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I. Centraalbureau Voor Schimmelcultures, Yeast Division, Delft, Julianalaan 67a, Netherlands. Communicated by Maudy Th. Smith.

Systematics of Hanseniaspora Zikes and Kloeckera Janke.

Sally A. Meyer[1,3], Maudy Th. Smith[2] and F. P. Simione Jr.[1]

1. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A.

2. Yeast Division of the Centraalbureau voor Schimmelcultures, Laboratory of Microbiology, University of Technology, Delft, The Netherlands.

3. Georgia State University, Department of Biology, Atlanta, Georgia 30303 U.S.A.

SUMMARY

The physiological and morphological characteristics of eighty-two strains of Hanseniaspora and Kloeckera, representing twenty-nine described species, were examined. These results along with DNA base composition and DNA/DNA reassociation experiments revealed that the genus Hanseniaspora comprises six distinct species, viz. H. valbyensis, H. uvarum, H. guilliermondii, H. occidentalis, H. osmophila and H. vinea, with K. japonica, K. apiculata, K. apis, K. javanica, K. corticis and K. africana, respectively, as their imperfect states.

This paper has recently been published in Antonie van Leeuwenhoek 44:79-96 (1978).

II. Georgia State University, Department of Biology, Atlanta, Georgia, 30303. Communicated by Sally A. Meyer.

The following are abstracts of papers presented at the Annual Meeting of the American Society for Microbiology, Las Vegas, May 14-19, 1978.

S. A. Meyer and F. P. Simione, Jr. Heterogeneity Within the Species Candida sake and Candida diddensii. Ga. State Univ., Atlanta, Ga. and American Type Culture Collection, Rockville, Md.

Thirty strains presently included in Candida sake (20) and C. diddensii (10) on the basis of physiological and morphological properties were examined for DNA base composition and degree of DNA relatedness with the type strain of the respective species. Percent guanine plus cytosine (%GC) for the C. sake strains ranged between 37.8 and 48.5%, and the C. diddensii strains showed a GC range between 33.9 and 40.5%. DNA reassociation studies, using the DNA-filter technique, revealed only ten of the C. sake strains to have a high degree of DNA reassociation (89-100%) with the C. sake type strain, and only one of the strains included in C. diddensii showed significant DNA reassociation (91%) with the C. diddensii type strain. Additional DNA reassociation experiments demonstrated that

three of the C. sake strains which did not exhibit significant DNA relatedness with the C. sake type strain have a high degree of DNA reassociation with the C. oleophila strain, presently included as a synonym of C. sake. Thus, these four strains represent a species distinct from C. sake.

The heterogeneity demonstrated within C. sake and C. diddensii is another example of the inability of the presently used physiological and morphological criteria to differentiate yeast species properly.

F. P. Simione, Jr. and S. A. Meyer. Genetic Relatedness Between Some Physiologically Similar Candida species with High GC Contents. American Type Culture Collection, Rockville, Md. and Ga. State Univ., Atlanta, Ga.

Relatedness between fourteen strains representing Candida brumptii (3), C. catenulata (1), C. iberica (1), C. ravautii (6) and C. zeylanoides (1) was investigated by molecular biological techniques. These five species are physiologically similar in the conventional tests with the excpetions of maltose and trehalose assimilation, glucose fermentation and growth at 37C. DNA thermal denaturation determinations revealed GC contents between 48 and 58%, broad melting profiles and compositional heterogeneity indicative of the presence of a secondary DNA component. Using the DNA-filter method for the detection of DNA reassociation, C. brumptii and C. catenulata demonstrated greater than 94% DNA reassociation with the C. ravautii type strain, whereas, C. iberica and C zeylanoides showed less than 20% DNA reassociation with the C. ravautii type strain.

Since the epithet, Candida brumptii has priority, C. catenulata and C. ravautii are considered synonyms of C. brumptii.

This is another example of the inability to differentiate yeast species on the basis of the currently used physiological tests.

III. Centraalbureau voor Schimmelcultures, P. O. Box 273, Baarn-The Netherlands. Communicated by J. A. von Arx.

Below follow abstracts of some recent papers on yeast-like organisms from this Institute.

von Arx, J. A. 1977. Notes on Dipodascus, Endomyces and Geotrichum with the description of two new species. Antonie van Leeuwenhoek 43:333-340.

The genus Endomyces is restricted to E. decipiens Reess, characterized by asci formed directly on hyphae and hat-shaped ascospores. The species forming asci from conjugating gametangial cells and having ellipsoidal ascospores are transferred to Dipodascus. A strain isolated from cladodes of Opuntia in Australia is described as Dipodascus australiensis von Arx et Barker and strains isolated from Armillaria mellea are classified as Geotrichum armillariae von Arx.

Weijman, A. C.M. 1977. Carbohydrate composition and taxonomy of the genus Dipodascus. Antonie van Leeuwenhoek 43:323-331.

Cabohydrates released during acid hydrolysis of intact cells of Dipodascus were studied by gas-liquid chromatographic analysis as their trimethylsilyl derivatives. In addition, cells were characterized by pyrolysis gas-liquid chromatography and pyrolysis mass spectrometry.

The data obtained support the classification of Dipodascus uninucleatus in a separate genus Dipodascopsis. Glucuronic acid is present in D. uninucleatus and, therefore, a possible affinity to fungi classified in the Zygomycetes is considered. Dipodascus aggregatus and Dipodascus australiensis were found to be rather different, but very close to Geotrichum candidum and related species.

The Application of Curie-Point Pryolysis Mass Spectrometry in Fungal Taxonomy

A. C. M. Weijman 1977. Centrallbureau voor Shimmelcultures, Baarn, The Netherlands. Elsevier Scientific Publishing Company, Amsterdam.

SUMMARY

Curie-point pyrolysis mass spectrometry (Py-MS) has been introduced as a promising tool for the biochemical analysis of fungi in relation to fungal taxonomy. In the complex spectra of intact fungal cells and cell walls, peaks can be detected characteristic of for example, proteins, polysaccharides, deoxyhexose polymers and phospholipids. The ability to discriminate between chitinous and non-chitinous cells is important in the taxonomy of yeasts and related fungi as is illustrated for Trichosporon aculeatum, Petriella setifera and Saccharomyces cerevisiae. Demonstration of deoxyhexoses has become important in the taxonomy of the genera Ophiostoma and Ceratocystis. Petriella setifera and Europhium aureum show a prominent peak at m/e 128 indicative for deoxyhexoses, in the spectra of intact cells. Py-MS can also be used to check the purity of cell wall preparations, based on the absence of peaks at m/e 48, 89, and 92 in the spectrum of cell walls, as is illustrated for Endomyces decipiens and also to monitor purification procedures. Attempts to isolate cellulose from Europhium aureum are described.

IV. Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pushchino, Moscow region, 142292, USSR. Communicated by V. M. Blagodatskaja.

In 1947 E. N. Odinzova described the new yeast species Endoblastomyces thermophilus. This organism formed pseudomycelium, asexual endospores, fermented glucose and grew well in a vitamin-free medium.

The latin diagnosis of this species was published in 1960 (in Kudriavzev) but the type strain was not designated. Thus, E. thermophilus is a nomen nudum.

L. do Carmo-Sousa (1970) suggested the similarity of this organism to Trichosporon captitatum. However, no cultures of E. thermophilus were available for her study.

We have 8 strains, isolated by E. N. Odinzova, and studied them. They did not form true mycelium and arthrospores, and thus are not members of the genus Trichosporon. The following investigation of physiological properties indicated, that this organism is similar to Candida krusei (Cast.) Berkhout, 1923. This name has priority, and E. thermophilus must be considered a synonym of C. krusei (Cast.) Berkhout. The formation of endospores in C. krusei was observed by Kurtzman and Smiley (1976). Thus, there are no differences between C. krusei and E. thermophilus.

1. Odinzova E. N. Microbiologya, Moscow, 16:273, 1947.

2. Kudriavzev V. I. Die Systematik der Hefen, Berlin, 1960.

3. L. do Carmo Sousa, Trichosporon, In: The Yeast, a taxonomic study, ed. J. Lodder, 1970.

4. Kurtzman C. P. and Smiley M. J., Antonie van Leeuwenhoek. 42:355, 1976.

V. Departmento de Microbiología, E.T.S. de Ingenieros Agrónomos, Universidad Politécnica, Ciudad Universitaria, Madrid-3 Spain. Communicated by J. Santa María.

The following are recent publications from our laboratory:

Santa María, J. and C. García Aser. "Pichia segobiensis sp. nov." Inst. Nac. de Inv. Agrarias, Anales, Serie General, Nr 5: 45-50. 1977.

Four strains of a new species, Pichia segobiensis, were isolated from insect sources.

Santa María, J., D. Vidal and Asunción R. Marín: "Gene controlled resistance and sensitivity to methyl violet 6B in Saccharomyces." Inst. Nac. de Inv. Agrarias. Anales, Serie General, Nr 5:51-62. 1977.

The sensitivity of 21 genera of ascosporogenous, 1 ustiliginales, 1 sporobolomycetaceae and 6 asporogenous yeasts to acid fuchsin, basic fuchsin, crystal violet, methyl violet 6B, rose bengal and Victoria blue has been determined. Resistance and sensitivity to methyl violet 6B divide yeasts into two classes, sensitive and resistant, and may be used as a criterion for distinguishing genera and as a physiological characteristic for delimiting species. In Saccharomyces, the response to methyl violet 6B is hereditary and under the control of a single mendelian gene.

Santa María, J. and Consuelo Sánchez: "Nitrogen compounds as sources of carbon and energy for yeasts." Inst. Nac. de Inv. Agrarias, Serie General Nr 5:63-73. 1977.

A total of 685 yeast strains, representing 137 species, belonging to 29 genera, were tested for their ability to grow in a medium containing casein hydrolyzate as the source of carbon and energy. Positive growth was observed in 76 species, 57 were unable to grow and only in 4 species positive as well as negative strains were found. Uniformity of test responses between strains of species studied suggested the usefullness of determining, as a taxonomic criterion, the ability to utilize nitrogen compounds as carbon and energy sources.

Santa María J.: "Effect of brilliant green and malachite green on yeast growth." Communicaciones INIA, Serie General, No. 3. 1978 (in press).

The effect of brilliant green and malachite green on the growth of 170 species of yeasts, representatives of 28 genera, was studied. The resistance to brilliant green is similar in yeasts and in Gram-positive bacteria, but Gram-positive bacteria exhibited greater resistance to malachite green than yeasts. Nevertheless, strains of 9 species are relatively resistant to both dyes. Sensitivity to malachite green may be a character of taxonomic significance as it appeared to be in the division of the strains now included in the species Sacch. uvarum.

Santa María J.: "A taxonomic reexamination of some species of the genus Saccharomyces." Communicaciones INIA, Serie General, No. 3. 1978 (in press).

With a view to improve the identification and characterization as easily and accurately as possible of some species of yeasts included in the classification of the genus Saccharomyces, a taxonomic re-examination of 25 species has been accomplished.

As a consequence, it has become possible to recognize the validity of the following taxospecies: S. florentinus, S. cidri, S. astigiensis nov. spec., S abulensis nov. spec., S. hispalensis nov. spec. and S. carlsbergensis(epithet restored, description emended and neotype strain designated) in the group of species which ferment glucose, galactose, sucrose, maltose and melibiose; a diagnostic key is given.

S. albasitensis nov. spec., S. kluyveri, S. microellipsodes, S. amurcae, S. mrakii and S. coreanus in the group which ferment glucose, galactose, sucrose, melibiose and raffinose completely, but not maltose or lactose; a diagnostic key is given.

S. capensis, S. onubensis and S. oxidans.
S. rouxii, S. prostoserdovi and S. hispanica.
S. bayanus, S. oviformis, S. beticus, S. fermentati(neotype strain designated) and Deb. dekkeri.
S. exiguus, S. chevalieri, S. vafer and S. cordubensis.
S. cerevisiae and S. gaditensis

The standard description and latin diagnosis of the new species is included.

Santa María J.: "Candida auringiensis species nova, a yeast from "alpechín". Communicaciones INIA. Serie General, No. 3. 1978 (in press).

Three strains of a new species, Candida auringiensis were isolated from "alpechin".

Cadahía, Esther, E. Cabrera, J. Santa María: "Flora zimógena en rumen de ovino y bovino." Inst. Nac. de Inv. Agrarias, Anales, Serie General, Nr. 5:75-84. 1977.

The strains of yeasts isolated from the rumen of 4 fistulated sheep and 1 cow were classified in 10 species and 5 genera. Trichosporon cutaneum in ovine rumen and Candida rugosa in bovine rumen are the species more frequently isolated.

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

Below follows some news from our laboratory. The following papers are in press:

J. B. Fiol & G. Billon-Grand. Osidases, nitrite-et nitrate réductases dans les genres Hanseniaspora et Kloeckera. Accepted for publication in Mycopathologia.

ABSTRACT

Intracellular osidases, nitrite reductase and nitrate reductase were found in some species of Hanseniaspora and Kloeckera. Taxonomical relationships between the 2 genera are discussed.

J. B. Fiol & G. Billon-Grand. Osidases, nitrite-et nitrate réductases dans les genres Dekkera et Brettanomyces. Submitted for publication to Mycopathologia.

PHYSIOLOGIE DES INSECTES - Étude comparée des osidases de Phoracantha semipunctata F. (coléoptère Cerambycidae xylophage) et d'une Levure du tube digestif de cet Insecte. Note (*) de Constantin Chararas, Marie-Claire Pignal et Guilane Vodjdani, présentée par M. Pieree-Paul Grassé. C. R. Acad. Sc. Paris, t.286 (20 mars 1978). Série D-p 867.

Yeast osidases in the digestive tract of xylophagous Insects have not yet been the subject of any research. We studied the osidases of a yeast Candida guilliermondii, isolated from the digestive tract of Phoracantha semipunctata F. larvae, Coleoptera Cerambycidae specific parasite of Eucalyptus trees. Phoracantha possesses a rich osidasic equipment acting on oligosaccharides, heterosides and polysaccharides, characterized by an activity on hemicellulose and cellulose.

Candida guilliermondii possesses an osidasic equipment acting on some oligosaccharides, some heterosides and some polysaccharides, such as starch and pectine, but its digestive enzymes have no effect on hemicellulose and on cellulose.

We can point out that this yeast partly interferes in the digestion of some of the wood compounds, but cellulases are secreted from the insects digestive tract tissues, even in the absence of the yeast. The yeast inhibition of antibiotic action does not change the insects' cellulasic activity.

J. B. Fiol, F. Jacob and R. Montrocher will attend the 6th International Specialized Symposium on Yeast, in Montpellier (France) in July 1978.

