Y E A S T

A News Letter for Persons Interested in Yeast

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The Editor wishes all readers of the Yeast News Letter a Happy and scientifically rewarding New Year.

I. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852. Communicated by Sally A. Meyer.

The Species Status of Hanseniaspora guilliermondii Pijper

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ABSTRACT

Selected yeasts classified as H. valbyensis were examined for their physiological and morphological properties and their DNA relatedness. The low degree of DNA reassociation with H. valbyensis, the assimilation of keto-2-gluconate, the growth at 37 C, and the formation of four ascospores per ascus, confirmed H. guilliermondii as a distinct species.

A manuscript regarding this topic has been submitted to the International Journal of Systematic Bacteriology.

II. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 20852. Communicated by D. S. King.

The strains listed below have been accessioned to the ATCC since the list that appeared in the June 1976 issue. Complete information for these strains may be obtained on request from the Mycology Department of the ATCC.

Bretannomyces intermedius
ATCC 34076

Candida albicans
ATCC 34133

Candida boidinii
ATCC 32929

Candida guilliermondii
ATCC 34134

Candida krusei
ATCC 34077, ATCC 34136

Candida parapsilosis
ATCC 34078

Candida podzolica
ATCC 34208

Candida pseudotropicalis
ATCC 34137

Hansenula anomala
ATCC 34080

Kloeckera apiculata
ATCC 32856

Kloeckera apiculata var. apis
ATCC 32857

Pichia pseudopolymorpha
ATCC 34023

Pichia strasburgensis
ATCC 34024

Rhodotorula glutinis var. salinaria
ATCC 34295

Rhodotorula sinensis
ATCC 34062

Saccharomyces capensis
ATCC 34081

Candida pulcherrima
ATCC 34079

Candida stellatoidea
ATCC 34138

Candida tropicalis
ATCC 34139

Candida veronae
ATCC 32898

Citeromyces matritensis
ATCC 34087

Cryptococcus albidus var. albidus
ATCC 34140

Cryptococcus albidus var. diffluens
ATCC 32899, ATCC 34141

Cryptococcus laurentii
ATCC 34142

Cryptococcus luteolus
ATCC 34143

Cryptococcus neoformans
ATCC 34144

Cryptococcus terreus
ATCC 34145

Debaryomyces hansenii
ATCC 34022

Saccharomyces cerevisiae
ATCC 34025, ATCC 34026, ATCC 34027, ATCC 34028, ATCC 34029, ATCC 34030, ATCC 34082, ATCC 34182, ATCC 34183, ATCC 34184, ATCC 34185, ATCC 34186, ATCC 34187

Saccharomyces chevalieri
ATCC 32900, ATCC 34083

Saccharomyces italicus
ATCC 34084

Saccharomyces ludwigii
ATCC 34085

Saccharomyces rosei
ATCC 34086

Saccharomyces rouxii
ATCC 32901

Saccharomycopsis lipolytica
ATCC 32935, ATCC 34017, ATCC 34018, ATCC 34088

Torula jeanselmei
ATCC 34123

Torulopsis glabrata
ATCC 32936, ATCC 34147

Trichosporon beigelii
ATCC 32902, ATCC 32967, ATCC 34148, ATCC 34181

Trichosporon penicillatum
ATCC 32968

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

Sporulation of the culture designated as Selenotila intestinalis Krassilnikov was achieved. On the basis of mode of ascus formation and ascospore morphology it is included in the genus Metschnikowia as new species M. lunata Golubev. The description of the latter is in preparation.

The following articles have recently been published:

Bab'eva, I. P., Guzeva, I. S., Dlusski, G. M., Golubev, W. I. 1975. Association of yeasts and ants in the forest biogeocenosis. In "Regularities of the Development of Soil Microorganisms", Leningrad, 16-25 (in Russian).

The yeast flora of ant-hills was investigated during three years. Two species of yeasts - Debaryomyces cantarellii and Deb. formicarius are the typical ectosymbionts of red forest ants. The maximum of growth of these yeasts coincides with the maximum of insect activity under conditions of low moisture and high stable temperature level.

Bab'eva, I. P., Golubev, W. I., Reshetova, I. S., Azieva, E. E., Blagodatskaja, V. M. 1976. Yeasts in high altitude regions of northern and southern hemispheres. Vest. MGU (ser. biol., soil sci.), N 2, 81-87 (in Russian).

In the soil and herb samples of high altitude regions of northern and southern hemispheres there are similar groups of yeast species among which cryptococci are dominant. The highest number of yeasts occurred on plates incubated at low temperatures (4-5 C).

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

1). We have so far reviewed ca. 1000 strains of our wine yeast collection. They were originally included in the following species:

saccharomyces cerevisiae	(600 strains)
Saccharomyces rouxii	(20 strains)
Saccharomyces uvarum	(16 strains)
Saccharomyces exiguus	(14 strains)
Torulaspora rosei	(128 strains)
Saccharomyces pastorianus	(49 strains)
Saccharomyces florentinus	(11 strains)
Saccharomyces oviformis	(129 strains)

The original taxonomic designation of ca. 20% of the strains of each species appeared to be incorrect. Since the majority of these yeast cultures were isolated and studied before the appearance of the first monograph by Lodder and Kreger-van Rij (1952) on yeast taxonomy, the cause for the mentioned mistakes is probably the fact that only the fermentation of five sugars was then taken into consideration. An additional source of errors could also be the difficulty in interpreting the results of raffinose fermentation.

2). Our laboratory participates in a multidisciplinary finalized research project on "Non-conventional sources of protein", supported by the Italian Council for Research (CNR). We have been given the task of preparing synchronized cultures of several yeast species actually used in SCP production. Other laboratories will analyze the chemical composition of these cells. Amounts of 50 to 100 grams (dry) are required for the analysis. Suggestions are welcome!

3). Below follow summaries of recently completed publications:

La flora blastomicetica dell'orecchio esterno dell'uomo. (Yeast flora of the external ear of man). A. Martini, G. Croce and L. Tuttobello. In press, Boll. Soc. It. Biol. Sper., 1976.

Summary: 22 normal ears, 9 with wax plugs and 16 characterized by various pathological conditions were studied. Yeast could be found only in 4 samples, namely one wax plug, one from an ear treated with antibiotics for a boil, one characterized by an eczematous dermatitis and one by an otomycosis by Aspergillus niger. Isolated cultures were found to belong to the species Candida robusta, Candida rhagii, Candida parapsilosis and Candida guilliermondi var. guilliermondii. A strange finding, which needs further confirmation, is the fact that only female subjects appeared to host yeasts in their ears.

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Osservazioni preliminari sulla effettivita di alcune tecniche per l'isolamento dei lieviti dal suolo. (Preliminary studies on the effectiveness of some procedures for the isolation of yeasts from soil). A. Martini, F. Federici and Chiara Luce Bibi. In press, Ann. Fac. Agr. Perugia, 1975.

Summary: Three different procedures of isolation were compared in the study of the yeast flora of soil. Obtained results indicate that rougher treatments are required for a complete release of the yeast cells from soil particles.

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I tweens come stimolanti della produzione di una proteinasi extracellulare nel lievito. (Tweens as stimulants of yeast proteinase production). A. Martini and F. Federici. In press, Ann. Fac. Agr. Perugia, 1975.

Summary: The addition of tweens 40 and 60 promotes the secretion into the medium of a proteolytic enzyme in a yeast of the genus Candida growing in shake culture. With tween 60 the yield is almost doubled.

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Gli agenti della fermentazione vinaria del "Cesanese del Piglio". (The yeasts causing the natural fermentation of "Cesanese del Piglio" wine). Rosini G. and Frances Victoria. Riv. Viticol. Enol. Conegliano, 28, 55-67, 1975.

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Applicazione della macerazione carbonica alla produzione del vino "Rosso Piceno" - Risultati di un biennio di esperienze: aspetti microbiologici. (Use of fermentation under anaerobic conditions in "Rosso Piceno" wine production: microbiological aspects. G. Rosini and Fantozzi P. Vini d'Italia, 17, 515-522, 1975.

V. Department of Biology, Faculty of Medicine, J. E. Purkyně University, Brno, Czechoslovakia. Communicated by Marie Kopecka.

The following are recent papers from this laboratory:

1. Nečas O. and Svoboda A.: Regeneration of yeast protoplasts. A freeze-etching study. Ztschr. Allg. Mikrobiologie 16, 615-625, 1976.

2. Gabriel M.: Formation and growth of spheroplasts of the blue-green alga Anacystis nidulans. Folia microbiol. 21, 195, 1976.

3. Gabriel M.; Havelková M., Kopecká M., Svoboda A.: Cell wall regeneration in protoplasts as a model for biogenesis of cell organelles. Proc. Ist Scient. Conf. Fac. Med., Acta Fac. Med. Univ. Brno 1976 (in press).

4. Svoboda A. and Kevei F.: Ultrastructure of a Dimorphic Yeast Trigonopsis variabilis studied by Freeze-etching. Folia microbiol. 20, 56-57, 1975.

5. Svoboda A.: Conjugation of yeast protoplasts. Arch. Microbiol. (in press).

6. Kopecká M.: The isolation of protoplasts of the fission yeast Schizosaccharomyces by Trichoderma viride and snail enzymes. Folia microbiol. 20, 273-276, 1975.

7. Kopecká M., Gabriel M. and Nečas O.: Isolation of nucleus-free yeast protoplasts and their inability to synthesize the glucan fibrils of the cell wall. Folia microbiol. 20, 56, 1975.

8. Kopecká M. and Horák J.: The effect of the antibiotic lomofungin on yeast cells and on synthesis of wall components of protoplasts. Folia microbiol. 21, 3, 194, 1976.

9. Kopecká M.: The use of antibiotic lomofungin for demonstration of nuclei in living yeast cells and protoplasts. Folia microbiol. 21, 406-408, 1976.

