Y E A S T

A Newsletter for Persons Interested in Yeast

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Herman J. Phaff
Editor

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

The strains listed below have been added to the ATCC since October 31, 1980. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

Schwanniomyces alluvius ATCC 44442 produces exocellular amylase	J. Augustin Slovak Academy of Science Czechoslovakia
Schwanniomyces occidentalis ATCC 44443 produces exocellular amylase	"
Leucosporidium capsuli ATCC 44444 produces exocellular amylase	"
Saccharomyces cerevisiae ATCC 44452 genetic	M.S.S. Murthy Bhabha Atomic Research Center India
Saccharomyces cerevisiae ATCC 44475 studies on yeast cell wall	Y. Koch Klement-Gottwald East Germany
Candida albicans ATCC 44476 studies on yeast cell wall	"
Cryptococcus neoformans ATCC 44477 isolated from spinal fluid	"
Cryptococcus neoformans ATCC 44478 - 44480 biochemical mutants	J. Rhodes NIH Bethesda, Maryland
Saccharomyces cerevisiae ATCC 44489 genetic	K. Kobayshi University of Tsukuba Japan
Candida albicans ATCC 44505 - 44506 reference strain	F.C. Odds University of Leicester England
Candida krusei ATCC 44507 reference strain	"

Candida tropicalis ATCC 44508 - 44509 reference strain	"
Torulopsis versatilis ATCC 42980 miso production	K. Kiuchi National Food Research Japan
Saccharomyces rouxii ATCC 42981 - 42982 miso production	"
Pityrosporum orbiculare ATCC 44031 cultured from tinia versicolor lesion	J. Faergemann Central Hospital Sweden
Cryptococcus neoformans ATCC 44023 - 44029 biochemical mutants	E. Jacobson McGuire Virginia Medical Center Richmond, Virginia
Saccharomyces cerevisiae ATCC 44067 - 44075 genetic studies	R. Wickner Dept. Health & Human Services Bethesda, Maryland
Saccharomyces cerevisiae ATCC 44076 - 44077 genetic studies	L. Clark University of California Santa Barbara, California
Cryptococcus laurentii ATCC 44096 producer of killer toxin	E.J. Middelbeek University of Nijmegen The Netherlands
Torulopsis sp. ATCC 44097 producer of killer toxin	"
Cryptococcus neoformans ATCC 44104 - 44105 isolated from animal digestive tract	H. Saëz Museum National d'Historie France
Saccharomyces cerevisiae ATCC 44106 - 44108 study of carbohydrate structure of yeast invertase	C. Ballou University of California Berkeley, California
Schizosaccharomyces pombe ATCC 44145 - 44157 genetic stocks	K. Wolf University of Munich Germany

Saccharomyces cerevisiae ATCC 44158 mutation in mitochondrial gene	T. Fox University of Basel Switzerland
Saccharomyces cerevisiae ATCC 44162 - 44164 mitochondrial mutants	D.K. Hanson Indiana University Bloomington, Indiana
Saccharomyces cerevisiae ATCC 44216 genetic	T. Fukasawa Keia University Japan
Saccharomyces cerevisiae ATCC 44222 genetic	G. Zetterburg University of Uppsala Sweden
Schwanniomyces occidentalis ATCC 44239 - 44256 genetic	E. Johannsen CSIR South Africa
Saccharomycodes ludwigii ATCC 44294 - 44304 genetic	Y. Oshima Osaka University Japan
Pichia mississippiensis ATCC 44357 - 44360, 44364 haploid complementary	C.P. Kurtzman USDA Peoria, Illinois
Pichia amylophila ATCC 44361 - 44363 haploid complementary	"
Candida albicans ATCC 44373 - 44374 serotype B	P. Auger University of Montreal Canada
Saccharomyces cerevisiae ATCC 44376- 44383 genetic	M. Kielland-Brandt Carlsberg Laboratory Denmark
Cryptococcus neoformans ATCC 44384 testing cell	K. Nishimura Chiba University Japan
Saccharomyces cerevisiae ATCC 44427 osmotic-sensitive mutant	P. Venkov Bulgarian Academy of Science Bulgaria
Cryptococcus luteolus ATCC 44440 produces exocellular amylase	J. Augustin Slovak Academy of Science Czechoslovakia

Endomycopsis (Saccharomycopsis) capsularis
ATCC 44441
produces exocellular amylase "

II. University of Lyon, Laboratoire de Biologie Végétale. Bat. 405 43 Blvd. du 11 Novembre 1918, 69622 Villeurbanne Cedex, France. Communicated by M.C. Pignal.

Since the last issue of the Yeast Newsletter the following three articles have been accepted for publication in Mycopathologia:

1. Contribution à la Systematique du genre Pichia. II. Recherche d'osidases intracellulaires, des nitrites et des nitrates reductases, des besoins vitaminiques et des teneurs en guanine + cytosine des ADN chez 18 espèces nouvelles: Consequences systematiques.

Geneviève Billon-Grand

ABSTRACT

The presence of intracellular osidases, nitrite and nitrate reductases, vitamin requirements and GC content in the DNA, have been investigated in 18 species of Pichia.

According to their typical characters and their biocharacters, a pattern is suggested for the integration of these new species into the formerly existing groups of the genus Pichia. A new way of classifying the species of the genus Pichia is proposed.

2. Biosystematique des Pichia. J.B. Fiol & G. Billon-Grand.

Nitrite and nitrate reductases were found in numerous species of Pichia. The specific activities of these enzymes are at the same level of activity as in 13 species of yeasts classified in the genus Hansenula. Taxonomic implications are discussed.

3. Les cytochromes des Kluyveromyces: Determination et implications systématiques.

J.B. Fiol et M. Claisse*

*Centre de Génétique moleculaire, Laboratoire propre du C.N.R.S., associé à l'Université Pierre et Marie Curie, Paris VI. 91190 Gif Sur Yvette.

SUMMARY

Low temperature spectrophotometric analysis carried out directly on whole cell pastes reveals either similarities or differences in the cytochrome absorption spectra of the different yeast species belonging to the genus Kluyveromyces. This analysis confirms the division of the genus into two groups, K_1 and K_2, and the existence of five different species in the K_1 group these systematic divisions have been previously based on physiological and biochemical criteria. Furthermore the spectrophotometric data sustain a division of the K_2 group into three main types, and suggest a phylogenetic link between K_1 and K_2 groups through K. africanus and K. aestuarii.

III. Centraalbureau voor Schimmelcultures, Yeast Division, Julianalaan 67A, 2628BC Delft, the Netherlands. Communicated by M. Th. Smith.

The following paper has been published recently. Smith, M. Th., Batenburg-v.d. Vegte, W.H. and Scheffers, W.A. 1981. Eeniella, a New Yeast Genus of the Torulopsidales.. International Journal of Systematic Bacteriology 31:196-203.

ABSTRACT

We found that three yeast strains previously assigned to the genus Brettanomyces differed in reproduction and morphology from the known Brettanomyces species, and we transferred them to the new yeast genus, Eeniella, with the single specis Eeniella nana sp. nov. The type strain of E. nana is CBS 1945.

IV. The Institute of Enology & Viticulture, Yamanashi University, Kitashin-machi, 1-13-1, Kofu, 400 Japan. Communicated by S. Goto.

Reproduction by multilateral budding of two strains of Kloeckera corticis. Submitted to J. Gen. Appl. Microbiology.

S. Goto

Reproduction by multilateral budding was discovered in two strains of Kloeckera corticis, one of the bipolarly budding yeasts, isolated from botrytised grapes. In addition to the usual bipolar budding, several buds, viz., three, four and five buds, developed at different places on a single yeast cell. The daughter-cells may be formed in basipetal succession presumably with annellation. Now in progress are further detailed studies on the conidium ontogeny in Kloeckera spp.

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

1. Dr. Yuzo Yamada returned to Japan in December 1980 after spending a year in our laboratory to learn the technique of genome comparison for yeast taxonomy. He taught us the procedure for determining CoQ in yeast.

2. Dr. Eveline Guého from the Institut Pasteur in Lyon, France, arrived in Davis in January, 1981 to learn the technique of DNA isolation and DNA-DNA homology for the systematics of yeast. She will stay for one year in our laboratory.

Publications:

1. Starmer, William T., Henry W. Kircher, and Herman J. Phaff. January 1980. Evolution and speciation of host plant specific yeasts. Evolution 34(1):137-146.

2. Phaff, H.J., W.T. Starmer, Mary Miranda, and M.W. Miller. July 1980. Candida mucilagina, a new species of yeast found in decaying cladodes of Opuntia inermis and in necrotic tissue of cereoid cacti. International Journal of Systematic Bacteriology 30(3):596-600.

3. Phaff, H.J. and W.T. Starmer. 1980. Specificity of natural habitats for yeast and yeast-like organisms. p. 79-102, IN: F.A. Skinner, Susan M. Passmore and R.R. Davenport (eds.), Biology and Activities of Yeasts, Society for Applied Bacteriology Symposium Series No. 9, Academic Press, New York. (reprints are not available).

4. Holzschu, Donald L., Joanne Tredick, and Herman J. Phaff. April 1981. Validation of the yeast Sporidiobolus ruinenii based on its deoxyribonucleic acid relatedness to other species of the genus Sporidiobolus. Current Microbiology 5(2):73-76.

5. Meyer, M.Th. and H.J. Phaff. 1981. An enzymatic method for the determination of yeast cell wall glucan in foods. J. Food Science (in press).

ABSTRACT

Processed cell wall β-glucans prepared from strains of baker's or brewer's yeast can be used as a thickening agent in aqueous food systems and they provide a fat-like mouthful. This article describes an analytical procedure for the determination of the glucan content added to a variety of food

products. The method is based on measurement of reducing sugars produced when the food sample, after appropriate pretreatment, is treated with a commercial microbial β-glucanase (Zymolyase) that is specific for the β-linkages present in the glucan polysaccharide of the added yeast cell walls.

6. M. Miranda, D.L. Holzschu, H.J. Phaff and W.T. Starmer. Pichia mexicana, a new heterothallic yeast from cereoid cacti in the North-American Sonoran Desert. Internat. Jour. Syst. Bacteriol. (in press).

ABSTRACT

A description is given of a novel member of the yeast genus Pichia that was recovered 13 times in the Sonoran Desert from necrotic tissue of cereoid cacti. Most of the isolates came from organpipe cacti. The new yeast occurs in the cactus "rot pockets" in the haploid condition and is heterothallic. Upon mixing of appropriate mating types, zygotes develop with hat-shaped ascospores. Physiologically, the haploid strains resemble Candida tenuis, but this species has a different habitat and shares only 9.2% of its nuclear deoxyribonucleic acid (DNA) base sequences with Pichia mexicana. The sexual state is physiologically similar to Pichia stipitis and an as yet undescribed cactus-specific species of the genus Clavispora, but their DNA sequence complementarity is less than 7% compared with P. mexicana. The base composition of the nuclear DNA of P. mexicana ranges from 42.2-43.0 mol% G+C (5 strains). The type strain of P. mexicana is UCD-FS&T 76-308A (=ATCC 42175=CBS 7066) and the complementary mating type is UCD-FS&T 391B (=ATCC 42176=CBS 7067).

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

The following abstract was published in the Society for General Microbiology Quarterly Vol. 8, p. 30, 1980. A full paper will appear elsewhere.

Spatial distribution of Two Membrane-bound Enzymes during the Cell Cycle of Sterigmatomyces halophilus. I. Salmon and R.K. Poole.

Sterigmatomyces halophilus is an unusual budding yeast in which daughter cells are formed remote from the mother cells on fragile projections called sterigmata (1). This property makes S. halophilus uniquely suitable for the study of the spatial distribution of cellular components since these sterigmata are easily disrupted by mild mechanical means (e.g. ultrasonication) allowing the easy detachment of immature buds from their mother cells.

Fractions containing cells at different stages of the cell cycle were obtained by isopycnic fractionation of exponentially growing cultures (2), and divided into two. One portion was treated ultrasonically to produce a mixture of immature buds, mothers and mother-bud doublets. Rate-sedimentation of this suspension using sucrose gradients produced three discrete bands corresponding to each of these populations. The activities of succinate dehydrogenase and cytochrome c oxidase (marker enzymes for the inner mitochondrial membrane) were measured in extracts prepared from (a) the cells recovered from the slowest sedimenting band (i.e., immature buds)and (b) the retained original population (i.e., mother-bud doublets).

The activities of both these enzymes, expressed on a per cell basis, varied in phase with the observed activity in the mother-bud doublets during the cell cycle (2), and when expressed as either specific activity [substrate transformed min^{-1} $(pg\ protein)^{-1}$] or activity per unit cell volume [substrate transformed min^{-1} μm^{-3}] was the same in both daughter and mother-daughter pairs. This indicates that these two enzymes (and by implication mitochondria) are evenly distributed between mother and developing daughter cells during the cell cycle.

These observations are consistent with those of Galdiero (3) who demonstrated that proteins and lipids were evenly portioned between mother and daughter cells during the cell cycle of Saccharomyces cerevisiae.

1. Salmon, I. and Poole, R.K. (1978) Bulletin of the British Mycological Society 12,121.
2. Salmon, I. and Poole, R.K. (1980) Society for General Microbiology Quarterly 7,85.
3. Galdiero, F. (1973) Experientia 29,496.

VII. Institute of Biochemistry and Physiology of Microorganisms, USSR Acad. of Sciences, Pushchino Moscow region 42292, U.S.S.R. Communicated by W.I. Golubev.

The correlation between pulcherrimin production and ploidy in Metschnikowia pulcherrima.

The production of red pigment, pulcherrimin, by the yeast M. pulcherrima was the subject of rather numerous investigations which showed that environmental factors may influence pigment formation. At the same time it was noted that pigment production was unstable in identical growth conditions. This yeast tends to split along two different lines - into red and white races. My recent observations suggest that such variations may depend on ploidy of cultures.

During a survey of the yeast flora on the bark of trees, several isolates were identified as haploid mating types of M. pulcherrima. In single cultures these isolates did not form chlamydospores but abundant conjugation and zygote formation were observed when the compatible strains were mixed on malt-extract agar. The diploid cultures were isolated from these mixtures by plating and selection of colonies in which formation of numerous pulcherrima cells was discovered microscopically. Diploid cultures obtained in this way produced asci and ascospores by incubation on dilute V-8 agar at 15°C.

All haploid cultures produced pulcherrimin on mannose-peptone agar and on acidified agar for starch formation. Fresh isolates gave red colonies without diffusion. After a year of maintenance in the laboratory, the intensity of colonial pigmentation decreased while diffusion of the pigment into the medium increased. All diploid cultures gave white colonies on the mentioned media without any trace of color in the agar. Some of them remained pure white during observations while minute red colonies or red sectors appeared in entirely white streaks and colonies of other cultures upon aging. Subcultures from these developed red portions seem to be haploid since they showed conjugation and zygote formation after mixing with compatible haploid strains.

The following articles have been published recently:

Gulevskaya, S.A., W.I. Golubev and A.R. Manukian, 1981. Ultrastructure of yeast capsules. Mikrobiologia, 50, N I, 110-113.

Golubev, W.I., V.M. Blagodatskaya, A.R. Manukian and O.L. Liss, 1981. The yeast florae of peats. Izvestiya Akademii nauk SSSR, ser. biol., N 2, 181-187.

