,6-dihydroxy-4-ribitylaminopyrimidine--to the class rib3. The mutants of the IV group, accumulating 2,6-dihydroxy-5-amino-4-ribitylaminopyrimidine were divided into three complementation classes: rib4, rib5, and rib6; the mutants of the V group, accumulating 6,7-dimethyl-8-ribityllumazine, corresponded to the class rib7. Two mutants of the IV biochemical group within complementation classes rib4 and rib5 were detected which could not grow in the medium with diacetyl without riboflavin. Intragenic complementation was found within classes rib6 and rib7. No linkage between mutations of different complementation classes was detected.

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6. Sibirny, A. A., Shavlovsky, G. M., Ksheminskaya, G. P., and Orlovskaya, A. G. Coordinate regulation of riboflavin permeade and α-glucosidase synthesis in the yeast Pichia guilliermondii. BIOKHIMIYA (Moscow) 44:1558-1568 (1979).

SUMMARY

Riboflavin permease of the yeast Pichia guilliermondii appears to be an inducible transport system. Its synthesis is induced by sucrose, maltose, α-methyl-D-glucoside, melezitose, and raffinose but not by D-glucose, trehalose, or cellobiose. The synthesis of riboflavin permease in the presence of sucrose or maltose is depressed by cycloheximide, actinomycin D, and 8-hydroxyquinoline. These results suggest that the synthesis of riboflavin permease is regulated on the transcription level. The inducers of riboflavin permease are also able to induce the synthesis of α-glucosidase. The mutants have been selected in which the synthesis of riboflavin permease occurs constitutively; the synthesis of α-glucosidase in these mutants is also constitutive. Growing of the yeast in a medium with a high content of glucose results in a parallel decrease of the riboflavin permease and α-glucosidase activities. These data are indicative of coordinate regulation of riboflavin permease and α-glucosidase in P. guilliermondii. Suboptimal or excessive content of vitamin B_2 in the medium does not affect the level of riboflavin permease in this yeast species.

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

F. C. Church, Jr.[1], and S. P. Meyers. Alpha-galactosidase from Pichia guilliermondii. MYCOLOGIA, in press.

SUMMARY

Examination of a variety of species of molds and yeasts, including those of the genus Pichia, have revealed significant production of the hydrolytic enzyme α-galactosidase in Pichia guilliermondii. Release of the enzyme in this ascosporogenous yeast was strongly affected by certain substrates such as melibiose and lactose, and soybean products, including casitone, soytone, and soybean oil meal. Maximal α-galactosidase was detected in media containing lactose and the soytone product at pH 7. Enzyme production was stimulated by the addition of metal salts to the culture broth.

[1]Present Address: Department of Food Science
North Carolina State University
Raleigh, North Carolina

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F. C. Church, Jr., S. P. Meyers, and V. R. Srinivasan. Isolation and Characterization of Alpha-galactosidase from Pichia guilliermondii. DEVELOPMENTS IN INDUSTRIAL MICROBIOLOGY, in press.

ABSTRACT

The hydrolytic enzyme, α-galactosidase (α-D-galactoside galactohydrolase) has been isolated from the ascosporogenous yeast Pichia guilliermondii. Optimal activity of the purified enzyme was at pH 4.8, with a range between 3.8 and 5.5. Our studies indicated a maximal activity at 70°C, with thermal inactivation at 80°C. Enzyme activity was strongly inhibited by the metallic cations Hg^{++}, Cu^{++}, and Zn^{++}, while the inhibitors dithizone and p-chloromercuribenzoate also partially inactivated the α-galactosidase. With p-nitrophenyl-α-D-galactopyranoside as the substrate, enzyme kinetics revealed a Km of 7.14×10^{-7}M. The molecular weight of the enzyme is 143,000, as estimated by sucrose density gradient centrifugation. Possible utilization of the yeast α-galactosidase in removal of soymilk oligosaccharides is noted.

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

The following are abstracts of papers accepted for publication:

N. van Uden, C. Cabeca-Silva, A. Madeira-Lopes, and I. Spencer-Martins. Selective isolation of de-repressed mutants of an α-amylase yeast by the use of 2-deoxyglucose. BIOTECHNOLOGY AND BIOENGINEERING.

2-Deoxyglucose repressed the formation of extracellular α-amylase by a strain of Lipomyces kononenkoae. Only de-repressed mutants grew on cornstarch medium containing 0.01% of this non-metabolizable glucose analog. From 10 plates inoculated with up to 7 million viable cells

each and irradiated with UV light in doses that reduced viability to about 30%, a total of 15 mutant strains were obtained which were derepressed and hyper-productive with respect to α-amylase. The best mutant produced 3.7 times more enzyme than the wildtype strain.

M. Vidal-Leiria and N. van Uden. Thermal dissociation in the psychrophilic yeast Candida curiosa. ZEITSCHRIFT FUER ALLGEMEINE MIKROBIOLOGIE.

The psychrophilic yeast Candida curiosa displayed a dissociative thermal profile. The optimum and the maximum (T_{max}) temperature for sustained growth were at about 16°C and 17°C, respectively. Transient cell number growth occurred up till about 28°C and transient mass growth up till about 33°C. Thermal death was not detectable at and near T_{max}, occurred only at temperatures several degrees higher and was not coupled with the termination of transient mass growth. It is concluded that T_{max}, transient mass growth, and thermal death of C. curiosa are governed by different targets of thermal inactivation.

XXX. Laboratoire de Structure et Fonction des Biomembranes, C.N.R.S. E.R. 143, 13288 Marseille, Cedex 2, France. Communicated by E. Azoulay.

Below follows the summaries of very recently published papers in the field of yeast physiology:

Jean Claude Bertrand, Hermine Bazin, Marcelle Zacek
Michele Gilewicz, and Edgard Azoulay

NADPH-Cytochrome c Reductase of
Candida tropicalis Grown on Alkane

EUROPEAN JOURNAL OF BIOCHEMISTRY 93, 237-243 (1979)

During growth on n-tetradecane, Candida tropicalis synthesizes a NADPH-cytochrome c reductase whose level is 4-times higher than that of cells grown on glycerol and 20-times higher than that of cells grown on glucose. An original method of solubilisation of this enzyme based on an osmotic shock is described. Studies of the purified enzyme showed that it is a flavoprotein having an apparent molecular weight of 67,000 (±1,000) containing 1 mol each of FMN and FAD per mol of enzyme. Dichloroindophenol and ferricyanide are reduced by this enzyme. NADPH is reduced in the presence of menadione. The Michaelis constant for cytochrome c and NADPH are respectively equal to 4.55 μM and 29 μM. The maximal activity is obtained at a pH comprised between 7.6 and 8. It is influenced by ionic strength and inhibited by thiol inhibitors.

The NADPH-cytochrome c reductase present in the cells grown on glycerol was isolated and purified. Its properties were found identical to those of the enzyme present in the cells grown on tetradecane, including its apparent molecular weight. However, its physiological function remains to be determined. Effectively, we have shown that the cells grown on glycerol do not synthesize cytochrome P-450; and the microsomal fraction prepared from these cells does not have any hydroxylase activity with dodecanoic acid as substrate.

This flavoprotein involved in the hydroxylation of the alkanes is a cytochrome c reductase, probably a cytochrome P-450 reductase.