10. Kreger D. R. and Kopecká M.: On the nature and formation of the fibrillar nets produced by protoplasts of Saccharomyces cerevisiae in liquid media. An electronmicroscopic, X-ray diffraction and chemical study. J. Gen. Microbiol. 92, 207-220, 1975.

VI. Department of Biochemistry, The Royal Dental Highschool DK 8000 Aarhus, Denmark. Communicated by Sven Darling.

Polyethylene Glycol-Induced Reversion of Yeast Protoplasts in Liquid Medium

The following is a summary of a recent work done in this laboratory. A manuscript for a paper is prepared and will soon be submitted for publication.

Protoplasts of Hansenula anomala and Saccharomyces cerevisiae were prepared by digestion with snail enzyme. The resulting protoplast suspension always contained some viable cells (0.5-2%). These were removed from the protoplasts on an isotonic, linear metrizamide density gradient. Ten ml PEG medium (peptone, yeast extract, glucose in 0.6 M KCl containing 30% (W/V) polyethylene glycol 6000) was inoculated with 0.2 ml purified protoplast suspension and incubated at 30°C. (Initial cell number: $N = 4 \times 10^6$ cells per ml PEG medium). At suitable times samples were withdrawn for phase contrast microscopy. After one hour growth started. After 12-16 hours the cells were osmotically stable, and cell division took place, resulting in normal cells.

The reversion of a single or a few protoplasts was observed in a Thoma counting chamber which was kept at 30°C and protected against evaporation. The observations were documented by micrographs taken at different times.

Agglutination of protoplasts was observed like agglutination of plant protoplasts and protoplasts of filamentous fungi as demonstrated in other laboratories.

Examination of several micrographs gave evidence that fusion of protoplasts occurred. Whether the progeny are normal cells was not investigated.

Experiments are planned to find the optimum condition of fusion.

VII. Laboratory of Applied Microbiology, Kochi University, Nankoku, Kochi, Japan. Communicated by Susumu Nagasaki.

The following is a report of our recent work on yeast or related subjects.

1. Published; Purification and properties of lytic β-1,3-glucanase from Flavobacterium dormitator var. glucanolyticae. S. Nagasaki, Y. Nishioka, H. Mori and S. Yamamoto, Agr. Biol. Chem., 40, 1059-1067 (1976).

2. In press; Purification and characterization of an exo β-1, 3-glucanase from a Fungi Imperfecti. S. Nagasaki, K. Saito and S. Yamamoto, Agr. Biol. Chem.

Abstract; An exo β-1,3-glucanase has been purified from the culture fluid of a Fungi imperfecti which had been used for the production of an yeast cell lytic enzyme (reports on which have been published previously). The method for the purification of the enzyme involved ammonium sulfate fractionation, column chromatography on DEAE-Sephadex A-50, BioGel P-150 and SE-Sephadex C-50, and affinity chromatography using powdered pachyman prepared from a chinese medicine "Bukuryo" Poria cocos Wolf. The molecular weight of the enzyme was determined to be 43,000 by the molecular sieve method using BioGel P-60. The enzyme removes single glucosyl residues succesively from the non reducing terminus of β-1,3-glucan with inversion of configuration. The enzyme has a comparatively narrow pH optimum with an optimum pH of 6.0 and is most active at 50°C. The Km values of the enzyme for laminarin, laminarihexaose, laminaripentaose, and laminaritetraose are 0.066, 0.83, 2.30, and 18.60 ($\times 10^{-3}$ M), respectively. Some other properties of the enzyme are also described.

3. In preparation; Multiple forms of the lytic glucanase of Flavobacterium dormitator var. glucanolyticae and the properties of the main component enzyme. H. Mori, S. Yamamoto and S. Nagasaki.

VIII. National Research Council, Div. of Biological Sciences, Genetics Section, Ottawa, Canada. K1A 0R6. Communicated by A Nasim.

(1). Division-Delay and Radiation Sensitivity in Yeast. (A. Nasim and M. A. Hannan.)

In an earlier study (Radiation Res., in press) it was demonstrated that, in certain mutants of Schizosaccharomyces pombe, the lack of induced division-delay could account for enhanced sensitivity to ultraviolet irradiation. This observation led us to believe that impaired physiological factors other than repair enzymes/processes may be responsible for radiation sensitivity in some of the mutants. A large number of radiation sensitive mutants have been isolated in the budding yeast, Saccharomyces cerevisiae. While a few of these mutants have been biochemically characterized as being deficient in the excision of pyrimidine dimers or the repair of DNA strand breaks, others have been indirectly assigned to different repair pathways. We are, at present, analyzing all the available mutants for the presence/absence of UV-induced division delay. Preliminary data show that the wild type and the mutants deficient in excision repair exhibit significant division-delay induced by UV while mutants like rad 9 divide rapidly after UV-irradiation. The experiments are in progress to identify a group of mutants lacking the property of induced division-delay in order to correlate it with their relative sensitivity to UV regardless of their proficiency or deficiency in repair enzymes/processes.

(2). Genetic Effects of Antitumor Antibiotics.
(M. A. Hannan and A. Nasim.)

Many antitumor antibiotics are known to interact with (and form lesions in) DNA. Studies are in progress in this laboratory to investigate the effects of such drugs on different genetic parameters such as forward and reverse mutations, gene conversion and mitotic recombination in Saccharomyces cerevisiae. In addition, yeast mutants deficient/defective in different repair pathways are being studied for their cross-sensitivity to these drugs in order to find (i) if such drug-induced lesions in DNA are repairable and (ii) if the drugs can be classified on the basis of the involvement of different repair processes for elucidating the mechanism(s) of their action on DNA. Data obtained with Bleomycin show that the wild type and the excision-deficient strains are equally sensitive to this drug. However, a mutant (rad 52) which is sensitive to ionizing radiations and reported to be deficient in DNA double strand break repair was found to be extremely sensitive to Bleomycin. These results indicate that Bleomycin-induced lesions are not repaired through excision and confirm the earlier suggestions that Bleomycin acts on DNA much like ionizing radiations.

Recent publications from this laboratory:

1. Hannan, M. A. (1975), Isolation and Analysis of Conidiation-Defective Mutants in Aspergillus niger. Molec. Gen. Genet. 142, 333.

2. Hannan, M. A., Duck, P. D. and Nasim, A. (1976) UV-induced Lethal Sectoring and Pure Mutant Clones in Yeast. Mutation Res. 36, 171.

3. Hannan, M. A., Miller, D. R. and Nasim, A. (1976) Changes in UV-inactivation Kinetics and Division Delay in Schizosaccaromyces pombe strains in different growth phases. In Press. Radiation Res.

4. Duck, P. D., Nasim, A. and James, A. P. (1976) A Temperature-Sensitive Mutant of Schizosaccharomyces pombe Exhibiting Enhanced Radiation Sensitivity. In Press. J. Bacteriol.

5. Hannan, M. A. (1976) Stepwise Mutational Improvement of Aspergillus niger for Citric Acid Productivity in Cane Molasses. In Press. Folia Microbiol.

IX. Dept. Molecular Biology and Biochemistry, University of California, Irvine, CA 92717, USA. Communicated by Steve Oliver.

1. Steve Oliver has been appointed to the faculty of the University of Kent at Canterbury. His address from Jan. 1, 1977 will be: The Biological Laboratory, The University, Canterbury, Kent CT2 7NJ, England.

2. The following papers from our laboratory have recently been accepted for publication:

C. N. Gordon and S. G. Elliott: 'Fractionation of yeast cell populations by centrifugal elutriation' (J. Bact., in press).

ABSTRACT

An exponential population of yeast cells has been fractionated by centrifugal elutriation, using water as the elutriating liquid. Evidence that the population had been fractionated according to age in the cell cycle was obtained by examining the fractions for their size distribution, microscopic appearance following Giemsa staining, and their ability to initiate synchronous growth.

S. J. McCready, B. S. Cox and C. S. McLaughlin: 'The extra-chromosomal control of nonsense suppression in yeast: an analysis of the elimination of [psi+] in the presence of a nuclear gene PNM⁻'. (Molec. Gen. Genet., in press).

ABSTRACT

When a [psi-] strain of yeast mutates to [psi+], the efficiency of suppression by certain ochre suppressors is increased. The [psi+], phenotype is inherited extrachromosomally. There is a nuclear gene, PNM, which, when mutant, causes loss of the [psi+] phenotype. PNM- is dominant to PNM+ and a heterozygous diploid gradually loses the ability over successive generations, to produce PNM+ [psi+] spores. This paper describes the kinetics of this elimination and the data obtained are discussed in relation to two models of the molecular nature of the [psi] genetic determinant - one considering the [psi] determinant as an autonomous nucleic acid, the other treating the possibility that the [psi] nucleic acid is that which codes for rRNA in the nuclear genome.

Stephen G. Oliver: 'On the mutability of the yeast mitochondrial genome' (J. Theoret. Biol., in press).

ABSTRACT

A mechanism is proposed to explain how a mutation in a single molecule of mitochondrial DNA (mitDNA) can come to affect all the other mitDNA molecules of a yeast cell. It is suggested that an initial mutation may be 'amplified' by a process which is, in fact, intended to ensure the identity of the cell's complement of mitDNA molecules. It is postulated that this process involves a small number of 'reference' copies of mitDNA to which all other ('derived') copies are compared and corrected once per cell cycle. Asymmetric gene conversion is proposed as the correction mechanism and the means of 'amplifying' mutations. The model is shown to be compatible with current data on spontaneous and induced mitochondrial mutation in Saccharomyces cerevisiae.