Golubev, W.I., 1980. Variability of Cryptococcus spp. in the ability for pseudomycelium formation. Mikrobiologia, 49, N 4, 599-603.

Golubev, W.I., I.V. Golubeva, 1980. Effect of carbon and nitrogen sources on the cellular form of Metschnikowia lunata. Mikologia i phitophatologia, 14, N 6, 486-489.

Golubev, W.I., T.M. Loginova, V.S. Turin, 1980. Ultrastructure of cell walls and septa in glucuronate-positive Candida spp. Mikrobiologia, 49, N 6, 942-944.

VIII. Georgia State University, University Plaza, Atlanta, Georgia 30303. Communicated by D.G. Ahearn.

The following are recent publications from our laboratory.

Ahearn, D.G., and R.L. Schlitzer. 1981. A pyrimidine sporulation medium for Filobasidiella. Mycologia 53 (2): 343-345.

* * *

Cerniglia, C.E. and S.A. Crow. 1981. Metabolism of aromatic hydrocarbons by yeasts. Arch. Microbiol. 129:9-13.

ABSTRACT

Six yeasts were examined for their ability to metabolize naphthalene, biphenyl and benzo(a)pyrene. All of the organisms tested oxidized these aromatic hydrocarbons. Candida lipolytica oxidized naphthalene to 1-naphthol, 2-naphthol, 4-hydroxy-1-tetralone and trans-1,2-dihydroxy-1,2-dihydronaphthalene. The major metabolite was 1-naphthol. C. lipolytica oxidized biphenyl to produce 2-,3-, and 4-hydroxybiphenyl, 4,4'-dihydroxybiphenyl and 3-methoxy-4 hydroxybiphenyl. 4-Hydroxybiphenyl was the predominant metabolite formed. C. lipolytica oxidized benzo(a)pyrene. Metabolites were isolated and identified by absorption spectrohotometry, mass spectrometry and thin-layer, gasliquid and high-pressure liquid chromatography. Where possible the structures of these metabolites were confirmed by comparison with authentic compounds.

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Hagler, A.N. and D.G. Ahearn. 1981. Rapid diazonium blue B test to detect basidiomycetous yeasts. Int. J. Syst. Bacteriol. 31:204-208.

ABSTRACT

Over 100 known eucaryotic microorganisms were examined for their capacity to form red to violet pigments with diazonium blue B (DBB) following an alkaline hydrolysis and ethanol wash. All 50 basidiomycetes gave rapid and unequivocal positive reactions with 1- to 6-day-old cultures, whereas all ascomycetes and algae were negative. Extracellular enzymes, generally considered to be typical of basidiomycetous yeasts, were detected in several ascomycetous taxa.

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

The following papers from our laboratory are currently in press:

1. Douglas, L.J. and J. McCourtie. Adherence of Candida albicans to denture acrylic as affected by changes in cell-surface composition. Proc. 5th Int. Symp. Yeasts, 1980. Pergamon of Canada.

2. McCourtie, J. and L.J. Douglas. Relationship between cell-surface composition of Candida albicans and adherence to acrylic after growth on different carbon sources. Infect. Immun., June 1981 issue.

ABSTRACT

The adherence of Candida albicans to acrylic was measured in vitro after growth of the yeast to stationary phase in defined media containing glucose, sucrose, galactose, fructose or maltose as the carbon source. In each case, yeast adherence was proportional to the concentration of sugar in the growth medium but equimolar concentrations of different sugars promoted adherence to different extents. In vitro adherence was further increased by the addition of divalent cations to assay mixtures but was inhibited when saliva-treated acrylic strips were used or when yeasts were suspended in mixed saliva during the assay. The rate of spheroplast formation of yeasts grown in media containing a 500 mM concentration of the different sugars correlated well with the relative adherence of the cells to acrylic. Galactose-grown yeasts were most resistant to spheroplast formation with Zymolyase-5000 and most adherent to acrylic, while fructose-grown organisms were least resistant to spheroplast formation and least adherent to acrylic. These results indicate that when grown to stationary phase in media containing high concentrations of certain sugars, C. albicans undergoes a change in cell-surface composition which facilitates its adherence to acrylic surfaces. Electron microscopy of yeasts harvested from such media revealed the presence of an additional surface layer which may be responsible for this adherence.

3. Harrington, C.R. and L.J. Douglas. Effects of nucleotides and sugar nucleotides on mannosyltransferase activity in Saccharomyces cerevisiae. J. Gen. Microbiol., 1981.

ABSTRACT

A survey was made of the effects of different nucleotides, sugar nucleotides and sugars on mannosyltransferase activity in Saccharomyces cerevisiae to assess their possible role in the regulation of mannan synthesis. Mannosyltransferase activity in spheroplast lysate and washed membrane preparations was not markedly affected by sugars, nucleoside monophosphates or by most of the sugar nucleotides tested. GDP and GTP both inhibited enzyme activity. ATP caused a significant stimulation, and analysis

of the mannan formed suggested that this was due mainly to increased synthesis of the polysaccharide moiety. GDP-glucose was a competitive inhibitor of total mannan synthesis and also inhibited the formation of mannolipids.

X. Laboratory of Comparative Biochemistry, Janssen Pharmaceutica, Research Laboratories, B-2340 Beerse, Belgium. Communicated by Hugo Van den Bossche.

Below follows an abstract to be published in "Archives Internationales de Physiologie et de Biochimie". The paper was presented at the 117th meeting of the Belgian Society of Biochemistry. June 13, 1981. An extended paper is in preparation.

H. Van den Bossche, G. Willemsens, W. Cools and W.F. Lauwers.

Effects of miconazole on the fatty acid pattern in Candida albicans.

ABSTRACT

The antimycotic activity of miconazole may be due to altered membrane properties because of the interference with ergosterol biosynthesis and consequent accumulation of C-14 methylated sterols. Ergosterol, the main yeast sterol, enables yeast to grow optimally with a variety of unsaturated fatty acid sources.

Candida albicans was grown for 6 hours in the presence of 10^{-7} M miconazole in order to know if the drug-induced effects on the ergosterol synthesis also affect the nature of free and esterified fatty acids. In the phospholipids a shift from mono- to di-unsaturated fatty acids was seen. The linoleate (18:2) and oleate (18:1) contents of phosphatidyl choline obtained from control cells were 15% and 46.5%, respectively. Phosphatidyl choline (PC) from treated cells contained 27.9% of 18:2 and 29.4% of 18:1. Almost similar changes were seen in phosphatidyl ethanolamine (PE). Smaller changes were observed in the quantitatively less important diphosphatidyl glycerol (diPG) fraction. Triglycerides of treated cells also contained a higher 18:2 content than control cells. The sterol esters of control cells contain 21% of palmitate (16:0), 7% of stearate (18:0) and 43% oleate (18:1), whereas the miconazole treated cells showed a 18:1 content of 13% only and the 16:0 and the 18:0 contents increased up to 42% and 14%. Similar alterations were seen in the free fatty acids.

At miconazole doses up to 10^{-8} M and a contact time of 16 h, the fatty acid pattern of the 4 lipid fractions investigated, revealed a shift from 18:1 to 18:2. In the triglyceride, free fatty acid and phospholipid fraction an

increase in palmitoleate (16:1) was also observed. However, at doses $\geq 10^{-8}$ M a higher level of saturated fatty acids mainly 16:0 and to a lesser extent 18:0 was found. Regarding the phospholipids the enrichment in saturated fatty acids is most pronounced in PC and PE. No significant change in the 16:0 content of diPG was found so far.

The observed shift to more unsaturated fatty acids may reflect an attempt by C. albicans to increase membrane fluidity, compensating for the miconazole-induced alteration of ergosterol synthesis. However, this improvement of the fluidity is opposed by an increased level of saturated fatty acids, indicative for an effect on the desaturation system. The accumulation of saturated fatty acids may be at the origin of miconazole's effects on certain membrane-bound enzymes, e.g., cytochrome oxidase, known to require a fluid environment provided by unsaturated fatty acids.

XI. University of Groningen, Laboratory of Electron Microscopy and Department of Microbiology, Biological Centre, 9751 NN HAREN, Kerklaan 30, The Netherlands. Communicated by M. Veenhuis and K.B. Zwart.

Below follow abstracts of papers recently published or submitted for publication.

M. Veenhuis, J.P. van Dijken, W. Harder 1980.
A new method for he cytochemical demonstration of phosphatase activities based on the use of cerous ions. FEMS Microbiology Letters 9:285-291.

M. Veenhuis, K.B. Zwart, W. Harder 1981.
Biogenesis and turnover of peroxisomes involved in the concurrent oxidation of methanol and methylamine in Hansenula polymorpha. Arch. Microbiol. 129:35-41.

M. Veenhuis, W. Harder, J.P. van Dijken, F. Mayer.
The substructure of crystalline peroxisomes in methanol-grown Hansenula polymorpha: evidence for an in vivo crystal of alcohol oxidase. Molecular and Cellular Biology, submitted for publication.

At the 22nd Dutch Federation Meeting, held from 22-24 April at Utrecht, The Netherlands, the following communication was presented: Subcellular events during degradation of crystalline peroxisomes in Hansenula polymorpha by M. Veenhuis, A. Douma, W. Harder.

ABSTRACT

Transfer of methanol-grown yeasts into glucose- or ethanol-containing media results in a rapid inactivation of

both alcohol oxidase and catalase in these cells (1,2). These enzymes are located in - partly or completely crystalline - peroxisomes and electron microscopical studies have shown that the decrease in enzyme activities is associated with a decrease of the peroxisomal volume density in these cells (1,2). The mechanisms of degradation of peroxisomes was studied in methanol-grown cells of the yeast H. polymorpha after the transfer of cells to glucose media. The first visible response of the cells to this change of the carbon source was the formation of dark membraneous layers derived from the ER around the peroxisomes to be degraded.

Subsequently one or several vacuolar vesicles became incorporated within the dark membranes. After sequestration the membranes of the vacuolar vesicles disrupted, thereby exposing the peroxisomes to attack by the vacuolar hydrolases. At this stage of the degradation process the peroxisomal crystalloids disappeared and the organelles became round of shape. Cytochemical staining experiments indicated that at least part of the vacuolar fluid was not present in the organelles since activities of vacuole-specific hydrolases, like glucose-6-phosphatase and acid phosphatase, which are absent in intact peroxisomes, were now present in the desintegrating peroxisome. As degradation progressed, small areas of desintegration became visible, which subsequently increased in volume and number. In such organelles activities of alcohol oxidase and catalase were no longer detected by cytochemical methods. Finally, the matrix of the organelle became finely granular and turned into an organelle, which may be considered to be a vacuole. The mechanisms underlying the process of degradation, resemble those of the autophagic process in plant cells. A major difference with the latter is that in methanol-grown H. polymorpha the vacuole is involved in supplying the hydrolytic enzymes required for degradation of redundant peroxisomes.

(1) Bormann, C. and H. Sahm, Arch. Microbiol. 117, 67-73 (1978).
(2) Veenhuis, M., K.B. Zwart and W. Harder, FEMS Microbiol. Lett. 3, 21-28 (1978).

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Archives of Microbiology 129:in press.

ABSTRACT

Growth of Hansenula polymorpha in shake flasks and chemostat cultures in the presence of methanol as the sole source of carbon and methylamine as the sole source of nitrogen was associated with the development of peroxisomes in the cells. The organelles were involved in the concurrent oxidation of these two compounds, since they contained both alcohol oxidase and amine oxidase, which are key enzymes in methanol and methylamine metabolism, respectively. In

addition catalase was present. Peroxisomes with a completely crystalline substructure were observed in methanol-limited chemostat-grown cells. Amine oxidase probably formed an integral part of these crystalloids, whereas catalase was present in a freely diffusable form.

Transfer of cells, grown in a methanol-limited chemostat in the presence of methylamine into glucose/ammonium sulphate media resulted in the loss of both alcohol oxidase and amine oxidase activity from the cells. This process was associated with degradation of the crystalline peroxisomes. However, when cells were transferred into glucose/methylamine media, amine oxidase activity only declined during 2 hours after the transfer and thereafter increased again. This subsequent rise in amine oxidase activity was associated with the development of new peroxisomes in the cells in which gradation of the crystalline peroxisomes, originally present, continued. These newly formed organelles probably originated from peroxisomes which had not been affected by degradation. When in the methanol-limited chemostat methylamine was replaced by ammonium sulphate, repression of the synthesis of amine oxidase was observed. However, inactivation of this enzyme or degradation of peroxisomes was not detected. The decrease of amine oxidase activity in the culture was accounted for by dilution of enzyme as a result of growth and washout.

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Submitted to: Molecular and Cellular Biology.

ABSTRACT

The substructural organization of completely crystalline peroxisomes present in cells of Hansenula polymorpha grown under methanol-limitation in the chemostat was investigated with different cytochemical and ultrastructural techniques. Time-dependent cytochemical staining experiments indicated that activities of the two main constituents of these organelles, namely alcohol oxidase and catalase, were present throughout the crystalline matrix. Catalase was completely removed from isolated peroxisomes by osmotic shock treatment. After such treatment the ultrastructure of the crystalline matrix of the oranelles remained virtually intact. Since alcohol oxidase activity was still present in this matrix it was concluded that alcohol oxidase protein is the only structural element of the peroxisomal crystalloids.

The molecular architecture of the crystalloids was investigated in ultrathin cryosections which permitted recognition of individual molecules in the crystalline matrix. Depending on the plane of sectioning, different crystalline patterns have been observed. Tilting experiments indicated that these images were caused by superposition of

octameric alcohol oxidase molecules, arranged in a tetragonal lattice.

A 3-dimensional model of the crystalloid is presented. The repeating unit of this structure is composed of 4 alcohol oxidase molecules. The crystalloid represents an open structure which may explain the observed free mobility of catalase molecules.

XII. Instituto de Enzimologia y Patologia Molecular del C.S.I.C., Facultad de Medicina de la Universidad Autonoma, Calle Arzobispo Morcillo n'4, Madrid-34, Spain. Communicated by R. Serrano.

Francisco Malpartida and Ramon Serrano 1981. Reconstitution of the Proton-Translocating Adenosine Triphosphatase of Yeast Plasma Membranes. J. Biol. Chem. May 1981.

SUMMARY

The plasma membrane ATPase of eukaryotic cells of the vegetable type (fungi, plants and algae) have been postulated to operate as proton pumps which generate membrane potentials and drive the uptake of nutrients by proton co-transport (Poole, R.J., 1978, Ann. Rev. Plant Physiol. vol. 29, pp. 437-460). In order to verify this important physiological role, a purified preparation of the yeast plasma membrane ATPase has been reconstituted with soybean phospholipids by a freeze-thaw-sonication procedure. The reconstituted proteoliposomes catalyzed a ^{32}Pi-ATP exchange partially sensitive to proton ionophores (uncouplers) and to the proton-potassium exchange carrier nigericin. The reaction was completely inhibited by the non-specific ionophore gramicidin and by the combination of uncouplers with the potassium ionophor valinomycin. These results are interpreted as evidence for two types of proton transport catalyzed by the enzyme preparation: electrogenic proton transport and electroneutral proton-potassium exchange.

XIII. University of Maryland Baltimore County, Department of Chemistry, Catonsville, Maryland 21228. Communicated by Richard Karpel.

Richard L. Karpel and Ann C. Burchard. A Basic Isozyme of Yeast Glyceraldehyde-3-Phosphate Dehydrogenase with Nucleic Acid Helix-Destabilizing Activity. Biochim. Biophys. Acta, in press, 1981.