VII. Research Institute of Fermentation, Yamanashi University, Kofu, 400, Japan. Communicated by Shoji Goto.

The following papers have been published:

1. S. Goto and I. Yokotsuka (1977). Wild Yeast Populations in Fresh Grape Musts of Different Harvest Times. J. Ferment. Technol. 55:417-422.

SUMMARY

The yeast flora in freshly crushed grape musts obtained at different harvest times was studied qualitatively and quantitatively using two varieties of grape, a white variety of the Koshu and a red variety of Muscat Bailey A, both grown in Yamanashi Prefecture, Japan.

Total counts of yeasts varied with harvest times, vineyards, and production year; the Koshu variety showed $10\text{-}10^5$ yeast cells/ml in musts and the Muscat Bailey A variety $10^3\text{-}10^6$ yeast cells/ml. Wild yeasts isolated were divided into six major groups; 1) apiculate

yeasts, 2) Saccharomyces, 3) Torulopsis, 4) film yeasts, 5) Rhodotorula, and 6) other yeasts.

The population patterns of these 6 groups did not change markedly at the different harvest times. That is, 40-72% of apiculate yeasts, 0-18% Saccharomyces, 13-19% Torulopsis, 3-22% film yeasts, and 1-4% Rhodotorula and other yeasts. The six groups were composed of 22 species of 10 genera. It was interesting that the Saccharomyces group disappeared during the late harvest times, in November. Further, this study suggested that harvest times, grape maturity, and trimming of vines were important factors controlling the yeast flora of freshly crushed grape musts.

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2. S. Goto, M. Yamazaki, Y. Yamakawa and I. Yokotsuka (1978). Decomposition of malic acid in grape must by wine and wild yeasts. Hakkokogaku 56:133-135.

SUMMARY

Fifty-five strains from 29 species in 9 genera were examined for ability to decompose malic acid in grape must. Potent activity to decompose malic acid during fermentation of grape must was detected in Saccharomyces bailii and Kloeckera javanica, several strains of Schizosaccharomyces pombe and the film-forming yeasts. Three strains of S. bailii decomposed 45-64% of malic acid, but titratable acidity was increased; three strains of Kl. javanica decomposed 69-95% of malic acid, and titratable acidity was decreased.

VIII. Institute of Fermentation Technology, Ankara University, Ziraat Fakültesi, Ankara/Turkey. Communicated by M. Hilmi Pamir.

The following study has been published in Gida 1:88-100 (1976): A study on the lactic acid bacteria and the yeast strains isolated from turkish fruit and tomato juices.

I. Sahin. A. U. Ziraat Fakultësi

Ankara/Turkey

The yeasts isolated from various juices consist of 35 strains. They have been divided into two groups by morphological characters: Asporogenous and sporogenous yeasts.

All these yeast strains were also distinguished and identified by their physiological characters according to Lodder and tested against the sugars including glucose, galactose, saccharose, lactose, melibiose, raffinose, arabinose, xylose, trehalose, cellobiose and melezitose, dextrin, starch, α-methyl-D-glucoside, malic acid, lactic acid and KNO_3.

Two of the strains which failed to sporulate belong to Kloeckera africana and Torulopsis candida.

As to sporogenous yeast strains they were identified as Schizosaccharo-

myces pombe, Saccharomyces cerevisiae and S. bailii.

The following study will be published in the Yearbook of Faculty: A study on the yeast strains isolated from turkish pickles.

I. Sahin. A. U. Ziraat Fakultësi

Ankara/Turkey

The purpose of this study was to isolate and to identify the yeast strains which cause spoilage of pickles. 71 samples of rinsing waters and brine yielding 147 yeast strains were investigated. They have been identified by Lodder and classified as follows:

Candida boidinii (isolated from pickles of pepper, cucumber, cabbage, tomato, carrot, cornel).

C. guilliermondii var. guilliermondii (isolated from pickles of pepper).

C. lambica (isolated from pickles of cucumber)

Debaryomyces marama (isolated from pickles of cucumber)

Hansenula anomala var. anomala (isolated from rinsing water).

Pichia kudriavzevii (isolated from pickles of pepper, tomato and cucumber).

P. membranaefaciens (isolated from rinsing water and pickles of tomato, cucumber.

Saccharomyces cerevisiae (isolated from pickles of pepper, plum, and carrot).

S. vafer (isolated from pickles of pepper, tomato and cabbage).

Torulopsis candida (isolated from pickles of tomato and carrot).

Trichosporon penicillatum (isolated from pickles of cucumber, cabbage and tomato).

A great number of the yeast strains broke down the DL-lactic acid. As a result of acid degradation, the pickles have been spoiled. In addition, the film-forming yeasts, especially of the genus Trichosporon have been responsible for softening of pickles.

Brief News Items from the Department of Fermentation Technology, Faculty of Agriculture. Ankara/Turkey.

We have built up a multi-purpose biotechnological pilot plant with 250 l capacity and have a project supported by Central Treaty

Organization (CENTO) to enrich it with microbial protein or to increase the biological value of straw which is used for livestock in Turkey.

IX. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852. Communicated by Kathryn A. Schmeding.

The strains listed have been added to the ATCC since November 1, 1977. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

Torulaspora phaffii ATCC 20499, Released Patent Long chain dicarboxylic acid production	Gulf Oil Co.
Candida ingens ATCC 36119, Potential fodder protein	D. P. Henry Univ. Queensland Brisbane, Australia
Saccharomyces rouxii ATCC 36141, Osmophilic spoilage yeast	A. R. Isaacs Queensland Dept. of Primary Industries Queensland, Australia
Kluyveromyces fragilis ATCC 36142, Sporulation studies	B. E. Kirsop NCYC Brewing England
Cephaloascus fragrans ATCC 36174, ATCC 36175 Cell Wall studies	C.A.N. van Oorschot CBS
Candida utilis ATCC 36178, zinc accumulation	E. D. Weinberg Indiana University Bloomington, IN
Saccharomycopsis fibuligera ATCC 36213, Glucoamylase production	K. Matsumoto IFO
Torulopsis pintolopesii ATCC 36231, Choline Bioassay	H. Baker N. J. Med. School East Orange, N.J.
Candida stellatoidea ATCC 36232, Miconazole bioassay	A. Espinel Ingroff VCU Richmond, VA
Dekkera bruxellensis ATCC 36234, Type Culture	CBS
Dekkera intermedia ATCC 36235, Type Culture	"
Rhodotorula marina ATCC 36236, Type Culture	"

Saccharomycopsis malanga ATCC 36237, Type Culture	"
Torulaspora cidri ATCC 36238, Type Culture	CBS
Torulaspora hansenii ATCC 36239, Type Culture	"
Torulaspora inconspicuus ATCC 36240, Type Culture	"
Torulaspora manchuria ATCC 36241, Type Culture	"
Torulaspora mrakii ATCC 36242, Type Culture	"
Torulaspora nilssonii ATCC 36243, ATCC 36244, Type Culture	"
Torulaspora pretoriensis ATCC 36245, Type Culture	"
Torulaspora vafer ATCC 36246, Type Culture	"
Torulaspora vanriji ATCC 36247, Type Culture	"
Candida albicans ATCC 36263 Cyclic 3'5' nucleotide phosphodiesterase activity	M. Gunsekaran St. Jude Children's Research Hospital Memphis, Tennessee
Hansenula jadinii ATCC 36264, Phosphorylation activity of respiration deficient mutants	A. Kimura Kyoto University Japan
Saccharomyces carlsbergensis ATCC 36265-36267, Phosphorylation activity of respiration deficient mutants	"
Candida zeylanoides ATCC 36275, isolated from tussock moth of Douglas-fir	NRRL
Rhodotorula ulzamae ATCC 36307, Type Culture	"
Saccharomycopsis capsularis ATCC 36308, Type Culture	"

Saccharomycopsis fibuligera ATCC 36309, Type Culture	"
Torulaspora eupagyca ATCC 36310, Type Culture	"
Torulaspora florentina ATCC 36311, Type Culture	NRRL
Candida boidinii ATCC 36351 Isolated from defatted soybean flakes	T. Nakase Ajinomoto Co. Kawasaki, Japan
Candida intermedia ATCC 36352 Isolated from defatted soybean flakes	"
Candida krusei ATCC 36353 Isolated from defatted soybean flakes	"
Candida tropicalis ATCC 36354 Isolated from defatted soybean flakes	"
Candida valida ATCC 36355 Isolated from defatted soybean flakes	"
Kloeckera apiculata ATCC 36356 Isolated from defatted soybean flakes	"
Pichia nonfermentans ATCC 36357 Isolated from defatted soybean flakes	"
Rhodotorula rubra ATCC 36358 Isolated from defatted soybean flakes	"
Saccharomyces cerevisiae ATCC 36359 Isolated from defatted soybean flakes	"
Saccharomyces exiguus ATCC 36360 Isolated from defatted soybean flakes	"
Torulaspora microellipsoides ATCC 36361 Isolated from defatted soybean flakes	
Saccharomyces uvarum ATCC 36362 Isolated from defatted soybean flakes	"

Saccharomyces cerevisiae ATCC 36375 Bioassay of 5-flurocytosine	Smith Shadomy Med. Coll. of Virginia Richmond, VA
Aessosporon salmonicolor ATCC 36395, Type Culture	CBS
Aessosporon salmonicolor ATCC 36396, haplophase of ATCC 36395	"
Aessosporon salmonicolor ATCC 36397, diplophase of ATCC 36395	"
Sporidiobolus ruinenii ATCC 36398, Type Culture	"
Sporobolomyces japonica ATCC 36399, Type Culture	"
Sporobolomyces salmonicolor ATCC 36400, Type Culture	"
Sporobolomyces salmonicolor ATCC 36401-37402	"
Itersonilia pastinacae ATCC 36403, Type Culture	"
Itersonilia perplexans ATCC 36404	"
Tilletiopsis minor ATCC 36405-36408	"
Torulopsis apis var. galacta ATCC 36475, Type Culture	W. I. Golubev Moscow State Univ. Moscow USSR
Schizosaccharomyces pombe ATCC 36478 Uracil requiring mutant	U. Leupold Institut für Mikrobiologie Bern, Switzerland
Cryptococcus neoformans ATCC 36480, γ-irradiated mutant	Erna Alture-Werber Montefiore Hosp. & Med. Ctr. Bronx, New York
Tilletiopsis washingtonensis ATCC 36488	CBS
Tilletiopsis washingtonensis ATCC 36489, Type Culture	"
Saccharomyces cerevisiae ATCC 36490 Copper resistant yeast	N. Naiki Gifu University Gifu, Japan

Kluyveromyces fragilis ATCC 36534 Potential food yeast from whey	Alan G. Lane CSIRO Div. of Food Res. Australia
Tilletiopsis sp. ATCC 36535, Isolated from powdery mildewed leaves of cucumber	Harvey C. Hoch N. Y. State Agric. Exp. St. Geneva, N.Y.

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

1. The following paper was recently published: Genome Comparison in Yeast Systematics: Delimitation of Species within the Genera Schwanniomyces, Saccharomyces, Debaryomyces, and Pichia. C. W. Price, Gayle B. Fusan, and H. J. Phaff. Microbiol. Rev. 42(1):161-193, 1978.

PROPERTIES AND KINETICS OF GLUCAN PHOSPHORYLASE OF THE AMYLOSEFORMING YEAST CRYPTOCOCCUS LAURENTII

M. S. Foda and H. J. Phaff. Zeitschr. f. Allgemeine Mikrobiol. 2:95, 1978.

Cell extracts of Cryptococcus laurentii, a yeast which synthesizes amylose when grown at low pH, contained an α-glucan phosphorylase (α-1→4) glucan: orthophosphate glucosyl transferase (EC 2.4.1.1). The enzyme, which is very labile, showed greatest stability at pH 8.0 in the presence of 0.1 M β-glycerophosphate and 0.1 M sodium fluoride. Based upon visible colour development with iodine a cell homogenate, centrifuged at 32.000 g to remove cell debris, synthesized starch-like polymers upon incubation with glucose-1-phosphate in a pH range 5.6-6.1. Based upon incorporation of labelled glucose from glucose-1-phosphate the pH for maximum activity was 5.9-6.2. The enzyme was partially purified by glycogen complexing, precipitation, and adsorption on hydroxylapatite, followed by elution. The purified enzyme has a K_m for glucose-1-phosphate of 1.6 x 10^{-2} M. Mutants impaired in their ability to synthesize amylose contained approximately one fourth of the phosphorylase activity as compared to the wild type. The possible role of the glucan phosphorylase in amylose biosynthesis is discussed.

2. The following are abstracts of papers in press:

β- GLUCANASES OF THE YEAST KLUYVEROMYCES PHASEOLOSPORUS

Partial Purification and Characterization

Tomas G. Villa, Marc-André Lachance, and Herman J. Phaff
Experimental Mycology 2: April issue, 1978

Four β-glucanases have been isolated from cell-free extracts and supernatants of lysed protoplasts of the yeast Kluyveromyces phaseolosporus after chromatography on Sephacryl S-200. β-Glucanase I was an endo-β-(1-3)-glucanase with a random action pattern upon its substrates. The main hydrolysis products from

laminarin were D-glucose and laminaribiose. The K_m with laminarin as substrate was 1.1 mM and the optimum pH was 4.5. The enzyme had an apparent molecular weight of 180,000, and was inhibited slightly by adenine and approximately 50% by 2.7 mM Hg^{2+}. Inhibition by Hg^{2+} was reversed by L-cysteine. β-Glucanase II was also an endo-β-(1-3)-glucanase, producing D-glucose and laminaribiose as hydrolysis products. The K_m estimated with laminarin as substrate was 0.6 mM and the optimum pH was 6.0. Its apparent molecular weight was 45,000. The inhibitions by adenine and Hg^{2+} were more pronounced than for β-glucanase I. The inhibition by Hg^{2+} was reversed by L-cysteine. β-Glucanase III was a typical exo-β-glucanase similar to those described in the literature for other yeast exo-β-glucanases. It hydrolyzed laminarin, releasing only D-glucose from the nonreducing end, and was unable to hydrolyze periodate-oxidized laminarin. The enzyme has a K_m value (laminarin as substrate) of 0.3 mM and a pH optimum of 6, and it rapidly hydrolyzed laminaridextrins to D-glucose. This β-glucanase was able to hydrolyze either β-(1-3) or β-(1-6) bonds. Its estimated molecular weight was 18,500. The enzyme was partially inhibited at high concentrations of adenine or Hg^{2+}. Inhibition by Hg^{2+} was not restored by L-cysteine. β-Glucanase IV released only D-glucose from laminarin but showed significant activity when tested on periodate-oxidized laminarin. Its molecular weight was 8700 and the optimum pH was 6.0. The K_m with laminarin as substrate was 0.6mM. The enzyme was inhibited partially by adenine and strongly by Hg^{2+}. Inhibition by Hg^{2+} was partially restored by L-cysteine. The intracellular β-glucanases showed the same molecular weight as those isolated from cell-free extracts. β-Glucanases I, II and IV showed lytic activity toward yeast cell walls, and a combination of the three enzymes produced spheroplasts of K. phaseolosporus.