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

Our investigations into the problem of genetics of carbon catabolite repression in Saccharomyces cerevisiae have resulted in the following publication: P. Haussmann and F. K. Zimmermann: "The role of mitochondria in carbon catabolite repression in yeast." Acriflavine induced, cytoplasmic respiratory deficient mutants are less sensitive to carbon catabolite repression of maltase and invertase. This is not due to a decreased utilization of sugars. The difference between a wild type ρ^+ and a mutant ρ^- strain after growth on maltose is quite drastic in the case of invertase (20 - 60-fold). This decreased carbon catabolite repression could not be mimicked with KCN (inhibition of respiration), dinitrophenol (inhibition of oxidative phosphorylation), both inhibitors, nor with erythromycin or chloramphenicol (both inhibitors of mitochondrial protein synthesis). It is suggested that the participation of mitochondria in carbon catabolite repression is not due to a mere metabolic effect but to the formation of a factor that does not require mitochondrial protein biosynthesis. To appear in Molec. gen. Genet. 1976.

One recessive mutant called cat1-1 has been obtained. It prevents fermentation of maltose even in the presence of a glucose resistant maltase synthesis; it slows down derepression of maltase synthesis in a constitutive, but still glucose-repressible maltase mutant. No derepression was observed with isocitrate lyase and fructose-1,6-diphosphatase; malate dehydrogenase is derepressible but much slower than in wild type. Two revertants have been obtained in a cat1-1 mutant strain. One mutation was dominant and allelic to cat1-1, it was called CAT1-2^d. It allows for a much faster (3-6 h) derepression of maltase, isocitrate lyase, fructose-1,6-diphosphatase and malate dehydrogenase. Another revertant carried the mutant allele cat2-1 which is recessive but epistatic over cat1-1; otherwise, it has the same properties as CAT1-2^d. Repressed and derepressed levels of those enzymes are the same as in wild type, it is only the time or onset of derepression which is affected by cat2-1 and CAT1-2^d. No additive effects were observed in CAT1-2^d cat2-1 haploids. The following model of carbon catabolite repression is proposed: Carbon catabolite repression regulates enzyme levels in two ways. The direct way is the repression of enzymes, and an indirect and novel way is the

control of the derepression process. Genes CAT1 and CAT2 are involved in this process. The function of CAT1 is to repress CAT2, whereas CAT2, directly or via other genes, blocks derepression. CAT1 is only functional when there is no carbon catabolite repression. Therefore, CAT2 is active and derepression blocked. After alleviation of carbon catabolite repression, CAT1 becomes active, and CAT2 is repressed. A hypothetical gene product of CAT2 is still present in the cell and has to be eliminated in a process that takes about 3-5 h. This leads to a delayed derepression. A cat1-1-mutant produces the CAT2-gene product permanently and therefore, derepression is blocked or slowed down. On the other hand, CAT1-2^d is considered to be a mutant allele the function of which is not affected by carbon catabolite repression, and consequently, the derepression process can start immediately after the alleviation of repression. The same situation in cat2-1 can be explained by the total absence of the CAT2-gene product. At present, we are using strains with CAT1-2^d and cat2-1 to isolate mutants with a broad spectrum of effects on glucose-repressible enzymes. Investigators: K. D. Enzian, P. Haussmann, I. Kaufmann and H. Rasenberger.

The role of mitochondria in carbon catabolite repression (Investigators: P. Haussmann and F. K. Zimmermann). Respiratory deficient, RD, mutants were induced with acriflavine, and shown to be mitochondrially inherited in crosses. Synthesis and induction of maltase was studied in the presence of MAL2. Induced maltase levels were always slightly higher in the RD mutants than in their respiratory competent, RC, ancestors. Maltose fermentation as measured by CO_2 production in a Warburg apparatus was the same or slightly reduced. In another set of haploid strains, MAL2-8^c MAL3 (inducible maltase) SUC3 (glucose repressible invertase), the specific activities of invertase were studied depending on the carbon sources: glucose, maltose, sucrose and raffinose. RD strains always had higher invertase levels than RC types, the highest activities were found in RDs grown on raffinose. The most significant difference between RD and RC was observed with growth on maltose (around 20 fold). This situation was exploited to identify the mitochondrial function involved in the aggravation of carbon catabolite repression. Anaerobic growth or RC cells for 24 h had no effect on invertase synthesis. This was also true for dinitrophenol. A limited release from repression was observed with KCN, but htis did not yield the same invertase levels as found in RDs (around fivefold stimulation). This eliminated a direct metabolic effect of mitochondrial function. Erythromycin and chloramphenicol were used as inhibitors of mitochondrial, macromolecular synthesis. After 24 h on media supplemented with these antibiotics, respiration was almost as low as in RD mutants. No induction of RD mutants was observed. However, invertase levels were as low as in normally maltose-grown RCs with erythromycin and about five times higher with chloramphenicol. From this, it is concluded that mitochondria contribute to the severity of carbon catabolite repression by (a) factor(s) made as long as they are maintained no matter whether metabolically active or not.

The role of cAMP in carbon catabolite repression (Investigators: H. Eckhardt and W. Katz). cAMP was measured using a cAMP binding protein (Buchler, Germany) and radioactive cAMP. No free cAMP could be found in crude extracts by this competition assay. After boiling, cAMP could readily be measured. This suggested that cAMP is present only bound, probably to proteins. A correlation between cAMP levels between and

the degree of carbon catabolite repression, as determined by enzyme determinations of repressible invertase, was not obvious in all strains neither in log phase cells growing on various carbon sources nor during derepression. Therefore, cAMP binding proteins were investigated. Gelfiltration on Sephadex G1-100 separates a high from a low molecular fraction. Further work is directed toward elucidating the role of binding proteins in carbon catabolite repression.

Study of the maltase reaction (Investigators: W. Katz and C. Preuss). It is possible that maltose is not the real inducer of maltase synthesis but only a derivative of it produced by an isomerization or transglycosylation reaction. Uniformly ^{14}C-labeled maltose was used as a substrate for maltase in crude extracts or purified preparations. During the course of the reaction, samples were subjected to thin layer chromatography using a solvent system that separated maltose, maltotriose, trehalose, isomaltose and glucose. Only maltose and glucose spots carried radioactivity and at the end, 99.5% of the input radioactivity was found at the glucose position. This was so even in the presence of glucose-6-phosphate. Maltase from yeast completely hydrolyses maltose to glucose, no transglycosylation could be demonstrated, and no isomerization to isomaltose or trehalose occurred.

Genetic tinker toys (F.K. Zimmermann). To study meiotic nondisjunction with the formation of haploid disomics the following strain was constructed: D9

$$\frac{\text{ade5} \quad + \quad \text{aro2} \quad + \quad \text{cyh2} \quad + \quad \text{leu1}}{+ \quad \text{lys5} \quad + \quad \text{met13} \quad + \quad \text{trp5} \quad +} \text{ 0 } \frac{+ \quad \text{ade3}}{\text{ade6} \quad +} \qquad \frac{\text{ura1}}{+} \text{ 0}$$

Spores can be plated on a minimal medium with uracil and selected for prototrophies for various requirements depending on the presence of additional supplements. Selection for prototrophy for all requirements for markers on chromosome VII gave 13 presumptive disomics which were crossed. Seven crosses yielded enough viable spores for tetrad analysis. Five were disomic for chromosome VII but normally haploid for two more chromosomes tested.

XI. Brooklyn College of the City University of New York, Dept. of Biology, Bedford Avenue and Avenue H, Brooklyn, New York 11210. Communicated by Norman R. Eaton.

James R. Paterniti has completed his doctoral dissertation. A summary of the work, entitled Thiazolidine-4-carboxylic Acid: Properties and Modes of Action in the Yeast Saccharomyces cerevisiae, follows:

Cells of Saccharomyces cerevisiae showed differential growth inhibition when cultured on various carbon sources in the presence of the proline analogue thiazolidine-4-carboxylic acid (TZ). On 0.5% yeast extract, 2% glucose and TZ (10 mg/ml) medium, growth lags from 8-10 hours were observed. After this initial period of inhibition recovery occurred, accompanied by slower rates of growth in exponential phase, and lowered cell titres in stationary phase. This growth inhibition could be largely reversed by the addition of proline to the medium. Similar patterns were observed in TZ-treated cultures using 0.5% acetate or 3% glycerol as the carbon source. By contrast, cell growth was totally inhibited on a medium containing 3% ethanol as the

carbon source. This inhibition was not proline reversible and appeared to have a different basis from that seen on other carbon sources. Indeed, TZ was shown to have two probable modes of action in yeast. It was found to be a non-competitive inhibitor of yeast alcohol dehydrogenase. This inhibition could not be reversed by proline *in vitro*. TZ was also found to be incorporated into protein.

In studies using radiolabeled analogue, it was found that TZ incorporation into protein was greatest during the lag phase of the growth cycle, and declined as cells progressed through the recovery phase. Thus the amount of TZ incorporation into protein could be related to the degree of growth inhibition observed. In an effort to explain the changes in TZ incorporation with the growth cycle, it was discovered that TZ entered the cell by way of a specific proline transport system. Changes in the activity of this system parallelled changes in both inhibition and incorporation patterns. Furthermore, changes in the activity of the proline permease system could occur under conditions of nitrogen starvation. Similar results have been reported in other fungal systems where nitrogen repression appears to control proline uptake (Kuznar, *et al.*, *Biochim. Biophys. Acta*, 318:273 (1973); Arst and MacDonald, *Nature*, 254:26 (1975).

The multidimensional aspects of TZ action were exploited in studies which used the analogue as a probe to select mutants in the proline system of yeast. One class of mutants was selected to be resistant to TZ on glucose and ethanol medium. Genetic and biochemical characterizations of a dominant mutant of this type showed that the mutation probably affected derepression of the proline uptake system.

Using a novel selection technique, mutants which were hypersensitive to TZ on glucose medium were obtained. These mutants were incapable of growing on glucose-TZ medium. Characterization of one such recessive mutant suggested that the affected gene produced an altered proline permease. The relationship between both classes of mutants and the control of proline uptake in fungi was discussed.