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Michèle Gilewicz, Marcelle Zacek
Jean Claude Bertrand, and Edgard Azoulay

Hydroxylase Regulation in *Candida tropicalis* Grown on Alkanes

CANADIAN JOURNAL OF MICROBIOLOGY 25, 201-206 (1979)

Candida tropicalis synthesizes a hydroxylase (3 to 5 nmol of product formed per minute per milligram of protein) and a cytochrome P-450 (0.10 to 0.12 nmol per milligram of protein) during growth on n-tetradecane. A three- to four-fold increase in the level of NADPH cytochrome c reductase is also observed in those cells as compared to the level of cells grown on glycerol. The most efficient inducers of the hydroxylase and of cytochrome P-450 are straight-chain alkanes having at least 10 carbon atoms. Alkenes and higher alcohols are also good inducers. There is little or no growth on ramified hydrocarbons such as pristane and on long-chain aldehydes and fatty acids.

The partial inhibition of growth on decane is probably due to the denaturation of the microsomal electron carrier systems by the fatty acid formed by hydroxylation of the decane in the yeast.

Partial Purification of Cytochrome P-450 of *Candida tropicalis* and Reconstitution of Hydroxylase Activity

J. C. Bertrand, M. Gilewicz, H. Bazin
M. Zacek, and E. Azoulay

FEBS LETTERS, Vol. 105, Number 1, 143-146 (1979)

1. Introduction

The various animal cytochromes P-450 have been widely studied and purified [1-5], but very few results have been obtained in this field concerning the cytochromes P-450 synthesized by yeasts [6-8].

The microsomal membranes of *Candida tropicalis* grown on hydrocarbons catalyse the transformation of lauric acid to ω-hydroxydodecanoic acid and then to 1, 12-dodecandioic acid [9]. This transformation is due to a monooxygenase comprising a cytochrome P-450 and a NADPH-cytochrome c reductase on one side and to alcohol- and aldehyde-dehydrogenases on the other. In this yeast, these enzymatical systems are specifically induced by alkanes [10]. We have established that it is possible to liberate 70% of the NADPH-cytochrome c reductase by an osmotic wash of the microsomal membrane [11]. This made it possible to purify and characterize a soluble form of this flavoprotein [11].

This paper describes a method for the isolation and partial purification of cytochrome P-450, the other proteic component of the monooxygenase induced by the alkanes, in *Candida tropicalis*. This method based on the solubilization of the microsomal membranes by a detergent is different from that in [12] where a solubilization of the cytochrome P-450 from a very particular strain of *C. tropicalis* was obtained. Effectively, the cytochrome P-450 of this strain was liberated from the microsomal membrane by French press treatment of the cells of this strain in presence of 30% glycerol. This possibility was not met again in any other strain of *Candida tropicalis*.

XXXI. Departamento de Microbiologia, Sección de Bioquimica de Microorganismos, CSIC, Facultades de Ciencias y Farmacia, Universidad de Salamanca, Salamanca, Spain. Communicated by J. R. Villanueva.

The following papers have been published or will appear very soon:

a) Structure and function of β-glucanases in yeast.

1) Villa, T. G., V. Notario, and J. R. Villanueva. Occurrence of an endo-1, 3-β-glucanase in culture fluid of the yeast Candida utilis. Purification and characterization of the enzyme activity. BIOCHEMISCAL JOURNAL 177:107-114, 1978.

2) Rey, F. del, I. Garcia-Acha, and C. Nombela. The regulation of β-glucanase synthesis in fungi and yeast. JOURNAL OF GENERAL MICROBIOLOGY 110:83-89, 1979.

3) Santos, T., F. del Rey, J. Conde, J. R. Villanueva, and C. Nombela. A mutant of Saccharomyces cerevisiae defective in the production of exo-1,3-β-glucanase. JOURNAL OF BACTERIOLOGY 139:333-338, 1971.

4) Rey, F. del, T. Santos, I. Garcia-Acha, and C. Nombela. Synthesis of 1,3-β-glucanases during the mitotic cycle, mating, and sporulation in the yeast Saccharomyces cerevisiae. JOURNAL OF BACTERIOLOGY 139:924-931, 1979.

5) Villa, T. G., V. Notario, and J. R. Villanueva. Direct chemical proof of different glycosylation patterns for yeast Exo- and Endo-1,3-β-glucanases. JOURNAL OF GENERAL MICROBIOLOGY 190:371-374, 1978.

The main results in this topic area are as follows:

An endo-1,3-β-glucanase from Candida utilis has been purified to homogeneity. It behaves as an acidic glycoprotein with an unusually low molecular weight (21,000) as compared with other yeast glycoproteins.

Regulation of 1,3-β-glucanases appears to follow a different pattern in different fungi. Glucose repressed the synthesis of 1,3-β-glucanase in Neurospora crassa; but in Trichoderma viridae or Saccharomyces cerevisiae the enzymes were produced in the presence of glucose and increase in specific activity during the exponential growth.

Saccharomyces cerevisiae produces an endo- and an exo-1,3-β-glucanase. Cells were synchronized in a zonal rotor. Synthesis of both enzymes took place during the mitotic cycle at the end of the S-phase. The hormonal stimulus which precedes mating did not activate the synthesis of 1,3-β-glucanases, and the specific activity of both enzymes remained constant until zygote formation was completed.

A mutant of Saccharomyces cerevisiae which is defective in the production of exo-1,3-β-glucanase has been isolated. This mutation does not seem to alter the normal physiology of the cell, so its role is not critical but accesory and dispensable.

Work carried out with the yeast Phichia polymorpha indicates two different glycosylation patterns for exo- and endo-1,3-β-glucanases. The predominant sugar in the endo-cleaving form was mannose. By contract, exo-1,3-β-glucanases contain mainly glucose.

b) Regulation of synthesis of external yeast glycoprotein in Saccharomyces cerevisiae.

1) Elorza, M. V., L. Rodriguez, J. R. Villanueva, and R. Sentandreu. Regulation of acid phosphatase synthesis in Saccharomyces cerevisiae. BIOCHIMICA ET BIOPHYSICA ACTA 521:342-351, 1978.

In Saccharomyces cerevisiae^{-136}ts, de-repressed acid phosphatase was almost exclusively located outside the permeability barrier. Only a minor part of the activity was associated with the protoplast; about half of it (48%) in the soluble fraction, the rest bound to the internal (45%) and plasma (7%) membranes.

The activity found in the membranes of de-repressed cells decreased by 30-40% after addition of inorganic phosphate or cyclo-heximide suggesting that this activity is the precursor of the external enzyme.

The alkaline phosphatase activity level could not be modified by changes in the concentration of inorganic phosphate.

Acid phosphatase was not synthesized if the cells were transferred to a low phosphate medium at the moment of incubation at 37°C or in the presence of cycloheximide at 23°C. The data suggested that enzyme formation is the result of the transcription and translation of a proenzyme.

Inorganic phosphate did not inhibit the translation of mRNA though it may act at the level of transcription.

c) Chitin synthesis in Saccharomyces cerevisiae.

1) Duran, A., and E. Cabib. Solubilization and partial purification of yeast chitin synthetase. Confirmation of the zymogenic nature of the enzyme. JOURNAL OF BIOLOGICAL CHEMISTRY 253:4419-4425, 1978.