PICHIA AMETHIONINA, A NEW HETEROTHALLIC YEAST ASSOCIATED WITH THE DECAYING STEMS OF CEREOID CACTI

W. T. Starmer, H. J. Phaff, Mary Miranda, and M. W. Miller
Internat. J. Syst. Bacteriol. 28(July issue) 1978.

A new species of the genus Pichia has been recovered 38 times in the Sonoran Desert from "rot pockets" of cereoid cacti and from Drosophila species which utilize the cacti. We have named the species Pichia amethionina due to its absolute requirement for methionine or cysteine. P. amethionina is heterothallic and demonstrates an agglutination reaction when opposite mating types are mixed. Two varieties are designated based on the combination of mannitol and ecological habitat. P. amethionina var. amethionina, the type variety, was recovered from cacti in the subtribe Stenocereinae and cannot assimilate mannitol, while P. amethionina var. pachycereana was found in cacti of the subtribe Pachycereinae and can assimilate mannitol. Results are given which demonstrate that the assimilation of mannitol is controlled by a single genetic locus. An evaluation of the interfertility and postmating viability among the two varieties and possibly identical organisms was made. The base composition of the nuclear deoxyribonucleic acid (average of 10 strains) is 33.05 ± 0.19 mol% G + C. The type strain of P. amethionina and of

the type variety, P. amethionina var. amethionina, is UCD-FST 76-401B (= ATCC 36080 = CBS 6940). The type strain of P. amethionina var. pachycereana is UCD-FST 76-384A (= ATCC 36079 = CBS 6943).

An Examination of "Hydrocarbon-Utilizing Mutants of Saccharomyces cerevisiae"

J. Bassel[1]
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Internat. J. Syst. Bacteriol. 28 (July issue) 1978.

Taxonomic and genetic studies were carried out on hydrocarbon utilizing yeasts received from the Department of Genetics and Selection, A.A. Zhdanov Leningrad State University, Leningrad, USSR. These strains have been referred to by Inge-Vechtomov and colleagues as "hydrocarbon-utilizing mutants of Saccharomyces cerevisiae." However, we were unable to confirm this identification. According to the taxonomic criteria in general use, including deoxyribonucleic acid base composition, these strains closely resemble those of Candida maltosa.

3. We are well along with the identification of some 500 strains of yeast isolated by Professor J. S. F. Barker (University of Sydney, Australia) from rots of various Opuntia cacti in Australia. Some of the species are identical to those found in the Sonoran Desert of North America (e.g. Pichia cactophila, Torulopsis sonorensis, Torulopsis sp.A, and Cryptococcus cereanus), while others have diverged somewhat (e.g. Pichia amethionina and Pichia sp.Q). Several other species, non-specific for cacti, were also identified.

XI. Department of Food and Nutrition, Osaka City University, Sumiyoshi, Osaka 585, Japan. Communicated by Akira Misaki.

The following are abstracts of two papers in press.

1. Yoshiaki Sone and Akira Misaki. Purification and Characterization of β-D-Mannosidase and β-N-Acetyl-D-Hexosaminidase of Tremella fuciformis. J. Biochem. 83:1135-1144 (1978).

ABSTRACT

β-D-Mannosidase (EC 3.2.1.25) and β-N-acetyl-D-hexosaminidase (EC 3.2.1.30) were purified approximately 500- and 200-fold, respectively, from the cell extract of Tremella fuciformis. Both glycosidases showed single protein bands in disc gel electrophoresis and the molecular weights of β-D-mannosidase and β-N-acetyl-D-hexosaminidase

were about 140,000 and 125,000, respectively, as estimated by Sephadex gel exclusion chromatography. The substrate specificities and kinetics of the two enzymes were tested with p-nitrophenyl glucosides and related oligosaccharides. The β-D-mannosidase hydrolyzed p-nitrophenyl β-D-mannoside, with K_m 2.1 mM and V_{max} 0.21 μmol per min per mg protein. 4-O-β-D-Mannosyl-D-mannose was readily hydrolyzed, but β-(1→4)-linked mannotriose and mannotetraose were hydrolyzed much more slowly. Unlike other known β-D-mannosidases, its activity was not inhibited by D-manno-γ-lactone, but was strongly inhibited by p-chloromercuribenzoate. The β-D-mannosidase tended to be inactivated in the presence of oxygen but was reactivated by dithiothreitol. The β-N-Acetyl-D-hexosaminidase hydrolyzed p-nitrophenyl β-N-acetyl-D-glucosaminide, with K_m 0.31 mM and V_{max} 4.6 μmol per min per mg protein. It was active on N, N'-diacetyl-chitobiose and higher saccharides (DP up to 5) and liberated N-acetyl-D-glucosamine. The glucosidase preparation was also slightly active on p-nitrophenyl β-N-acetyl-D-galactosaminide (K_m 0.19 mM, and V_{max} 0.6 μmol per min per mg protein). Both β-D-mannosidase and β-N-acetyl-D-hexosaminidase had an optimum pH of 5.0. Inhibition of both glycosidases by various metal ions was tested and their stabilities were investigated.

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2. Yoshiaki Sone and Akira Misaki. Structures of the Cell Wall Polysaccharides of Tremella fuciformis. Agricultural Biological Chemistry, 42:(No. 4) (1977).

ABSTRACT

The cell wall fraction prepared from the yeast-like cells of Tremella fuciformis was fractionated by proteinase digestion, hot-water and alkaline extractions. The acidic polysaccharide, which may originate from the outer layer of the cell wall, was purified from the hot-water extract. It was composed of D-glucuronic acid, D-mannose and D-xylose (molar ratio, 0.5:3.8:1.0). The methylated polysaccharide yielded on acid hydrolysis 2,3,4-tri-O-methyl-D-xylose, 2,3,4,6-tetra-O-methyl-, 2,4,6-tri-O-methyl-and 4,6-di-O-methyl-D-mannose, and 2,3,4,-tri-O-methyl-D-glucuronic acid (molar ratio, 1.0:0.8:2.7:2.3:0.5) together with a trace of 3,4-di-O-methyl-D-xylose, suggesting that the polysaccharide consists of a backbone of (1→3)-linked D-mannose residues, some of which are substituted at the C-2 positions with single or short side chains of D-xylose, D-mannose and D-glucuronic acid residues.

The alkali-insoluble residue of the cell wall was composed of D-glucose, D-glucuronic acid, D-mannose and D-xylose (molar ratio, 4.3:0.6:2.5:1.0). Methylation and periodate oxidation studies suggested it to comprise two polysaccharide moieties, i.e., β-D-glucan and glucurono-xylo-mannan, the structure of the latter resembling that of the cell surface acidic polysaccharide. The glucan moiety was shown by methylation and enzymatic degradation to have a branched structure consisting of β-(1→3)- and β-(1→6)-linkages (approx. ratio, 2:3). The controlled Smith degradation of the alkali-insoluble polysaccharide yielded an insoluble, degraded, predominantly (1→3)-linked gluco-mannan which may represent the

backbone of the cell wall polysaccharide. On the basis of these findings, the constitution of the cell wall is discussed.

A part of this work was presented at the Annual Meeting of the Agricultural Chemical Society of Japan, in Kyoto, April, 1976.

ABSTRACT

Immunochemical studies on the polysaccharides of Tremella and Cryptococcus

Yoshiaki Sone, Akira Misaki and Mitsuo Torii (This work was presented at the annual meeting of the Biochemical Society of Japan, in Tokyo, October, 1977, and the annual meeting of the Agricultural Chemical Society of Japan, in Nagoya, April, 1978.

SUMMARY

Immunochemical studies using quantitative preciptin, quantitative agglutination and double diffusion techniques confirmed the previous results deduced from structural studies of acidic heteropolysaccharides of Tremella sp. and Cryptococcus sp., that they have close phylogenic relationship.

It was also found that the two strains of T. fuciformis (strain T-7 and T-19) are serologically different from each other. Species of Cryptococcus appear to have very close phylogenic relationship to T. fuciformis strain T-7, and most species of Tremella tested in the present study have rather close relationship to the strain T-19. This must be attributed to either the absence or presence of (1→2)-linked D-xylose side chains in the cell-surface polysaccharide of individual microorganism.

XII. Institut für Allgemeine Biochemie der Universität Wien und Ludwig Boltzmann-Forschungsstelle für Biochemie, Währinger Straße 38, A-1090 Wien, Austria. Communicated by M. Breitenbach.

One of the research groups of the institute is working on yeast sporulation. Our main interests are: The role of mitochondria in sporulation, glucose repression of sporulation and mutants lacking this regulatory mechanism, adenylate cyclase and intracellular cAMP. The following is an abstract of a paper we are just writing:

In the wild type A-1160, we found a glucose sensitive phase at the beginning of premeiotic DNA synthesis. At the same time (13 hours in the system used), the cells show a prominent peak of intracellular cAMP (FEMS lett. 1:79-82 (1977), which can be completely suppressed by 0.2% glucose. A peak of respiratory activity comes much earlier (at 6 hours) and occurs also in nonsporulating a/a and α/α diploid cells. Results with an isogenic system consisting of a wild type strain (respiratory competent), a diploid strain homozygous for op-1 (deficient in the mitochondrial adenine nucleotide translocator) and the corresponding rho⁻ petite strain (part of this was presented at the 7th Spore Conference, Madison, Wisconsin, 1977): The oxygen uptake of the diploid op-1 strain on 1 % KAc is 0.22 nM/min. 10^6

cells as compared to 4.9 for the wild type and 0.06 for rho⁻. The op-1 strain is unable to grow on a synthetic medium containing acetate as a source of carbon. Yet, it sporulates on 1 % KAc. If the cells are kept in pure nitrogen, they do not sporulate. Similarly, a concentration of antimycin A which is just sufficient to suppress respiration in the diploid op-1 strain (5 ppb) is also sufficient to suppress sporulation. The isogenic rho strain is not able to sporulate. From this we conclude that the low oxygen uptake in op-1 is mitochondrial and is essential for sporulation. Energy production from acetate, however, seems to be not necessary for sporulation. So we may hypothesize that some other mitochondrial function, which is lacking in rho⁻ mitochondria, but not in op-1 mitochondria, seems to be essential for sporulation.

Intracellular cAMP: op-1 and the isogenic wild type show a low but significant level of cAMP. In the rho⁻ strain cAMP cannot be detected with the radioimmunoassay used by us.

XIII. Institut für Allgemeine Biochemie der Universität Wien, Währinger Strasse 38, A-1090 Wien, Austria. Communicated by Helmut Ruis.

Below follows the summary of a manuscript from our laboratory which has recently been submitted for publication.

Regulation of Catalase Formation in Saccharomyces cerevisiae by Carbon Catabolite Repression

H. S. Cross and H. Ruis

SUMMARY

A number of strains of Saccharomyces cerevisiae, wild type or respiratory deficient, were grown on different carbon sources. During and after growth, activities of catalase T and catalase A were determined in the cells. Specific activities of catalase T were about tenfold higher in late stationary wild type cells grown on glucose than in wild type cells harvested when glucose had just disappeared completely from the medium, or in respiratory deficient strains (rho⁻, mit⁻, pet) grown to stationary phase.

Catalase A activity is completely absent in wild type cells grown to zero percent glucose or in respiratory-deficient cells grown on glucose to stationary phase. High catalase A activity was detected in respiratory-competent cells grown to late stationary phase on one of the three sugars. The same result was obtained with a strain carrying the op-1 (pet 9) mutation, although this strain is unable to grow on nonfermentable carbon sources. All respiratory-deficient strains tested have low, but significant catalase A activities after growth on galactose or raffinose.

Wild type cells harvested during growth on glucose and rho⁻-cells grown on low glucose to stationary phase contain catalase A protein, which is enzymatically inactive. The apoprotein of the enzyme is apparently accumulated in rho⁻-cells whereas wild type cells seem to contain a mixture of apoprotein and heme-containing catalase A monomer during growth on glucose.

The results obtained show that energy, probably in the form of ATP, is required for derepression of yeast catalases from catabolite repression. At least in the case of catalase A, energy produced by respiration is required if catabolite repression is caused by glucose. If sugars less effective in carbon catabolite repression are utilized, ATP derived from fermentation seems to be sufficient for partial derepression. Depending on genetic and other factors, formation of the active enzyme is apparently influenced by glucose repression at different points: (1) at the level of protein synthesis, (2) at the stage of heme incorporation, (3) at the level of formation of the enzymatically active tetramer.

XIV. Universidad Nacional Autonoma De Mexico, Instituto De Biologia, Departamento De Biologia Experimental, Apartado Postal 70-600, Mexico 20, D. F. Communicated by Antonio Peña.

The following paper has been accepted for publication in the Journal of Membrane Biology: "Effect of Ethidium Bromide on Ca^{2+} uptake by yeast." Antonio Peña.

SUMMARY

Ethidium bromide and some other cationic dyes have been found to inhibit monovalent cation uptake. This dye produces also, in a K+-free medium, an efflux of K+ which could be of the electrogenic type. The study of the same cationic dye on Ca^{2+} uptake showed a large stimulation of the uptake rate of the divalent cation of more than tenfold. The analysis of the effects of the cationic dyes on Ca^{2+} uptake indicated that the efflux of K+ is of the electrogenic type, and can drive the uptake of the divalent cation.

Kinetic data on Ca^{2+} uptake indicate that both under "normal" or under stimulated conditions, the divalent cation is taken up by the same transport system. The addition of ethidium bromide, besides, can stimulate the uptake of Mn^{2+} and ^{14}C-glycine, and could be a good tool to magnify and study some of the characteristics of ion transport in yeast.

XV. Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan. Communicated by Kenji Soda.

The following is the summary of our paper on a yeast oxygenase published recently.

PROPERTIES OF 2-NITROPROPANE DIOXYGENASE OF HANSENULA MRAKII. Formation and Participation of Superoxide.

Toshiko Kido and Kenji Soda
From the Laboratory of Microbial Biochemistry
Institute for Chemical Research
Kyoto University, Uji, Kyoto-Fu 611, Japan

Kozi Asada
From the Research Institute for Food Science
Kyoto University, Uji, Kyoto-Fu 611, Japan

The Journal of Biological Chemistry, 253:226-232 (1978).