The following is a summary of a talk entitled *Control of α-glucoside Utilization in Saccharomyces* given by N. R. Eaton at a symposium on "The Genetics of Eucaryotic Microorganisms" sponsored by the British Microbiology and Genetical Societies and held at Swansea, Wales, in March, 1976:

Studies on the mechanisms regulating utilization of α-glucosides by yeasts have been hampered by the inability to obtain mutants in the structural genes for the hydrolases involved. Comparisons of the rates of thermal inactivation of maltases from temperature sensitive mutants and the parental strains from which they were derived have led various investigators to conclude that all of these maltases have comparable first order decay constants and are probably products of the same, as yet unidentified, structural gene. These studies have usually been done at temperatures of 50° or higher, at which virtually complete inactivation occurs within 8-10 minutes. At lower temperatures, however, where the process can be analyzed more precisely, heat inactivation of maltase preparations shows a marked deviation from the linear relationship expected of inactivation according to first order reaction kinetics. The curve obtained can be resolved

into three linear components, reflecting the presence of at least three distinct maltases, each with its characteristic first order decay constant and each comprising a characteristic fraction of the total maltase of the cell. Five mutants, temperature-sensitive for maltose fermentation, have been found to lack the relatively heat-stable maltase. Temperature sensitivity, therefore, appears to result from the absence of this maltase. The production of all three components is normally coordinately controlled, and it is concluded that the temperature-sensitive strains carry mutations in the structural gene for the missing maltase rather than in a regulatory gene.

XII. Brooklyn College of the City University of New York, Dept. of Biology, Bedford Avenue and Avenue H, Brooklyn, New York 11210. Communicated by Nasim A. Khan.

Below follow the summary of a paper which has been accepted for publication in Molecular General Genetics and a summary of recent results obtained by Mr. Richard Hackel of my laboratory on Genetic Control of Invertase Formation in Yeast.

Effect of the Petite Mutation on Maltose and Alpha-Methylglucoside Fermentation in *Saccharomyces cerevisiae*

Nasim A. Khan and Alan Greener

SUMMARY

Several hundred petite mutants were isolated from yeast strains of different genotype to examine the effect of the petite mutation on maltose and alpha-methylglucoside fermentation. In most cases petite mutants isolated retain the ability to ferment maltose and alpha-methylglucoside, although at a slower rate. In one strain (1403-7A), however, the ability to ferment alpha-methylglucoside is completely lost in all petite mutants isolated from this strain. It is suggested that mitochondrial factors may be involved in the utilization of alpha-methylglucoside in strain 1403-7A.

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Invertase Hyperproducer Mutants in a Yeast Strain Bearing the SUC3 Gene

Richard A. Hackel

Mutants resistant to glucose repression for invertase formation have been isolated in a strain of *Saccharomyces* bearing the SUC2 gene (Montencourt, Kuo and Lampen, 1973. J. Bacteriol. 114:233-238). Since no genetic analyses were performed with these mutants, it was not clear whether the hyperproduction of invertase in these mutants was due to a mutation in the SUC2 gene or some other gene. This problem was reinvestigated in our laboratory using the strain EK-6B, carrying the tightly linked genes SUC3 and MAL3. This strain is unable to utilize raffinose (a β-fructoside like sucrose which can be hydrolyzed by invertase) in the presence of the sugar 2-deoxy-D-glucose (2DG). Mutants able to utilize raffinose in the presence of 2DG normally produce higher levels of invertase when grown in glucose media. Therefore, this was the basis of a selection method for isolating invertase hyper-producers (F. K. Zimmermann, personal communication).

Several mutants able to use raffinose in the presence of 2DG (designated DGR mutants) were isolated. These mutants produce up to forty-fold more invertase when grown in YEP + 2% glucose (YEPD) compared with the parental strain EK-6B. Two such stable DGR mutants were selected for further biochemical and genetic studies and the results are summarized below:

(1) DGR mutants were crossed to a segregational sucrose and maltose nonfermenter (Z1-2D). Diploids isolated from these crosses had the same levels of invertase as the parental strain EK-6B.

(2) DGR mutants were crossed to a SUC3 mal0 strain (R.S. 5-1) and diploids were isolated. These diploids had the same levels of invertase as the diploid EK-6B x R.S. 5-1. The mutation responsible for hyperproduction of invertase in both DGR mutants (DGR2 and DGR3) was unlinked to the SUC3 gene on the basis of tetrad analysis.

(3) Both DGR mutants were found to be allelic to one another. The same gene was affected in each mutant.

(4) Invertases produced by these DGR mutants were indistinguishable from invertase of the parental strain EK-6B, with respect to Km for sucrose and heat inactivation at 65°C.

(5) These DGR mutants had 2-5 fold more alpha-glucosidase when grown on YEPD compared with the parental strain EK-6B.

Therefore, on the basis of the above studies, we conclude that the gene responsible for hyperproduction of invertase in these DGR mutants is a regulatory gene which affects the level of invertase and alpha-glucosidase formation.

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

Below follow abstracts of five recent papers.

1. K. Kobayashi and T. Ito: Further invivo studies on the participation of singlet oxygen in the photodynamic inactivation and induction of genetic changes in Saccharomyces cerevisiae. Photochem. Photobiol., 23, 21-28 (1976).

SUMMARY

In vivo participation of singlet excited oxygen ($^1O_2$, $^1\Delta_g$) in the photodynamic inactivation and induction of genetic changes (gene conversion) in acridine orange-sensitized yeast cells was investigated by using N_3^-, an efficient $^1O_2$ quencher, and D_2O, a known agent for the enhancement of the lifetime of $^1O_2$. The addition of N_3^- protected the cells from both photodynamic actions. From an analysis of the concentration-dependent protection, about 80% of the induction of the genetic change is explainable

on the basis of $^1O_2$ mechanism. The quantitative estimation of the N_3^- protection in the inactivation was not possible because of the sigmoidal nature of the inactivation curve. The replacement of H_2O with D_2O during illumination was effective in enhancing the photodynamic inactivation but almost completely ineffective for the gene conversion induction. The deuterium effect with the cell system was clearly not as large as would be expected from *in vitro* experiments. This, however, could be explained from the kinetic consideration that natural quenchers of $^1O_2$ in the cell would mask the deuterium effect. By experiments with different cell stages it was demonstrated that these two modifying effects were dependent on the intracellular reaction environment. The conclusion is that $^1O_2$ must be the major intermediate responsible for the photodynamic actions in acridine orange-sensitized yeast cells.

2. T. Ito: Toluidine blue: The mode of photodynamic action in yeast cells. Photochem. Photobiol., (1976) (in the press).

SUMMARY

Toluidine blue, a thiazine dye, was shown to have *in vivo* photodynamic activity through singlet oxygen (O_2, $^1\Delta_g$) production. This was based mainly on the effective protection by N_3^- and the marked enhancement in D_2O for the sensitized inactivation of yeast cells. The mode of the *in vivo* activity was, however, quite different from that of acridine orange, for which the singlet oxygen mechanism has also been proposed. The most characteristic feature in the toluidine blue-sensitization was the total lack of the induction of gene conversion (at *trp* 5), while the survival went down below 10%. The non-induction of genetic changes was confirmed at several pH's in the neutral region, whereas the inactivation was seen in parallel to the reported pH dependence of singlet oxygen production *in vitro*. Direct measurements by microspectrophotometry showed none of the toluidine blue was accumulated in the cell. It was also ascertained from acridine-sensitized induction of gene conversion that toluidine blue never interfered with the binding of acridine orange to cellular DNA. These findings suggested that the unique mode of photodynamic activity of toluidine blue is attributable to its action from outside of the cell. Furthermore, comparisons between the photodynamically treated cells (with toluidine blue) and nontreated cells with respect to the response to UV irradiation excluded certain cell functions relating to the expression of gene conversion from the possible damage sites.

The photoreactivation process of UV- induced gene conversion was not disturbed by the pre-toluidine blue sensitition. In view of the foregoing results, the plasma membrane was tentatively suggested as the most likely site of damage.

3. K. Kobayashi and T. Ito: Wavelength dependence of singlet oxygen mechanism in acridine orange-sensitized photodynamic actions of yeast cells: Experiments with 470 nm. Photochem. Photobiol., (in the press).

SUMMARY

Singlet oxygen mechanism was evaluated for acridine orange-sensitized photodynamic induction of gene conversion in Saccharomyces for 470 nm illumination by using a concentration dependent protective action of added N_3^- to the system. It amounted to 96% of the total effect. This value is to be contrasted with 76% previously observed for 510 nm illumination.

4. T. Ito and K. Kobayashi: In vivo evidence for the photodynamic membrane damage as a determining step of the inactivation of yeast cells sensitized by toluidine blue. Photochem. Photobiol., (in the press).

SUMMARY

According to the previous work (Ito, 1976), yeast cells are photodynamically inactivated in the presence of toluidine blue in spite of the fact that they do not take up the sensitizer appreciably. Genetic analysis with the same system showed no induction of changes in the gene in contrast with that of the cells sensitized by acridine orange (Kobayashi and Ito, 1976). These results, and others as well, obtained in the previous paper (Ito, 1976) have suggested that the cell may be inactivated by the damage occurring in the cell envelope through the reaction with singlet oxygen ($O_2\ ^1\Delta_g$) which was produced outside of the cell. In this short note the evidence of the induced membrane damage in the toluidine blue-sensitized yeast cells is presented.

5. T. Ito and K. Kobayashi: Induction of lethal and genetic damage by vacuum-ultraviolet (163 nm) irradiation of aqueous suspensions of yeast cells. Radiation Res. (1976), (in the press).