2) Duran, A., E. Cabib, and B. Bowers. Chitin synthetase distribution on the yeast plasma membrane. SCIENCE 203:363-365, 1979.

Chitin synthetase was solubilized with digitonin from a particulate yeasts fraction. The solubilized enzymes which did not sediment at 200.000 xg and emerged after the void volume in a Sepharose 6 B column was active only after treatment with a protease. A 20-fold purified enzymic preparation showed a requirement for a phospholipid; phosphatidyl-serine and lysophosphatidylserine were the best activators. The solubilized enzyme catalyzed the formation of insoluble chitin in the absence of added primer. The synthetic polysaccharide was examined by electron microscopy and found to consist of losenge-shaped particles about 60 nm long and 10 nm wide.

Purified intact yeast plasma membranes were allowed to synthesize chitin, and the nascent chains of polysaccharide were observed by the fluorescence produced by a brightener or by autoradiography. Chitin synthetase was present in many different locations in the membrane.

d) Inositol deficiency in yeasts.

1) Dominguez, A., J. R. Villanueva, and R. Sentandreu. Inositol deficiency in Saccharomyces cerevisiae NCYC. ANTONIE VAN LEEUWENHOEK, JOURNAL OF MICROBIOLOGY AND SEROLOGY 44:25-34, 1978.

2) Dominguez, A., M. V. Elorza, E. Santos, J. R. Villanueva, and R. Sentandreu. Inositol deficiency in yeast: metabolic, enzymatic and autoradiographic studies. ANTONIE VAN LEEUWENHOEK, JOURNAL OF MICROBIOLOGY AND SEROLOGY 44:341-352, 1978.

When Saccharomyces cerevisiae NCYC 86, an inositol-dependent strain is grown at suboptimal concentrations of inositol the buds are apparently unable to separate from the parent cells. Thin sections of such cells show an irregularly thickened cell wall. These morphological features may be due to a continuation or increase in the production of glucan while the synthesis of DNA, RNA, phospholipids, and protein is greatly inhibited. Additon of inositol resulted in initiation of growth. Inositol was first incorporated into phoshatidyl-inositol. RNA was the first macromolecule synthesized. High resolution autorradiography showed that inositol was first incorporated into internal membranes and later transferred to the plasma membrane.

XXXII. University of New South Wales, School of Food Technology, Kensington, P. O. Box 1, NSW 2033, Australia. Communicated by Graham H. Fleet.

Below follows the abstract of a M.Sc. thesis by Bonni Yee Reichelt, completed August 1979. This material is currently being prepared for publication.

ABSTRACT

The fission yeast, Schizosaccharomyces pombe, was examined for the presence of glucanases likely to be involved in the degradation of cell wall polysaccharides during growth and morphogenesis. No (1→3)-β-D- or (1→6)-β-D-glucanase activities were detected in any of the subcellular fractions examined. Two distinct endo-(1→3)-β-D-glucanases, designated glucanase I and glucanase II, were found exclusively with the cell walls of S. pombe. Glucanase I consisted of two subunits of MW 78,500 and 82,000 daltons and had 67% carbohydrate associated with the purified enzyme. Glucanase II was a single polypeptide of MW 75,000 daltons. Both enzymes exhibited low K_m and high V_{max} values for laminarin. Although the purified enzymes had the same substrate specificity and similar action pattern on laminarin, glucanase II had a much higher hydrolytic activity on isolated cell walls.

The physiological role and regulation of these two enzymes was studied. Glucanase II occurred in high levels only in conjugating and sporulating cells. Evidence was obtained indicating that this enzyme was synthesized in a latent or less active form during conjugation and was activated by proteinases during sporulation.

A mutant of S. pombe was obtained which, unlike the wild type, lysed at 37°C and contained glucanase II during logarithmic growth. This mutant appeared to be constitutive for the lytic glucanase II and may prove to be a regulatory mutant.

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

The following is a list of our work published since June 1979:

Pyruvate Holo- and Apocarboxylase Content of Biotin-Deficient Baker's Yeast and the Characteristics of the Holoenzyme Formation in Permeabilized Cells ?

Sampsa Haarasilt, Erkki Oura, and Heikki Suomalainen

YEAST 12-79

ARCHIVES OF MICROBIOLOGY 112:121-127 (1979)

The holo- and apocarboxylase proteins in yeast grown in a chemostat at different biotin concentrations and on different carbon sources were assayed.

The pyruvate carboxylase activity of the cells grown on glucose was almost constant from the lowest biotin concentration up to a biotin concentration of 10 µg/l. The content of the apocarboxylase protein was maximal at 0.5 µg/l biotin exceeding the level of the active pyruvate carboxylase protein by a factor of about 2.5. With increasing biotin concentration in the medium, the content of apocarboxylase protein decreased and was negligible at biotin concentrations higher than 100 µg/l. The total content of pyruvate carboxylase protein (i.e., apo- + holo-enzyme) was roughly constant over a wide biotin concentration range. At a biotin concentration 50 µg/l, where the maximum yield was reached, the cells still contained pyruvate apocarboxylase. The rapid increase in yield found around a biotin concentration of 10 µg/l correlates more with the appearance of activity of glyoxylate clycle enzymes than with the increase in the activity of pyruvate carboxylase. When cells were growing on ehanol with biotin as the growth limiting factor, the cells still used biotin for the formation of pyruvate holocarboxylase; and proportionally more of the total content of pyruvate carboxylase protein was in the form of holoenzyme that in the corresponding cultivation on glucose.

The existence of urea amidolyase apoprotein in yeast cells grown with urea as the nitrogen source under biotin deficiency is reported. The presence of acetyl-CoA apocarboxylase in biotin-deficient cells could not be demonstrated.

* * *

Qualitative Yeast Enzyme Analysis by Electrophoresis

Anssi Saura, Juhani Lokki, Erkki Oura, and Heikki Suomalainen

EUROPEAN JOURNAL OF APPLIED MICROBIOLOGY AND BIOTECHNOLOGY (in press)

Baker's yeast (Saccharomyces cerevisiae) was cultivated under different intensities of aeration on glucose and on ethanol. Seventeen enzymes of the Embden-Meyerhof pathway and the TCA cycle or related reactions were then assayed by starch gel electrophoresis. There were both qualitative and quantitative differences in many enzymes, most notably in glyceraldehyde-3-phosphate dehydrogenase, alcohol dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, and fumarase. Enzyme electrophoresis seems to offer a promising method for rapidly obtaining information about many yeast enzymes from a large number of samples.

* * *

Estimation of the Suitability of Molasses as Yeast Substrate

Erkki Oura

Abstract of paper presented at the Symposium--Problems
with Molasses in the Yeast Industry
August 31-September 1, 1979, Helsinki

Molasses are very heterogeneous by-products of the sugar industry that are used as raw materials in the fermentation industries. The difficulties of analysing molasses for their main components are indicated by the somewhat diverging results obtained for the same lot of molasses in different laboratories. Because of the big variety of different components in molasses, a complete chemical analysis is troublesome to make; and in practice it is almost impossible to determine all the compounds that can interfere with yeast growth or fermentation. As a consequence, the suitability of molasses has in general been estimated by studying its effects on yeast growth and fermentation directly. In this paper, one method of this kind will be described. Shortages of some compounds, that are normal constituents of good molasses and can be regarded necessary for yeast growth, was estimated by using an aerobic batch-fed cultivation, where the molasses wort, supplemented with a nitrogen source, phosphate, and magnesium salts, and biotin are fed using a 15 hour program. After this, the yeast yield was determined. In this cultivation the final concentration of molasses constituents was quite low, including that of possible inhibitory substances; the dilution factor in this cultivation being lower than normally used in industrial practice. For this reason, an anaerobic cultivation was also performed to detect hte presence of inhibitory compounds rather than the shortage of useful compounds. Here the amount of molasses corresponded to a dilution factor of about 1:5; and in this case the concentration of inhibitory substances, if present, was quite high at the start of the cultivation, and yeast was in contact with these substances for 2 days. At this moment, when the fermentation was not completed, the content of ethanol and yeast cell material was determined.