2-Nitropropane dioxygenase, purified to homogeneity from a yeast, Hansenula mrakii, is significantly inhibited by superoxide dismutase and various scavengers for superoxide anion such as cytochrome c, epinephrine, NADH, thiols, and polyhydric phenols. The reduction of cytochrome c and the oxidation of epinephrine and NADH are concomitant with the inhibition of enzymatic oxygenation. Neither the oxidation nor the reduction occurs in the presence of superoxide dismutase or in the absence of 2-nitropropane or oxygen. Superoxide anion added externally induces the oxygenation. These findings indicate the generation of superoxide anion and its participation in the oxygenation of 2-nitropropane.

The difference spectrum of the binding of NADH to 2-nitropropane dioxygenase exhibits a negative peak at 353 nm. One mole of NADH is bound to 1 mol of the enzyme and the pro-R hydrogen of the nicotinamide moiety of bound NADH predominantly is transferred to superoxide anion formed enzymatically or given externally. Thus, the diastereotopic hydrogen of NADH is discriminated by the enzyme, although not completely.

XVI. Centre National de la Recherche Scientifique, Laboratoire D'Enzymologie, 91190 Gif Sur Yvette, France. Communicated by J. Schwencke.

The following is the summary of a recently published article:

1. "Localization of Polyphosphate in Vacuoles of Saccharomyces cerevisiae"

K. Urech, M. Durr, Th. Boller and A. Wiemken, Institute für Allgemeine Botanik der ETH, Labor, für Pflanzenphysiologie, Sonneggstr. 5, CH-8092 Zürich, Switzerland.

J. Schwencke, Laboratoire d'Enzymologie, C.N.R.S., F-91190 Gif-sur-Yvette, France

ABSTRACT

Virtually all of the polyphosphate (PP) present in yeast protoplasts can be recovered in a crude particulate fraction if polybase-induced lysis is used for disrupting the protoplasts. This fraction contains most of the vacuoles, mitochondria and nuclei. Upon the purification of vacuoles, the PP is enriched to the same extent as are the vacuolar markers. The amount of PP per vacuole is comparable to the amount of PP per protoplast.

The possibility that PP is located in the cell wall is also considered. In the course of the incubation necessary for preparing protoplasts, 20% of the cellular PP is broken down. As this loss of PP occurs to the same extent in the absence of cell wall degrading enzymes, it is inferred that internal PP is metabolically degraded, no PP being located in the cell walls.

It is concluded that in Saccharomyces cerevisiae most if not all of the PP is located in the vacuoles, at least under the growth conditions used.

Arch. Microbiol. 116:275-278 (1978)

Request for reprints may be addressed to Dr. A. Wiemken in Zurich or to J. Schwencke in Gif-sur-Yvette.

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2. "Characteristics and integration of the yeast vacuole with cellular functions"

Jaime Schwencke, Physiol. Veg. 1977, 15 (3) 491-517

SUMMARY

Methods for the isolation of yeast vacuoles and the determination of their contents of micrometabolites are discussed. The transport of arginine and of S-adenosyl-methionine into isolated vacuoles share a number of common characteristics, among which the apparent lack of a requirement of metabolic energy is most interesting.

In the review of the lysosomal functions of the yeast vacuole the role of proteases in protein turnover, the control of some enzymatic activities, cell-wall synthesis and cellular differentiation are examined. Present hypotheses concerning the role of glucose and the vacuolar membrane in the control of these processes are also discussed.

Some of the characteristics of the vacuolar membrane are compared to those of the plasmalemma. In particular, the elasticity of the tonoplast which appears related to its high content of phospholipids. Resistance to nystatin is probably due to its low content of sterols.

Recent advances in the cytochemical visualization and in the knowledge of the ontogeny of some of the hydrolytic vacuolar enzymes are described.

XVII. Carlsberg Foundation Biological Institute, DK-2200 Copenhagen, N. 16, Tagensvej, Denmark. Communicated by Erik Zeuthen.

Our laboratory submits two communications as indicated by the following abstracts of talks to be presented at the VIth International Specialized Symposium on Yeasts to be held in Montpellier, France in July, 1978.

Rates of Glycolytic Activity during the Division Cycle of Schizosaccharomyces pombe.

Kirsten Hamburger and Birte Kramhøft

The glycolytic activity of S. pombe was studied during the division cycle. Synchronously growing cultures were produced by a selection procedure (1). The cells were grown in a defined medium with 1% glucose (2). The glycolytic activity was measured with a gasometric method, the gradient diver (3). Our results show that the glycolytic activity increases in a linear fashion between successive divisions. At each division, the rate of linear increase doubles. Similar results are obtained with cultures synchronized by heat and with cloned cells in natural synchrony (4).

With the gasometric method the glycolysis is measured in the presence of a small, however significant, consumption of oxygen. It is therefore of interest to study the glycolytic activity of cells with suppressed respiration. In many eukaryotic cells the drug, D(-)-threo-chloramphenicol (CAP) inhibits the mitochondrial protein synthesis whereas the protein synthesis in the cytoplasm is less affected. S. pombe has been grown for more than 100 generations in the defined medium containing 2 mg CAP per ml. The oxygen consumption of cells treated this way is reduced by 85%, the glycolytic activity by 13% and the generation time, measured by cell multiplication is increased from 140 minutes to about 200 minutes. These effects of CAP are fully reversible. After transfer of the cells to the normal growth medium the respiration, the glycolysis, and the cell multiplication return to the normal levels in a course of a few generations. Apparently CAP in S. pombe specifically inhibits the mitochondrial protein synthesis. The glycolytic activity of selection synchronized CAP-treated cells will be reported.

References

1. J. M. Mitchison and W. S. Vincent, Nature 205:987-989, 1965.

2. J. M. Mitchison, in: Methods in Cell Physiology, vol. IV, pp. 131-164. D. M. Prescott ed. Academic Press, 1970.

3. B. A. Nexo, K. Hamburger and E. Zeuthen, Compt. rend. trav. lab. Carlsberg 39:33-63, 1972.

Heat Shock Synchronization and the Cell Division Cycle in Schizosaccharomyces pombe

Birte Kramhøft and Kirsten Hamburger

In previous work from our laboratory, it has been demonstrated that it is possible to induce synchronous growth in suspension cultures of Schizosaccharomyces using a cyclic heat treatment (1, 2, 3).

The work was done using cultures growing in a complex medium (YEG) with an exponential generation time of 110 minutes at 32°C, using 6 heat shocks each lasting 30 minutes at 41°C spaced 110 minutes apart at 32°C.

The present work demonstrated that the same synchronizing technique can be applied to cultures of S. pombe growing in a chemically defined medium (EMM2, ref. 4) in which the exponential generation time is 140 minutes at 32°C, provided the various parameters of the heat program (temperature and duration of shocks, interval between shocks and number of shocks) are chosen accordingly to prescription. Thus, a series of 5-6 heat shocks each lasting 1 hr at 41°C and spaced 140 minutes apart at 32°C will induce two synchronous cell divisions in the culture. Each division period is preceded by a period of DNA synthesis (6).

Determination of the timepoint of the occurrence of the two synchronous divisions (time points of 50% increase in cell numbers) in cultures synchronized either in the YEG medium or EMM2 shows that these divisions occur at identical time points relative to the end of the synchronizing heat treatment in both systems. This means that irrespective of the difference in generation time in exponentially multiplying cultures in YEG and EMM2 media, the free running generation time of heat synchronized cultures is the same and independent of the exponential generation time. A comparison of both types of heat synchronized S. pombe cultures with the equivalent cultures synchronized by the selection method of Mitchison and Vincent (5), demonstrated that the relative positioning of the cell cycle markers, nuclear division, cell plate formation, DNA synthesis and cell division is very similar in all systems. We, thus, conclude that the induction of synchronous division using heat shocks does not perturb the cell cycle in any serious way.

References

1. B. Kramhøft and E. Zeuthen, Compt. rend. trav. lab. Carlsberg, 38:351-368, 1971.

2. B. Kramhøft and E. Zeuthen, in: Methods in Cell Biology, vol. XII, pp. 373-380, D. M. Prescott ed., Academic Press, 1975.

3. B. Kramhøft, S. B. Nissen and E. Zeuthen, Carlsberg Res. Commun. 41:15-25, 1976.

4. J. M. Mitchison, in: Methods in Cell Physiology, vol. IV, pp. 131-164. D. M. Prescott ed., Academic Press, 1970.

5. J. M. Mitchison and W. S. Vincent, Nature 205:987-989, 1965.

6. B. Kramhøft, K. Hamburger, S. B. Nissen and E. Zeuthen, in preparation.

XVIII. Biological Institute, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464, Japan. Communicated by N. Yanagishima.

The following papers have been published recently or will appear soon.

1. Sakurai, A., S. Tamura, N. Yanagishima and C. Shimoda. Isolation and chemical characterization of the peptidyl factor, α substance-I_A inducing sexual agglutinability in Saccharomyces cerevisiae. Agric. Biol. Chem. 41:389-393. 1977.

2. Sakurai, A., S. Tamura, N. Yanagishima and C. Shimoda. Structure of a peptidyl factor, α substance-I_A inducing sexual agglutinability in Saccharomyces cerevisiae. Agric. Biol. Chem. 41:395-398. 1977.

3. Hagiya, M., K. Yoshida and N. Yanagishima. The release of sex-specific substances responsible for sexual agglutination from haploid cells of Saccharomyces cerevisiae. Exp. Cell Res. 104:263-272. 1977.

4. Yanagishima, N., T. Shimizu, M. Hagiya, K. Yoshida, A. Sakurai and S. Tamura. Physiological detection of a binding substance for the agglutinability-inducing pheromone, α substance-I in Saccharomyces cerevisiae. Plant & Cell Physiol. 18:1181-1192. 1977.

5. Yanagishima, N. Sexual cell agglutination in Saccharomyces cerevisiae: Sexual Cell recognition and its regulation. Bot. Mag. Tokyo Special Issue 1:43-53. 1978.

6. Shimoda, C., N. Yanagishima, A. Sakurai and S. Tamura. Induction of sexual agglutinability of a mating-type cells as the primary action of the peptidyl sex factor from α mating-type cells in Saccharomyces cerevisiae. Plant & Cell Physiol. 19. 1978 (in press).

7. Kawanabe, Y., M. Hagiya, K. Yoshida and N. Yanagishima. Effect of concanavalin A on the mating reaction in Saccharomyces cerevisiae. Plant & Cell Physiol. 19. 1978 (in press).

The main recent results which are not included in the above papers are as follows.

We have succeeded in isolating and purifying the a mating-type agglutination substance responsible for sexual agglutination (Yoshida, Y., M. Hagiya and N. Yanagishima. Biochem. Biophys. Res. Commun. 71:1085. 1976). A factor thought to be proteinase C (carboxypeptidase Y) was found to destroy the a mating-type agglutination substance specifically (Matsushima, Y., C. Shimoda and N. Yanagishima Plant & Cell Physiol. 17:621. 1976; Shimoda, C., Y. Matsushima and N. Yanagishima. Antonie van Leeuwenhoek 42:511, (1976). a Cells were known to produce a factor which induced sexual agglutinability of α cells (Yanagishima, N., K. Yoshida, K. Hamada, M. Hagiya, Y. Kawanabe, A. Sakurai and S. Tamura. Plant & Cell Physiol. 17:439, (1976).

Two kinds of genes controlling inducibility of sexual agglutinability were found. The action of these genes is not mating-type-specific. a and α cells carrying one of these genes are inducible for sexual agglutinability. Manuscripts on these results and chemical characterization of α matingtype agglutination substance and the binding

substance for α substance-I inducing sexual agglutinability of a cells are in preparation.

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

Below follows the summary of a paper that is in the press and should be published in the Journal of General Microbiology soon. Then follows an abstract of a paper presented at the recent symposium on "Wall and Hyphal Growth" organized by the British Mycological Society at Queen Elizabeth College, London. It will be published later in the Bulletin of the British Mycological Society (1978) 12, part II.

R. K. Poole and Ian Salmon. The Pool Sizes of Adenine Nucleotides in Exponentially-growing, Stationary Phase and 2'-Deoxyadenosine-Synchronized Cultures of Schizosaccharomyces pombe 972 h^-. J. Gen. Microbial. (in press).

The intracellular pools of adenylates and the value of the adenylate energy charge, i.e. ([ATP] + 1/2 [ADP]) x ([ATP] + [ADP] + [AMP])$^{-1}$, in extracts of Schizosaccharomyces pombe have been determined. Dilution of extracts before enzymically converting AMP and ADP to ATP was necessary for the quantitative measurement of all three adenylates. In trichloroacetic acid or perchloric acid extracts of exponentially-growing cultures, the energy charge was 0.8-0.9, and rose to about 0.95 on glucose exhaustion in the stationary phase of growth. Lower energy charge values (0.6-0.64) were obtained using chloroform extracts. During the last 2.5 h of a 4 h pulse with 2'-deoxyadenosine of an exponentially-growing culture, and during the induced synchronous growth that followed removal of the inhibitor by centrifugation, the pool sizes of all three adenylates, particulary ADP, oscillated. Minimal ATP/ ADP ratios (and energy charge values) occurred concurrently with maximal rates of respiration that were relatively insensitive to stimulation by carbonylcyanide m-chlorophenyl hydrazone. In cultures not treated with 2'-deoxyadenosine but centrifuged and resuspended in fresh medium, a transient extensive increase in the ATP/ADP ratio occurred 1-2 h after resuspension. However, rates of O_2 uptake, in the absense or presence of carbonylcyanide-m-chlorophenyl hydrazone, increased smoothly throughout this period. The results suggest that in 2'-deoxyadenosine-induced synchronous growth, respiration rates may be controlled by the intracellular ATP/ADP ratio, and demonstrate that division synchrony is not induced by depletion of ATP pools.

I. Salmon and R. K. Poole. Growth and Cell Division of a Novel Budding Yeast, Sterigmatomyces halophilus.

Sterigmatomyces halophilus has an unusual mode of growth, daughter cells being formed remote from mother cells on fine projections (sterigmata), which can be mechanically disrupted permitting separation of the cells. Rate zonal separation on sucrose equivolumetric gradients has allowed the fractionation of exponential cultures

into various size classes, enabling the cell cycle to be studied. Measurements of DNA per cell in successive fractions indicates that DNA replication must occur either very early (i.e. in the unbudded mother cell) or very late in the cyle (i.e. just preceeding cytokinesis).

XX. School of Biological Sciences, University of East Anglia, University Plain, Norwich NR4 7TJ, England. Communicated by J. A. Barnett.