SUMMARY

Yeast cells suspended in distilled water were irradiated with monochromatic 163 nm photons by immersing a specially designed discharge tube into the suspension. This was thought to be a useful means of investigating in vivo effects of radiation-induced water radicals on wet

cells in the complete absence of ionic species, since 163 nm photons can dissociate water only via excitation. These experiments showed that (1) the water radicals excluding e_{aq}^-) exerted both lethal and genetic (gene-conversion) effects quite potently, and (2) the characteristic protection against these effects was observable when 2-mercaptoethanol or, in particular, p-aminobenzoic acid, a specific scavenger for OH radicals, was added to the medium prior to irradiation. Nearly complete protection from both lethal and genetic effects was observed in some cases with p-aminobenzoic acid. These results establish unequivocally that the OH radical, and not the hydrogen atom (H radical), possesses the damaging potency in the cell. Comparisons with γ-ray experiments revealed several differences between 163 nm photons and γ-rays in the protective actions of radical scavengers, which may be attributable to reactive species other than OH radicals produced by the γ-rays.

XIV. Institute of Microbiology, Federal Institute of Technology, 8092 Zurich, Switzerland. Communicated by R. Hütter.

Miozzari, G. F.
"Tryptophan synthesis in Saccharomyces cerevisiae - regulation of the capacity of tryptophan synthesis in vivo."
Dissertation Nr. 5698, ETH-Zurich 1976, 103 p.

SUMMARY

This report presents an analysis of the mechanisms involved in the control of the flux to tryptophan in Saccharomyces cerevisiae.

All of the typtophan biosynthetic enzymes are synthesized in excess. The flux through the pathway is adjusted to the rate of protein synthesis through feedback inhibition of the first enzyme by the end-product tryptophan. Increasing or lowering the concentration of individual enzymes, or of chorismate, the first substrate of the pathway, has no noticeable influence on the overall flux to tryptophan.

Tryptophan limitation relieves feedback inhibition of the first enzyme and uncovers the uninhibited synthetic capacity of the pathway, which exceeds the rate of consumption of the amino acid by a factor of three. In addition, tryptophan limitation causes derepression of 4 of the 5 tryptophan-enzymes. Although it does not appear to serve as an instrument for the specific regulation of the flux to tryptophan, this derepression entails a two- to three-fold increase in the synthetic capacity of the pathway.

A number of tryptophan- and indole-analogues were analyzed as to their effects "in vivo". A method was developed for the determination of enzyme activities in permeabilized cells.

Miozzari, G. F., P. Niederberger, P. Hasler, R. Hütter
"Determination of Enzyme Activities in Permeabilized Cells of Various Microorganisms."

Abstract of paper given at the meeting of the Swiss Society for Microbiology, June 18, 1976 at Geneva, Switzerland, to be published in Experientia.

For investigating metabolic pathways in microorganisms, simple methods for determining enzyme activities are required. We have developed a procedure for permeabilizing cells which allows "in situ" measurement of enzyme activities using minimal cell mass. The method was originally devised for assaying amino acid biosynthetic enzymes in Saccharomyces cerevisiae but has also proved to be successful for assaying the analogous enzymes in Schizosaccharomyces pombe and Escherichia coli.

Cells, preferably grown on minimal medium, are harvested during any growth phase by centrifugation. After washing the cells with distilled water and potassium phosphate or Tris-HCl buffer (both 0.1 M, pH 7.6), they are resuspended in the respective buffer containing 0.05% Triton X-100 to provide a final cell concentration of 100 mg cells (wet weight) per ml buffer. After thorough mixing, the suspension is frozen at -20°C. The cells could be stored in this state for several weeks without loss of activity for most of the enzymes tested. Prior to the enzyme assays, the cell suspension is thawed carefully in a water bath at 30°C and then placed in an ice bath.

Using this method we tested biosynthetic enzymes of the arginine, histidine, and tryptophan pathways. Enzyme activities in the permeabilized cells proved to be generally higher and significantly more stable than those in crude extracts.

XV. Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh , EH9 3JT, Scotland. Communicated by J. M. Mitchison.

Papers Published in 1976:

1. Fraser, R. S. S. and Carter, B. L. A. Synthesis of polyadenylated messenger RNA during the cell cycle of Saccharomyces cerevisiae. J. Mol. Biol. 104, 223-242 (1976).

2. Fraser, R. S. S. and Moreno, F. Rates of synthesis of polyadenylated messenger RNA and ribosomal RNA during the cell cycle of Schizosaccharomyces pombe. J. Cell sci. 21, 497-521 (1976).

3. Nurse, P., Thuriaux, P., Nasmyth, K. Genetic control of the cell division cycle in the fission yeast Schizosaccharomyces pombe. Molec. gen. Genet. 146, 167-178 (1976).

4. Sissons, C. H. Improved technique for accurate and convenient assay of biological reactions liberating $^{14}CO_2$. "Analytical biochemistry." 70, 454-462 (1976).

5. Smith, H. T. B. and Mitchison, J. M. Anaesthetics delay and accelerate division in the fission yeast Schizosaccharomyces pombe. Exp. Cell Res. 99, 432-435 (1976).

Paper in Press:

1. P. A. Fantes. Control of cell size and cycle time in Schizosaccharomyces pombe. J. Cell Sci. Accepted September 1976.

SUMMARY

Steady state and perturbed cells of Schizosaccharomyces pombe have been observed through several division cycles by time lapse photomicrography. Perturbed cells were produced by the use of a conditional cell division cycle mutant in which nuclear division is reversibly blocked at high temperature. These experiments show that in both populations cell length at division and cell cycle duration are homeostatically controlled, probably by a primary size control mechanism. Cycle time is indirectly controlled, as cells which have an extended cycle are on average larger at division, so the daughter of such cells need to grow by a smaller amount and for a shorter period, before dividing again. In general, deviations from the mean are corrected within a single cycle, but in the case of very long cells the control breaks down because the cycle cannot be shortened by more than a quarter under the conditions used. These cells take more than one cycle to return to normal.

2. J. M. Mitchison. Enzymes synthesis during the cell cycle. In Leopoldina Symposium on "Cell Differentiation in Microorganisms, plants and animals". VEB G. Fischer Verlag, Jena, GDR, 1977.

SUMMARY

Out of nineteen enzymes examined in synchronous cultures of the fission yeast Schizosaccharomyces pombe, nearly all show continuous increases in activity through the cell cycle. Only one, TMP kinase, an enzyme of DNA synthesis, shows a clearcut pattern of periodic synthesis. Asynchronous controls of the gradient selection technique show that some enzymes can be perturbed by the synchronising technique and that these perturbations can resemble periodic synthesis once per cycle. A short review of other systems emphasizes the importance of carrying out controls of the synchronising procedures.

Some of the enzymes in S.pombe show a linear pattern of activity increase with a doubling in rate once per cycle. This pattern is also shown by rRNA, mRNA and CO_2 production. The rate changes differ in their timing in the cycle and in their dependence on DNA synthesis and division.

Theories for the control of periodic enzyme synthesis do not explain the large number of cases where synthesis appears to be continuous. Gene dosage may be important. There is evidence for translational control and stable mRNA in yeast. The control of unstable enzymes needs to be considered.

Ph.D. Thesis, University of Edinburgh, 1976:

J. Creanor. Title: Oxygen consumption and carbon dioxide evolution during the cell cycle of Schizosaccharomyces pombe.

SUMMARY

Oxygen uptake and CO_2 evolution were measured in synchronous cultures of the fission yeast Schizosaccharomyces pombe, growing in a minimal medium. The rate of oxygen uptake increased abruptly at the middle of the cycle and again at the end of the cycle. During the intervening periods the rate remained constant. Oxygen uptake was measured manometrically and polarographically.

The rate of CO_2 evolution doubled sharply at the time of nuclear division (0.75 of the way through the cycle). For the remainder of the cell cycle, the rate remained constant. Similar step-wise increases in the rate of CO_2 evolution could be induced by adding glucose to late exponential cultures. So various attempts were made to show that the steps in the rate of CO_2 evolution in synchronous cultures were not artefacts induced by the synchronisation technique.

The addition of DNA synthesis inhibitors and a nuclear division inhibitor to synchronous cultures had no effect on the patterns of oxygen uptake and CO_2 evolution. Similarly in an induced synchronous culture, in which DNA synthesis, nuclear division and cell division - but not "growth", were synchronised, oxygen uptake and CO_2 evolution showed a continuous increase and not a "synchronous" pattern.

Evolution of $^{14}CO_2$ was measured in synchronous cultures. Various technical problems made the interpretation of these results difficult but there is a similarity between the measurement of $^{14}CO_2$ evolution and CO_2 evolution measured manometrically in synchronous cultures.

Experiments with protein and RNA synthesis inhibitors suggested either that an increase in oxygen uptake and CO_2 evolution was dependent on continued protein and RNA synthesis; or that in the absence of either protein or RNA synthesis, the demand for energy decreased and hence the flux through the energy yielding pathways which result in oxygen uptake and CO_2 evolution also decreased.

Other experiments showed that optical density increase doubled in a synchronous culture at about the same time that CO_2 evolution doubled in rate. Both hexokinase and ATP were shown to increase exponentially in synchronous cultures and are therefore not obvious regulators of, or obviously regulated by, oxygen uptake or CO_2 evolution.

The control of respiratory and fermentative activity is discussed and it is suggested that the levels of intermediates in the EMP pathway and the TCA cycle control the activities of these pathways.

XVI. University of East Anglia, School of Biological Sciences, University Plain, Norwich NOR 88C, England. Communicated by James A. Barnett.

The following are recent publications from our laboratory:

Barnett, J. A. (1975). The entry of D-ribose into some yeasts of the genus Pichia. Journal of General Microbiology 90, 1-12.

Barnett, J. A. (1976). The utilization of sugars by yeasts. Advances in Carbohydrate Chemistry and Biochemistry 32, 125-234.