Using these methods, some normal beet and cane molasses, as well as some Quentin molasses were tested. It was observed that although some molasses lots behaved rather normally in the aerobic cultivation, they obviously contained some inhibitory compounds that could be seen by the decreased yeast growth and the limited ability to produce ethanol. A satisfactory correlation between this decrease and the chemical analyses of the molasses has not yet been found.

* * *

Evidence for Essential Arginine in Yeast Adenylate Cyclase

Kaija Varimo and John Londesborough

FEBS LETTERS 106:153-156 (1979)

Reagents highly specific for arginine, 2,3-butanedione, 1,2-cyclohexanedione, and phenylglyoxal inactivate yeast adenylate cyclase. Reaction is pseudo-first order and substrate analogue, adenylylimidodiphosphate (AMP-PNP), protects the enzyme against the inactivation. Arginine is suggested to be involved in the binding of substrate to yeast adenylate cyclase.

* * *

Multiple Forms of Carboxylesterases in Baker's Yeast

Elke Parkkinen

Submitted for publication in CELLULAR AND MOLECULAR BIOLOGY

The esterases were isolated from disintegrated cells by centrifugation and ammonium sulphate precipitation. About 2/3 of the esterase activity was present in 55% ammonium sulphate precipitates and 1/3 in 55%-75% ammonium sulphate precipitates. Five different esterase activity peaks E_{1A}, E_{1B}, E_2, E_3, E_4 were found by chromatography on Sephdex G 100 and Sephadex G 200. The ratio of the sizes of the peaks $E_{1A}:E_{1B}:E_2:E_3:E_4$ was 9:0:69:13:9 for the 55% precipitate and 0:18:66:2:14 for the 55%-75% precipitate. Each of these esterase activities was identified on both sides of the plasma membrane; i.e., in cell wall digest fractions and in sphaeroplast lysate fractions.

The molecular weights, the pH optima, the temperature optima, and heat stabilities were determined. On the basis of substrate specificities using p-nitrophenyl esters and β-napthyl esters with increasing length of carbon chain in the acid moiety (C_2-C_{12}), and on the basis of sensitivity to different esterase inhibitors, the esterase peaks are believed to reflect multiple esterase activities within the cells.

* * *

The following publications have appeared since the last issue of the Yeast Newsletter. The abstracts of these reports have been given in Yeast Newsletter 28 (1979):1, 30, 31.

Heikki Suomalainen and Matti Lehtonen, The production of aroma compounds by yeast, JOURNAL OF THE INSTITUTE OF BREWING 85 (1979), 149-156.

Solbodan Grba, Erkki Oura, and Heikki Suomalainen, Formation of trehalose and glycogen in growing baker's yeast, FINNISH CHEMICAL LETTERS 1979, No. 2, 61-64.

John Londesborough and Kaija Varimo, The temperature-dependence of adenylate cyclase from baker's yeast, THE BIOCHEMICAL JOURNAL 181 (1979), 539-543.

XXXIV. Agricultural Microbiology Section, Dairy and Food Science Department, University of Saskatchewan, Saskatoon, Canada S7N OWO. Communicated by W. M. Ingledew.

W. M. Ingledew and J. D. Burton

Chemical Preservation of Spent Brewer's Yeast

JOURNAL OF THE AMERICAN SOCIETY OF BREWING CHEMISTS 37:140-144, 1979

Many smaller breweries are concerned with economic disposal of spent brewer's yeast and are searching for methods that do not depend on complete moisture removal. Because of recent interest in liquid animal feeds or feed supplements, a study was made of the effect of several grain "pickling" agents on the viability both of yeast and of possible bacterial contaminants such as Salmonella. The chemical preservatives sorbic acid, sodium benzoate, formaldehyde, propionic acid, and mixtures of acetic/propionic acid, isobutyric/propionic acid, formaldehyde/propionic acid, and formaldehyde/formic/acetic/propionic acid were evaluated by standard microbiological techniques. The last two preparations were very effective on yeast slurries within 10 hours of contact time. They also lowered the viability of yeast, Salmonella, Escherichia coli, and Lactobacillus that had been added to heat-treated yeast slurry at approximately 5×10^4/ml. Other preservatives not capable of killing the large numbers of yeast in slurry were more useful to control the lower numbers of bacteria and yeast in heated slurry.

* * *

G. S. Menegazzi and W. M. Ingledew

Heat Processing of Spent Brewer's Yeast Slurry

JOURNAL OF FOOD SCIENCE (accepted September 13, 1979)

Moist heat treatments of brewer's yeast at cell populations of approximately 10^6/ml resulted in biphasic but otherwise typical survival curves. Brewer's yeast was shown to suffer either thermal injury or death over the tested temperature range of 47°C-53°C. Recovery from thermal injury was possible in liquid media at 20-30°C. Death kinetics were predictable and reproducible. At cell concentrations found in industrial yeast slurries ($\sim 5 \times 10^8$/ml), industrial pasteurization of spent yeast could not be planned by assuming normal first order kinetics, as dying cells released materials which altered the thermo-resistance of survivors. Under simulated yeast slurry conditions, however, with protective cell supernatant (menstrum) obtained after vigorous heating of slurried yeast, a phantom thermal death time curve was obtained which could be used to accurately predict yeast kill (decimal reduction time) at various slurry concentrations and at any temperature.

XXXV. Université de Bordeaux II, Institut d'Oenologie, 351 Cours de la Libération, 33405 Talence, France. Communicated by Mrs. S. Lafon-Lafourcade.

The following papers have been published:

1. S. Lafon-Lafourcade et P. Ribereau-Gayon. "Quelques observations sur les problèmes microbiologiques de la vinification en blanc." CONNAISSANCE DE LA VIGNE ET DU VIN, 1979, 13, 51-76.

ABSTRACT

White wine vinification imposes on yeast cells special metabolic constraints, which frequently concern the inhibition of fermentation activity in non-proliferating populations. In such a case, growth activators are ineffective; and the value of inoculation lies in the quality of the variety used. However, the newly applied notion of "survival factor" for yeasts fermenting in white grape musts of high sugar concentration may eventually lead to a practical solution of fermentation stops.

2. S. Lafon-Lafourcade. "Origine de l'acidité volatile des vins." CONGRÈS INTERNATIONAL DE MICROBIOLOGIE ET INDUSTRIE ALIMENTAIRE, Paris, Octobre 1979.

ABSTRACT

In sweet wines, a high volatile acidity content can arise exclusively from activities of yeasts. In such cases, the deviation of yeast metabolism has two causes:

a. The high sugar concentration.

b. The specific action of substances produced by Botrytis cinerea in infected grapes during the surmaturation period.