The following are recent publications from this laboratory:

Barnett, J. A. and Sims, A. P. (1976). Some physiological observations on the uptake of D-glucose and 2-deoxy-D-glucose by starving and exponentially-growing yeasts. Archives of Microbiology 111:185-192.

Barnett, J. A. and Sims, A. P. (1976). A note on the kinetics of uptake of D-glucose by the food yeast, Candida utilis. Archives of Microbiology 111:193-194.

Barnett, J. A. (1977). The nutritional tests in yeast systematics. Journal of General Microbiology 99:183-190.

Sims, A. P. and Barnett, J. A. (1978). The requirement of oxygen for the utilization of maltose, cellobiose and D-galactose by certain anaerobically fermenting yeasts (Kluyver effect). Journal of General Microbiology (in press).

The substance of the last paper is as follows. Of the yeasts that ferment D-glucose anaerobically, over 40% can use certain glycosides and D-galactose oxidatively, but cannot ferment them. This phenomenon is called the Kluyver effect. More than half the yeast species described which exhibit this effect do so with more than one substrate. Yeasts showing the effect with maltose, cellobiose and D-galactose are compared with fermenting strains, to determine whether enzyme inactivation or cessation of sugar uptake is responsible. The different responses of the yeasts to anaerobic conditions, with respect to their enzymic activity, sugar uptake and CO_2 production, consistently showed that the Kluyver effect resulted from the requirement of transport for oxygen, and this seems to be the common explanation throughout the yeasts.

Work on the Kluyver effect is continuing here.

XXI. Department of Biochemistry, James Cook University, QLD, 4811, Australia. Communicated by K. Watson.

Research has continued in our laboratory on the biochemistry of psychrophilic and thermophilic (psychrophobic) yeasts. The following are summaries of results of recent communications.

Kenneth Watson and Helen Arthur. Cell surface topography of Candida and Leucosporidium yeasts as revealed by scanning electron microscopy. J. Bacteriol. 130:312-317 (1977).

A summary of this paper was presented in Yeast Newsletter XXVI, p. 10.

H. Arthur, K. Watson, C. R. McArthur and G. D. Clark-Walker. Naturally occurring respiratory deficient Candida slooffii strains resemble petite mutants. Nature 271:750-752 (1978).

Summary

C. slooffii is a naturally occurring respiratory deficient yeast originally isolated from the anaerobic environment of horse intestine but which has since been found in the guts of several domestic animals (van Uden and Buckley, The Yeasts (Lodder, J. ed.), p.1049-1051, 1970). This yeast has been confirmed as lacking respiration; in addition, it has been shown to lack cytochrome aa_3, to have poorly defined mitochondrial profiles (Arthur and Watson, J. Bacteriol. 128:56-68, 1976) and to never revert to respiratory competent forms. When circular DNA is prepared from mitochondrial-like particles of three strains of C. clooffii, a single buoyant density peak is found in each of the strains. However, each circular DNA has a different buoyant density which ranges from 1.680 to 1.674 g cm^{-3}. Confirmation that these circular DNAs are different was obtained by electron microscopy which showed that each preparation has a unique circular DNA size profile ranging from 0.56 to 1.76 um. The results indicate that the mitochondrial DNA has suffered large deletions and that each strain is independently derived. In these respects they resemble true petite mutants. On the basis of the above results, we suggest that petite mutants of many yeast species, rather than being laboratory curiosities, may indeed be widespread in habitats which do not select for respiratory competence.

K. Watson, H. Morton, H. Arthur and M. Streamer. Membrane lipid composition: a determinant of anaerobic growth and petite formation in psychrophilic and psychrotolerant yeasts. Biochem. Soc. Trans. (in press).

Summary

Previous studies have established that membranes of psychrophilic yeasts are rich in polyunsaturated fatty acids, in contrast to thermophilic species (Arthur and Watson, J. Bacteriol. 128:56-68, 1976). Furthermore, two of the latter species are naturally occurring respiratory-deficient yeasts and one of them, Candida slooffii, has recently been shown to resemble petite (cytoplasmic) mutants (Arthur, Watson et al. Nature, 271:750-752 1978). We have been prompted by these observations to investigate the possible correlation of polyunsaturated fatty acids in yeast membranes with (a) ability to form spontaneous and drug-induced petite mutants and (b) ability to grow anaerobically. Examination of a range of psychrophilic, mesophilic and thermophilic yeasts has led to the conclusion that membrane lipid composition, in particular linolenic acid, is related to petite formation and anaerobic growth. Psychrophilic yeasts appear to be obligate aerobes and were unable to undergo more than 2-3 divisions under anaerobic conditions. Stable repiratory-deficient (petite) mutants were also not formed by these yeasts. In contrast, thermophilic species grew well anaerobically and formed spontaneous petite mutants or could be induced to form such mutants by treatment of cells

with ethidium bromide or euflavine. Mesophilic species, on the other hand, could be divided into two groups on the basis of the presence or absence of polyunsaturated fatty acids. The presence of polyunsaturated fatty acids. The presence of polyunsaturated fatty acids in cell membranes correlated well with inability to grow anaerobically and inability to form stable petite mutants. From these results, it is tentatively proposed that there is a trend, in eucaryotic microorganisms in general, from fermentative to respiratory metabolism in going from thermophilic, mesophilic to psychrophilic species. Such a trend may also be operative, but to a more limited extent, in procaryotic microorganisms.

XXII. Institute of Physics, College of General Education, University of Tokyo, Komaba 3-8-1, Meguroku, Tokyo, 153, Japan. Communicated by Takashi Ito.

Below follow abstracts of two recent papers on yeast cells.

1. T. Ito and K. Kobayashi: A survey of *in vivo* photodynamic activity of xanthenes, thiazines, and acridines in yeast cells. Photochemistry and Photobiology, 26:581-587 (1977).

SUMMARY

In view of the recent interest in the possibility of a singlet oxygen mechanism playing an important role in photodynamic action, a number of different types of dyes were surveyed with respect to cell inactivation and induction of genetic changes in yeast cells. These comprise three xanthene dyes, three thiazine dyes, three acridine dyes and ethidium bromide. Rhodamine B in the first group and methylene blue in the second group were inactive under the present conditions. Both were found to be non-penetrable into the cell. However, since toluidine blue is active, non-penetrability is not a determining factor in photodynamic action. Ethidium bromide was inactive under the present conditions, even though it was penetrable into the cell. The survey showed that the dye must be bound to DNA in order to be active in the induction of a genetic change (gene conversion). All dyes which were active in either inactivation or induction or both were modified in their effectiveness both by the addition of N_3^- (suppresion) and in deuterated medium (enhancement) indicating that the sensitization mechanism involves singlet oxygen. The deuterium effect was generally observable to a lesser extent in the *in vivo* situation than *in vitro*, in particular for genetic changes by proflavine and acriflavine in which the sensitizer binds to DNA.

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2. T. Ito: Cellular and subcellular mechanisms of photodynamic action: The $^1O_2$ hypothesis as a driving force in recent research. Photochemistry and Photobiology, (1978) in press). Special issue for International Conference of Singlet Oxygen and Related Species at Pinawa, Canada, August, 1977.

SUMMARY

Since 1972, the protective (quenching) effect of N_3^- and the enhancing effect of D_2O have been used in combination to demonstrate $^1O_2$ ($^1\Delta_g$) as the major reactive intermediate in the dye-sensitized photooxygenation of biomolecules in the in vitro system. Extended application to the in vivo system has recently begun producing some results which generally support the involvement of $^1O_2$ pathway in the photosensitization processes. The use of other $^1O_2$ quenchers and acceptors has also been increasing. In the application of these techniques as a diagnostic tests for $^1O_2$ participation in the in vivo system, careful examination of the experimental conditions should be made with respect to the quenching capability and accessibility of D_2O in the specified cellular environment. Furthermore, in view of the diffusive nature of $^1O_2$, generation sites, generation efficiencies, hence the location of the type of sensitizers, and the reactivity of $^1O_2$ with presumed target structures in the cell should also be taken into account in the interpretation. Recent studies illustrating the importance of these factors are discussed. Finally, a tentative picture of the mode of in vivo photodynamic activity of common dye sensitizers is outlined.

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

"Mathematical Analysis of Mutation Yield Data in Unicellular Systems." Presented at the Annual Meeting of the Radiation Research Society in Toronto, May 15, 1978.

R. H. Haynes and Friederike Eckardt

Induced mutation frequency for any mutagen dose x is given by $M(x) = Y(x)/S(x)$ where $Y(x)$ is the mutant yield per cell assayed and $S(x)$ is the surviving fraction of cells. For homogeneous cell populations and assay systems for which mutation and killing are stochastically independent observables, and the mutation yield can be expressed formally as $Y(x) \doteq \{1-\exp[-H_m(x)]\} \exp[-H_k(x)]$, where H_m and H_k are the expected numbers of mutational and lethal hits, respectively. If both H_m and H_k contain only linear (single-hit) terms, the initial slope of $Y(x)$ is proportional to the mutability of the system and the maximum yield of mutations occurs at the one lethal hit dose (LD_{37}). However, if killing is single-hit, but mutagenesis purely quadratic, the initial slope of the yield curve is zero and the yield maximum is shifted to twice the one lethal hit dose. If the survival curve has a shoulder such that H_K has

both linear and quadratic terms, the maximum mutational yield for linear mutation induction occurs at one half of LD_{14} dose; for purely quadratic induction the maximum yield is at one half the dose for four lethal hits (1/2 LD_2). Additional shifts in the yield maxima occur if mutation and killing are not stochastically independent. Such shifts in mutational yield maxima occur for UV mutagenesis in yeast. For risk estimation it is more informative to plot mutation yield and survival independently rather than mutation frequency alone. (Supported by NRC Canada).

Paper to be presented to Canadian Genetics Society Annual Meeting, Halifax, N.S., June 16, 1978.

"Yields and Frequencies in Mutation Research"

R. H. Haynes and Friederike Eckardt

In mutation research mutation frequencies (mutants per survivor) are reported more commonly than mutation yields (mutants per cell assayed). Induced mutation frequency is the ratio of yield to survival and when this quantity is plotted double logarithmically against mutagen dose it is possible to determine by inspection of the graph whether mutagenesis is a 'one-hit', 'two-hit' or some more complex kinetic process. However, for purposes of risk estimation as well as theoretical analysis it is more informative to plot yield and survival independently. We have worked out the expected mathematical form of yield curves for various kinetic situations: exponential or shouldered survival curves, accompanied by linear, quadratic or biphasic mutation induction both with and without 'δ-effects'. The dose giving the maximum mutational yield depends on the particular combination of mutation and survival kinetics involved. Examples of various types of yield curves as found in UV mutagenesis of yeast will be presented.

XXIV. Bhabha Atomic Research Centre, Division of Radiological Protection, Trombay, Bombay 400 085, India. Communicated by M. S. S. Murthy.

M. S. S. Murthy, P. Subrahmanyam, B. S. Rao, N. M. S. Reddy, V. V. Deorukhakar, N. Sankaranarayanan and U. Madhvanath.

In our laboratory we have been using several strains of yeast for many investigations with ionising radiation, hyperthermia and chemical mutagenesis. The following is a brief report of the results recently obtained.

Ionising radiation:

i) Role of oxygen in the repair of high LET radiation: Oxygen is known to have a dual role in the radiobiological aspects of low LET radiation i.e. it enhances the radiation effects (Lethality, gene conversion, mutation etc.) when present at the time of irradiation, but it also aids recovery when present during post irradiation treatment. The oxygen enhancement ratio (OER) widely used in radiobiology refers only to the enhancing effect of oxygen and is known to decrease with the increasing LET of the radiation. However,

the role of oxygen in the repair of damage caused by high LET radiation has not been studied. We have suspended diploid yeast cells in phosphate buffer containing 70% enriched Boron at non-toxic concentration. When such a suspension is exposed to a beam of thermal neutrons in a nuclear reactor (40 MW power), intense high LET (200 KeV/μm) radiation will be produced in situ, causing cell lethality. The nuclear reaction involved is $^{10}B + {}^{1}_{0}n \; T \; {}^{4}_{2}He + {}^{7}Li$. Samples were exposed in both euoxic and anoxic conditions to graded doses covering survival down to 1%. After irradiation, they were both plated immediately and also allowed to undergo liquid holding recovery (LHR) in both euoxic and anoxic conditions. The results of these experiments can be summarized as follows:

a) Oxygen present during irradiation still has an enhancing effect on the high LET radiation studied in this investigation (DMF = 1.29 at 10% survival level).

b) The high LET damage can also undergo LHR (DP/IP survival - 2.5 at 30% survival).

c) The LHR takes place only in the presence of oxygen and is totally inhibited in its absence.

ii) Nature of damage involved in LHR. After treatment by gamma and alpha radiation, the diploid yeast cell suspensions were subjected to LHR under various conditions. These experiments show that a) the cells which have undergone LHR are saturated with sublethal damage; b) elimination of sublethal damage of growing cells in nutrient broth for various periods of time prior to LHR, progressively reduces their ability to undergo LHR. These results are taken to indicate that there is a close relationship between sublethal and potentially lethal damage; it supports the hypothesis that potentially lethal damage may constitute that part of the sublethal damage which interacts to form lethal damage.

iii) Spontaneous induction of gene conversion: The condition under which the spontaneous induction of gene conversion occurs is studied in diploid yeast strain BZ 34 which is hetero-allelic at the argino succinase locus. These studies show that sporulation is a necessary condition for the spontaneous induction of gene conversion. Any agent which inhibits sporulation such as anoxia, low temperature, caffeine and radiation can also inhibit the spontaneous induction of gene conversion.

iv) We have been studying the LHR and budding cell resistance of a number of mutant strains of yeast. About 20 such strains have been obtained from the Yeast Genetic Stock Centre, Berkeley. The purpose of this investigation is to elucidate the genetic control of these repair processes. Preliminary results show that intragenic recombination pathway controlled by the REC 4 gene in strain 2C8 has no influence on the budding resistance. Further investigations are in progress.

Hyperthermia

1) Interaction of damage caused by hyperthermia and ionising radiation: Hyperthermic treatment at temperatures above 50°C is lethal to yeast cells. Anoxic cells are more sensitive to hyperthermic treatment than euoxic cells. This is exactly opposite to the situation in radiation and hence is of specific interest in the treatment of cancer. We have investigated some aspects of the interaction of damage caused by the two agents. These experiments show that:

a) Hyperthermia enhances the damage caused by ionising radiation in both euoxic and anoxic cells.

b) In euoxic cells, hyperthermic potentiation decreases continuously with the increasing time gap between heat treatment and exposure to radiation. After about 16 h the potentiation is completely lost. This suggests that hyperthermic damage is being repaired in the euoxic cells.

c) The hyperthermic potentiation did not show any decrease with increasing time gap in anoxic cells. This suggests that repair of hyperthermic damage requires oxygen. Since the oxygen status of cells in a solid tumor follows a very complex course, this finding is important in evaluating the usefulness of a combination therapy of solid tumors.