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

Barnett, J. A. & Sims, A. P. (1976). A note on the kinetics of uptake of D-glucose by the food yeast, Candida utilis. Archives of Microbiology (in the press).

Barnett, J. A. The nutritional tests in yeast systematics. (Submitted to the Journal of General Microbiology.)

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

Below follow abstracts of work published or accepted for publication since the last issue of the Yeast News Letter.

THE REVERSIBLE BINDING OF A LOW K_m CYCLIC AMP PHOSPHODIESTERASE BY CRUDE MICROSOMES OF YEAST. John Londesborough. Abstract of paper presented at the Tenth International Congress of Biochemistry, July 25-31, 1976, Hamburg.

Baker's yeast contains a cAMP phosphodiesterase which is reversibly bound by the particulate fraction sedimenting between 12000 g and 10500 g. The enzyme is eluted from the particles by 0.3M KCl pH 7.2, and re-binds when the KCl is removed. Both the molecular weight and catalytic activity of the solubilized enzyme depend upon its concentration, so that assays routinely are performed close to a standard concentration. At about physiological ionic strength there is an equilibrium between bound enzyme, free enzyme and particles, which depends on the pH but does not change greatly between 3°C and 20°C. At physiological pH, ionic strength, and concentration of "microsomal" particles, about 50% of the enyzme is bound, and the distribution between free and particle-bound states may respond to physiological changes within the yeast cell. The effects of pH, ionic strength and temperature indicate that the binding is mainly by ionic interactions, and is probably specific. Removal of RNA does not prevent binding, and the role of other chemical components is being investigated.

COMPARISATION OF METHODS FOR THE DETERMINATION OF CELL VIABILITY IN STORED BAKER'S YEAST. Elke Parkkinen, Erkki Oura and Heikki Suomalainen. Journal of the Institute of Brewing 82, 283-285, 1976.

The classical plate method for discriminating between viable and nonviable yeast cells in stored baker's yeast was compared with dead cell staining techniques using methylene blue and three fluorochrome stains. The increase of dead yeast cells during storage of baker's yeast for up to 16 days at 5°C, 20°C and 35°C was determined. It was found that during prolonged storage, especially at 35°C, the death rate increased rapidly and the yeast cake began to become soft because of autolysis. Under these conditions the choice of method for determining the proportion of dead cells proved to be of great importance. Useful results for yeast stored for some time at 35°C could be obtained only by the fluorochrome technique using primuline, acridine orange or acriflavine as fluorochromes.

SOLUBILIZATION AND OTHER STUDIES ON ADENYLATE CYCLASE OF BAKER'S YEAST. Kaija Varimo and John Londesborough. Submitted for publication in the Biochemical Journal.

1. The adenylate cyclase content of Saccharomyces cerevisiae changed only 2-fold between aerobic yeast grown at less than 0.005% glucose and anaerobic yeast grown on 10% glucose, and did not correlate with the activity of NADH-oxidase. 2. The enzyme sedimented from mechanical disintegrates of yeast over an unusually wide range of centrifugal forces. 3. The enzyme was readily solubilized by Ficoll and by Lubrol PX. Lubrol caused a 2-fold activation. 4. Both particle-bound and Lubrol-solubilized enzyme had an apparent Km for ATP of 1.6 mM in the presence of 0.4 mM cyclic AMP and 5 mM $MnCl_2$ at pH 6.2 and 30°C. 5. The Lubrol-solubilized enzyme behaved on gel-filtration as a monodisperse protein with an apparent molecular weight of about 450 000.

CHANGES IN CYTOCHROME LEVELS DURING THE STORAGE OF BAKER'S YEAST AT DIFFERENT TEMPERATURES. Risto Bergelin, Erkki Oura and Heikki Suomalainen. Journal of the Institute of Brewing 82, 286-287, 1976.

The content of cytochromes determined per g fresh yeast was found to stay constant during nearly 3 months' storage of baker's yeast at 5°C. When stored at 24°C and 35°C the content of cytochromes apparently increased during the first 14 and 4 days of storage, respectively, and then decreased continuously.

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The following publications have appeared since the last communications. The abstracts of these reports have been given in Yeast News Letter 24:2, 77, 79, 1976.

Londesborough, J., Quantitative estimation of 3'5' cyclic AMP phosphodiesterase using anion exchange resin in a batch process. Analytical Biochemistry 71, 623-628, 1976.

Oura, E., The effect of aeration intensity on the biochemical composition of baker's yeast: activities of enzymes of the glycolytic and pentose phosphate pathways. Biotechnology and Bioengineering 18, 415-420, 1976.

XVIII. Laboratoire de Structure et Fonction des Biomembranes, Département de Biologie, U. E. R. Scientifique de LUMINY, 70, route Leon Lachamp, 13288-MARSEILLE. Communicated by Edgard Azoulay.

Y_{ATP} value in Candida tropicalis grown on n-alkanes, fatty acids, and acetate

Michel Gallo and Edgard Azoulay

The amount of ATP produced during n-alkane, fatty acid, or acetate metabolism in Candida tropicalis has been established from the P/O ratios measured on isolated mitochondria, yield on substrate and carbon balance. For these three kinds of substrates Y_{ATP} value has been found to be close to 4, although Y_{sub} on acetate is very different from those found with n-alkanes or fatty acids.

Published in Biotechnology and Bioengineering, Vol. XVII, pages 1705-1715 (1975).

Microsomal cytochromes of Candida tropicalis grown on alkanes

Michel Gallo, Bernadette Roche and Edgard Azoulay

A comparison of methods used in isolating microsomes and in measuring microsomal cytochrome P-450 demonstrated that separation following protoplast lysis gave the best results. By this latter technique a high amount of cytochrome P-450 (0.2-0.3 nmol/mg) was recovered, but cytochrome P-420, considered as the denatured form, was absent.

The alkanes specifically induce cytochromes P-450 and b_5 localized on the microsomes. The denaturation in vivo of cytochrome P-450 into cytochrome P-420 even occurs during storage at 1°C. This degradation is increased during preparation of subcellular fractions if no preventive measures are taken.

Published in Biochimica et Biophysica Acta, 419 (1976) 425-434.

Mise en évidence de deux systèmes de transport du phosphate chez Candida tropicalis

Francis Blasco, Gaston Ducet et Edgard Azoulay

Candida tropicalis has two phosphate transport systems, one of which is constitutive and has a low affinity for its substrate (Km_{app} $1.2.10^{-3}$ M). The other one characterized by a high affinity for $H_2PO_4^-$ (Km_{app} = $4.5.10^{-6}$ M) appears only under phosphate starvation conditions. The regulation of the latter would act on the one hand on the synthesis of binding proteins for P_i (repression-derepression) and on the other on the activation or inactivation of the carriers.

Published in Biochimie, Extrait du Tome 58, n° 3, 1976, p. 351.

XIX. Kyoto University, Faculty of Agriculture, Department of Food Science and Technology, Kyoto 606, Japan. Communicated by Akira Kimura.

The following is a summary of our recent work:

Akira KIMURA and Masami OKUDA. Disappearance of Phosphorylation Activity of Nucleotides from the Mitochondria-rich Cells of a Yeast, Hansenula jadinii; a Modified Pasteur Effect. Agr. Biol. Chem., 40, 1373 (1976).

SUMMARY

A yeast, Hansenula jadinii, which was one of the best producers of CDP-choline in our system, lost its activity when cultured in a jar fermenter. This phenomenon was also produced in flasks. Cells cultured aerobically in a medium containing 1% glucose (A-cells) could not phosphorylate nucleotides although development of mitochondria was observed, whereas cells cultured less aerobically in a medium containing 5% glucose (D-cells) could phosphorylate CMP to CTP and finally produce CDP-choline although they had only poorly developed mitochondria. Further study revealed that the A-cells were unstable in hexokinase activity, although they had a dense cytosol, whereas the D-cells remained stable, and they had many round particles. Glycolytic activity was about 4 times stronger in the D-cells than in the A-cells. The phenomenon that respiration (development of mitochondria) suppresses fermentation (glycolysis) has been known as the Pasteur effect. However, in our system, phosphofructokinase which has been thought to be the primary enzyme inhibited in the Pasteur effect was active in the A-cells. Therefore, our phenomenon seems to be a modified Pasteur effect.

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

Characterization of Schizosaccharomyces pombe as a "Petite Negative" Yeast

Roger Bohman and T. W. James

Work in progress is aimed at establishing the basis for the operational characterization of Schizosaccharomyces pombe as a "petite negative" yeast. We have described the relationship between growth and respiratory parameters in both batch and chemostatic cultures grown under a variety of conditions. Under batch culture conditions the expression of glucose repression is dependent not only on the growth carbon source but the carbon source used to challenge the cells. We argue that the obtained relationships are dependent on the relative rates of cellular growth and the rate of synthesis of respiratory machinery. We have shown that the transient nature of these two variables makes the use of the traditional batch culture system both inadequate and deceptive for this type of study because the two rates can vary quite independently. Control studies on glucose repression in batch cultures of Saccharomyces cerevisiae further demonstrate that glucose repression in batch cultures is not

seen on a minimal medium supplemented with glucose, but can be demonstrated, as originally shown, in a broth medium supplemented with glucose.

By using chemostatic cultures we hold the cellular growth rate constant while monitoring the effect of increasing glucose concentration on the rate of synthesis of new respiratory machinery. We have obtained data showing that respiration rates under these conditions for both S. pombe and S. cerevisiae are sensitive to increasing concentrations of glucose. By monitoring the effect of inhibitors of mitochondrial transcription and translation and cytoplasmic translation on the respiratory parameters in a chemostat under steady state conditions as well as on the release from glucose repression, we have demonstrated that the maintenance of respiratory competence in S. cerevisiae, a "petite-positive" species, shows dependence on both mitochondrial and cytoplasmic processes. On the other hand, in S. pombe this competence is almost exclusively dependent on the mitochondrial processes. We argue that whether it be at the level of information storage or translation that the basis for the "petite negative" character of S. pombe is this greater dependence on the mitochondrial processes.