These substances are thought to be similar to "botryticine" (described in previous works) as a fermentation inhibitor. An active extract of them can be obtained by fractional precipitations with ethanol. The main components of this active precipitate are heteropolysaccharides, described as a phytotoxin by Dubourdieu and Kamoen (1978).

These inhibiting substances have the following action on yeast metabolism:

a. Glycero-pyruvate fermentation is promoted with a correlative increase in acetic acid production.

b. Acetic acid production is promoted towards the end of the fermentation.

Papers accepted for publication:

1. S. Lafon-Lafourcade, F. Larue, and P. Ribereau-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. APPLIED AND ENVIRONMENTAL MICROBIOLOGY.

2. S. Lafon-Lafourcade et A. Joyeux. Techniques simplifiées pour le dénombrement et l'identification des microorganismes vivants dans les moûts et les vins. CONNAISSANCE DE LA VIGNE ET DU VIN.

3. P. Ribereau-Gayon, S. Lafon-Lafourcade, D. Dubourdieu, V. Lucmaret et F. Larue. Métabolisme de Saccharomyces cerevisiae dans le moût de raisins parasités par Botrytis cinerea. Inhibition de la fermentation; formation d'acide acétique et de glycérol. COMPTES RENDUS ACADEMIE DES SCIENCES, PARIS.

XXXVI. Research Institute for Viticulture and Enology, Matúškova 25, 886 15 Bratislava, Czechoslovakia. Communicated by E. Minárik.

The following paper had been presented and was published at the International Microbiology and Food Industry Congress in Paris organized by A.P.R.I.A., (Association pour la Promotion Industrie-Agriculture) Paris, October 9, 1979:

Pesticides and Alcoholic Fermentation

SUMMARY

Fungicides used in vine protection may be classified into three groups according to their inhibitory activity on sporogenous yeast species and influence on preferential development of asporogenous yeasts of the microflora of spontaneously fermenting musts. Under normal fermentation conditions, the ratio of sporogenous and asporogenous yeasts had been established at 2.5-3:1. In the presence of fungicide residues of the first group (folpet, captafol, captan, dichlofluanid), this ratio changed to 1-1.5:2 in favor of asporogenous yeast species. Fungicides of the second group (e.g., thiram, copper fungicides) show less toxic activity on sporogenous yeast species than those of the first group. The third group includes indifferent fungicides with no toxic influence on yeasts even at higher application concentrations (dithiocarbamates, systemic fungicides, etc.). Practical aspects of wine making are discussed in this connection from the point of view of reducing fungicide residue concentration in must and wine.

The following is a summary of a paper accepted for publication in VITIS (GFR):

Ľ. Drobnica, E. Šturdik, and E. Minárik

Effect of N-trichloromethylthio-tetrahydrophtalimide on Wine Yeasts

SUMMARY

N-trichloromethylthio-tetrahydrophtalimide (NTT), an active component of many commercial fungicide preparations used in viticulture, is a powerful inhibitor of yeast fermentation due to the inactivation of some glycolytic enzymes. In experiments with respiring yeasts, the results obtained show also interference of NTT with the oxidative phosphorylation. The inhibition of these processes and glycolysis, too, is a result of modification of proteins requiring thiol groups for their catalytic activity. The multitarget effect of NTT on yeast energy metabolism is the reason for inhibition of biosynthetic processes and also of yeast growth blocking. Based on these facts, we point out the results obtained from a study of the effect of NTT on incorporation of ^{14}C-labelled precursors into protein and nucleic acids. Included in the paper are data that compare the anti-yeast activity of different types of commercial

fungicides. The importance of yeasts as a model for elucidation of the mode of action of antifungal substances is emphasized.

XXXVII. Meetings.

1. VTH INTERNATIONAL SYMPOSIUM ON YEASTS (ISY)--(1980)

[In Conjunction with the VIth International Fermentation Symposium (IFS)]

London, Ontario, Canada, July 20-25, 1980

ISY Scientific Committee: G. G. Stewart (Chairman), B. Johnson, M. A. Lachance, C. Robinow, I. Russell E. A. Tustanoff

Invited Scientific Speakers in Vth ISY

PLENARY LECTURES: Chairman--G. G. Stewart

A. H. Rose--University of Bath, Bath, England. "Saccharomyces cerevisiae as a model experimental eukaryote"

F. Sherman--The University of Rochester, Medical Center, Rochester, New York. "Why use yeast in studies of molecular biology and genetics?"

H. J. Phaff--University of California, Davis, California. "The species concept in yeast: physiological, morphological, genetical, and ecological parameters"

C. Robinow--University of Western Ontario, London, Ontario, Canada. "The view through the microscope"

INDUSTRIAL AND AGRICULTURAL USES OF YEAST: Chairmen--A. H. Rose and R. C. Righelato

H. Suomalainen--Alko, Helsinki, Finland. "Yeast--alcohol production and bread making"

C. Panchal--The Labatt Brewing Company Limited, London, Ontario, Canada. "Regulatory factors in alcohol fermentations"

Name of speaker undecided. "Ethanol--a renewable energy source"*

C. Ratledge--University of Hull, Hull, England. "Yeasts as sources of oils and fats"

B. Kirsop--Food Research Institute, Norwich, England. "Brewing yeasts--control growth and metabolism during beer fermentations"

R. Kunkee--University of California, Davis, California. "Wine yeasts: old and new; wild and tame"

R. D. Seeley--Anheuser-Busch, Inc., St. Louis, Missouri. "Yeast food products"

G. G. Stewart--The Labatt Brewing Company Limited, London, Ontario, Canada. "The genetic manipulation of industrial yeast strains"

GENETICS OF YEAST: Chairmen--A. James and R. S. Woods

M. Crandell--University of Kentucky, Lexington, Kentucky. "Genetics of _Hansenula wingei_"

H. Bussey--McGill Universtiy, Montreal, Quebec, Canada. "Killer yeasts--their plasmids and to xin"

G. Rank--University of Saskatoon, Saskatoon, Saskatchewan, Canada. "Isolation and characterization of the plasma membrane"

G. R. Fink--Cornell University, Ithaca, New York. "Analysis of genetic control by transformation"*

P. Slonimski--C.N.R.S., Gif-sur-Yvette, Sein-et-Oise, France. "Organization, expression, and regulation of mitochondrial genes in yeast"

F. Lacroute--C.N.R.S., Strasbourg, France. "Genetic regulation in yeast"*

C. P. Hollenberg--Max-Planck Inst. für Biology, Tübingen, West Germany. "The expression in _Saccharomyces cerevisiae_ of bacterial genes integrated in a 2μm DNA vector"

M. V. Olson--University of Washington, Seattle, Washington. "t-RNA genes"

CONJUGATION AND SPORULATION: Chairman--B. F. Johnson

N. Yanagishima--Nagoya University, Nagoya, Japan. "Mechanism and regulation of sexual cell agglutination in _Saccharomyces cerevisiae_"

J. J. Miller--McMaster University, Hamilton, Ontario, Canada. "Sporulation in _Saccharomyces_ cerevisiae"

V. L. MacKay--Rutgers Universtiy, New Brunswick, New Jersey. "Conjugation in _Saccharomyces_"*

C. B. Calleja--National Research Council, Ottawa, Ontario. "Developmentally regulated proteins associated with conjugation and sporulation in the fission yeast"