Chemical mutagenesis:

i) Genotoxicity of food colors: Yeast can be conveniently used as a microbial system for detecting the genotoxic effects of chemical substances in the environment. Radiation and a number of chemicals such as EMS, MNNG, HNO_2, 4 NQO, AF-2 etc. induce gene conversion at a high frequency in the diploid strain BZ-34. We have been investigating the genotoxicity of a number of food coloring agents-both permitted and non-permitted (but still widely used in India) in this strain.

Preliminary results show that the permitted colors, without any further metabolic activation do not incude gene conversion even at high concentration (5 mg/ml). This work is in further progress.

ii) Determination of REC value for EMS: One of the short-cut methods of quantitating the mutagenic hazards of a chemical is to compare its effectiveness with that of radiation. The unit proposed for this is Rem-equivalant-chemical (REC). We have estimated the REC value for the chemical mutagen EMS in diploid yeast strain BZ-34. This compares favorably with similar values obtained in a number of other species, emphasising that a constant REC value may be expected for a given chemical, irrespective of the test organism.

iii) Radiation response of yeast strains D4 and D7: These strains have been extensively used to detect mitotic gene conversion and mitotic crossing over caused by chemical mutagens. We have studied the response of these strains to gamma radiation with lethality, gene conversion and crossing over as the end points. These results show a number of differences in the response to gamma radiation and to chemicals.

iv) Correlation between gene conversion and mutation: Since gene conversion in yeast is a simple method of testing the genotoxicity of chemicals, it is necessary to establish a correlation between gene conversion and mutation. We have compiled a list consisting of nearly 150 chemical substances which have been tested for gene conversion in different yeast strains all over the world. This compilation shows that substances which are a) non-convertogenic are also non-mutagenic; b) weak convertogens are also weak mutagens and c) strong convertogens are also strong mutagens, thus establishing a firm correlation between the two.

A. Presentation of Reports at Conferences

No.	Title(Author)	Conference details
1.	Use of yeast Saccharomyces cerevisiae in radiobiology (P. Subrahmanyam)	18th Annual Conference of Association of Microbiologists of India, Madurai, 21-23 Dec. 77.
2.	Influence of rad mutations on budding cell resistance and liquid holding recovery in diploid strains of Saccharomyces cerevisiae (B.S. Rao and M.S.S. Murthy)	"

B. Technical Publications

1.	On the nature of damage involved in liquid holding recovery in diploid yeast after gamma and alpha irradiation (B.S. Rao and M.S.S. Murthy)	Int. J. Radiat. Biol. 1978 (in press)
2.	OER of fission neutrons for induction of heteroallelic reversion in diploid yeast (P. Subrahmanyam, B. S. Rao and U. Madhanath)	Radiat. Res. 1978 (in press)
3.	Determination of radiation equivalance of Ethyl Methane Sulphonate (EMS) for induction of gene confersion in diploid yeast (M. S. S. Murthy and N. Sankaranarayanan)	Mutat. Res. 1978 (in press)
4.	Induction of gene conversion in Saccharomyces cerevisiae by the nitrofuran derivative furylfuramide (AF-2) (M. S. S. Murthy and N. Sankaranarayanan).	Mutat. Res. 1978 (in press)

C. Papers in preparation

I. Modification of radiation induced damage and repair in yeast by hypoxia (P. Subrahmanyam, B. S. Rao, N. M. S. Reddy, M. S. S. Murthy and U. Madhvanath)

ABSTRACT

The lethal response of diploid yeast strain BZ 34 to densely ionising radiation from $^{10}B(n, \alpha)^{7}Li$ has been compared to that of ^{60}Co gamma radiation.

The relative biological effectiveness (RBE) was found to be 3.6. The values of oxygen enhancement ratio (OER) for the two radiations have been shown to be respectively 1.3 and 2.8. Liquid holding recovery of the irradiated yeast was also studied for different combination of aeration and hypoxia during irradiation and recovery. The investigations point to the following conclusions.

If hypoxia is maintained throughout the irradiation and recovery, the high LET induced damage does not undergo recovery whereas that for gamma radiation is depressed significantly.

Oxygen is essential for the recovery from the damage induced by both the radiations irrespective of whether the irradiation was carried out in aerobic or hypoxic conditions.

The magnitude of recovery is generally low for $\alpha + {}^{7}Li$ induced damage than gamma induced damage.

II. Determination of radiation equivalence of the chemical furylfuramide (AF-2) for the induction of gene conversion in diploid yeast and estimation of genetic risk to Japanese population (M.S.S. Murthy and N. Sankaranarayanan)

ABSTRACT

Dose-effect relationship for the end point of induction of gene conversion by AF-2 in Saccharomyces cerevisiae strain BZ 34 has been reported. On the basis of this, radiation equivalent chemical (REC) value for AF-2 for the induction of gene conversion has been calculated to be 0.085 µg/ml.hr. This compares well with the data obtained with other test systems and other genetic endpoints. An inadequacy in the estimation of genetic risk to human population on the basis of REC values obtained from laboratory test systems has been discussed.

XXV. Institut für Mikrobiologie, Technische Hochschule Darmstadt Schnittspahnstr. 10, 6100 Darmstadt, Federal Republic of Germany. Communicated by M. Ciriacy.

Current research in progress by our group:

(1) Genetic and metabolic regulation of carbohydrate utilization in Saccharomyces cerevisiae (M. Ciriacy, K. D. Entian, M. Großmann, H. Raenberger, U. Bohn and I. Breitenbach)

(2) Clonal Aging and related problems in yeast (R. Kern and D. Breilmann)

M. Ciriacy and U. Bohn are currently investigating a dominant regulatory mutant (CCR80^{r}) affecting glucose repression of oxidative

enzyme formation. Glucose-resistant enzyme formation was most prominent with regard to the mitochondrial enzymes succinate dehydrogenase and NADH: cytochrome c oxidoreductase. The effect of CCR80^r mutations was rather small but still significant on the gluconeogenetic enzymes isocitrate lyase, malate synthase and fructose-1, 6-bisphosphatase and on invertase. Aerobic fermentation of glucose was almost completely inhibited in this mutant; anaerobic fermentation was reduced. Therefore, the mutant appears to be altered in the regulation of glycolysis. The glucose-resistant synthesis of oxidative enzymes seems to be a corollary of this alteration. Furthermore, we determined levels of glycolytic intermediates in glucose-grown wild-type and mutant cells. Only the contents of fructose-1, 6-bisphosphate and of pyruvate were significantly reduced in the CCR80 mutant. However, the differences were not sufficiently large to provide with a final explanation of the mutant's phenotype. A further observation indicated a regulatory disorder of glycolysis and/or glucose uptake: CCR80^r is epistatic over the pyruvate kinase-less phenotype (pyk$^-$ -mutant was kindly provided by Dr. D. Fraenkel, Harvard Medical School). pyk$^-$ cells are completely inhibited by glucose, whereas CCR80^r pyk double mutants can grow on glucose. It was suggested by others, that glucose inhibition in pyk mutants is induced by accumulation of glycolytic intermediates. However, the double mutant accumulates 3-phosphoglycerate and PEP in considerable amounts, too. Although the primary defect in CCR80^r mutants is still undiscovered, their existence provides evidence for a common control site of metabolic regulation at the level of glucose uptake and/or glycolysis, and of the derepression of oxidative enzymes (Published in part: Molec. gen. Genet. 159, 329-335, 1978).

Mr. Ciriacy and Ingrid Breitenbach started recently with the isolation of mutants unable to utilize glucose as a sole carbon source. Out of 104 mutants, 18 were unable to ferment glucose or maltose. Most of them were strongly inhibited by glucose. Until now, this phenotype could be attributed to enzymatic defects with respect to pyruvate kinase (allelic to pyk-mutants found by others), phosphoglycerate mutase, pyruvate decarboxylase, resp. Two mutants are pleiotropically affected in some glycolytic enzymes.

Carbon catabolite repression of enzymes involved in disaccharid-fermentation was investigated by K. -D. Entian. As reported in Vol. XXVI, we isolated three types of mutants resistant to catabolite repression, notably of the enzymes invertase and maltase. The first class with reduced hexokinase levels was previously described (Entian et al., Molec. Gen. Genet., 156, 99-105, 1977). In addition, catabolite inactivation was affected, too (Entian, Molec. gen. Genet., 158, 201-210, 1977). The second class has elevated hexokinase activity and was inhibited in the presence of maltose, even if glucose was present in the media. 3 to 4 h after maltose addition, fermentation was strongly reduced, whereas respiration was only slightly affected. Growth stopped 3 h after maltose addition. There was, however, no remarkable difference in any of the glycolytic enzymes between mutant and wild type. Hexose phosphates were slightly increased, triose phosphates were slightly decreased, ATP,

ADP, NAD and NADH were normal. When maltose was added to cells pre-grown on ethanol medium, there was a drastic effect on the level of intracellular glucose. Whereas wild type level was about 15 to 20 µmoles/g dry weight, mutant level ranged about 330 to 360 µmoles/g dry weight. The kinetic of intracellular glucose accumulation increased drastic 2 to 3 h after maltose addition. This increase could be observed at the same time, when growth stopped. After feeding with labeled maltose, cells were fractionated. Most of the activity was found in the low molecular fraction, whereas the protein fraction was only poorly labeled. A quarter of the activity could be detected in the cell debris. Up to now, we expect that the huge increase of intracellular glucose is responsible for the inhibitory effect of maltose.

Heat inactiviation of the elevated hexokinase showed no difference to wild type. It is supposed, that the increased hexokinase activity is caused by a regulatory defect in hexokinase synthesis.

H. Rasenberger currently investigates mutants, which do not ferment glucose and which are inhibited in the utilization of other carbohydrates in the presence of glucose. The uptake and utilization of different sugars and glycerol was tested by manometric and enzymatic means. Two of the mutants seem not to be affected in glycolytic enzymes. One of the mutants seems to have a defect in phoshoglucoisomerase.

M. Groβmann examines the role of proteinase B in enzyme inactivation. Addition of glucose to yeast cells growing on non-sugar carbon sources results in a loss of activity of fructose-1, 6-bisphosphatase (FBPase), isocitrate lyase (ICL) and malate dehydrogenase (MDH). The proteinase B inhibitor phenylmethylsulfonyl fluoride (PMSF) was added 40 minutes prior to glucose addition to the culture medium (final concentration 2 mM). For the FBPase this resulted in a complete stop of inactivation. This was monitored by the total activities which represent the enzyme activity per ml of crude extract. Therefore, the increasing protein content during cell growth has no influence on the activity values. The decrease of specific activity of FBPase was caused by PMSF-unaffected cell growth. A very small decrease of specific activity was recognized for ICL and MDH, indicating that sunthesis of these enzymes was not completely stopped.

After switching off the catabolite inactivation, we now can see that there is a strong repression of FBPase. Prepression of ICL and MDH is weak during the first hour after glucose addition, but after 90 minutes it seems to be as strong as the FBPase repression. The unaffected repression of malate synthase showed that the proteinase B inhibitor does not interfere with repression mechanism. Furthermore, PMSF had no influence on glucose uptake which is necessary for starting the repression process. This was revealed by investigating cell fermentation and respiration after glucose addition to ethanol pregrown cells. There was no difference between a 2 mM PMSF containing culture and an untreated one. With regard to these results, it

seems that enzyme inactivation compensates weak repression (MDH, ICL) and magnifies strong repression (FBPase) to avoid ATP-splitting cycles.

Dagmar Breilmann and Rudolg Kern are presently concerned with various aspects of clonal ageing in Saccharomyces cerevisiae. Dagmar Breilmann is working with mutants carying a simple nutritional requirement and now tries to find a mutation which leads to some kind of inaccuracy in the translation process, thereby functioning as a missense suppressor which restores a certain amount of wild-type enzyme activity. It is anticipated that altered ribosomes are involved. For this purpose, a strain of Saccharomyces cerevisiae was treated with EMS and red ade mutants were isolated. Rich medium containing cycloheximide is now used to find resistant white, slow growing colonies, which are again adenine independent. Both of these traits, resistance and adenine independence, should be due to one and the same mutation, which is supposed to result in a ribosomal alteration. The exact character of these mutants then has to be defined in tetrad analysis.

Rudolf Kern is engaged in the isolation of successive daughter cell generations from immobilized mother cells. Logarithmically growing Saccharomyces cerevisiae cells are bound to a concanavalin A-derivatived nylon matrix after the method of Edelman et al. (Proc. nat. Acad. Sci. 68, 2153-2157, 1971). A special chamber was used which allows for microscopic observation of the binding process and, after the cells are supplemented with a continuous flow of nutrient broth, of successive budding of the fixed cells. Released daughter cells can be isolated by filtration or centrifugation of outflown medium. Attempts are now made to construct a chamber for isolating ca. 10^8 daughter cells from immobilized mother cells every 2 hours. This allows for testing the accuracy of the translation process and the genetic stability. The genetic test system is D7 (Zimmermann et al.. Mutation Res. 28, 381-388, 1975).

XXVI. Department of Physiology, Carlsberg Laboratorium, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark. Communicated by M. C. Kielland-Brandt.

Below follows the abstract of a recently published paper:

S. Holmberg and M. C. Kielland-Brandt: A Mutant of Saccharomyces cerevisiae Temperature Sensitive for Flocculation. Influence of Oxygen and Respiratory Deficiency on Flocculence. Carlsberg Res. Commun. 43:37-47 (1978).

A flocculent strain of Saccharomyces cerevisiae, containing the dominant gene for flocculence FL04, was mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine. Non-flocculent mutants were isolated with a selection procedure based on the slower sedimentation of non-flocculent cells. One closer studied mutant was due to an unlikened suppressor mutation for FL04. This suppressor gene is designated sufl. The gene sufl

is neither centromere linked nor linked to his4 or the mating type locus. The gene sufl behaves as a recessive in some diploids and as a dominant in others, illustrating the genetic complexity of the flocculation phenomenon. The mutant was found to be non-flocculent after growth at 30°C whether aerated or not. At 22°C the mutant was flocculent in the absence of aeration during growth, but non-flocculent with aeration or supplementation of the growth medium with ergosterol and unsaturated fatty acids. None of several inhibitors of mitochondrial functions had any effect on the expression of flocculence. A number of petites induced in the mutant strain with ethidium bromide had altered flocculation phenotypes.