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

Current research in our laboratory is concerned with the mechanism of temperature adaptation in yeast. The following is a summary of work which has been recently accepted for publication.

Thermal Adaptation in Yeast: Growth Temperatures, Membrane Lipid and Cytochrome Composition of Psychrophilic, Mesophilic and Thermophilic Yeasts. H. Arthur & K. Watson. J. Bacteriol. 128: In Press.

ABSTRACT

The temperature limits of growth of a number of yeast species were examined and on this basis the organisms were classified into different thermal categories. The following species were examined:

Leucosporidium frigidum and Leu. nivalis, psychrophilic, temperature limits of growth -2 to 20 C. Candida lipolytica, mesophilic, temperature limits of growth 5 to 35 C. C. parapsilosis and Saccharomyces telluris, thermotolerant, temperature limits of growth 8 to 42 C. Torulopsis bovina and C. slooffii, thermophilic, temperature limits of growth 25 to 45 C and 28 to 45 C, respectively.

The membrane lipid and cytochrome composition of mitochondrial fractions isolated from these yeasts were compared. There was a direct correlation between the growth temperature and the degree of membrane lipid unsaturation; the lower the temperature, the greater the degree of lipid unsaturation. The membrane lipid composition of the thermophilic yeasts were distinguished by the high percentage (30 - 40%) of saturated fatty acid as compared with the mesophilic and psychrophilic yeasts. The latter contained approximately 90% unsaturated fatty acid, 55% of which was linolenic acid, $C_{\alpha\text{-}18:3}$. Changes in

phospholipid composition in relation to temperature were also noted. The respiratory-deficient thermophile, C. slooffii, was characterized by the absence of cardiolipin (sensitivity 0.1 µg phosphorus) and cytochrome aa_3. The absence of conventional mitochondrial structures in this thermophilic microorganism is tentatively suggested although low concentrations of cytochromes b, c and c_1 were detected by low temperature spectroscopy. On the other hand, the respiratory-competent thermophile, T. bovina, was characterized by a high cardiolipin (25% of the total phospholipid) and cytochrome aa_3 content (1 nmole/mg mitochondrial protein). Low temperature spectra showed the presence of one b-type cytochrome in the thermophilic yeasts, two b-type cytochromes in the mesophilic yeast and three b-type cytochromes in the psychrophilic yeasts. It was concluded that a knowledge of the properties of the biological membrane is fundamental to an understanding of the ability of a microorganism to grow and reproduce in different temperature environments.

Leucosporidium Yeasts: Obligate Psychrophiles which Alter Membrane-lipid and Cytochrome Composition with Temperature. K. Watson, H. Arthur and W. A. Shipton. J. Gen. Microbiol. 1976. In Press.

SUMMARY

The temperature limits of growth of three psychrophilic yeasts, Leucosporidium frigidum, Leu. gelidum and Leu. nivalis, were examined. All species grew well at subzero temperatures (-1°C). The maximum temperature of growth was significantly higher with glucose as substrate (18-20°C) as compared with ethanol (17-18°C). There was a direct correlation between the growth temperature and the degree of fatty acid unsaturation of the cell lipids; the lower the temperature the greater the degree of fatty acid unsaturation. At subzero temperatures (-1°C) with ethanol as substrate 90% of the total fatty acid was unsaturated with linolenic (35-50%) and linoleic (25-30%) the predominant fatty acids. Linolenic acid accounted for less than 20% of the total fatty acid at temperatures close to the maximum for growth with oleic (20-40%) and linoleic (30-50%) the major fatty acids. Difference spectra of intact cells showed marked changes in the ratios and amounts of individual cytochromes as a function of growth temperature. In Leu. frigidum with glucose as substrate, the ratios of cytochromes $a + a_3$:b:c at 8°C and 19°C were 1:1.1:2.9 and 1:2.3:16.7 respectively. Similar changes in the ratios of the cytochromes were noted for Leu. gelidum but much less marked changes were noted for Leu. nivalis. The temperature effects were interpreted as supporting the view that membrane structure and composition are fundamental to temperature adaptation in psychrophilic yeasts.

Abstract presented at the FEBS-Symposium on the Biochemistry of Membrane Transport, Zurich, July 18-23, 1976.

Membrane Transport: A Controlling Factor in Thermal Adaptation in Yeasts

K. Watson, P. Watson, H. Arthur and W. A. Shipton

The ability of microorganisms to adapt to different temperature environments has attracted considerable attention but the mechanisms underlying this phenomenon are not understood. The yeast cell with

its wealth of membrane organization, together with its rapid growth, reproduction and relative genetic simplicity is an attractive system in which to study the mechanism of thermal adaptation in eucaryotic microorganisms. The temperature limits of growth of a number of yeast species were examined and on this basis the organisms were classified into psychrophilic, mesophilic and thermophilic yeasts. The transport of various metabolites, including amino acids, was examined in intact cells. The amino acid transport systems of psychrophilic yeasts were active at low temperatures (2°C) but were relatively inactive (10% of the activity at 2°C) at 40°C. Rapid inactivation of metabolite transport was observed at the latter temperature. By contrast, active transport was observed in thermophilic yeasts at 40°C with little activity at low temperatures. Mesophilic yeasts showed little transport activity at low temperatures and activity was rapidly diminished at 40°C. The variations in metabolic transport were correlated with differences in membrane structure and lipid composition. Scanning and transmission electron microscopy revealed marked differences in cell surface and membrane topography of these yeasts. Psychrophilic and thermophilic yeasts were characterized by a high and low degree of membrane lipid unsaturation, respectively. It is proposed that alterations in membrane lipid unsaturation and hence membrane fluidity are important factors in determining the ability or inability of yeasts, adapted to different thermal domains, to transport metabolites across the cell wall.

XXII. Université De Lyon, Laboratoire De Biologie Végétale 43, Boulevard Du 11 Novembre 1918, 69621, Villeurbanne, France. Communicated by M. C. Pignal.

The following are recent publications from this laboratory:

Fiol, J. B. Systématique des Saccharomyces: osidases et besoins vitaminiques. Mycopathologia 58:49-58, 1976.

Fiol, J. B. and Billon-Grand, G. Nitrite réductase des Saccharomyces (groupe Torulaspora) et des Debaryomyces. Implications systématiques. Accepted for publication in Mycopathologia.

Thesis:

A thesis (Doctorat de Spécialité) was defended on 6 July 1976. F. Toukourou - A contribution to the study of interaction between yeasts and tannins: Action of tannins on the fermentation; - study of the tannase of Pichia strasburgensis and P. pseudopolymorpha.

SUMMARY

If yeasts contain a tannase, hydrolysable tannins are less inhibitory on the fermentation of glucose than condensed tannins; for yeasts lacking tannase the reverse situation holds. For the fermentation of sugars requiring the induction of enzymes (permeases, hydrolases, etc.), the presence of tannins is inhibitory under all conditions, even at low concentration. Experiments on the "tanning" of yeast cells have shown that the inhibition of glucose fermentation increases with the concentration of tannin and the time period of "tanning", but the intracellular fermentative potential of the cells is not

affected. Other experiments involved the testing of the action of certain monomeric phenolic compounds, which are constituents of tannin molecules.

The tannase of Pichia strasburgensis has been studied (K_m, pH optimum for activity) and compared with the enzyme from Asperigillus niger. The latter has twice the esterase and "depsidasique" activity. In contrast, the enzyme from Pichia pseudopolymorpha has only "depsidasique" activity.

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

Recent publications

1. H. Schneider, F. Gmünder and A. Fiechter: n-Alkane Oxidation by Candida tropicalis. I. Hypothetical Role of Catalase Enzyme and the Microbodies in Hydrocarbon-Assimilating Yeast. European J. App. Microbiol. 2, 221-230 (1976).

   The specific activity of the enzyme catalase was investigated in batch cultures of Candida tropicalis on the following substrates: Gelsenberg 14/18, n-hexadecane, 1-octadecene, oleyl alcohol, oleic acid and glucose. The catalase activity does not change with the different oxidation levels of the hydrocarbon substrates. A correlation between specific activity and growth rate was established.

2. O. Käppeli: Funktion und Bau der Zelloberfläche von Candida tropicalis bei der Assimilation von Kohlenwasserstoffen. Swiss Federal Institute of Technology, Dissertation No. 5661, Zurich (1976).

   The growth of Candida tropicalis on hydrocarbons requires the transport of the substrate by direct contact between cells and dispersed phase. The cell surface shows an affinity towards the insoluble substrate. It was shown that the fixation is an adsorption not involving any enzyme. Based on the results a model for transport is described.

3. O. Käppeli and A. Fiechter: The Mode of Interaction between the Substrate and Cell Surface of the Hydrocarbon-Utilizing Yeast Candida tropicalis. Biotechnol. Bioeng. 18, 967-974 (1976).

   A method for the measurement of the affinity of the cell surface to hydrocarbons is described. The affinity was basically unaffected by different pH values and temperature as well as by the chain length of the substrate. The contact time required for saturation of the cell surface with substrate was 30 sec. Cells grown on glucose showed a 25% lower adsorption capacity compared to those grown on n-alkane. The glucose grown cells showed also a more distinct dependence of the amount of adsorbed hydrocarbon on the quality of the emulsion. The interaction between the substrate and cell surface turned out to be an adsorption that did

not involve an enzymatic reaction. These results led to the conclusion that a lipopolysaccharide moiety present at the cell surface is responsible for the affinity.

4. A. Fiechter: Continuous Cultivation of Yeasts. Methods in Cell Biology, 11, 97-130 (1975).

   The aim of this article was to demonstrate the usefulness of continuous cultivation methods in microbiological studies.