We have isolated, from S. cerevisiae, a nucleic acid helix-destabilizing protein, i.e., a protein which lowers the melting temperature (Tm) of nucleic acids. This protein has been identified as a basic isozyme of glyceraldehyde-3-

phosphate dehydrogenase. Following is an abstract of this work:

A nucleic acid helix-destabilizing protein has been purified from S. cerevisiae using affinity chromatographic techniques. Crude protein extracts at low ionic strength (~0.05 M) were applied sequentially to tandem columns of native DNA-cellulose, aminophenyl-phosphoryl-UMP-agarose, poly (I C)-agarose, poly(U)-cellulose and denatured DNA-cellulose. The 2 M NaCl eluant of the poly(U)-cellulose column was dialyzed to low ionic strength and recycled through native DNA-cellulose, poly(I C)-agarose and poly(U)-cellulose. Purified helix-destabilizing protein eluted from the poly(U)-cellulose between 0.1 and 0.5 M NaCl. On the basis of enzymatic activity, immunological cross reactivity, mobility on SDS gels, amino acid analysis and preliminary peptide mapping experiments, this material was identified as an isozymic fraction of glyceraldehyde-3-phosphate dehydrogenase. The major crystallizable isozyme of this enzyme from yeast is, however, considerably more acidic than the helix-destabilizing protein, and displays significantly lower helix-destabilizing activity.

Stoichiometric levels of the isolated protein at low (~0.01) ionic strength depress the Tm of poly(A-U) and polyd(A-T) by as much as 28 and 22°C, respectively. Longer double helices, poly(A U) and Cl. perfringens DNA, are also denatured by the helix-destabilizing protein, but at relatively slow rates. The binding of this protein to [^{3}H]poly(U) on nitrocellulose filters is [Na^+]-dependent, with a 50% reduction at 0.09 M NaCl. Based on its effect on the circular dichroism spectrum of poly(A), the protein was shown to distort the conformation of the polynucleotide chain. An analogous protein from mammalian cells, P8, was also shown to depress poly(A-U) Tm.

XIV. Laboratorium Voor Microbiologie Technische Hogeschool, Delft 2628 BC, Holland. Communicated by W.A. Scheffers.

Carrascosa, J.M., Viguera, M.D., Nunez de Castro, I. and Scheffers, W.A. 1981. Metabolism of acetaldehyde and Custers effect in the yeast Brettanomyces abstinens.

Antonie van Leeuwenhoek 47 (in the press).

Brettanomyces abstinens growing on different initial glucose concentrations showed an anaerobic inhibition of fermentation. This Custers effect decreased as the initial glucose concentration in the medium increased. Two aldehyde dehydrogenases, one NAD^+-linked and the other $NADP^+$-linked were observed. The results suggest that the NAD^+-linked enzyme is involved in the production of acetic acid and is

repressed by glucose. The $NADP^+$-linked enzyme seems to be a constitutive enzyme. Acetyl-CoA synthetase activity also was not greatly affected by the growth conditions. The results support the earlier hypothesis that the Custers effect in Brettanomyces is provoked by the reduction of NAD^+ in the conversion of acetaldehyde to acetic acid.

XV. Department of Biophysics, All India Institute of Medical Sciences, New Delhi 110029, India. Communicated by V.K. Jain.

The following paper was recently published:

I. Gupta, V.K. Jain and R.K. Mishra. 1981.

Studies on the Effects of 2-Deoxy-D-Glucose on Glucose Uptake and Glycolysis in Respiratory-Deficient Yeast Cells. Indian Journal of Experimental Biology 19: 231-237.

ABSTRACT

Modifications induced by the glucose antimetabolite, 2-deoxy-D-glucose (2-DG) on glucose uptake and glycolysis were studied under different environmental and physiological conditions in respiratory-deficient mutants of yeast cells. The following observations have been made: (a) Glucose uptake shows a biphasic response as a function of time and also as a function of initial glucose concentration. 2-DG causes inhibition of glucose uptake but does not change the uptake behaviour qualitatively. This inhibition increases under conditions of starvation, radiation injury and growth. (b) In contrast to glucose uptake, glycolysis as a function of glucose concentration follows simple saturation kinetics expressed by Michaelis-Menten equation. Effects of 2-DG on rate of glycolysis show in general, non-competitive type of inhibition, but at low concentration of glucose (<1 mM) and 2-DG (<0.2 mM) slight stimulation of glycolysis is observed. 2-DG-induced inhibition of glycolysis increases in presence of ammonium ions but is not influenced by starvation or radiation injury by UV-light. Inhibition of glycolysis persists even after removal of 2-DG. (c) Quantitatively, 2-DG-induced inhibition of glycolysis is more than that of glucose uptake under similar conditions. Various mechanisms which might give rise to the effects described have been discussed.

XVI. Laboratory of Microbiology, Gulbenkian Institute of Science, 2781 Oeiras Codex, Portugal. Communicated by N. van Uden.

The following are abstracts of papers published recently or accepted for publication:

A. Madeira-Lopes and N. van Uden. Effects of chloramphenicol on the thermal profile of Saccharomyces cerevisiae. Zeitschrift Fur Allgemeine Mikrobiologie, 21, 53-55, 1981.

Chloramphenicol decreased the maximum temperature for growth of petite mutant of Saccharomyces cerevisiae, shifted the Arrhenius plot of thermal death to lower temperatures and shortened correspondingly the Arrhenius plot of growth, while an associative thermal profile was maintained. At saturating concentrations (about 5 mg per ml) of chloramphenicol in liquid mineral medium with vitamins and glucose the final maximum temperature for growth was depressed from about 40°C to about 37°C. The results suggested that chloramphenicol acted in the mutant on targets other than mitochondrial ribosomes and that these targets are identical or associated with the death and T_{max} sites of the yeast.

* * *

N. van Uden and H. da Cruz Duarte. Effects of ethanol on the temperature profile of Saccharomyces cerevisiae. Zeitschrift Fur Allgemeine Mikrobiologie (accepted for publication).

Ethanol at concentrations above 3% (w/v) decreased the maximum temperature for growth of Saccharomyces cerevisiae in batch culture. At 9% (w/v) (the highest concentration tested) the maximum temperature suffered a decrease of about 10°C. At effective concentrations ethanol shifted the Arrhenius plots of growth and death in the superoptimal temperature range to lower temperatures while an associative thermal profile was maintained. Thus at a concentration of 6% (w/v), ethanol depressed the optimum temperature for growth from 37°C to 25°C, the final maximum temperature for growth from 40°C to 33°C and the initial maximum temperature for growth from 44°C to 36°C. The results indicate that during alcoholic batch fermentation these three cardinal temperatures are variables, the values of which decrease with increasing ethanol concentration. When the ethanol concentration becomes high enough to depress them successively below the process temperature, the yeast population becomes increasingly subject to ethanol-enhanced thermal death. Implications of the findings for the production of fermentation ethanol in batch and continuous processes are discussed.

* * *

I. Sa-Correia and N. van Uden. Production of biomass and amylases by the yeast Lipomyces kononenkoae in starch-limited continuous culture. European Journal of Applied Microbiology and Biotechnology (accepted for publication)

In strain IGC 4052 of the amylolytic yeast Lipomyces kononenkoae growing in starch-limited chemostat cultures the

critical dilution rate was reduced to about 0.5 of its theoretical value due to severe catabolite repression of amylase formation while its value in a repression resistant mutant was near its theoretical value. The enzyme yield coefficients and the specific production rates of α-amylase and glucoamylase passed through maxima at intermediate dilution rates. The shapes of the respective curves were partly determined by catabolite repression (parent strain) or its absence (mutant strain) while induction did not seem to play a role. An additional growth-linked regulatory mechanism seemed to be involved. The use of continuous culture as compared with batch culture, increased the maximum biomass productivity by a factor of 2.2 in the mutant strain and a factor of 1.4 in the parent strain.

XVII. Universita di Parma, Istituto di Genetica, 43100 Parma, Italy. Communicated by I. Ferrero.

Below follow abstracts of three recent papers from our laboratory.

1. Iliana Ferrero[1], Anna-Maria Viola[1], and A Goffeau[2] 1981.

[1]Istituto di Genetica, Universita di Parma, 43100 Parma, Italy.
[2]Laboratoire d'Enzymologie, Universite Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium.

Induction by glucose of an antimycin-insensitive, azide-sensitive respiration in the yeast Kluyveromyces lactis. Antonie van Leeuwenhoek 47:11-24.

Increasing the glucose concentration from 0.1 to 10% in exponentially growing cultures of Kluyveromyces lactis CBS 2359 does not repress the antimycin-sensitive respiration (Q_{O2} of 80 μ l O_2 h^{-1} mg^{-1} dry weight) but raises the antimycin-insensitive respiration from 3 to 12 μ l O_2 h^{-1} mg^{-1} dry weight. Antimycin A inhibits the growth of K. lactis on a variety of substrates with the exception of glucose at concentrations equal to or higher than 1% where substantial antimycin-insensitive respiratory rates are induced. It can be concluded that a minimal antimycin-insensitive Q_{O2} is necessary for cellular growth when the normal resiratory pathway is not functional.

The antimycin-insensitive respiration elicited by growth in high glucose concentrations is poorly inhibited by hydroxamate and is inhibited 50% by 90 μM azide or 1 mM cyanide. These concentrations are much higher than those necessary to inhibit cytochrome c oxidase which is not involved in the antimycin-insensitive respiration as was demonstrated by spectral measurements. A pigment absorbing at

555 nm is specifically reduced after addition of glucose to antimycin-inhibited cells. The same pigment is reoxidized by further addition of high concentrations of sodium azide indicating its participation in the antimycin-insensitive, azide-sensitive respiration.

* * *

2. Ferrero, I., Rossi, C., Marmiroli, N., Donnini, C. and Puglisi, P.P. 1981.

Effect of chloramphenicol, antimycin A and hydroxamate on the morphogenetic development of the dimorphic ascomycete Endomycopsis capsularis. Antonie van Leeuwenhoek (accepted for publication).

Mitochondrial protein synthesis, primary (antimycin-sensitive) respiration and secondary (antimycin-insensitive, salicyl-hydroxamic-sensitive) respiration, have been characterized in the dimorphic yeast Endomycopsis capsularis.

The inhibition by chloramphenicol (CAP) of the morhogenetic development from the yeast-like form to the mycelial structure in this yeast could represent the intervention in the morphogenetic process of mitochondrial protein synthesis, since chloramphenicol blocks in vivo and in vitro mitochondrial protein synthesis. In fact, other functions such as primary and secondary respiration, do not seem to play a role in the morphogenetic development since their inhibition by antimycin A (AA) or by salicyl-hydroxamic acid (SHAM) does not affect the process. In addition, mitochondrial protein synthesis has been shown not to be inhibited by the two respiratory inhibitors.

* * *

3. Ferrero, I., Saccani, M.G., Rossi, C., Viola, A.M. and Puglisi, P.P.

The Role of the Mitochondrial Particle in the Regulation of the L- and D-Lactate Ferricytochrome C Oxidoreductases in the Yeast Saccharomyces cerevisiae. Microbiologia (in press).

SUMMARY

It is known that in S. cerevisiae the synthesis of L-lactate ferricytochrome c oxido-reductase (L-LRC) (E.C.1.1.2.3) and of D-lactate ferri cytochrome c oxidoreductase (E.C.1.1.2.4) (D-LCR) is repressed by glucose and that two enzymes are absent in cells grown under anaerobic conditions. Some authors report that in 'petite' mutants L-LCR is absent, whereas others report that it is present but at a lower level than in the in the wild type strain. This suggests that the mitochondrial alteration due to rho⁻

condition interferes with the L-LCR synthesis or function. We have analyzed the role played by the two key mitochonrial functions altered in rho$^-$ mutants: i.e., a) mitochondrial protein synthesis (MPS) and b) respiratory activity.

The L-LCR and D-LCR activities measured in isolated mitochondria from cells grown in the presence or absence of either a mitochondrial protein synthesis (MPS) inhibitor, chloramphenicol (CAP) or of a respiratory inhibitor, antimycin A (AA) show that the block of MPS and of respiration does not inhibit the synthesis of a 'basal' level of the two enzymes but inhibits their induction by lactate. Similar results are obtained with the mit$^-$ mutants, whereas the rho^o mutation has a more drastic effect particularly on L-LCR which cannot be detected in our rho^o mitochondria.

XVIII. Instituto de Enzimologia & Patologia Molecular Del C.S.I.C., Facultad de Medicina de la Universidad Autonoma. Arzobispo Morcillo, S/N, Madrid-34-Spain. Communicated by C. Gancedo.

J. Perea and C. Gancedo have isolated a mutant defective in phosphoenolpyruvate carboxykinase. The mutation segregates 2:2 and causes inability to grow on gluconeogenic substrates below the level of trioses. Sporulation in homozygotes carrying the mutation is abolished on acetate but remains normal on glycerol. A search for mutants of the other gluconeogenic enzyme fructose 1,6-bisphosphatase has been unsuccessful since the phenotype of these mutants is similar to that exhibited by petites, this making the identification impracticable by conventional means.

Maria J. Mazon, Juana M. Gancedo and C. Gancedo have continued the work on the physiological inactivation of fructose 1,6-bisphosphatase. It has been found that growth on ammonium prevents the very rapid inactivation of the enzyme (reversible inactivation). The inactivation is paralleled by an incorporation of phosphate into the fructose1,6bisphosphatase protein. Work is progressing to elucidate the characteristics of the reversible inactivation and its possible relation with the irreversible inactivation described previously in this laboratory.

Rosario Lagunas has continued her research into the Pasteur effect and its causes. Here is an abstract to be presented at the VII International specialized Symposium on yeast cell surface (Valencia 23-26 September, 1981).

YEAST 6-81

Lagunas, R., A. Busturia, and C. Dominguez.

Inactivation of Sugar Transport Systems in S. cerevisiae and its Relation to the Pasteur Effect.

Pasteur effect (activation of sugar consumption by anaerobiosis) is irrelevant in growing S. cerevisiae. The phenomenon appears only after exhaustion of the nitrogen source in the medium (R. Lagunas. T.I.B.S. in press). The reasons for this different behaviour of growing and resting cells have been studied in various yeast strains and the results suggest the following conclusions

Contribution of respiration to sugar catabolism is small in growing yeast. However, upon disappearance of a nitrogen source the ability to ferment sugars greatly decreases and respiration becomes predominant. A shift to anaerobiosis of these cells would decrease the formation of ATP which tends to be compensated by an increase of the rate of sugar consumption.

The responsible mechanism for the decrease of fermentation in resting cells has also been studied. Two factors contribute to this decrease: a direct effect of the lack of ammonium on phosphofructokinase and a rapid inactivation of the sugar transport systems due to their rapid turnover. The following approximate half-lives have been observed.

Glucose transport system, 7 hr.
Maltose transport system, 3 hr.
Galactose transport system, 3 hr.

These values contrast with the 70 hr half-life of the pool of proteins in yeast and suggest a preferential degradation of the sugar carriers.

Addition of ammonium to resting yeast restores sugar transport activities as well as glycolytic flux in about two hours. Since the increase of total proteins is negligible during this period, it appears that yeast synthesizes the transport systems before resuming normal growth.