A method of measuring flocculence using the spectrophotometer is described.

XXVII. Department of Genetics and Interdepartmental Developmental Biology Program, The Ohio State University, Columbus, Ohio 43210. Communicated by Philip S. Perlman.

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

C. William Birkey, Jr., Robert L. Strausberg, Philip S. Perlman and Jean L. Forster. Vegetative Segregation of Mitochondria in Yeast: Estimating Parameters Using a Random Model. Department of Zoology, Duke University, Durham, North Carolina 27706. Molec. gen. Genet. 158:251-261 (1978).

Summary

Yeast zygotes which are heteroplasmic for mitochondrial genes reproduce vegetatively to form clones of diploid progeny which are homoplasmic. This vegetative segregation of mitochondrial genes has been interpreted in terms of a random distribution of mitochondria or mitochondrial genomes between mother and bud at cell division. We have developed equations which permit calculation of the number of segregating units in the zygote and the number of those units which enter the bud, assuming that segregation of the units is genetically random and numerically variable or equal. Use of the equations requires data from partial pedigree analyses: we isolate zygotes, separate the first bud, then determine the frequency of mitochondrial alleles among the progeny of mother cells whose first buds were homoplasmic. Application of this method to data from five crosses suggests that most zygotes have a small number of segregating units (usually less than a dozen) and only one or two enter the first bud. Analysis of the frequency of buds which are nearly but not quite homoplasmic indicates that the segregating units may be mitochondria or portions thereof which include many mitochondrial genomes, all the genomes in a unit being genetically identical in most but not all cases. These results are compatible with, but do not prove, the hypothesis of random vegetative segregation of mitochondria.

XXVIII. Miller Brewing Company, 3939 West Highland Blvd., Milwaukee, Wisconsin 53208. Communicated by Michael C. Barney.

Below follow two abstracts of papers to be presented at the May 1978 Annual Meeting of the American Society for Microbiology in Las Vegas and at the Am. Soc. for Brewing Chemists, respectively.

Genetic Transformation of Saccharomyces cerevisiae

G. P. Jansen, M. C. Barney and J. R. Helbert

DNA extracted from Saccharomyces diastaticus (ATCC 28339, α haploid) was used to transform cells of α haploid, multiple auxotrophic recipients, S. cerevisiae (YGSC Nos. R251-4A & D160-2C). Genetic transformation was successfully accomplished by incubating DNA extracted from the donor with spheroplasts of the recipient. Agents known to affect membrane permeability were added to the reaction mixture. Following exposure of spheroplasts to DNA, cell walls were regenerated in hypertonic media containing 3% agar. Transformants were scored by their ability to grow in minimal media lacking the auxotroph requirements, by colonial pigment changes, and by morphological differences. These transformants were further characterized by fermentation and assimilation patterns. Failure of the transformants to sporulate and quantitations of DNA indicated that the genetic changes were not due to hybridization. A transformation efficiency of 0.2% was obtained.

Use of Spheroplast Fusion and Genetic Transformation to Introduce Dextrin Utilization into S. uvarum

M. C. Barney, G. P. Jansen and J. R. Helbert

Using two methods of genetic manipulation, the ability to utilize dextrin was introduced into the brewer's yeast, Saccharomyces uvarum. Both methods involved combining the genetic information of S. diastaticus with that of S. uvarum. The fusion technique involved the formation of spheroplasts by enzymatic digestion of the cell wall followed by a fusing of the two cell types. After the spheroplasts were fused, a cell wall was regenerated by incubating cells in a hypertonic medium containing 3% agar. The second technique involved extracting DNA from S. diastaticus. The S. uvarum recipients were spheroplasted and were incubated with extracted DNA in the presence of sarkosyl. Subsequently, cell walls were regenerated by incubating the spheroplasts in a hypertonic medium containing 3% agar. With both methods, the progeny were shown to be able to utilize dextrin.

XXIX. 10 West Grove, London SE10 8QU, England. Communicated by J. F. T. Spencer.

The following papers have been accepted for presentation at the 6th International Specialized Symposium on Yeasts, to be held at Montpelier, July 2-8, 1978.

1. Aberrant Mating Behavior in Some Industrial Yeast Strains.

As is known, brewing and distillers' yeasts are frequently homothallic and, in addition, sporulate poorly and show very low spore viability. These strains do, however, exhibit mating behavior and frequently mate with one or both mating types of haploid tester strains, though the frequency of mating may be low. We observed earlier that strains isolated as segregants from a hybrid between laboratory diploid mating strains and a distiller's yeast frequently showed not only apparent bisexual mating behavior in mass mating, but, in addition, all four spores from one ascus were initially of the same mating type. Recently, similar behavior has been observed in direct matings between haploid tester strains and a number of industrial yeast strains. Also, hybrids obtained from these matings showed major differences in the viability of the spores formed, viability being much higher in spores formed in crosses with the a mating type, for instance. The relationship of these observations to the known genes for homothallism will be discussed.

2. Dominant and Recessive Alleles of Genes Controlling Sensitivity and Resistance in Brewing Yeasts to Media Having Elevated Osmotic Tensions.

Recently a relationship between sensitivity to high osmotic tensions and the presence of suppressors in yeasts have been reported by Singh. Tests of a number of segregants between brewing and distillers' yeasts and laboratory strains showed that some of the segregants from osmotic-resistant parent strains were osmotic sensitive, the frequency of appearance of sensitive segregants varying with the parent strains. In addition, an osmotic-sensitive brewing yeast, which is also somewhat unusual in producing relatively large numbers of viable spores, yielded osmotic-resistant segregants. Further, segregants obtained from these strains have so far been osmotic-sensitive. The study is being extended to allow further investigation of these phenomena. The relationship, if any, to the presence of suppressors in the parent strains will also be investigated.

We should like to report the isolation of a Metschnikowia species, probably M. bicuspidata, from water obtained from a salt marsh on the Gower Peninsula, near Swansea, Wales. To the best of our knowledge, this is the first report of the isolation of this species in Britain. The samples were taken from the neighborhood of a growth of Spartina townshendii, on the edge of the tidal part of the estuary. The species is tentatively taken as M. bicuspidata on the basis of the size of the cells and asci, and the habitat. Further studies, to confirm the specific identification, are in progress.

XXX. Department of Microbiology, University of Guelph, Guelph, Ontario Canada N1G 2W1. Communicated by K. F. Gregory.

The following is the abstract of a paper currently in press:

Momose, H. and K. F. Gregory. Temperature-sensitive mutants of Saccharomyces cerevisiae variable in the methionine content of their protein. Appl. Environ. Microbiol. 1978.

ABSTRACT

Temperature-sensitive mutants were derived from Saccharomyces cerevisiae Y5α, by ethyl methane sulfonate mutagenesis, in a search for mutants which would produce methionine-rich protein at the non-permissive temperature. A total of 132 mutant strains was selected which showed adequate growth on minimal medium at 25°C but showed little or no growth on the same medium supplemented with a high concentration (2 mg/ml) of L-methionine at 37°C. Several of these mutants were found to increase the proportion of methionine in their protein to much higher levels than that of the wild-type parent after a temperature shift from 25°C to 37°C. Two strains, 476 and 438, which were temperature sensitive only in the presence of methionine, produced cellular protein with methionine contents as high as 3.6% and 4.3%, respectively, when incubated in the presence of methionine. The former strain contained 2.5% methionine even when incubated at 37°C in the absence of methionine. Wild strain Y5α, on the other hand, had 1.75% methionine under all conditions tested. Most temperature-sensitive mutants isolated had the same methionine content as the wild strain. It is concluded that the proportion of a specific amino acid, such as methionine, in S. cerevisiae protein can be altered by culturing certain temperature-sensitive mutants at an elevated temperature.

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

The following is a summary of a yeast project going on in my laboratory.

Environmental Studies on Tryptophan Overproduction in a Methanol-Utilizing Yeast

R. Longin, C. L. Cooney and A. L. Demain

L-tryptophan production by a 5-fluorotryptophan-resistant mutant of Hansenula polymorpha is being studied in batch culture, continuous culture and in a washed cell-suspension system; all systems are chemically-defined. In batch culture at 0.5% (v/v) methanol concentration, tryptophan overproduction amounted to 25mg/l and 36mg/g DCW. Phosphate limitation resulted in production of 27mg/l and 65mg/g DCW. In both methanol-limited and phosphate-limited chemostats, overproduction increased with decreasing dilution rate. At the low dilution rate of 0.045h^{-1}, phosphate limitation

led to production of 100mg/l but specific production was only 25mg/g DCW. The highest specific productivity observed in the chemostat was 1.3mg tryptophan/g.h. A phosphate-deficient, washed-cell suspension system was used to investigate additional factors regulating overproduction. Tryptophan overproduction values in this system using 2.5% (v/v) methanol in a 4-day incubation were 230mg/l, 60mg/g DCW and a maximum productivity of 1.5mg/g.h. The addition of 5mM methionine markedly stimulated productivity to 2.5mg/g.h.

XXXII. Department of Food Science and Technology, University of California, Davis, California 95616. Communicated by Eric A. Johnson.

The following is an abstract of the paper, "A simple method for the isolation of astaxanthin from the basidiomycetous yeast Phaffia rhodozyma" which is to appear in the June 1978 issue of Applied and Environmental Microbiology.

ABSTRACT

A method is described for the quantitative, and possibly, large scale extraction of astaxanthin from the yeast Phaffia rhodozyma. The method utilizes extracellular enzymes produced by the bacterium Bacillus circulans WL-12, which partially digests the yeast cell wall and renders the carotenoid pigments extractable by acetone or ethanol. Complete recovery of astaxanthin from heat killed P. rhodozyma cells was obtained after growing B. circulans WL-12 on these yeast cells for 26 h and then extracting the yeast/bacterium mixture with acetone. A bacteria-free lytic system, which gave quantitative extraction of astaxanthin from P. rhodozyma, was obtained by concentrating the culture broth from the growth of B. circulans WL-12 on P. rhodozyma cells. Hydrolytic enzyme activities detected in this concentrate included β-(1→3)-glucanase, β-(1→6)-glucanase, α-(1→3)-glucanase, xylanase and chitinase. The lytic system was found to work most efficiently at pH 6.5 and with low concentrations of yeast.

XXXIII. The Brewing Research Foundation, Nutfield, Redhill, Surrey, England. Communicated by Brian H. Kirsop and Barbara Kirsop.

Much of the work carried out in the Microbiology Department is now related to the lipids of yeast, for these exert a controlling influence on yeast growth and on the accumulation of minor metabolites in beer. Papers published recently include:

1. Vivienne Aries and B. H. Kirsop. Sterol Synthesis in Relation to Growth and Fermentation by Brewing Yeasts inoculated at different concentrations. Journal of the Institute of Brewing 83:220, 1977.

2. Vivienne Aries and B. H. Kirsop. Sterol Synthesis by strains of Saccharomyces cerevisiae in the presence and absence of dissolved oxygen. Journal of the Institute of Brewing. (in press).

3. G. T. Taylor and B. H. Kirsop. The origin of the medium chain length fatty acids present in beer. Journal of the Institute of Brewing 83:241, 1977.

4. B. H. Kirsop. Oxygen and sterol synthesis during beer fermentations. Proc. Euchem. Conference on Metabolic Reactions in the Yeast Cell in Anaerobic and Aerobic conditions, Helsinki 41, 1977.

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Factors Influencing the Survival of Yeast Cells During Lyophilisation

Barbara Kirsop

SUMMARY

The NCYC has found that maintenance of cultures by subculturing over a period of 25 years has resulted in an unacceptable degree of change, whereas cultures freeze-dried for the same period remain stable (Kirsop, J. Inst. Brew. 1974, 80:565-570). In the light of these results, a major program of freeze-drying has been undertaken and all cultures in the NCYC have now been lyophilised. Nearly 1000 strains have been processed using the method described earlier (Kirsop, J. Inst. Brew. 61(6):466-471, 1955, modified by the use of older cells for the inoculum.

The results obtained have enabled an assessment to be made of factors affecting the resistance of strains to lyophilisation. It is clear from the viability figures that there is little relationship between survival and taxonomic position and that survival is strain specific.

With this in mind, strains showing high and low survival figures have been treated in a number of ways to try to establish the optimum conditions for survival and the factors that distinguish high-surviving strains. It has been shown that post-logarithmic cells survive better than younger cells.

Proportionate numbers of cells survive when inoculum size is varied. Washing cells before resuspending in the menstrum has no effect on survival. Resuscitation methods have been varied but have so far failed to affect survival.

A significant difference in survival has been obtained, however, when strains are grown aerobically (in McCartney bottles on a reciprocal shaker) or microaerophilically (in standing McCartney bottles). Cells generally survive better after growth in oxygen-limited conditions. It has been established that the availability of oxygen determines the sterol and unsaturated fatty acid content of cells and it therefore seemed of interest to examine cells from high- and low-surviving cultures for the presence of these substances. Gas-liquid chromatographic analysis was used. Although differences were found, there seemed to be no logical pattern that could distinguish high-from low-surviving cultures. Addition of unsaturated fatty acids by supplementing the growth medium with Tween 80 paralled the effect obtained from aerobic growth of cultures.

Survival has also been increased by incubating cells on sporulation medium before lyophilisation. Eighteen low-surviving strains from the genera Saccharomyces, Brettanomyces and Candida all showed significantly higher survival following incubation on potassium acetate agar for 72 hours. This may offer a useful method for increasing the viability of non-sporulating species, but would not be recommended for sporulating species because of the undesirability of encouraging meiotic division.

Survival can be expected to depend on such parameters as membrane strength, resistance to osmotic stress and conformation of cells and variation in these may be the underlying cause of the observed strain specific response to lypophilisation. Viability remains little altered following storage of ampoules over a period of 25 years.

XXXIV. Research Institute for Viticulture and Enology, Matuskova 25, Bratislava, Czechoslovakia. Communicated by E. Minárik.

The following publications appeared recently:

1. E. Minárik, M. Emeriaud and O. Jungová: Importance of preferential glucose and fructose fermentation by wine yeasts for natural sweet wines (in Slovak). Kvas. prum. Prague 23:281-284 (1977).

2. E. Minárik, O. Jungová and M. Emeriaud: Fructophilic yeasts and their influence on natural sweet wines (in German). Wein-Wiss. (Wiesbaden) 33:42-47 (1978).