YEAST CELL CYCLE: Chairman--G. C. Johnston

J. M. Mitchison--University of Edinburgh, Edinburgh, Scotland. "Cell cycle controls in fission yeast"

E. Cabib--National Institutes of Health, Bethesda, Maryland. "Regulation of chitin and glucan synthesis during the yeast cell cycle"

A. E. Wheals--University of Bath, Bath, England. "Approaches to _Saccharomyces_ cell cycle"*

L. Hereford--Brandeis University, Waltham, Massachusetts. "Regulation of histone synthesis in the cell cycle"

TAXONOMY AND ECOLOGY: Chairman--J. F. T. Spencer

S. A. Meyer--Georgia State University, Atlanta, Georgia. "The contribution of DNA reassociation studies to yeast taxonomy"

J. P. van der Walt--C.S.I.R., Pretoria, South Africa. "Hybridization studies in Debaryozyma, Kluyveromyes, Schwanniomyces, and Schizosaccharomyces"

W. T. Starmer--Syracuse University, Syracuse, New York. "The evolutionary ecology of yeasts found in decaying stems of cacti"

J. P. Utz--Georgetown University, Washington, D.C. "Disease in man from yeast"

R. Hurley--Institute of Obstetrics and Gynaecology, Queen Charlotte's Hospital, London, England. "Candida mycoses"

BIOCHEMISTRY: Chairmen--E. R. Tustanoff and J. E. Cummins

V. N. Luzikov--Moscow State University, Moscow, U.S.S.R. "Regulation of mitochondrial biogenesis in yeast: role of mitochondrial and cytoplasmic proteinases"*

P. K. Maitra--Tata Institute of Fundamental Research, Bombay, India. "Genetics of glucose phosphorylation in yeast"

A. W. Linnane--Monash University, Clayton, Victoria, Australia. "Mitochondrial DNA in the control of mitochondrial protein synthesis"*

A. D. Panek--Cidade University, Rio de Janeiro, Brazil. "Genetic relationships between maltose and trehalose metabolism"

P. Borst--University of Amsterdam, Amsterdam, The Netherlands. "Yeast mitochondrial DNA"*

L. W. Parks--Oregon State University, Corvallis, Oregon. "Correlation of sterol structural changes on membrane fluidity and enzyme activity"

W. E. M. Lands--University of Michigan, Ann Arbor, Michigan. "Effect of fatty acid structure on yeast cell physiology"

V. P. Cirillo--State University of New York, Stoney Brook, New York. "Unresolved questions dealing with transport mechanisms in yeast"

D. S. Beatty--Mount Sinai School of Medicine, New York City, New York. "Regulation of mitochondrial protein synthesis in yeast"

G. S. Gettz--University of Chicago, Chicago, Illinois. "Role of phospho-lipids in mitochondrial biogenesis"

S. A. Henry--Albert Einstein College of Medicine, Bronx, New York. "Genetic regulation of phospho-lipid synthesis in yeast"

R. O. Poyton--University of Connecticut, Farmington, Connecticut. "Coupling between nuclear and mitochondrial genomes in the assembly of yeast cytochrome c oxidase"

PHAFF SYMPOSIUM (in honor of Herman Jan Phaff): Organized--S. A. Meyer and A. L. Demain

S. A. Meyer--Georgia State University, Atlanta, Georgia. "Introduction"

F. M. Rombouts--Agricultural University, Wageningen, The Netherlands. "Pectic enzyme biosynthesis and roles in fermentation and spoilage"

A. T. Abdelal--Georgia State University, Atlanta, Georgia. "Regulation of carbamyl phosphate metabolism"

H. Tanaka--Niigata University, Nigarashi, Niigata, Japan. "Concerted induction of cell-wall lytic enzymes in Bacillus circulans"

M. W. Miller--University of California, Davis, California. "Yeasts from honey bees"

M. A. Lachance--University of Western Ontario, London, Ontario, Canada. "Yeasts associated with black knot disease of trees"

L. Mendonça-Hagler--Institute of Microbiology, University of Rio de Janeiro, Rio de Janeiro, Brazil. "Yeast systematics: modern day approach"

A. L. Demain--Massachusetts Institute of Technology, Cambridge, Massachusetts. "Summing Up"

*Subject area to be discussed not the exact title.

Enquiries regarding the Scientific Program should be addressed to:

Dr. G. G. Stewart, Chairman, Vth ISY
The Labatt Brewing Company Limited
150 Simcoe Street
London, Ontario, Canada N6A 4M3
Telephone No.: 1(519)673-5326
Telex No.: 064 7244

Enquires regarding registration, accommodations, and the submission of abstracts should be addressed to:

Mr. K. Charbonneau
Executive Secretary, IFS/ISY
National Research Council of Canada
Ottawa, Ontario, Canada K1A 0R6
Telephone No.: 1(613)993-9009
Telex No.: 053 3145

2. THE TWELFTH ANNUAL MEETING OF YEAST

GENETICS CONFERENCE--JAPAN

The Twelfth Annual Meeting of Yeast Genetics Conference--Japan--was held from September 27 to 29, 1979, at the Luke Hall of Mochida Pharmaceutical Company, Ltd., Tokyo, Japan. The following topics were presented and discussed:

The next annual meeting of the Yeast Genetics Conference--Japan--will be held at Ginza Hall of Sapporo Breweries Ltd., in Tokyo from October 21 to 23, 1980.

Session 1: Structure and Function of Cell Organelles (Chairpersons, K. Takeo and K. Tanaka)

M. Osumi and M. Nagano (Department of Biology, Japan Women's University, Bunkyo-ku, Tokyo 112). Ultrastructure of yeast cell membrane. I. Analysis of paracrystalline arrays.

M. Nagano and M. Osumi (Japan Women's University). Ultrastructure of yeast cell membrane. II. Effects of antifungal agents.

Y. Tani, Y. Yamada, and T. Kamihara* (Seibo Women's Junior College, Neyagawa-shi 572; *Department of Ind. Chem., Faculty of Engineering, Kyoto University). Studies on the dimorphism of *Candida tropicalis* pK 233.

Y. Ohsumi and Y. Anraku (Department of Bot., Faculty of Science, Tokyo University, Bunkyo-ku, Tokyo 113). Amino acids transport system of the vacuoles from *Saccharomyces cerevisiae*.

Y. Kakinuma, Y. Ohsumi, and Y. Anraku (Department of Bot., Faculty of Science, Tokyo University). On the vacuole bound Mg-ATPase from *Saccharomyces cerevisiae*.

Session 2: Radiation and Mutation (Chairpersons, K. Kobayashi, T. Saeki, and M. Dohi)

A. Ito and T. Ito (Institute of Physics, College of General Education, Tokyo University, Meguro-ku, Tokyo 153). Enhancing effect of ascorbate on toluidine blue-photosensitization of yeast cells.

T. Ito (Institute of Physics, College of General Education, Tokyo University). Dependence of photodynamic efficacy of acridine orange and toluidine blue on the degree of sensitizer-cell interaction.

T. Saeki (Division Genetics, National Institute Radio. Sci., Chiba-shi 280). The effect of caffeine on survival of UV-irradiated diploid yeast strains defective in different repair pathways.