XIX. Institut für Allgemeine Biochemie Der Universität Wien, Währinger Strasse 38, A-1090, Vienna, Austria. Communicated by M. Breitenbach.

Below follow abstracts of two papers from our group that were presented at the Cold Spring Harbor meeting "Mitochondrial Genes" (May 13-17, 1981). Dr. Renee Schroeder is now working with Rudolf Schweyen of the Munich Institute of Genetics and will start postdoctoral work with Piotr Slonimski (C.N.R.S., Gif-sur-Yvette) at the beginning of 1982.

R. Schroeder[1,2], M. Breitenbach[1], R.J. Schweyen[2]

[1]Institut für Allgemeine Biochemie und Ludwig Boltzmann Forschungsstelle für Biochemie der Universität Wien;

[2]Genetisches Institut der Universität München.

Mitochondrial Transcription and Translation During Yeast Differentiation

Recently we have presented genetic and biochemical evidence (1) that the mitochondrial system plays a specific role in yeast sporulation and germination. In the present communication we shall compare vegetative, sporulating, and germinating wild type cells with respect to mitochondrial transcription and translation. During sporulation a new transcript could be detected in the oxi3 gene cluster. In the cob/box locus the 10S circular transcript is missing. The maturation process of the cob transcripts is more advanced in sporulation than in vegetative growth. The 11S transcript of the cytochrome oxidase subunit 2 gene is completely missing, whereas the concentration of the 20S transcript is increased. It is possible that during sporulation the 20S transcript is translated. Subunit 2 could be detected in pulse labeling experiments during sporulation.

Pulse chase studies during sporulation and vegetative growth show that most of the mitochondrially synthesized polypeptides are very stable. The ribosome-associated var-1 protein, however, is degraded very rapidly both during sporulation and vegetative growth. This protein seems to be synthesized to a lesser extent during sporulation than the other mitochondrial polypeptides.

(1) R. Schroeder and M. Breitenbach: The role of the mitochondrial genome in yeast differentiation. In "Sporulation and Germination" (H.S. Levinson et al. eds.) Proceedings of the 8th International Spore Conference. ASM Publications, in the press (1981).

* * *

M. Breitenbach and R. Schroeder.

Mitochondrially Inherited Germination-Deficient Mutants of Yeast.

Institut für Allgemeine Biochemie und Ludwig Boltzmann Forschungsstelle für Biochemie der Universität Wien, Austria.

We have isolated mutants which are inherited mitochondrially, respire and grow normally on nonfermentable substrates, but are deficient in the outgrowth of ascospores (1). One of the mutants maps between oli2 and cob on the mitochondrial genome. The morphology of the spores which contain both nuclear and mitochondrial DNA, seems to be quite normal.

In the present communication we have used pure spore preparations (isolated by a new method) to compare the behaviour of wild type and mutant spores on germination media:

Rousseau and Halvorson have defined the following stages of wild type germination. stage 1) phase darkness (T_{30}; "germination proper"), 2) elongation (T_{120}; "outgrowth") and 3) budding (T_{150}; the spores need, however, several budding cycles to return to a true vegetative cell cycle). Protein synthesis starts during stage 1, DNA synthesis during stage 3. The mitochondria seem to be in a non-respiring state during stages 1 through 3. The synthesis of spectroscopically detectable cytochromes begins only in stage 3, cytochrome a/a_3 being synthesized later than cytochromes b and c. The mutant is blocked in stage3 as shown by microscopy, low temperature cytochrome spectra, DAPI-fluorescent staining, DNA- and protein-synthesis measurements. The phenomena described here provide an example for the participation of an organellar gene in cell differentiation.

(1) R. Schroeder and M. Breitenbach: The role of the mitochondrial genome in yeast differentiation. In "Sporulation and Germination" (H. Levinson et al. eds.) Proceedings of the 8th International Spore Conference. ASM Publications, in the press (1981).

XX. Biochemisches Institut der Universität Freiburg i. Brsg. Hermann-Herder-Str. 7, D-7800 Freiburg i. Brsg., Federal Republic of Germany. Communicated by Dieter Wolf.

The following paper is in press in the Journal of Bacteriology.

Dieter H. Wolf and Claudia Ehmann. 1981.

Carboxypeptidase S Mutants of Yeast and Studies on Mutants with Multiple Proteinase Deficiencies.

SUMMARY

A new carboxypeptidase - carboxypeptidase S - was found in a Saccharomyces cerevisiae strain lacking carboxypeptidase Y (D.H. Wolf and U. Weiser, Eur. J. Biochem. 73:553-556, 1977). Mutants devoid of carboxypeptidase S activity were isolated from a mutant strain that was also deficient in carboxypeptidase Y. The four mutants analyzed in detail fell into one complementation group. The defect segregated 2:2 in meiotic tetrads. Gene dosage experiments indicated that the mutation might reside in the structural gene of carboxypeptidase S. The absence of both enzymes, - carboxypeptidase Y and carboxypeptidase S -, did not affect mitotic growth. Ascopore formation was only slightly affected by the absence of both carboxypeptidases. Protein degradation under conditions of nutrient deprivation and under sporulation conditions showed no obvious alteration in the absence of carboxypeptidase Y and carboxypeptidase S. When a proteinase

B mutation, which led to absence of proteinase B activity and which resulted in partial reduction of sporulation, was introduced into a mutant lacking both carboxypeptidases, the ability of diploid cells to sporulate was nearly completely lost. Mutants lacking both carboxypeptidases were unable to grow on the dipeptide benzyloxycarbonyl-glycyl-L-leucine as a sole nitrogen source, which indicates an additional function for carboxypeptidase Y and carboxypeptidase S in supplying nutrients from exogenous peptides. Catabolite inactivation of fructose-1, 6-bisphosphatase, cytoplasmic malate dehyrogenase, phosphoenolpyruvate carboxykinase and inactivation of NADP-dependent glutamate dehydrogenase, - events, which have been proposed to involve proteolysis in vivo - were not dependent on the presence of carboxypeptidase Y and S. In a mutant lacking both carboxypeptidases four new proteolytic enzymes with carboxypeptidase activity were detected.

XXI. Universidade Federal do Rio de Janeiro, Dept. de Bioquimica, Instituto de quimica, Rio de Janeiro, Brasil. Communicated by Anita Panek.

Below follows the abstract of a MSc thesis recently defended in our laboratory.

Cyclic AMP Activation of Trehalase. Effects of the glc-1 Mutation in Saccharomyces cerevisiae.

Claudio Henrique Dias Ortiz

Yeast contains two forms of the hydrolytic enzyme trehalase: active and cryptic. The latter appears to be converted to active trehalase by action of a cAMP-dependent protein kinase. In the present work cAMP-dependent phosphorylation of cryptic trehalase by ATP Mg was demonstrated in extracts of a normal strain, whereas in extracts from a glc-1 mutant practically all of the trehalase is already in the fully activated state. These results are consistent with the hypothesis that the already documented, defective accumulation of both trehalose and glycogen in glc-1 mutants, results from abnormal activation of a protein kinase, by-passing its normal cAMP-dependent regulation.

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V.L.A. Costa-Carvalho, M.H.M. Rocha-Leão and A.D. Panek

Glycogen Accumulation by Yeast Mutants Defective in Phosphoglucomutase.

IRCS Medical Science, 8, 563 (1980).

Yeast cells bearing the gal5 gene lack PGM-II, the main phosphoglucomutase isozyme. Phosphoglucomutase is involved in

the synthesis of UDPG, a substrate of glycogen synthase, yet these mutant cells are capable of accumulating glycogen in nonproliferating conditions in the presence of glucose. Moreover, when compared with isogenic revertants for the gal5 character, no alteration in the amount of accumulated glycogen was observed. Both gal5 mutant and revertant cells show identical behaviour during growth. These facts indicate that the absence of the main phosphoglucomutase isozyme, PGM-II, in Saccharomyces cerevisiae does not affect glycogen metabolism.

* * *

I would like to obtain some efficient wine and beer yeasts (those that produce high alcohol levels) which I would like to test for temperature resistance. Any available strains would be gratefully received.

XXII. Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558, Japan. Communicated by C. Shimoda.

1. The following papers were recently published.
C. Shimoda 1980. Differential effect of glucose and fructose on spore germination in the fission yeast Schizosaccharomyces pombe. Can. J. Microbiol. 26 741-745.
H. Inoue and C. Shimoda 1981. Changes in trehalose content and trehalase activity during spore germination in the fission yeast Schizosacharomyces pombe. Arch. Microbiol. 129. 19-22.

2. The following is in press.
H. Inoue and C. Shimoda. Induction of trehalase activity on a nitrogen-free medium. A sporulation-specific event in the fission yeast Schizosaccharomyces pombe.

SUMMARY

Kinetic experiments with synchronously sporulating cultures of a homothallic h^{90} strain of Schizosaccharomyces pombe showed that trehalase activity abruptly increased in the late sporulation process, coinciding with the appearance of visible spores. Trehalase activity was absent in vegetative cells. A set of strains different in genetic constitution at the mating-type loci was tested for induction of trehalase on the nitrogen-free sporulation medium. The appearance of trehalase activity on the sporulation medium was observed only in sporulating cultures cultures of homothallic strains (h^{90}) and diploid strains heterozygous for mating-type (h^+/h^-), and mixed cultures of heterothallic h^+ and h^- strains. Trehalase activity was not induced in nonsporogenic strains heterothallic haploid strains (h^+ and h^-), diploid strains

homozygous for mating-type (h^+/h^+ and h^-/h^-) and the homothallic strain harboring a mutation in the mat2 gene, which was unable to undergo the first meiotic division. Trehalose accumulation on the sporulation medium was observed solely in the sporulating cultures. These results led us to conclude that the induction of trehalase activity as well as the accumulation of trehalose in the medium lacking nitrogen sources was a sporulation-specific event under the control of the mating-type genes.

XXIII. Institut für Mikrobiologie, Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, 6000 Frankfurt/Main, Federal Republic of Germany. Communicated by M. Brendel.

Below follow abstracts of papers recently published or submitted.

R. Toper, W.W. Fath, and M. Brendel. Nucleic acid metabolism in yeast: II. Metabolism of thymidylate during thymidylate excess death. Mol.Gen.Genet, in press (1981).

A discrete class of strains of Saccharomyces cerevisiae, able to utilize, highly efficiently, exogenous deoxythymidine-5'-monophosphate (dTMP), was found to be sensitive to concentrations > 10 µM dTMP in an otherwise complete growth medium. Excess dTMP is cytostatic and cytotoxic: 90% of exponentially growing cells lose colony forming ability within 1h of exposure to excess dTMP in a growth medium. Uptake of dTMP, adenine, histidine, and leucine does occur during this thymidylate excess death (TED). dTMP is anabolized to higher phosphorylated nucleotides and catabolized to thymidine intracellularly. DNA synthesis is blocked under TED-conditions but not RNA and protein biosynthesis.

A. Ruhland, E. Haase, W. Siede, and M. Brendel. Isolation of yeast mutants sensitive to the bifunctional alkylating agent nitrogen mustard. Mol.Gen.Genet, 181, 346-351 (1981).

Mutants of Saccharomyces cerevisiae with enhanced sensitivity to the DNA cross-linking agent nitrogen mustard (HN2) have been isolated and partially characterized with respect to their phenotypic and genetic properties. The screening technique, based on HN2-sensitivity as sole criterion, yields approximately 1 sensitive isolate in 200 clones when applied to an intensively mutagenized population of a resistant parent strain. Mutants characterized so far are all due to recessive nuclear genes and represent at least seven complementation groups. They exhibit different degrees as well as different patterns of sensitivity towards monofunctional and bifunctional alkylating agents, and ultraviolet light.

A. Ruhland, M. Kircher, F. Wilborn, and M. Brendel. A yeast mutant specifically sensitive to bifuntional alkylation. Mutation Res. Lett., in press (1981).

From a large batch of mutants sensitive to nitrogen mustard we have isolated a mutation that specifically confers sensitivity to bi- and trifunctional alkylating agents. No or little cross-sensitivity to radiation or monofunctional agents could be detected. Sensitivity does not seem to be due to preferential alkylation of mutant DNA as parent and mutant strain exhibit the same amount of DNA alkylation and the same pattern of DNA lesions including interstrand crosslinks. The mutation is due to a defect in a nuclear gene which has been designated SNM1 (sensitive to nitrogen mustard), it may control an important step in the repair of DNA interstrand crosslinks.

XXIV. Department of Genetics, Institute of Molecular Biology, Bulgarian Academy of Science, 1113 Sofia, Bulgaria. Communicated by P.V. Venkov and L.I. Stateva.

Below follows the abstract of the Ph.D. dissertation of L.I. Stateva, completed in May 1981.

An osmotic-dependent Saccharomyces cerevisiae mutant VY1160 has been genetically and electron microscopically characterized. Mannan structure has also been studied.

The mutant strain VY1160 has the following phenotypic characteristics: temperature sensitivity, rifampicin susceptibility, respiratory deficiency, dependence on the presence of 10% sorbitol as osmotic stabilizer in the nutritional media and capability for lysis of cells, suspended in water or buffers lacking the osmotic stabilizer.

Genetic analysis of the mutant has shown that it carries three recessive nuclear mutations, designated $sorb^-$, ts1 and ts2. Two of these ($sorb^-$ and ts1) are closely linked to each other. The mutation $sorb^-$ determines the cell lysis, while the mutation ts1 increases the ability for lysis of sorbitol-dependent cells. The presence of two nuclear mutations involved in the cell lysis is the most likely explanation of the genetic stability of this feature. The second temperature sensitive mutation ts2 confers the susceptibility of VY1160 mutant cells to rifampicin.

The mutation $sorb^-$ is not centromere linked. It is located on chromosome I and maps approximately 31 recombination units distal from ade1. ts1 Mutation is also located on chromosome I and is shown to map approximately 37 recombination units from ade1.

Electron microscopic investigations by freeze-etching technique of the cell wall and membrane of the mutant cells and wild type cells have been carried out. These experiments showed that in contrast to the wild type cells, the osmotic dependent mutant cells are characterized by local alterations in the structure of the cell wall and membrane, which are revealed under osmotic shock. Local ruptures in the cell wall and membrane are clearly visible, forming holes through which cellular contents are flowing out.

Mannan has been isolated from strains: wild type, triple mutant VY1160 (sorb$^-$ ts1 ts2) and single segregants with genotype: sorb$^-$ or ts1. Mannan structure has been studied by mild acid hydrolysis, β-elimination and acetolysis.

The obtained results have shown that mannan from VY1160 is characterized by more short side chains and less long side chains, the same defect being observed in ts1 single mutant and not in sorb$^-$ mutant. Therefore, we conclude that ts1 is most probably the mutation which determines this alteration in mannan structure of the VY1160 mutant strain.

The following paper was recently published:

Lubomira I. Stateva and Pencho V. Venkov, 1981. Meiotic Mapping of the Nuclear Determinant of Cell Lysis of the Osmotic Dependent Saccharomyces cerevisiae Mutant VY1160, Mol. Gen. Genetics, 181:414-415.

SUMMARY

A recessive nuclear mutation sorb$^-$, which determines the ability for lysis of the osmotic dependent Saccharomyces cerevisiae mutant VY1160 has been mapped on the right arm of chromosome I. sorb$^-$ is not centromere linked and is approximately 31 recombination units from ade1.

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

Below follow summaries of two papers recently published or in press.