SUMMARY

Most wine yeast species show preferential glucose fermentation in mixture of glucose and fructose of the grape juice. This is important for natural sweet wines for the type of Tokay, Sauternes, Moscato d'Asti etc. In the unfermented portion of the residual sugar of the wine fructose, displaying substantially higher sweetening capacity than glucose, prevails. Fructophilic yeasts (e.g. Torulopsis stellata) occuring in spontaneously fermenting grape juice originating either from Botrytis-contaminated grapes, or from grapes treated by anti-Botrytis fungicides, preferentially ferment fructose leaving the less sweet glucose in the unfermented sugar portion of the wine. The importance of glucophilic yeast selection for the fermentation of natural sweet wines is evident.

The 14th Conference of the Subcommission "Microbiology of Wine" of the Office International de la Vigne et du Vin is due to be held in Paris May 24, 1978. The following papers are presented:

1. E. Minárik (Czechoslovakia): Practical testing methods of enological properties of wine yeasts.

2. C. Llaguno (Spain): Enzyme equipment of yeasts - possibilities of distinction of wine yeasts used in enology.

3. V. Kovács (Jugoslavia): Grape oxydase inactivation by chemical and physical methods.

4. - : Practical aspects of active dry yeasts in wine making.

XXXV. Department of Food Science, Faculty of Agriculture, ELFATEH University, P. O. Box 2547, Tripoli, Libya. Communicated by Amin S. El Nawawy.

Yeast Biomass from Corn Steep Water (C.S.W.)

by

Mohamed A. Fouda, Amin S. El Nawawy, Montaser Roushdi and Hamdey Emara (Agriculture Research Centre, Giza, Egypt)

SUMMARY

Corn steep water resulting from starch mills was used for yeast protein production. In this way, a protein of high biological value was obtained, and environmental pollution hazards were minimized. Candida utilis was used, besides two new isolates obtained from corn steep liquid. Continuous cultivation methods were adopted in most of the experiments using a "bioflo" chemostat. No great variation occurred in the quantity of protein, but conversion from corn protein to yeast protein could be traced by tryptophan determinations. Comparison between two different doubling times (3 and 6 hrs) was held throughout the work, the longer being more favorable for the conversion. The conversion possibility into yeast protein also enhanced the separation of most dry matter from corn steep liquid, especially the colloidal matter normally found in fresh C. S. liquid.

(Paper presented at the first Arab Conference of Food Science and Technology, Cairo, November 1976).

XXXVI. New Books

1. Microbial growth on C_1-compounds. Abstracts of the International Symposium held Sept. 12-16, 1977, Pushchino. Publ. by Scientific Centre for Biological Research USSR Academy of Sciences, Pushchino, 1977. USSR.

2. Proceedings of the Fifth International Specialized Symposium on Yeasts, Keszthely, Hungary, Sept. 12-15, 1977. Part II, Invited papers and agenda. E. K. Novak, T. Deak, T. Török, and J. Zsolt, eds. (For further information write to: Dr. E. K. Novak, Dept. of Mycology, Natl. Institute of Hygiene, H-1097 Budapest, Gyáli ut 2-6, Hungary.

3. The Life of Yeasts (2d enlarged and revised ed.) H. J. Phaff, M. W. Miller, and E. M. Mrak. Harvard University Press 329 pp. (July, 1978).

4. Journal of Applied Biochemistry Pre-Publication Announcement

The International Union of Biochemistry is pleased to announce that its Executive Committee has recently approved the formation of a new IUB-sponsored publication, the Journal of Applied Biochemistry. The Editor-In-Chief is Dr. J. J. Marshall, Laboratories of Biochemical Research, Howard Hughes Medical Institute, P. O. Box 520605, Miami, Florida 33152, USA., who is also Chairman of the Special Membership Committee of I.U.B. An Editorial Board consisting of distinguished biochemists from universities, research institutes and industry has been organized and negotiations are in progress with Academic Press to Publish the journal for I.U.B. Publication will commence in January 1979.

A summary of the prospectus of the journal is attached and comments on this will be welcomed by the Editor-in-Chief. A list of Editors is also attached. All Editors will be expected to maintain the highest of standards for acceptance of papers for Journal of Applied Biochemistry.

Contributions to the Journal of Applied Biochemistry will be accepted after May 1, 1978. These should be sent directly to the Editor-in-Chief. Papers should be in the format of Archives of Biochemistry and Biophysics. Detailed "Instructions to Authors" will be available during May 1978.

XXXVII. National and International Meetings.

1. August 13-18, 1978, 29th Annual Meeting, Society for Industrial Microbiology at Rice University, Houston, Texas. Information-Ms. Ann Kluback, Society for Industrial Microbiology, 1401 Wilson Blvd., Arlington, VA 22209.

2. The following Yeast Discussion Session was sponsored by the U. S. Federation of Culture Collections and held during the May 1978 Annual Meeting of the American Society for Microbiology.

"Problems in the Indetification of Yeasts, particularly Medically Important Yeasts"

Discussion Leader: Sally Ann Meyer, Georgia State University, Atlanta

Commercially available identification products
Billy H. Cooper, Baylor Universty, Dallas, Tx.

So what is a heterobasidiomycetous yeast?
Jack W. Fell, University of Miami, Florida

Identification of Prototheca species in tissue and culture
William Kaplan, CDC, Atlanta, Ga.

The Genus Issatchenkia: Taxonomy and imperfect states
Cletus P. Kurtzman, Northern Regional Research Center, Peoria, Ill.

Gas Chromotographic Detection of Candida antigens
Geoffrey A. Land, Wadley Institute of Molecular Medicine, Dallas, Tx.

Dilemma of the Black yeasts in the clinical laboratory
Michael R. McGinnis, North Carolina Memorial Hospital, Chapel Hill. N.C.

"Funny" yeasts from the oncologists
William G. Merz, John Hopkins University, Baltimore, Md.

How uncommon are 'uncommon' yeasts?
Christine Pinello, Analytab Products, Plainview, N.Y.

Is there a 'real' Candida stellatoidea?
Glenn Roberts, Mayo Clinic & Foundation, Rochester, N.Y.

Atypical Candida albicans in the diagnostic laboratory: the elusive recluse, i.e., germ tube negative
Ronald Schlitzer, Georgia State University, Atlanta, Ga.

3. Tenth Annual Conference of the Czechoslovak Yeast Commission, held in Smolenice 14th-16th, February 1978. Communicated by A. Kocková-Kratochvílová.

D. Michaljaničová: Transport systems for disachharides of Saccharomyces cerevisiae.

S. Janda: The influence of metabolic inhibitors on the transport of saccharides by Rhodotorula glutinis.

A. Knotková: The source and inhibition of the formation of pH gradient in yeasts.

G. Kellová: Yeast glutathione reductase and the function of thiol groups in its catalytic activity.

D. Vraná: The cell cycle in continuous and batch culture of Candida utilis.

V. Polachová: The variability and numerical taxonomy in the species Candida utilis.

J. Páca: The growth induction and changes in the formation of secondary products of P_{O_2} (oxygen partial pressure) on the cultivation of Candida utilis on ethanol.

V. Kováčová: The influence of nitrosomethylurea on the formation of RD-mutants in Saccharomyces cerevisiae.

J. Šubík and G. Takáczová: Biochemical features of yeast mitochondrial mutants resistant to mucidin.

G. Takáczová and J. Šubík: Biochemical features of yeast nuclear mutants resistant to mucidin.

A. Kocková-Kratochvílová, L. Hronská, M. Jurčová: Model strains in Czechoslovak Yeast Collection.

A. Kocková-Kratochvílová, J. Zemek, J. Augustin and Ľ. Kuniak: Cellulases in yeasts and yeast-like organisms.

J. Zemek, Ľ. Kuniak, J. Augustín, A. Kocková-Kratochvílová: The determination of the activity of hydrolytic enzymes in yeasts.

J. Augustín, L. Kuniak, A. Kocková-Kratochvílová, J. Zámocký: Mannanases and mannosidases in yeasts and yeast-like organisms.

M. Černáková, J. Zemek, A. Kocková-Kratochvílová, Ľ. Kuniak and J. Augustín: The enzyme contents in Aureobasidium pullulans.

Z. Krátký: The growth of Aureobasidium pullulans in hemicellulose waste water.

P. Biely: The regulation of the synthesis of endo-beta-1, 4-xylanase in Cryptococcus albidus.

Y. Kochová: Modern aspects in medical mycology.

A. Tomšíková: Recent yeast mycoses.

J. Šandula, A. Kocková-Kratochvílová, L. Masler: The selection of yeast for the utilization of lignin-cellulose materials.

J. Grones, J. Šandula and A. Vojtková: The utilization of xylose oligosaccharides in yeasts.

B. Košíková, J. Zemek, J. Augustín, D. Janiak, A. Kocková-Kratochvílová: The influence of lignin and lignin fragments on the growth of S. cerevisiae, Hansenula anomala, Candida utilis, Trichosporon cutaneum and other organisms.

J. Zámocký, M. Švábová, J. Augustín, J. Zemek and A. Kocková-Kratochvílová: Phosphatases in yeasts and yeast-like organisms.

Z. Sestáková: The selection and preservation of brewing yeasts.

R. Barták and J. Brabec: The kinetics and optimalization of the hydrolysis of pig excrements in laboratory conditions.

J. Farkaš: Investigations on new preservation agents from the viewpoint of their inhibition effect on microorganisms and health safety.

E. Streiblová: The biosynthesis of fissions in thermosensitive mutant of Schizosaccharomyces pombe.

M. Sipiczki and V. Farkaš: The influence of 2-deoxyglucose on the morphology and chemical composition of cell wall in Rhodosporidium toruloides.

M. Kopecká and M. Gabriel: The new cytological method of nuclear staining in yeasts and molds using lomofungin.

M. Kopecká and V. Farkaš: The influence of lomofungin on the regeneration of cell wall in yeast protoplasts.

A. Svoboda and D. Piedra: The yeast protoplast reversion in polyethylene media.

M. Gabriel: The study of growing protoplasts of Rhizopus nigricans by the partial enzymatic inhibition of cell wall biosynthesis.

M. Havelková: The origin of extracellular vesicles localized on cytoplasmic membrane of yeast protoplasts.

Z. Holan: The precise localization of chitin in Saccharomyces cerevisiae.

A. Navara and E. Minárik: The influence of yeasts on the quality of red wines.

E. Minárik and A. Navara, O. Jungová: The preference of glucose and fructose fermentation in wine yeasts.

V. Stollárová: The frequency of natural yeast and yeast-like species on fruits in South Slovakia.

L. Švorcová: The noncaloric sweetening matter USAL and its utilization by yeasts.

XXXVIII. Brief News Items

1. On September 1, 1977 Professor Dr. Torsten O. Wikén retired as Professor of General and Applied Microbiology and as Director of the Laboratory of Microbiology, University of Technology, Delft, The Netherlands. At the same time, he returned to his native country Sweden, where his address is:

   Professor Emeritus Dr. T. O. Wikén
   Runristarvägen 53
   18600 Vallentuna, Sweden

2. Dr. Gerald Reed informs all his friends and acquaintances that he has joined Amber Laboratories of Juneau, Wisconsin 53039, as Vice President of Corporate Development. This follows his retirement from Universal Foods for whom he has worked for the last 22 years, first as Director of Research and then as Vice President of Research.

3. On April 27, 1978, the dedication of Carl C. and Gertrude Lindegren Hall took place at Southern Illinois University at Carbondale, Illinois. It gives the Editor much pleasre to inform the readers of the Yeast Newsletter of this well-deserved honor bestowed upon this husband and wife team, who contributed so much to the science of yeast genetics.

4. Dr. Martha D. Berliner, Simmons College, 300 The Fenway, Boston, Massachusetts 02115, participated in a round-table discussion on fungal protoplasts at the first meeting of the New England Mycological Society at the University of Rhode Island on April 15. Her topic was: "Yeast protoplasts - past, present and future."

5. News from the Medical Mycology Group at the Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia, 23298.

   1. Dr. H. Jean Shadomy, Department of Microbiology, has been promoted, effective 1 July 1978 to Professor of Microbiology.

   2. Mr. Dennis Dixon will be completing his Ph.D. program, under Professor H. J. Shadomy, in August 1978 at which time he will accept an appointment as Assistant Professor at Loyola College, Baltimore, MD. Thesis: Isolation of Pathogenic Fungi from a Region Endemic for Blastomycosis: Significance of Woody Plant Material.

6. The following publications have appeared since the last communication. The abstracts of reports have been given in Yeast Newsletter 25 (1976):1, 11-12, 26 (1977):2,28.

E. Oura and H. Suomalainen, Qualitative requirements and utilization of nutrients: Yeast, in CRC Handbook Series in Nutrition and Food ed. by M. Rechcigl, Jr., Sect. D: Nutritional Requirements, vol. 1, CRC Press, Cleveland, Ohio 1977, pp. 171-192.

Elke Parkkinen, Erkki Oura and Heikki Suomalainen, The esterases of baker's yeast. I. Activity and localization in the yeast cell, J. Inst. Brew. 84 (1978), 5-8.

Dr. Heikii Suomalainen Alco, Box 350
SF-00101, Helsinki, Finland

7. Recent publication from our laboratory follows below:

A. Fiechter, O. Käppeli, A. Einsele, H. Hug und H. Schneider:

Uptake of n-alkanses by yeast cells

DECHEMA Monogr. Biotechnologie: 81, 157, 1977

Uptake of n-alkanes by cell walls from Candida tropicalis is mediated by a strong lipophilic mannan-fatty acid complex as demonstrated by partial pronase degradation studies (Käppeli, 1976). Involvement of carrier proteins in substrate transport can be excluded.

A. Fiechter.
Mikrobiologisches Institut,
Swiss Federal Institute of Technology,
Weinbergstrasse 38, CH 8092 Zürich
Switzerland.

8. Below follows some recent work from this laboratory:

-I. Delpech, C. Bizeau, R. Bonaly et P. Galzy

Etude de l'action des gènes de la série PLi sur la structure de la paroi de Saccharomyces cerevisiae HANSEN.

A study of the composition of cell walls extracted from a wild strain and three mutant strains "smooth colony" of Saccharomyces cerevisiae had been done.

It points out differences in the mannose/glucose ratio and in the concentration of some other components of the cell wall in the mutant strains. The immunological study of the cell wall shows that "smooth colony" mutants and wild strains differ in their mannoproteins.

P. Galzy
Ecole Nationale Supérieure
Agronomique de Montpellier

Chaire de Génétique
et de Microbiologie

34060 Montpellier Cedex, France

9. Postdoctoral position available. Starting June 1, 1978 for research on the genetics of yeast cell recognition. Candidate must have expertise in electron microscopy. Salary $10,000 to $13,000. Send curriculum vitae with referee names to:

Dr. Marjorie Crandall
School of Biological Sciences
University of Kentucky
Lexington, KY 40506