K. Kobayashi (Institute of Biology Science, University, Tsukuba, Ibaragi-ken 300-31). DNA damages and induction of gene conversion and reverse mutation.

S. Nakai and I. Machida (Division of Genetics, National Institute Radio. Sci., Chiba-shi 280). Production of intra- and intergenic recombination during commitment of meiotic processes in rad-mutants of *Saccharomyces cerevisiae*.

Y. Nakatomi (Res. Lab., Oriental Yeast Company Ltd., Itabashi-ku, Tokyo 174). Detection, with a color differential medium, of variants in yeast stock cultures.

M. Takagi, M. Kawamura, Y. Kawaguchi, and K. Yano (Department of Agricultural Chemistry, Faculty of Agriculture, Tokyo University, Bunkyo-ku, Tokyo 113). Mutants of *Candida tropicalis* deficient in assimilation of *n*-alkane.

T. Mizunaga, L. Rodriguez*, and J. O. Lampen* (Department of Agricultural Chemistry, Faculty of Agriculture, Tokyo University, Bunkyo-ku, Tokyo 113; *Waksman Institute of Microbiology, Rutgers University). Small invertase-less mutant of *Saccharomyces cerevisiae*.

Session 3: Gene Regulation, Recombination, and Mapping (Chairpersons, I. Takano, S. Harashima, and H. Tamaki)

Y. Nogi and T. Fukasawa (Department of Mol. Genet., Faculty of Medicine, Keio University, Shinjuku-ku, Tokyo 160). Isolation and characterization of temperature-sensitive mutants for *gal3* gene in *Saccharomyces cerevisiae*.

T. Fukasawa, T. Segawa, Y. Nogi, and K. Obonai (Department of Mol. Genet., Faculty of Medicine, Keio University). Properties of purified galactose-metabolizing enzymes in *Saccharomyces cerevisiae*.

K. Matsumoto and Y. Oshima* (Department of Ind. Chemistry, Tottori University, Tottori-shi 680; *Department of Fermentation Technology, Osaka University). The regulatory mechanism of galactose utilization in *Saccharomyces cerevisiae*.

H. Tamaki (Doshisha Women's College, Kyoto 602). Genetic basis on the acquisition of starch fermentability in *Saccharomyces*.

Y. Nakagawa and N. Yanagishima (Biology Institute, Faculty of Science, Nagoya University, Chigusa-ku, Nagoya 464). Gene regulation of inducibility of sexual agglutinability. II.

N. Yanagishima (Biology Institute, Faculty of Science, Nagoya University, Chigusa-ku, Nagoya 464). Mode of action of *a* pheromone in *Saccharomyces cerevisiae*.

I. Takano and T. Oshima (Suntory Institute of Biomedical Res., Shimamoto-cho, Mishima-gun, Osaka 618). A model for mating-type differentiation and novel genes for homothallism in *Saccharomyces* yeasts.

Y. Kaneko, A. Toh-e, and Y. Oshima (Department of Fermentation Technology, Osaka University, Yamadakami, Suita 565). Positive regulatory gene for the production of alkaline phosphatase.

T. Takahashi (Ctr. Res. Laboratory, Asahi Brew. Ltd., Ohta-ku, Tokyo 143). Genetic analysis of tetraploid yeast.

T. Yamazaki (Department of Fermentation Technology, Yamanashi University, Kofu-shi 400). Linkage grouping of auxotrophic markers in Saccharomycodes ludwigii.

Session 4: Cytoplasmic Inheritance (Chairpersons, A. Toh-e and K. Ouchi)

N. Kawakami, H. Tanaka, H. Mondo, S. Katamine, and H. Kawakami* (Faculty of Eng., Hiroshima University, Hiroshima 730; *Suzugamine Women's College) Endocytosis in yeast protoplasts.

S. Nagai and S. Ochi (Department of Biology, Faculty of Science, Nara Women's University, Nara 630). Counteractive effect of neutral red against ethidium bromide.

K. Suda, G. Nishimura, K. Tano*, and A. Uchida** (Department of Biology, Nara Ed. University, Nara 630; *Osaka City University; **Kobe University). Suppressiveness of ethidium bromide-induced petites.

Y. Miyata (Doshisha Women's College, Kyoto 602). Purification of the killer factor produced by Sake yeast.

T. Oshima and I. Takano (Suntory Institute, Biomedical Res., Shimamoto-cho, Mishima-gun, Osaka 618). Four different 2 μm DNA plasmids of Saccharomyces yeast.

N. Gunge, A. Tamaru, F. Ozawa*, and K. Sakaguchi** (Ctr. Res. Laboratory, Mitsubishi Chem. Ind., Midori-ku, Yokohama 227; *Japan Women's University; **Mitsubishi Kasei-Institute of Life Sciences). Plasmid-like DNAs in Kluyveromyces lactis.

Session 5: Biochemistry (Chairpersons, T. Fukazawa and M. Takagi)

B. Ono, K. Ishihara, and Y. Ishino (Department of Pharmaceutical Technology, Faculty of Pharmaceutical Science, Okayama University, Okayama 700). Omnipotent suppressors isolated in a ψ+ strain of yeast Saccharomyces cerevisiae.

J. Ishiguro and C. S. McLaughlin* (Konan University, Okamoto, Kobe 655; *University of California, Irvine). Study on yeast ribosomal proteins by two-dimensional gel electrophoresis using nonequilibrium pH gradient gel.

M. Yamamoto (Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606). Genetic analysis of resistant mutants to thiabendazole in Schizosaccharomyces pombe.

K. Nakura and T. Murayama (Biology Institute, Faculty of Science, Ehime University, Matsuyama-shi 790). The nature of ribosomal proteins from cadmium resistant strain of yeast.

Session 6: Sporulation and Life Cycle (Chairpersons, C. Shimoda and M. Tsuboi)

K. Morimoto and M. Tsuboi (Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558). pH Dependency of sporulation in Saccharomyces cerevisiae.

M. Tsuboi (Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558). Genetic studies on Saccharomyces cerevisiae strains which sporulate in nitrogen-rich medium.

P. K. Srivastava, S. Harashima, and Y. Oshima (Department of Fermentation Technology, Osaka University, Suita-shi 565). Formation of 2-spored asci by interrupted sporulation in Saccharomyces cerevisiae.

S. Okamoto and T. Iino (Department of Bot., Faculty of Science, Tokyo University, Tokyo 113). Analysis of a mutant which predominantly produces two-spored asci in Saccharomyces cerevisiae.

C. Shimoda (Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558). Genetic analysis of spore-germination-deficient mutants in the yeast Schizosaccharomyces pombe.

H. Inoue and C. Shimoda (Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558). Occurrence of trehalase activity during sporulation in the fission yeast Schizosaccharomyces pombe.

A. Hirata and K. Tanaka* (Institute of Applied Microbiology, Tokyo University, Tokyo 113; *Institute of Med. Mycol., Nagoya University). Fine structure of ascospore formation in Schizosaccharomyces pombe.

Session 7: Reports (Chairperson, N. Yanagishima)

A. Toh-e (Department of Fermentation Technology, Osaka University, Suita-shi 565). Genetics of killer yeast.

T. Hirano, N. Kawakami*, and I. Takano** (Tokyo Metropolitan Res. Isotope Center; *Hiroshima University; **Suntory Institute, Biomedical Res.). Report of the Fifth International Protoplast Symposium.