Cesira L. Galeotti, K.S. Sriprakash, Claire M. Batum and G.D. Clark-Walker

An Unexpected Response of Torulopsis glabrata Fusion Products to X-Irradiation. Mutation Research, 81(1981) 155-164.

Summary

Intra-species fusion products of Saccharomyces cerevisiae, Saccharomyces unisporus and Torulopsis glabrata have been isolated following polyethylene glycol-induced fusion of protoplasts and selection for prototrophic colonies. Staining with lomofungin showed that all fusion products were uninucleate. Measurement of DNA content mostly gave values between haploid and diploid levels indicating that the majority of fusion products were aneuploid. Nevertheless, fusion products of S. cerevisiae and S. unisporus were, as expected, more resistant to X-irradiation than their haploid parents. By contrast, the X-ray dose-response curve of all T. glabrata fusion products was indistinguishable from their progenitors despite the fact that mitotic segregants could be recovered amongst the survivors to X-rays. A possible explanation for the behaviour towards X-rays of T. glabrata fusion products is that this species lacks a DNA repair pathway involving recombination between homologous chromosomes. We conclude from this study that the shape of the X-ray dose-response curve should not be taken to indicate the ploidy of new yeast isolates without supporting data.

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

Partial Duplication of the Large Ribosomal RNA Sequence in an Inverted Repeat in Circular Mitochondrial DNA from Kloeckera africana. Implications for Mechanisms of the Petite Mutation. J. Mol. Biol. (1981) 147 (in press).

The 27,100 base-pair circular mitochondrial DNA from the yeast Kloeckera africana has been found to contain an inverted duplication spanning 8600 base-pairs. Sequences hybridizing to transfer RNAs and the large ribosomal RNA are present in the duplication, however, one end of this segment terminates in the large mitochondrial ribosomal RNA sequence so that at least 1000 base-pairs of the gene is not repeated. The large and small mitochondrial ribosomal RNAs have been shown to have lengths of 2700 and 1450 bases, respectively, and genes for these sequences are separated by a minimum of 1300 base-pairs and a maximum of 1750 base-pairs. Consequences of the large inverted duplication to mechanisms of the petite mutation are discussed in terms of previous hypotheses centred on intramolecular recombination in yeast mitochondrial DNA at sequences of homology or partial homology. Despite the long inverted duplication in K. africana mitochondrial DNA, this yeast has one of the lowest frequencies of spontaneous petite mutants amongst petite positive yeasts. One implication of these findings is that in this yeast intra-molecular mitochondrial DNA sequence homology may not be an important factor in the excision process leading to petite formation.

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

Below follows a summary of work conducted recently in our laboratory.

R.K. Vashishat and Anjly Kaushal

Studies on UV-Induced Mitotic Crossing Over and Gene Conversion in Saccharomyces cerevisiae.

SUMMARY

A study on the mechanism of UV-induced reciprocal and non-reciprocal mitotic recombination was made with stationary phase cells of strain D7 of Saccharomyces cerevisiae. Mitotic crossing over at the ade2 locus was detected visually by the appearance of pink and red twin sectored and pink, red and white triply sectored colonies on low adenine medium. Mitotic gene conversion at trp5 locus was monitored by the appearance of tryptophan non-requiring colonies on tryptophanless medium.

A UV-dose of 60 seconds giving 49 percent survival was selected for various experiments. This dose falls in the region of the shoulder of the dose-survival curve indicating that the yeast cells retain the capacity to repair the damage induced by UV-irradiation. Incubation of stationary phase cells with 40 mM hydroxyurea (HU) in liquid complete medium for varying intervals (1 to 12 h) had no effect on survival and percentage of mitotic crossovers. However, there was an increase in the frequency of mitotic gene convertants after 8 and 12 h of incubation. Treatment of UV-induced cells with HU also increased the frequency of UV-irradiated mitotic gene convertants with no measurable effects on survival and UV-induced mitotic crossing over. Since the cells were at two-strand stage, inhibition of DNA synthesis by HU alone and after UV-irradiation prolonged the pairing time, thereby increasing the frequency of mitotic gene conversion. On the other hand, mitotic crossing over in this particular strain, will only be detected if it occurs at four-strand stage. This may be the reason that HU treatment did not affect reciprocal mitotic recombination. This confirms the hypothesis that UV induces recombination by delaying DNA synthesis, which may lead to pairing of homologous chromosomes.

Incubation of yeast cells with cycloheximide (CH) in liquid complete medium, did not affect survival, spontaneous mitotic crossing over and mitotic gene conversion, indicating that most of the proteins essential for spontaneous induction of these events are present in the mitotic cells. Post-irradiation inhibition of protein synthesis with CH decreased survival and frequency of mitotic gene convertants. This shows that protein synthesis is required after UV-irradiation for full survival and production of recombinants. However, CH had no effect on UV-induced mitotic crossovers.

The differential effect of HU and CH on UV-induced reciprocal and non-reciprocal recombination is because of the occurrence of the former at the four-strand stage and that of the latter at the two-strand stage and not due to the existence of two different mechanisms for these events. The frequency of UV-induced mitotic gene convertants was increased if HU was added within 4 h after UV-irradiation. However, inhibition of protein synthesis within 3 h after UV-treatment decreased the frequency of UV-induced mitotic gene convertants. This implies that UV-induced non-reciprocal mitotic recombination is completed within 3-4 h after irradiation.

Recombinational repair was found to be involved in UV-induced reciprocal as well as non-reciprocal recombination because incorporation of caffeine in various post-irradiation selective media decreased the frequencies of both the events.

The following paper has been published:

Vashishat, R.K., Manjula Vasudeva and S.M. Kakar, 1980. Induction of mitotic crossing over, mitotic gene conversion and reverse mutation by epichlorhydrin in Saccharomyces cerevisiae. Indian Journal of Experimental Biology 18:1337-1338.

XXVII. Genetisches Institut, University of Munich, D-8000 Munich 19, Maria-Ward-Strasse 1a, West Germany. Communicated by K. Wolf.

Below follow the titles and references of several recent papers from our laboratory.

1. Thrailkill, K., Birky, C.W. Jr., Luckemann, G., Wolf, K: Intracellular population genetics evidence for random drift of mitochondrial allele frequencies in Saccharomyces cerevisiae and Schizosaccharomyces pombe. Genetics 96, 237-262 (1980).

2. Wolf, K., Del Giudice, L.: Effect of ethidium bromide on transmission of mitochondrial genomes and DNA synthesis in the petite negative yeast Schizoaccharomyces pombe. Current Genetics 1, 193 (1980).

3. Egel, R., Kohli, J., Thuriaux, P., Wolf, K.: Genetics of the fission yeast Schizosaccharomyces pombe. Annual Review of Genetics 14, 77-108 (1980).

4. Del Giudice, L., Wolf, K: Joint control of nuclear and mitochondrial DNA synthesis in fission yeast. Microbial Genetics Bulletin 48, 8 (1980).

5. Wolf, K., Del Giudice, L: Ethidium bromide induced reversible bias of mitochondrial marker transmission in fission yeast. Microbial Genetics Bulletin 48, 10 (1980).

6. Burger, G., Wolf, K.: Mitochondrially inherited resistance to antimycin and diuron in the petite negative yeast Schizosaccharomyces pombe. Molec. gen. Genet. 181, 134-139 (1981).

7. Del Giudice, L., Wolf, K., Buono, C., Manna, F.: Nucleocytoplasmic interactions in the petite negative yeast Schizosaccharomyes pombe. Inhibition of nuclear and mitochondrial DNA syntheses in the absence of cytoplasmic protein synthesis. Molec. gen. Genet., in press.

8. Seitz-Mayr, G., Wolf, K.: An extrachromosomal mutator inducing point mutations and deletions in the mitochondrial genome of fission yeast. Microbial Genetics Bulletin, in press.

9. Del Giudice, L., Wolf, K.: Synthesis of mitochondrial DNA during the cell cycle of the petite negative yeast Schizosacchromyces pombe. Microbial Genetics Bulletin, in press.

10. Del Giudice, L., Wolf, K.: Cloning of mitochondrial DNA from the petite negative yeast Schizosaccharomyces pombe in the E. coli plasmid pBR322. Microbial Genetics Bulletin, in press.

11. Del Giudice, L., Wolf, K., Manna, F., Pagliuca, N.: Synthesis of mitochondrial DNA during the cell cycle of the petite negative yeast Schizosaccharomyces pombe. Molec. gen. Genet., in press.

12. Wolf, K., Lang, B., Anziano, P.Q., Perlman, P.S.: Organization and expression of the mitochondrial genome in fission yeast. European Journal of Cell Biology 24, 27 (1981).

Books:

Wolf, K.: Genetik. Estermann Verlag, Braunschweig, in press.

Del Giudice, L., Wolf, K: Genetica, Biogenesi ed Evoluzione dei mitocondri. Piccin Editore, Padua, in press.

Wolf, K.: Die Gene. Ullstein-Verlag, Berlin, in press.

Genetics, Biogenesis and Bioenergetics of Mitochondria. W. Bandlow, R.J. Schweyen, D.Y. Thomas, K. Wolf, F. Kaudewitz, eds., Walter de Gruyter, Berlin - New York, 1976.

Mitochondria 1977. Genetics and Biogenesis of Mitochondria. W. Bandlow, R.J. Schweyen, K. Wolf, F. Daudewitz, eds., Walter de Gruyter, Berlin - New York, 1977.

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

Below follow abstracts of recent papers, presentations, and manuscripts from our laboratory.

1. A. Maraz and L. Ferenczy. Transfer of mitochondria via anucleate protoplasts in Saccharomyces cerevisiae. 8th Congress of the Hungarian Society of Microbiology, 27-29 August 1979, Budapest p. 135, 1979.

ABSTRACT

During protoplast formation in budding yeasts, the buds are frequently released as "mini-protoplasts". Although the majority of these "mini-protoplasts" do not possess nuclei, they do contain mitochondria. Enucleated "mini-protoplasts" are easily separated from those bodies containing nuclei by low-speed centrifugation. Only a small proportion (< 5%) of the "mini-protoplasts" obtained after fractionation contains nuclei.

In this laboratory, a "mini-protoplasts" fraction was prepared from an erythromycin-resistant (ery^R) auxotrophic mutant of S. cerevisiae. In this strain, erythromycin resistance is determined by a mitochondrial gene. Polyethylene glycol and Ca^{2+} were used to induce fusion between this "mini-protoplasts" fraction and nucleated protoplasts of a neutral petite (rho^0) mutant derived from an adenine-requiring strain of S. cerevisiae. Under partially-selective conditions, the majority of fusion products were haploid and ery^R, containing the nucleus of the recipient adenine-requiring strain and the mitochondrial genome from the ery^R respiratory-competent donor cells.

2. A. Maraz and L. Ferenczy 1980. Selective Transfer of Fungal Cytoplasmic Genetic Elements by Protoplast Fusion. Current Microbiol. 4:343-345.

ABSTRACT

An anucleate small-protoplast fraction was prepared from a respiratory-competent Saccharomyces cerevisiae strain that had mitochondrially inherited resistance to erythromycin, and used to transfer mitochondria selectively. Polyethylene glycol and Ca^{2+} were applied to induce fusion between these

respiratory-deficient S^o mutant derived from an adenine-requiring strain of the same species. The majority of fusion products were haploid and erythromycin resistant, containing the nucleus of the recipient adenine-requiring strain and the mitochondrial genome from the respiratory-competent donor cells. Selective transfer of mitochondria and other cytoplasmic genetic elements also seems possible in a wide variety of fungal and other cells.

3. A. Maraz and J. Subik 1981. Transmission and Recombination of Mitochondrial Genes in Saccharomyces cerevisiae after Protoplast Fusion. Mol. Gen. Genet. 181:131-133.

ABSTRACT

Protoplasts of auxotrophic stains of Saccharomyces cerevisiae of opposite and identical mating types carrying different mitochondrial drug-resistance markers, with both homosexual and heterosexual mitochnodrial backgrounds, were induced to fuse by polyethylene glycol. After selective regeneration of prototrophic fusion products, the transmission and recombination frequencies of mitochondrial genes in populations of cells were determined and compared with those obtained in mating processes. The frequencies obtained in the fusion experiments proved very similar to those found in the zygote clones. The behavior of mitochondrial genes was apparently affected neither by nuclear mating type background nor by the method of transfer of mitochondrial genomes (i.e., protoplast fusion or mating), making possible mitochondrial genetic studies by protoplast fusion irrespective of the mating type barrier of yeast strains.

4. M. Pesti, S. Paku and E.K. Novak 1981. Some characteristics of nystatin-resistant sterol mutants of Candida albicans. Acta Microbiologica Academiae Scientiarum Hungaricae (in the press).

ABSTRACT

After nitrosoguanidin treatment, various stable, auxotrophic and nystatin-resistant mutants of Candida albicans were isolated which showed a decreased growth yield and an increased cell volume. On media containing 0.01% of the carbon source, most of them could assimilate glycerol, α-methyl-D-glucoside, DL-lactic acid, L-sorbose, L-arabinose and ribitol to only a significantly reduced extent, or not at all, in comparison to the ergosterol-producing parental strains. It was suggested that the altered plasma membrane composition of sterol mutants leads to a quantitative decrease in the permeation efficiency of the solute, possibly to below the maintenance level.

5. M. Pesti, E.K. Novak, L. Ferenczy and A. Svoboda 1981. Freeze fracture electron microscopical investigation of Candida albicans cells sensitive and resistant to nystatin. Sabouraudia 19:17-26.

ABSTRACT

The plasma membrane ultrastructure of the nystatin-resistant ergosterol mutant having an altered lipid composition was not significantly different from that of the parental, ergosterol-producing, nystatin-sensitive strain. Nystatin treatment did not result in an ultrastructural change in the resistant cells, but led to the aggregation of membrane particles in the sensitive ones. A deepening and deformation of invaginations, atypical membrane fracture, certain changes in the structure of the cell wall and special ornamentation of the sensitive cell surface were considered to be nonspecific effects of nystatin treatment.

6. M. Pesti, J. McA. Campbell and J.F. Peberdy 1981. Alteration of ergosterol content and chitin synthase activity in Candida albicans. Current Microbiology 5:185-189.

ABSTRACT

Chitin synthase (EC 2.4.1.16) activity in total membrane fractions from two ergosterol deficient mutants of Candida albicans was significantly higher and the zymogenic component of the enzyme from a membrane preparation of stationary phase cells of mutants was more susceptible to trypsin digestion than in their ergosterol-producing parental strain. The observed increase in chitin synthase activity may be interpreted in terms of the greater rigidity of the ergosterol-deficient plasma membranes, resulting in the increased accessibility of activating factor or trypsin to the zymogenic from of chitin synthase and hence an increased quantity of active enzyme, whereby both the total and specific activity are enhanced.

7. M. Pesti and L. Ferenczy 1981. Hybrids of Candida albicans sterol mutants with different nystatin resistance obtained by protoplast fusion. The Journal of General Microbiology (submitted).

ABSTRACT

Intraspecific protoplast fusion was carried out in various pairings between auxotrophic, ergosterol-producing strains of Candida albicans and their ergosterol-less, nystatin-resistant mutants. Stabile, heterozygous, nutritionally completed diploid hybrids were obtained, which proved to be sensitive, semiresistant or resistant to the drug as a consequence of complementation or non-complementation for

ergosterol biosynthesis. Dominant mutation was not found. The cell volume of sensitive hybrids originating from two nystatin-resistant mutants was the same as for those which originated from two ergosterol non-producing strains. Nystatin-resistant, ergosterol non-producing hybrids, however, had a higher cell volume than the sensitive ones, proving the importance of ergosterol in the direct or indirect determination of the cell volume.

XXIX. Institut Curie-Biologie, Centre Universitaire, Batiment 110, 91405 Orsay, France. Communicated by E. Moustacchi.

The following articles have been recently published:

S. Hixon, H.L. Franks and E. Moustacchi, 1980. Yeast mitochondrial DNA characterization after ultraviolet radiation. Mutation Res. 73:267-277.

F. Fabre, 1981. Mitotic recombination and repair in relation to the cell cycle in yeast. In: "Molecular Genetics in Yeast", Alfred Benzon Symp. 16, Ed. D. Von Wettstein, J. Friis, M. Kielland-Brandt and A. Stenderup, Munksgaard, Copenhague, pp. 1-8.

E. Moustacchi, 1980. Mutagenicity testing with eukaryotic microorganisms. Arch. Toxicol. 46:99-110.

R. Chanet, N. Magana-Schwencke and E. Moustacchi, 1980. Genetic effects of formaldehyde in yeast. Current status and limitations of the radiation equivalence concept. In: "Radiobiological Equivalents of Chemical Pollutants", Ed. Int. Atomic Energy Agency, Vienna, pp. 45-59.

N. Magana-Schwencke and E. Moustacchi, 1980. Biochemical analysis of damage induced in yeast by formaldehyde. III. Repair of induced cross-links between DNA and proteins in wild type and in excision-deficient strains. Mutation Res. 70:29-35.

J.A.P. Henriques and E. Moustacchi, 1980. Sensitivity to photoaddition of mono- and bi-functional furocoumarins of X-ray-sensitive mutants of Saccharomyces cerevisiae Photochem. Photobiol. 31:557-563.

D. Averbeck et E. Moustacchi, 1980. Decreased photo-induced mutagenicity of mono-functional as opposed to bi-functional furocourmarins in yeast. Photochem. Photobiol. 31:475-478.

J.A.P. Henriques and E. Moustacchi, 1980. Isolation and characterization of pso mutants sensitive to photoaddition of psoralen derivatives in Saccharomyces cerevisiae. Genetics 95:273-288.

N. Magana-Schwencke, D. Averbeck, J.A.P. Henriques et E. Moustacchi, 1980. Absence de pontages inter-chaines dans l'ADN traité par le 3-carbethoxypsoralene et une irradiation a 365 nm. C.R. Acad. Sci. Paris, 291:207-210.

The following articles are in press or submitted.

The effects of three pso genes on induced mutagenesis: a novel class of mutationally defective yeast. C. Cassier, R. Chanet, J.A.P. Henriques and E. Moustacchi. Genetics (1980), December issue (in press).

Mutagenesis and repair in yeast mitochondrial DNA. E. Moustacchi et M. Heude. In: "Molecular and Cellular Mechanisms of Mutagenesis", Ed. A. Hollaender, Plenum Press, Basic Life Sciences serie, (1981) (in press).

Genetic effects and repair in yeast of DNA lesions induced by 3-carbethoxypsoralen and other photoreactive furocoumarins of therapeutic interest. D. Averbeck, N. Magana-Schwencke et E. Moustacchi. In: "Psoralens in cosmetics and dermatology", Pergamon Press, (1981) (in press).

Mutagenesis induced by Mono- and bi-functional alkylating agents in yeast mutants sensitive to photoaddition of furocoumarins (pso). C. Cassier et E. Moustacchi (submitted).

The fate of 8-methoxypsoralen photoinduced cross-links in nuclear and mitochondrial yeast DNA. Comparison of wild type and repair deficient strains. N. Magana-Schwencke, J.A.P. Henriques, R. Chanet et E. Moustacchi (submitted).

Interactions between mutations for sensitivity to psoralen photoaddition (pso) and to radiations (rad) in yeast. J.A.P. Henriques et E. Moustacchi (submitted).

Recently completed dissertation

Voies de réparation des lésions induites par la photoaddition de furocoumarines chez la levure. Isolement et caracterisation d'une nouvelle classe de mutants (pso). J.A.P. Henriques. Thèse de Doctorat d'Etat, Université Paris-Sud, n° d'ordre 2382 (soutenue le 30.1.1981).

XXX. Abteilung Genetik, Fachbereich Biologie, Technische Hochschule Darmstadt, D-6100 Darmstadt, German Federal Republic. Communicated by Friedrich K. Zimmermann.

Genetics of yeast phosphofructokinase: I. Breitenbach-Schmitt has exploited the fact that there are two genes for the formation of phosphofructokinase: PFK1 and PFK2. Recessive mutant alleles have been isolated which eliminate all phosphofructokinase activity as measured in vitro;

however, fermentation and alcohol production are still possible. A double mutant with defective pfk1 and pfk2 alleles is completely blocked in glycolysis. Starting with either pfk1 or pfk2 mutant strains, she has isolated mutants with a complete block in glycolysis and obtained in this way a large number of new pfk1 and pfk2 defective alleles.

Genetics of yeast pyruvate decarboxylase: Mutants with defects in pyruvate decarboxylase have been isolated and two genes identified: PDC1 and PDC2. All mutants have some residual enzyme activity. Pyruvate decarboxylase of mutant pdc1-14 shows abnormal substrate kinetics and also different affinity for the co-factor thiamine pyrophosphate. This suggests that PDC1 is the structural gene for pyruvate decarboxylase. pdc2 mutants with residual activity form enzyme that is not obviously different from wild type. The PDC1 gene has been cloned (Investigations by D. Schmitt).

Genetics of maltose fermentation in yeast: C. Stüwer has succeeded in cloning a maltose gene from a pool derived from a strain carrying MAL1. Furthermore, we have a selective system which allows us to isolate negative mutant alleles of MAL2. This is based on the fact that mutant allele hex2-3 causes not only a derepressed formation of a number of enzymes normally subject to carbon catabolite repression but also an extreme maltose toxicity in combination with various functional MAL2 alleles. Several mutants resistant to maltose derived from a hex2-3 MAL2-8^c strain had a defect in the MAL2 gene whereas others were affected in a number of genes involved in general derepression and thus unable to grow on maltose and other nonfermentable carbon sources.

Genetics of invertase formation in yeast: M.K. Groszmann has completed his thesis on the genetics of invertase formation in Saccharomyces cerevisiae. Through a combination of various mutant alleles he obtained a strain that formed on a glucose medium 63 units of invertase per mg protein in the crude extract. However, this activity was not found at that level after growth on a non-fermentable carbon source. An interesting feature were de novo fermenters which could be obtained from strains carrying no SUC genes. They formed invertase and were due to the activation of a silent gene now called SUC7. A systematic survey of various diploids heterozygous for different SUC genes in combination with either silent SUC7 or two different active alleles showed that there is an effect of SUC7 on the specific activities of invertase formed in the presence of other SUC genes. The only hypothesis that could accommodate the observed phenomena would be to postulate that the SUC genes carry not only a structural region coding for the protein moiety of yeast invertase but also carry a controlling region which forms a regulatory factor. Silent SUC7 would form a controlling factor that interferes with the positive action of the controlling factors of the other SUC genes. In the case of activated alleles of

SUC7, the controlling factor apparently no longer interferes strongly or may be not at all with the controlling factors of the other SUC genes.

XXXI. Department of Botany, University of Peshawar, Peshawar, Pakistan. Communicated by B.A. Siddiqi.

Below follows a summary of a recently completed research project that was sponsored by the Pakistan Science Foundation. We have been investigating a number of food additives for the induction of mitotic recombination and mutation in the diploid yeast Saccharomyces cerevisiae.

Mutagenicity- and Recombinogenicity-Testing of Some Food Colours and Flavours in Saccharomyces Cerevisiae.

B.A. Siddiqi and S.R. Chughtai*

*Department of Botany, University of Baluchistan, Quetta, Pakistan.

SUMMARY

Colouring and flavouring agents comprise two very important groups of food additives, but very little information is available about their genotoxicity. The present study was initiated to assess the mutagenic and possibly carcinogenic potential of a number of food colours and flavours. Altogether, 43 food colours and 20 flavours, widely used in Pakistan and elsewhere in the world, have been tested. The tested colours include Tartrazine, Sunset Yellow FCF, Orange H.K., Lemon Yellow, Metanil Yellow, Amaranth, Ponceau 4R, Ponceau 6R, Madeira Colour, Carminic Acid, Bixine, Indigotine, Syregul, Brilliant Blue FCF, Seelachfarbe, New Blue VN, Azo Rubine, Laksefarbe, Violet Acid 5B, Dunkel Blue, Diazolrein Blue 6B, Mocca Brown, Cherry Red, Patent Blue, Raspberry Red, Apple Green, Erythrosine, Egg Yellow, Lime Juice Yellow, Chocolate Brown, Orange Red, Direct Brown, Rhodamine B, Eriochrome Black T, Methyl Red, Orange G, Phloxine, Congo Red, Rose Pink, Rose Bengal, Eosin Y, Rouge S and Auramine O. The tested flavours include Strawberry, Raspberry, Orange, Sandal Wood, Vanilla, Afza, Mango, Elachi, Kewra, Lemon, Banana, Pine Apple, Green Rose, Almond, Zafran, American Ice Cream Soda, Cardamom, Peppermint, Coconut and Tuti Fruti.

The additives were tested using three genetic end-points: mitotic gene conversion, mitotic crossing over, and reverse mutation in the diploid strains D4 and D7 of S. cerevisiae. In stationary-phase tests, all colours, except Phloxine, Rose Pink and Auramine O, were tested for the induction of gene conversion at the ade2 and trp5 loci of D4. Rouge S was also tested in log-phase cells of D4. Eleven food colours viz:

Eriochrome Black T, Methyl Red, Orange G, violet Acid 5B, Rose Pink, Congo Red, Rose Bengal, Eosin Y, Phloxine, Rouge S and Auramine O were tested in stationary-phase cultures of D7 for the induction of gene conversion at the trp5, crossing over at the ade2 and reverse mutation at the ilv1 locus. Six colours viz: Congo Red, Rose Pink, Rose Bengal, Eosin Y, Rouge S and Auramine O were also tested in log-phase cells of D7. All the flavours were tested in stationary-phase cells of both the strains.

In stationary-phase tests, cells were treated with colour (10 mg/ml) or flavour (0.01 ml/ml) solution at three different pH levels: 5.91, 6.96 and 8.05, for 4 hours in the dark at 30°C. In log-phase tests, the test chemical was added after 24 hours and the treatment was terminated after 48 hours of growth at 30°C. Cultures with or without EMS (0.01 ml/ml), respectively, served as the positive and negative control.

The study revealed that all the tested food additives, except two colours Rouge S and Auramine O, without any further metabolic activation, were non-mutagenic in diploid yeast. They failed to increase significantly the frequency of gene conversion, crossing over and reverse mutation in the diploid strains. The food dyes Rouge S and Auramine O were detected as powerful mutagens and recombinogens. They induced a significantly high frequency of convertants, recombinants and revertants in both the strains. While all the non-mutagenic additives were non-toxic, the mutagenic dyes Rouge S and Auramine O were severely cyto-toxic even at comparatively low concentrations (0.1 and 0.05 mg/ml, respectively). They significantly inhibited the cell division and reduced the cell viability were active in stationary- and log-phase cells having dose dependent mutagenic and cyto-toxic effects. The positive control, EMS, exhibited mutagenic and recombinogenic activity which is an evidence for the reliability of the test systems.

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S.R. Chughtai, A.S. Mohmand and B.A. Siddiqi. Testing of Betal leaf extract for the induction of mitotic gene conversion in Saccharomyces cerevisiae.

ABSTRACT

A relationship between the habitual chewing of "betal quid" and the incidence of oral cancer has been established. Betal leaf (leaf of Piper betal) is a major constituent of betal quid. It has been shown to induce chromosomal aberrations in human leucocyte culture and plant cells. However, it did not induce mutation in Drosophila. Thus, conclusive evidence for the role of betal leaf in the induction of oral cancers remains to be established experimentally. The close relationship between carcinogenesis

and mutagenesis has prompted us to undertake an investigation in yeast on possible mutagenicity of betal leaf. We tested the aqueous extract of betal leaf for the induction of mitotic gene conversion in strain D4 of the yeast Saccharomyces cerevisiae. The results indicate that it failed to increase significantly the frequency of mitotic gene conversion at the ade2 and trp5 loci in the stationary - as well as in log-phase tests. Moreover, it neither caused cell killing nor inhibited the cell division. Further studies are in progress to investigate the possible mutagenicity of betal leaf and other constituents of betal quid.

XXXII. Maharashtra Education Society's Abasaheb Garware College, Microbiology Department, Karve Road, Pune 411004, India. Communicated by V.B. Rale.

Indian Fermented Foods

P. Akut, G. Dudhal, D. Pawar, A. Gole, S. Tembe, M. Rao, S.S. Joshi, A.D. Agate, V.B. Rale.

(Summary of a preliminary report of the project, published in Undergraduate Student's Association of Microbiology (USAM), July, 1980).

There is a great need for research on the use of microorganisms in the processing of foods, a long neglected field, and especially foods with which the Western World is not familiar. Research has been well under way on oriental foods like Tempeh, Shoyu, Koji, Angkak, Sufu, Lao-Chao, and a host of others. Hesseltine and Wang have recently outlined the advantages of fermented foods such as: (1) means of preservation, (2) reduction of material volume, (3) destruction of undesirable products, (4) enhancement in nutritional value, (5) improvement in appearance and flavor, (6) salvage of product, (7) reduction in fuel use for prefermented cooking, (8) enjoyability and safety. Microbiological data are available on a South Indian fermented food - Idli - used as main course dish. Fermentaiton is known to be carried out by Leuconostoc, Pediococcus spp. Jilebi and Anarsa are two more such foods, consumed by all strata of the socio-economic groups in India: the former a favorite sweet item in marriage feasts and the latter a typical item during festivals. This work was undertaken to (1) reveal the organisms responsible for fermentation, flavor and aroma, (2) speciate them, and (3) effect time reductions in the course of fermentation since normal fermentation times for Jilebi and Anarsa are 24h and 10 days, respectively.

Recipes:

Jilebi. Mix 1 Kg Wheat (Triticum aestivum L.) flour with 1/4 to 1/2 Kg (Cicer arietinum L.) flour. Add enough water to

make paste, followed by a small amount of buttermilk. Ferment for 24 h (at room temperature), mold into thick circular threads (with a mold), fry in Ghee, and soak in sugar syrup.

Anarsa: Soak rice (Oryza sativa L.) in water for 3 successive days, changing water every 24 h. Grind and make paste with water. Mix 3/4 Kg Gur (jaggery) per Kg of paste. Ferment for 10-15 days (at room temperature). Roll into circular discs and fry in vegetable oil.

Both products do not perish for several days.

Microbial Methodology:

Jilebi: Total Viable Counts (TVC) of fermented doughs were performed on Davis, Rogosa and SPC media after making suitable dilutions in phosphate buffer (pH 7.2). Dominant organisms were isolated, purified and biochemically characterized.

Anarsa: Enrichments of fermented doughs were required to be made in Davis' broth substituted by 60% (w/v) jaggery for glucose.