Kotaro Hieda
Rikkyo (St. Paul's) University
Department of Physics-
Biophysics Laboratory
Nishi-Ikebukuro 3, Toshima
Tokyo 171, Japan

Haruhiko Mori
Noda Institute
for Scientific Research
399 Noda, Noda-shi, Chiba-ken
278, Japan

3. SYMPOSIUM

PROBLEMS WITH MOLASSES IN THE YEAST INDUSTRY

A Symposium, Problems with Molasses in the Yeast Industry was held from August 31 to September 1, 1979, in Helsinki. The symposium was organized by the Research Laboratories of the Finnish State Alcohol Monopoly (Alko).

The following papers were presented and discussed. The proceedings of this Symposium will be published at the end of this year.

1. M. Maung [United Nations Industrial Development Organization (UNIDO) Lerchenfelderstr. 1-7, A-1070 Vienna]: Problems and prospects of fermentation alcohol industry in developing countries.

2. H. Schiwek (Süddeutsche Zucker-AG, Zentral-Laboratorium, Postfach 11 27, D-6718 Grünstadt 1): The influence of the different constituents of molasses on its suitability for yeast production.

3. S. Labendzinski (Institute of Fermentation Industry, ul. Rakowiecka 36, P-02-532 Warszawa): Quality and suitability of beet molasses as raw material for baker's yeast production.

4. F. Hill (Henkel KGaA, Postfach 1100, D-4000 Düsseldorf 1): The content of essential trace elements in beet molasses after clarification with flocculating agents.

5. H. Klaushofer (Institute of Food Technology, University of Agriculture, Peter Jordanstrasse 82, A-1190 Wien): Some unconventional raw materials for baker's yeast production.

6. E. Oura (Research Laboratories of the State Alcohol Monopoly (Alko), Box 350, SF-00101 Helsinki 10): Estimation of the suitability of molasses as yeast substrate.

7. H. Janshekar and A. Fiechter (Institute of Microbiology, Swiss Federal Institute of Technology, ETH-Hönggerberg, HPT, CH-8092 Zürich): Maximum biomass production rate from molasses, physical and biological restrictions.

8. H. Hongisto (Finnish Sugar Co. Ltd., SF-02460 Kantvik): Residual molasses from the chromatographic separation process.

9. B. P. Baker (United Molasses Company Ltd., Sugar Quay, Lower Thames Street, London EC3R 6DQ): The availability, composition, and properties of cane molasses.

Heikki Suomalainen
Finnish State Alcohol Monopoly
Helsinki, Finland

4. SECOND BILATERAL CONFERENCE ON YEASTS (GDR--CSSR)

The Second Bilateral Conference on Yeasts was held in Greifswald (GDR) on May 12-16, 1979. This conference was established by the Biological Society of GDR under the sponsorship of Ernst-Moritz-Arndt University in Greifswald. The chairman of the organizing committee was Professor R. Birnbaum and the scientific secretary was Professor F. Böttcher.

One hundred forty participants were present, including 20 Czechoslovaks and some invited guests from USSR, Hungary, Poland, and Vietman. Seventy-four posters were presented and five plenary discussions connected with introductory lectures were undertaken:

1. Regulatory mechanisms (Reuter; Jena, GDR)
2. Hybridization by protoplast fusion (Ferenczy; Szedged, Hungary)
3. Genetics of yeasts growing on n-alkanes (Böttcher; Greifswald, GDR)
4. Natural variability (Kocková-Kratochvílová; Bratislava, CSSR)
5. Biomass from nontraditional substrates (Babel; Leipzig, GDR)

Extensive discussions were held about each problem. Finally, it was decided that such conferences will be held every two years in Czechoslovakia, German Democratic Republic or in Hungary. Participants from other socialistic countries will be invited. The next conference will take place in Szeged in 1981. Publications will appear of all poster contributions at the beginning of 1980 (editor, Professor F. Böttcher, EMA University, Greifswald, GDR).

A. Kocková-Kratochvílová
809 33 Bratislava
Czechoslovakia

XXXVIII. Brief News Items.

1. Dr. E. K. Novak, Chairman of the Organizing Committee of the 5th International Specialized Symposium on Yeasts, held in Keszthely, Hungary, September 12-15, 1977, has sent the Editor of the Yeast Newsletter ten sets of the Proceedings of that conference. They are available to readers of the Yeast Newsletter on a first-come, first-serve basis for $10 per set. Checks should be made out to Yeast Newsletter, and requests mailed to Dr. H. J. Phaff, Department of Food Science and Technology, University of California, Davis, California 95616, U.S.A.

2. The following two articles have recently been published by workers in my laboratory:

G. Moulin and P. Galzy. Study of an amylase and its regulation in *Lipomyces starkeyo*. AGRICULTURAL AND BIOLOGICAL CHEMISTRY 43 (6), 1165-1171, 1979.

G. Moulin, Maguy Guillaume, and P. Galzy. Sélection de souches de levures en vue de la production d'alcool sur lactosérum. LE LAIT N° 558-589, 489-496, 1979.

P. Galzy
Ecole Nationale Supérieure
Agronomique de Montpellier
34060 Montpellier Cedex
France

3. The following paper was recently published:

J. Lodder and N. J. W. Kreger-van Rij. Debaryomyces or Debaryozyma. INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY 29:418, 1979.

N. J. W. Kreger-van Rij
Laboratory for Medical Microbiology R.U.
Oostersingel 59
9713 EZ Groningen
The Netherlands

4. The following papers have been published recently from my laboratory:

James J. Foy and J. K. Bhattacharjee. Biosynthesis and Regulation of Fructose-1,6-Bisphosphatase and Phospho-fructokinase in S. cerevisiae Grown in the Presence of Glucose and Gluconeogenic Carbon Sources. JOURNAL OF BACTERIOLOGY 136:647-656 (1978).

Jerome J. Kinzel and J. K. Bhattacharjee. Role of Pipecolic Acid in the Biosynthesis of Lysine in Rhodotorula glutinis. JOURNAL OF BACTERIOLOGY 138:410-417 (1979).

J. K. Bhattacharjee
Department of Microbiology
Miami University
Oxford, Ohio 45056

5. Doctors Richard Baldwin and Gilbert Dalldorf of Research Corporation (Brown-Hazen Program) visited our laboratories on October 23 to meet with Dr. Thomas Kerkering, recipient of the first Dalldorf Fellowship in Medical Mycology.

Dr. Awatar S. Sikhon, Mycologist with the Provincial Laboratory of Public Health in Edmonton, Alberta, Canada, spent the week of November 5-9 in our laboratory learning our methods for clinical laboratory studies with antifungal agents.

Dr. Gerald E. Wagner has left our laboratories to accept a position as Assistant Professor of Microbiology, Department of Microbiology, George Washington University Medical Center, Washington, D.C.

Dr. Smith Shadomy presented a lecture on "New Approaches to Antifungal Susceptibility Tests and Blood and Tissue Assays" on October 11 at the Program "Recent Advances in Medical Microbiology" sponsored by the Bronx-Lebanon Hospital Center in New York (Dr. V. Torcan, Director).

Dr. Smith Shadomy
Division of Infectious Diseases
Department of Medicine
Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia 23298