Starter culture experiment: Chosen isolates were grown in Davis' broth and Davis' broth + 60% w/v jaggery, on a rotary shaker. Various combinations of specific cultures (3.2×10^3/ml for yeasts and 2.6×10^3/ml for lactic cocci) were then mixed in a range of 6-24% (v/w) into sterilized doughs and visually monitored to complete fermentation.

Results:

Jilebi: Four types of yeasts (Isolate Nos. 1-4) and a lactic coccus (Isolate 5) were isolated and characterized. Isolate 2 and 4 had maximum fermentative activity.

As against natural fermentaiton time of 24 h, a culture combination of Nos. 2+4+5 at 24% (v/w) reduced it to only 4 h, with better aroma, flavor, and appearance.

Anarsa: Only one type of yeast (Isolate 6) was obtained. At a concentration of 20% (v/w), it reduced the fermentation time to only 2 days as against the normal 10 days.

Conclusion:

This project demonstrated the role of yeasts and lactic cocci in two of the popular Indian fermented foods - Jilebi and Anarsa. It also revealed that these foods can be processed in a shorter and better way when starter cultures of the natural microorganisms prevailing in them are made and introduced at proper concentrations. This would be a boon to housewives and enthusiastic entrepreneurs.

Further, we are interested in identifying these organisms and optimizing the processes.

XXXIII. Department of Biology, Guru Nanak Dev University, Amritsar-143005, India. Communiated by D.K. Sandhu.

Below follow abstracts of two recent papers from our laboratory.

1. D.K. Sandhu and M.K. Waraich: Protein and amino acid contents of 13 yeast strains grown on cheese whey: Indian J. Microbiol.

The protein content and amino acid composition were determined of 13 yeast strains (Brettanomyces anomalus, Candida curvata, C. humicola, C. kefyr, C. pseudotropicalis, Debaryomyces marama, Hansenula muscicola, Kluyveromyces fragilis, Pichia polymorpha, Torulopsis candida, Trichosporon cutaneum, Tr. pullulans, Wingea robertsii) grown on cheese whey. High contents of lysine, aspartic acid, threonine and serine and a deficiency in sulfur amino acids were the general characteristics of all the strains. Tr. cutaneum produced the maximum amount of protein and amino acids and C. humicola the minimum. In addition to Tr. cutaneum and K. fragilis, Wingea robertsii appeared to be a promising strain for the production of single cell protein using cheese whey as the substrate.

* * *

2. D.K. Sandhu and M.K. Waraich: Airborne yeasts in Amritsar (India). Antonie Van Leeuwenhoek.

An aerial survey of yeast in Amritsar has been carried out for a period of one year by the petri plate exposure method. A total of 14 yeast species appeared on the plates. Out of these Candida was the commonest yeast genus, representing 43.1% of the total colony count followed by Rhodotorula and Torulopsis. Maximum numbers of yeasts were recorded in the month of October and minimum during January-February.

XXXIV. Alko, Box 350, SF-00101, Helsinki 10, Finland. Communicated by Heikki Suomalainen.

The following paper was submitted for publication since the appearance of the December 1980 issue of the Yeast Newsletter.

Erkki Oura, Heikki Suomalainen and Risto Viskari.

Role of Yeast and other Microbes in Bread Making.

Submitted for publication in Economic Microbiology

This review article concentrates on the production figures and properties of baker's yeast as required for making bread. Also sour doughs are well covered and in that context other yeasts and bacteria are dealt with.

The article contains new estimates for consumption and production of baker's yeast. The world production was set a 1 430 000 tons of fresh yeast (28-32 % d.m.) and the highest consumption per person per year at 2.0 kg for Finland.

Yeast metabolism is discussed from the point of view of carbon dioxide evolution and the role of ATP hydrolysis as a possible rate limiting step in fermentation is emphasized. Environmental and chemical factors with the most pronounced effects on the leavening activity of yeast are: temperature, pH, osmotic pressure, organic acids, ethanol and mineral salts. The role of yeast in bread making, especially for aroma formation, is discussed.

Sour doughs are treated mostly from the microbiological point of view by detailing organisms responsible for souring and aroma formation in different types of sour doughs. Also the technological aspects of sour dough preparation are covered.

The following publication has appeared since the last communication. The abstract of report has been given in Yeast Newsletter 29(1980): 2, 66.

John Londesborough and Tiina-Maaria Lukkari 1980. The pH and temperature dependence of the activity of the high K_m cyclic nucleotide phosphodiesterase of bakers' yeast. The Journal of Biological Chemistry 255: 9262-9267.

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

We have shown that the yeast Pachysolen tannophilus can ferment the pentose, D-xylose. In our initial publication we showed that ethanol concentrations as high as 0.53% w/v could be obtained from a 2% solution of D-xylose. Allowing access of air to the medium increased the rate of ethanol production over that in sealed tubes. Subsequent work has shown that the ability of yeast to ferment D-xylose under the "semi-aerobic" conditions employed is probably common, since it occurred in yeast species belonging to six out of seven genera tested. D-xylose is thought to be metabolised to D-xylulose 5-phosphate (via xylitol), then to glucose 6-phosphate (via pentose cycle enzymes), after which the normal glycolytic pathway is followed.
Publication: Schneider, H., P.Y. Wang, Y.K. Chan and R. Maleszka. 1981. Conversion of D-xylose into ethanol by the

yeast Pachysolen tannophilus. Biotechnol. Letters 3:89-92.

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

The following is the summary of a paper accepted for publication in WEIN-WISSENSCHAFT (GFR) in 1981.

E. Minarik. Ecology of yeasts and yeast-like microorganisms in bottled wines. (In German).

The occurrence of Saccharomyces bailii var. bailii in bulk wine is very rare. Microbiological examination of bottled shelf wines in two wine regions of Czechoslovakia showed the dominance of this osmophilic, fructophilic and chemoresistant yeast. As the usual source of contamination in wineries concentrated grape juice as well as different devices of the filling line may be considered. The importance of efficient methods in wine handling and sanitary conditions in the winery and especially in the bottling center is emphasized.

* * *

The following are summaries of two papers accepted for publication in Kvasny prumysl (Fermentaion Industry) Prague in 1981:

E. Minarik. The yeast flora of bottled wines indicating biological stability of finished wine products (In Slovak).

Saccharomyces bailii var. bailii was found to be the dominant component of the microflora of sweet bottled table wines. Torulopsis sp. belonging to air contaminants of bottling centers may be regarded a harmless accompanying yeast of wines. The importance of effective preventative measures to achieve wine stability is described.

* * *

E. Minarik. Reasons, originators and prevention of film-formation on wines.

Results of long-term investigation on the ecology of film formation by film-forming yeasts in Czechoslovak wines are given. The asporogenous Candida vini and C. zeylanoides and the sporogenous Pichia membranaefaciens belong to the most widespread originators of film-formation on wines. They may be accompanied by C. krusei, Hansenula anomala var. anomala and Pichia fermentans. The most important characteristics of the yeast species are described and technological conclusions connected with the prevention of film-formation on wines are

drawn.

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

Below follow abstracts of recent papers from our laboratory. Participating laboratories in this research are.

Centre Technique Experimental de L'Institut Technique de la Vigne et du Vin, 46 bis Rue des Hauts Paves - BP 1141, 44024 Nantes Cedex (France).

Centre Technique Experimental de L'Institut Technique de la Vigne et du Vin, 12, Rue E. Pallu, 37033 Tours Cedex (France).

A. Poulard, L. Simon et C. Cuinier 1980. Variabilité de la microflore levurienne de quelques terroirs viticoles du Pays nantais. Conn. Vigne. Vin. 4: 219-238.

ABSTRACT

The authors studied fermentative and oxydative microorganisms of soil and of grape musts in different soils of the Nantes region. They showed that if the microorganisms are quantitatively and qualitatively homogeneous with a given type of soil, to the contrary there exists a certain heterogeneity among the microorganisms of different soils. The authors confirm the role of sulphiting on the microorganisms of grape musts.

* * *

L. Simon 1981. Scanning electron microscopic study of the early morphological stages of development of Aureobasidium pullulans in culture. Beitr. Biol. Pflanzen, 55: 273-283.

ABSTRACT

S.E.M. observations of the early development of the colonies in a particular pleiomorphic species of Hyphomycetes, Aureobasidium pullulans (de Bary) Arnaud, have been carried out. Six hours after inoculation only new blastospores are present in the culture that may be themselves conidiogenous. Chlamydospores germination is obtained by a minimal seven hour incubation and involve the mechanical disrupture of the outer melanized sporal layer. A fourteen-hour culture is necessary to detect both conidial and promycelial forms these latter become rapidly fertile (17 to 20 h), first on their proximal cell, then on intercalary cells. Sterigmata formation is never observed. The exogenous polyblastic conidiogenesis of

the blastospores, chlamydospores and hyphae is distinctively asynchronous. Early budding may partially explain the rapid colonization of numerous substrata by this saprophytic ubiquitous fungus.

* * *

A. Poulard et L. Simon. La microflore levurienne du Vignoble nantais. Cryptogamie. Mycologie (in press).

ABSTRACT

748 yeast strains representing 14 genera and 40 species have been isolated during the grape harvests of 1976 and 1977, from soil, inflorescences, black and white juices, and musts, in the main zones of the Nantes regional vineyards; some of these species are unknown or little known in viticultural ecosystems: Trichosporon penicillatum, Exophiala jeanselmei, Aureobasidium pullulans, Endomycopsis vini, Pichia rhodanensis, Pichia vini, Rhodotorula pilimanae, Torulopsis gropengiesseri, Trichosporon cutaneum.

The genus Saccharomyces was found predominant. Several species were observed to appear successively during the fermentation: Metschnikowia pulcherrima, Kloeckera apiculata, Saccharomyces ellipsoideus, Saccharomyces bayanus. Such a sequence is generally described in northern limits of the grapevine culture of european countries.

During the warm and dry summer of 1976, the fermentative species were more numerous in the juices and musts, but 1977, a cool and moist year, promoted oxydative yeast colonization.

XXXVIII. University of Strathclyde, Department of Applied Microbiology, Royal College Building, 204 George Street, Glasgow G1 1XW, Scotland. Communicated by John R. Johnston.

Dr. H.P. (Jim) Reader was awarded the Ph.D. degree during 1980 for a thesis entitled, "Genetic analysis of flocculation in Saccharomyces cerevisiae". He is now working in Oporto, Portugal, in the wine industry (John Harvey & Sons, Limited). A summary of the Ph.D. thesis is as follows:

1. Hybridisation and genetic analysis of flocculation has been carried out with strains of Saccharomyces cerevisiae of brewing and laboratory origin.

2. Isolation of ascospores from flocculent brewing yeasts gave a range of mating strains. Crosses of these maters with haploids of laboratory origin gave hybrids with spore viabilities ranging from 0-63%. Two brewing strains (AB80, NCYC 1055) produced maters giving hybrids with greatly improved spore viabilities. Tetrad analysis of these hybrids

aneuploid, and carried genes for homothallism. Heterothallic spore cultures from the hybrids were themselves crossed with haploids and spore viabilities of the subsequent hybrids ranged from 1.5% to 59%. These second-generation hybrids were not apparently diploid.

3. Hybrids derived from AB80 and NCYC 1055 showed fermentation rates and flocculation characterisitcs which were inferior, comparable or superior to those of the parental brewing strains. Two segregants of AB80 showed improvements in stability of flocculence and respiratory sufficiency.

4. The genes conferring flocculation, previously referred to as FL01, FL02 and FL04 are located about 37 cM from ade1 on the right arm of chromosome I, and probably constitute a single locus, to be known as FL01.

5. An apparently recessive independent flocculation gene (floX) is expressed in some diploid heterozygotes but not in others. This gene is apparently located on chromosome I and may be allelic with FL01, but is subject to complex control of expression. Many haploids and diploids carrying floX do not flocculate.

6. Hybridisation of a strain carrying the flocculation gene FL05 with a strain carrying the dominant mnn4 mutation indicated that cell wall mannosylphosphate groups played little part in flocculation conferred by this gene.

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

The following are abstracts of two recent papers from this laboratory.

Construction of a Hybrid Brewing Strain of Saccharomyces carlsbergensis By Mating of Meiotic Segregants

by
Claes Gjermansen

Department of Physiology, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby

and

Poul Sigsgaard

Department of Brewing Chemistry,
Carlsberg Research Laboratory,
Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby

Carlsberg Res. Commun. 46: 1-11, 1981

A brewing strain of Saccharomyces carlsbergensis yielded upon sporulation 1-, 2- and 3-spored asci. The spore viability was low, but a number of clones could be derived from the spores. Among these a- and α-maters were identified revealing that the parental brewing strain is heterozygous for the two mating type alleles. A number of lines with opposite mating type were crossed pairwise. One of the hybrids produced by mating of two presumably haploid or near haploid clones was equal to the parental strain in brewing performance on a pilot scale. The separation of the nuclear genomes of brewer's yeast into individual cells and their successful mating into hybrid strains opens the possibility to introduce genetic alterations in brewing strains by mutagenesis and genetic transformation of the haploid lines.

* * *

Genetic Differences Between Saccharomyces carlsbergensis and S. cerevisiae Analysis of Chromosome III by Single Chromosome Transfer

by

Torsten Nilsson-Tillgren

Institute of Genetics, University of Copenhagen
Øster Farimagsgade 2A, DK-1353 Copenhagen K

and

Claes Gjermansen, Morten C. Kiellad-Brandt,
Jens G. Litske Petersen and Steen Holmberg
Department of Physiology, Carlsberg Laboratory
Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby

Tetrad analysis of most Saccharomyces strains used in beer production is impossible due to a low yield of viable spores. The present paper describes the use of single chromosome transfer in the genetic analysis of a brewer's yeast.

The technique employs the kar1 mutation, which reduces karyogamy after conjugation. In rare cases kar1 x KAR crosses yield progeny resulting from the transfer of one chromosome or a limited number of chromosomes from a nucleus of one parent to one of the other. S. cerevisiae strains with an extra S. carlsbergensis chromosome III have thus been isolated from

crosses between spore-derived clones of the brewing strain and haploid karl S. cerevisiae strains carrying several auxotrophic markers. When the disomics were crossed to other haploid S. cerevisiae strains a normal spore viability was obtained, allowing tetrad analysis.

High functional homology was found between the transferred S. carlsbergensis chromosome and chromosome III of S. cerevisiae. All genes essential for viability on the latter are represented on the former as are also HIS4, LEU2, MAT and THR4. Despite the functional homology, the transferred chromosome had a structure that was substantially different from that of standard S. cerevisiae strains. It did not recombine with S. cerevisiae chromosomes III, except in a certain region, where recombination was normal. Furthermore, restriction endonuclease analysis showed that the variant chromosome has a nucleotide sequence in the HIS4 region different from that of S. cerevisiae. The S. carlsbergensis brewing strain is heterozygous for this sequence variation, containing also a HIS4 region with a sequence identical or close to that of S. cerevisiae.

XL. Pabst Brewing Company, Brewing Research & Development, Milwaukee, Wisconsin 53201.
Communicated by Michael F. Waxman.

M.F. Waxman. Mating type interconversions in the brewery yeast Saccharomyces cerevisiae. To be presented at the Joint Annual Meeting of the Genetics Society of America and the Gentics Society of Canada, June 15-17, 1981, at North Carolina State University, Raleigh, N.C.

ABSTRACT

Mating type interconversions in the brewery yeast Saccharomyces cerevisiae. Genetic analysis of brewer's yeast has been encumbered by the organism's inability to mate, low frequency of sporulation and low spore viability - all of which are assumed to be caused by its polyploid or aneuploid genomic constitution. In this study we describe the existence of the homothallic gene in brewery yeast, the timing of the initial switching event and the isolation and characterization of mutants that are incapable of switching mating types. Back crosses to tester strains have indicated that the HO gene is intact in these mutants and that a second genetic alteration has effectively "locked" these strains into the α mating type. We propose that the phenomenon of homothallism (or mating type interconversion) is also responsible for the impedance in the genetic analysis of brewer's yeast. We also propose that homothallism has evolved in brewery yeast by a process of "natural" selection to insure both genetic homogeneity and its consequence, beer flavor consistency.

XLI. Faculty of Science, Charles University, Prague, Czechoslovakia. Communicated by Olga Bendova.

Below follows the summary of a dissertation (DrSc. 1979) from our Department.

Typification of yeast strains Saccharomyces carlsbergensis Hansen

The knowledge of strain properties is of great importance for the selection of suitable brewer's yeast.

For this purpose results of strain typification are used, the subject of which is their evaluation according to the features followed.

From the theoretical point of view it is possible to evaluate brewer's strains on the basis of their different morphological, physiological and biochemical characters.

Among the morphological characters the cell size, the length-width ratios and their correlation coefficients are significant for brewer's yeast typification.

Among the physiological and biochemical characters the estimation of fermentation, floculation and sedimentation rate of yeast strains is of greatest importance. Further suitable characters to be followed are the growth rate, the ethanol tolerance and their osmophilic properties. The other characters used for yeast typification, i.e., ratio surface/volume, appearance of giant colonies, sporulation, growth factors requirements and the qualitative evaluation of maltotriose utilisation are variable to a very small degree and therefore of little importance for brewer's strains typification.

From the practical point of view the strains should be evaluated according to their ability to ferment the wort, the sedimentation rate, the rigidity of yeast sediment, according to the yeast crop and the influence on beer flavour, including the production of volatile compounds.

The typification of yeast strains on the combination of laboratory, semi-pilot plant, pilot plant and large scale trials guarantees the best possible choice of strains for practical use.

This conclusion was confirmed by evaluation of the quality for beers resulting from large scale trials in which strains were used and applied according to the proposed typification scheme.

* * *

Bendova, Olga - Charles University, Prague, Faculty of Science.

Kurzova, Vera - Research Institute of Brewing and Malting, Prague (Kvasny prumysl (in press)

Autolysis of brewer's yeast strains

Unsuitable technological beer production conditions and brewer's yeast may result in yeast autolysis which represents a serious problem for the beer quality concerning its flavour and biological stability. The article deals with the evaluation of various brewer's strains from the mentioned point of view. Some differences in autolysis rate measured by amino-acid nitrogen concentration and by yeast proteases activity were estimated among the strains tested.

Further experiments are in progress and their results will be published later on.

XLII. Brief News Items

1. The following contribution from our Laboratory occurs in Biology and Activities of Yeasts (F.A. Skinner, S.M. Passmore, and R.R. Davenport, eds.). Society for Applied Bacteriology Symposium Series No. 9. Academic Press London. N.J.W. Kreger-van Rij. 1980. Generic Differentiation in Yeasts, p. 29-52.

N.J.W. Kreger-van Rij
Laboratorium voor
Medische Microbiologie
R.U., Oostersingel 59
9713 EZ Groningen, the
Netherlands

2. The following recent papers have been published from our yeast research projects.

1. John H. Zwolshen and J.K. Bhattacharjee "Genetic and Biochemical Properties of Thialysine-resistant Mutants of S. cerevisiae" J. Gen. Microbiol. 122, 281-287 (1981).

2. James J. Foy and J.K. Bhattacharjee "Concentration of Metabolites and the Regulation of PFKase and FBPase in S. cerevisiae" Arch. Microbiol. 129 (in press) 1981.

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

3. I have moved from the School of Biological Sciences, University of Kentucky, Lexington, KY 40506 to: Department of Medicine, Box 15, Div. of Infectious Diseases, Harbor-UCLA Medical Center, Torrance, California 90509, where I hold the title of Associate Research Mycologist.

Marjory Crandall, Ph.D.

4. Professor A.H. Rose paid a visit to Hungary (4-15, May 1981) and delivered two lectures. a) The role of lipids in the yeast cell membrane, and b) On the ethanol tolerance of yeasts. Recent article: Lukacsovocs F., Hajnal E., Deak T.: Examination of the stability of wines bottled of lower alcohol content (in Hungarian), Borgazdasag, 29:30-33, 1981. A recent trend in this country is to decrease the alcohol concentration of wines to 9-11 vol %. This, however, caused some troubles with the shelf-life of bottled wines. The main factor causing turbidity and/or sediment proved to be a yeast identified as Zygosaccharomyces bailii. To overcome this difficulty more rigorous hygienic measures were suggested.

T. Deak, Budapest, Hungary

5. Department of Microbiology, Escuela Tec. Sup. de Ingenieros Agr., Univ. Politecnica, Ciudad Universitaria, Madrid 3, Spain.
The yeast collection (Microbiology Collection Yeast Cultures, MCYC) of this Department includes some 1,500 strains of yeasts other than pathogens. A catalogue which includes 638 cultures has been recently published.

J. Santa Maria

6. We have recently formalised our personnel working with yeast into a Yeast Technology Group. The group at present comprises 14 workers, led by Dr. D.R. Berry (Convener), Dr. E.A. Berry and myself. Our interests range within yeast physiology, genetics, biochemistry and yeast viruses, but our emphasis is upon applications of use or potential use to the Yeast Industries.
Presently, these include the whisky, beer and wine fermentations, food yeast and fermenter design, but we are beginning to consider yeast as a host for other products, including the use of recombinant DNA technology.
We would welcome increased contact with other yeast groups, particularly those with an emphasis upon

applied aspects. Any visitors to our laboratories and Department would also be made most welcome.

J.R. Johnston
Department of Applied Microbiology, Royal College Building, 204 George Street, Glasgow G1 1XW, Scotland

7. I have moved to the new Applied Molecular Biology Group at the University of Manchester Institute of Science and Technology from the University of Kent. Rick Ludwig has joined me here as a Post-doctoral Fellow, having completed his Ph.D. research in Cal McLaughlin's laboratory in the University of California at Irvine. Stephen Brown has completed his Ph.D. research in my laboratory and has moved to a Post-doctoral position in Professor Armin Fiechter's group at ETH-Honggerberg, Zurich, Switzerland.

The following two papers were recently published by my laboratory:

S.W. Brown, S.G. Oliver, D.E.F. Harrison and R.C. Righelato. 1981. Ethanol inhibition of yeast growth and fermentation: Differences in the magnitude and complexity of the effect. Eur. J. Appl. Microbiol. Biotechnol. 11:151-155.

S.G. Oliver. 1981. The coordination of transcription with translation in yeast. In 'The Fungal Nucleus' (Eds. K. Gull and S.G. Oliver) pp. 315-330, Cambridge University Press.

Steve Oliver, Department of Biochemistry, UMIST, PO Box 88, Manchester M60 1QD, England.

XLIII. Obituary

1. Øistein Strømnaes (1914-1980)

A typically Norwegian biologist, who had been representing his country in yeast research, passed away on July 21, 1980, after his life devoted to life science and to his fatherland. He was Professor of General Genetics at the University of Oslo for many years, and carried out active research work on yeasts, particularly the gene conversion and mitotic recombination in Saccharomyces cerevisiae.

His career in biology was seriously distorted by the notoriously known historical event, occupation of Norway by the Nazi Germans (1940-45). Along with his work on the surface as a young biologist, he fiercely and calmly led a military intelligence line, code-named XU, for the underground resistance movement (Norwegian Home Front). He willingly risked his life for liberation of his country.

He returned to science after the end of the war and renewed his study during his sojourn in the University of California at Berkeley. He efficiently absorbed new developments in yeast genetics there to proceed further on his own later in his home school of Oslo. He published many articles in this field in domestic and international journals. In the 1970's, he did much work for environment protection in Norway and her neighboring countries.

His old friends say that he used to relax and refresh himself by playing magic in times of serious difficulty. This trend of intelectual curiosity led him to start studying Chinese at the age of 63. He made pretty good progress until he fell fatally ill.

He was a man of respectable personality with scientific enthusiasm, courage, honesty, love of family life and warm hospitality. His entire life of 66 years may be depicted in my Oriental appreciation as a subtly shaped ellipsoid polished out of a gem of jade, one focus being the life science, and the other, Norwegian patriotism. In the physical appearance, he quite resembles Ignace Jan Paderewski (1860-1941), renowned pianist-statesman of Poland. Øistein may have had a somewhat similar genotype, on which science was superimposed.

Susumu Nagai, March 19, 1981, Nara, Japan

2. With deep sorrow, I inform you of the death of Dr. Masaya Hayashibe, former Professor of our Laboratories. He had moved to the Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558, Japan, on October 1974.

He died in August 1978, at the age of 59 years.

S. Katohda
Department of
Agricultural Chemistry,
Faculty of Agriculture,
Yamagata University,
Wakabacho, Tsuruoka 997,
Japan

XLIV. Meetings and Specialized Courses

1. A Trilateral Conference on Yeasts will be held in Budapest, 27-31 July, with participants from Czechoslovakia, East-Germany and Hungary. Topics will cover both the basic and the applied fields of yeast research. The Organizing Committee: Dr. T. Deak (Chairman), Dr. L. Ferenczy, Dr. E.K. Novak.

For further information contact:

Dr. T. Deak
Department of Microbiology
University of Horticulture
H-1118 Budapest, Somloi ut 14-16, Hungary

2. "The VIth International Symposium on Yeasts will be held at the University of Bath, England, from 18-21 September, 1984. Professor A.H. Rose will be the Chairman of the Organizing Committee. Further details will be announced as they become available".

3. We are organizing an international course on Biochemistry and Genetics of yeasts next year (1982) from July 5th to July 24th. Detailed information will be supplied on request after September 1981.

C. Gancedo
Instituto de Enzimologia
y Patologia Molecular del
CSIC. Facultad de
Medicina, Universidad
Autonoma. Arzobispo
Morcillo s/n. Madrid-34,
Spain.

4. International Training Course on "Taxonomy and Identification of Yeasts"

The Gulbenkian Institute of Science, Oeiras, Portugal will offer in 1982 (20 September - 15 October) an intensive international training course on yeasts. The course should be of special interest to research workers who wish to become acquainted with the state of modern yeast taxonomy and in addition want to receive practical training in the identification of yeast isolates.

The course will be based on the forthcoming 3rd edition of "The Yeasts" (N.J.W. Kreger-van Rij, editor) and will be taught by the following yeast taxonomists: Dr. N.J.W. Kreger-van Rij (Univ. of Groningen, the Netherlands), Dr. C.P. Kurtzman (NRRC, Peoria, Illinois), Dr. Sally A. Meyer (Georgia State Univ., Atlanta) and Professor Jack Fell (Univ. of Miami, Florida).

Requests for information should be directed to:

Prof. N. van Uden
Laboratory of
Microbiology, Gulbenkian
Institute of Science,
2781 Oeiras Codex,
Portugal.

5. Eleventh International Conference of Yeast Genetics and Molecular Biology

Coordinator: H.L. Roman, United States

Secretary: R.C. von Borstel, Canada

Finance Committee:

E.A. Bevan, Great Britain	C. Carter, Ireland
S. Fogel, United States	R.H. Haynes, Canada
F. Kaudewitz, Germany (BRD)	U. Leupold, Switzerland
L. Kovac, Czechoslovakia	G.E. Magni, Italy
R.J. Planta, Netherlands	A. Putrament, Poland
G. Simchen, Israel	P.O. Slonimski, France
T. Takahashi, Japan	J.M. Wiame, Belgium

It is very likely that the XI International Conference of Yeast Genetics and Molecular Biology will be organized by Professor P.P. Slonimski and Professor P. Galzy in Montpellier, France from the 13 to 17th, September 1982.

Definitive information will be forwarded in a few

months to those who indicate their interest by filling and sending the following form to:

XIth International Yeast Genetics Conference
Chaire de Génétique et Microbiologie
ENSAM Place Viala
34060 Montpellier Cedex, France

I am interested in further information concerning the XIth International Conference of Yeast Genetics and Molecular Biology.

NAME (capitals)

ADDRESS (capitals)

COUNTRY

6. Thirteenth Annual Conference of the Yeast Commission of the Czechoslovak Microbiological Society, held in the castle Smolenice, 11 to 13th February 1981. Communicated by Dr. A. Kocková-Kratochvilová.

(1) Reviews:

L. Drobnica: Regulation of the glycolysis and respiration in eukaryotic cells (read by E. Sturdik).

L. Kovac: The role of mitochondria in the regulatory mechanisms of yeasts.

A. Kotyk: Transport regulation processes in yeasts.

P. Biely: The regulation of the production and secretion of extra-cellular enzymes in yeasts.

D. Michaljanicova The maltotriose transport into cells of S. cerevisiae and S. uvarum.

V. Vacata: Membrane potentials of various yeasts.

B. Skarka, L. Kovaczova: The production of acids of the Krebs cycle in S. lipolytica cultivated on n-alkanes.

L. Silhankova: Repair processes in yeasts and their importance for mutagenesis.

G. Takaczova: Transport and recombination of mitochondrial genes in S. cerevisiae.

M. Sipiczky (Hungary): Homothallism and heterothallism of yeasts from the view-point of genetics.

A. Maraz (Hungary), J. Subik: The behavior of mitochondrial genes in S. cerevisiae after protoplast fusion of opposite and of the same mating types.

A. Svoboda: Interspecific yeast hybridization.

Y. Gbelska, V. Poliachova, L. Kovac, J. Subik: An S. cerevisiae mutant deficient in phosphatidylserine synthase.

O. Bendova: New trends in the preparation of industrially important yeast strains.

M. Havelkova New knowledge in yeast cytology

M. Hrmova: Morphogenesis of C. albicans and its regulation.

A. Kocková-Kratochvilová: Problems in taxonomy and identification of yeasts.

(2) Reports and posters

M. Sipiczky, J. Kucsera (Hungary): The transfer of nuclear and mitochondrial information in Schizosaccharomyces species.

J. Hasek, E. Streiblova: The structural characteristics of cdc mutant ED 115 of Schizosaccharomyces pombe.

Z. Leskova, E. Dudikova, J. Subik: The induced synthesis of α-glucosidase under the condition of ATP deficiency in S. cerevisiae mitochondria.

M. Havelkova: Extracellular vesicles in protoplasts.

A. Tomsikova: Candida-cidic factor.

Y. Kochova (GDR): Pathogenic yeasts in human environment.

M. Vrsanska: The binding locus of β-xylanase of Cryptococcus albidus.

J. Paca The changes in glucose dissimilation and respiration activity during the starvation of baker's yeast.

E. Minarik, A. Navara: The contaminating yeasts in bottled wines and their technological consequences.

A. Navara, E. Minarik: The importance of yeasts in the production of red wines with regard to the destruction of acids.

F. Malik,E. Minarik, G. Vojtekova, F. Karnis: The propagation of wine yeasts under technological conditions.

J. Cernoch, D. Halama: Metabolites of anaerobic bacteria serving as substrates for yeasts.

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