5. J. R. Pringle and J. -R. Mor: Methods for Monitoring the growth of Yeast Cultures and for Dealing with the Clumping Problem. Methods in Cell Biology, 11, 131-168 (1975).

   The article deals with biochemical, physiological, cytological and developmental studies on monitoring the growth of yeast cultures.

6. J. R. Pringle: Methods for Avoiding Proteolytic Artefacts in Studies of Enzymes and Other Proteins from Yeasts. Methods in Cell Biology, 12 149-184 (1975).

   Yeast cells contain a variety of different proteolytic enzymes. During the past several years, it has become increasingly apparent that these proteolytic enzymes pose a very serious problem for attempts to study other yeast proteins.

7. J. R. Pringle: Induction, Selection, and Experimental Uses of Temperature-Sensitive and Other Conditional Mutants of Yeast. Methods in Cell Biology, 12 233-273 (1975).

   In some areas of biological investigation (e.g., studies of the pathways of small-molecule metabolism), the contribution of genetic analysis has clearly been great; in other areas (e.g., studies of development), one still hears more of the potential than of the triumphs of genetic methods. The intrinsic power of genetic analysis is treated on the level of: (1.) Range. By the introduction of appropriate mutations, one can in principle, interfere with the synthesis or function of any cellular protein or RNA species (2.) Specificity. (3.) The possibility of isolating interesting mutants in the absence of any prior knowledge about the functions that will be affected.

XXIV. National Research Council Canada, Division of Biological Sciences, Ottawa, Canada, KIA OR6. Communicated by Byron F. Johnson.

Evidence of Discrete Size Fragments of Mitochondrial DNA Complexed with a Specific Nuclease from S. cerevisiae

Charles V. Lusena and Alberto Bernardi

Laboratoire de Génétique Moléculaire
Institut de Biologie Moleculaire
Paris 75005, France

Elsevier/North Holland Biomedical Press, Amsterdam 1976

Described in this report is the isolation of about 15 discrete fragments of mtDNA of molecular weight ranging from 2 to 0.25 x 10^5. These fragments were isolated as a complex with a specific nuclease.

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Volume-Related Mitochondrial Deoxyribonucleic Acid Synthesis In Zygotes and in Vegetative Cells of Saccharomyces Cerevisiae

Eng-Hong Lee and Byron F. Johnson

In press, J. Bacteriol.

ABSTRACT

The synthesis of mitochondrial deoxyribonucleic acid in Saccharomyces cerevisiae cells has been examined during conjugation, in preconjugal conditions, and in control cultures which were not exposed to obverse diffusible sex factors. The ratios of mitochondrial to nuclear DNA varied from about 0.1 in control cells, to about 0.3 in α cells exposed for 180 min to cell-free culture medium from a cells, and to about 0.4 in conjugating cells 150 min after mixing. The enhanced levels of mitochondrial DNA during preconjugal and conjugal conditions seem correlated with enhanced cell volumes. Likewise, amounts of mitochondrial DNA in vegetative cells were found to be correlated with cytoplasmic volumes.

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A comparison of quantitative methods for measuring yeast flocculation

G. B. Calleja and Byron F. Johnson

In press, Can. J. Microbiology

ABSTRACT

Two quantitative objective methods for measuring flocculation of a yeast culture were compared and correlated with subjective estimation by eye. One method involved counting in a haemocytometer the number of free cells (i.e., cells not found in flocs) and then the total number of cells after complete deflocculation by pronase. The number of cells in flocs was derived by subtracting the number of free cells from the total number of cells. The other method made use of the decrease in turbidity of a flocculated culture on standing. Gross flocs settled to the bottom of a tube within 5 min. The decrease in turbidity was measured by a photoelectric colorimeter. The difference in turbidity readings between 0 min and 5 min was assumed to represent the turbidity component due to cells in flocs. The methods were appropriately applicable to monitoring the time course of sex-directed flocculation of the fission yeast Schizosaccharomyces pombe. A culture at maximum flocculation contained 500 gross flocs ml^{-1}, which represented up to 70% of the total number of cells. A typical primary floc occupied a volume of 1 x 10^{-4} ml and contained 1 x 10^5 cells.

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

Below is a summary of recent work in this laboratory on carotenoid pigment production in a yeast (cf. Andrewes, Phaff and Starr, Phytochemistry 15, 1003, 1976).

Production of Carotenoid Pigments by Phaffia rhodozyma

E. A. Johnson and M. J. Lewis

The production of carotenoid pigments by an imperfect yeast Phaffia rhodozyma is growth associated. On defined media the yield of carotenoid pigments approaches 500 µg/g yeast. The yield depends on the strain used and the cultural conditions. Maximum pigment biosynthesis occurs during the exponential phase of growth but the maximum value of the xanthophyll to carotene ratio occurs after glucose is exhausted. High aeration rates promote a high xanthophyll (mainly astaxanthin) to carotene ratio. High initial glucose concentrations decreased cell yield and pigment production but these were increased by high aeration rates and increased nitrogen concentration of the medium and selection of optimum pH. When grown at pH 3.5 or at an initial glucose concentration of 5%, lycopene and β-zeacarotene were detected in this species, indicating two possible points for cyclization in the pathway of β-carotene biosynthesis in this yeast.

XXVI. Laboratory of Cell Biology, Janssen Pharmaceutica, B-2340, Beerse, Belgium. Communicated by S. De Nollin.

Below follow abstracts of several recent contributions from this laboratory.

Mykosen: In Press.
"An ultrastructural and cytochemical study of Candida albicans after in vitro treatment with imidazoles". Sonja De Nollin and M. Borgers.

SUMMARY

The ultrastructural changes in C. albicans after exposure in vitro to different doses of clotrimazole and miconazole have been investigated. The changes obtained with low dose levels of both drugs concerned preferentially the cell periphery with eventual hyperactivity of the central vacuolar system. With high dose levels, on the other hand, the morphologic changes differed markedly between the two drugs with respect to the number of cells involved, the intensity of the lesions and their subcellular location.

This discrepancy in response of C. albicans towards high doses of the imidazoles, shed some doubts on the previously propagated mode of action of these drugs. Additionally to the morphological description of the involutionary processes in C. albicans, the behavior of some oxidative and peroxidative enzymes has been investigated after miconazole treatment. In the light of these findings, an alternate hypothesis is formulated proposing hydrogen peroxide intoxication as the cause of cell death.

Antimicrobial Agents and Chemotherapy: In Press.
"Cytochemical and biochemical studies of yeasts after *in vitro* exposure to miconazole". Sonja De Nollin, H. Van Belle, F. Goossens, F. Thone and M. Borgers.

ABSTRACT

Yeast cells exposed to different doses of the antimycotic agent miconazole reveal important cytochemical changes in the topographic distribution of the phosphatases. A strong effect has been observed on the behavior of oxidative and peroxidative enzymes. A decreased activity of cytochrome c oxidase and - peroxidase and an increased activity of catalase is seen after treatment with a fungistatic dose of miconazole, whereas a complete disappearance of these enzymes is observed after treatment with a minimal fungicidal dose of miconazole. This is in complete agreement with the quantitative biochemical data. A hypothesis is advanced on the possible involvement of the latter enzymes in the mechanism of action of this drug.

Thesis: Ultrastrukturele en biochemische eigenschappen van gisten en de invloed van antimycotica. By Sonja De Nollin.

SUMMARY

The application of conventional fixatives for the visualization of the ultrastructure of different yeast species and of *C. albicans* in particular is described. The problem of inadequate permeation of chemical fixatives was solved by sectioning solidified pellets of the yeasts in the presence of the fixative, a procedure yielding fairly well-preserved subcellular structures. A comparison was made with protoplasts of *C. albicans*. The *Candida* cells were also examined using scanning electron microscopy and the morphologic appearance of the surface is discussed.

The new preparation procedure was also used for the demonstration of enzyme activities of *C. albicans* and *S. cerevisiae*. The distribution pattern of subcellular organelle markers is generally at variance with that found in mammalian cells. Reactivities towards non-specific and specific phosphatase substrates were mostly confined to the central vacuolar apparatus. Oxidative and peroxidative activities were demonstrated in mitochondria, whereas catalase was present in the peroxisomes. The possible function of the various organelles in relation to their enzymatic content is discussed.

Along with this cytochemical visualization of enzymes, and after evaluating an adequate method for the homogenization of the yeasts, biochemical studies of yeast enzymes were done. The appearance and the behaviour of the non-specific phosphatases and the oxidative and peroxidative enzymes under different experimental conditions of *S. cerevisiae* and *C. albicans* are reported.

Beside this descriptive part on the morphology, cytochemistry and biochemistry of enzymes in untreated control cells, the ultrastructural changes in *C. albicans* after exposure to different doses of miconazole, clotrimazole, R 34000 and amphotericin B have been investigated.

In vitro exposure to fungistatic doses of miconazole revealed that the plasmalemma and cell wall are the organelles that undergo changes. After exposing cultures to minimal fungicidal doses of miconazole, degradation of plasmalemma and cytoplasmic organelles was observed. Injured parts of cellular material were sequestered from the rest of the cytoplasm and extruded into the central vacuole. A similar degradation occurred at the cell periphery. Exposure to a fungicidal dose, resulted in a completely necrotic cell interior, which was, however, surrounded by an intact cell wall. From the morphologic point of view a clear dose relationship could be established. These results were compared with those seen with scanning electron-microscopy. Cells exposed to fungistatic and minimal fungicidal doses of miconazole, presented rough surfaces and multiple desoriented buds and budscars, whereas in control cultures, the cells appeared well separated, the treated ones forming small clusters of interconnected cells. After exposure to a fungicidal concentration, most of the remaining cells showed smooth surfaces and were covered with numerous vesicular structures, probably representing cytoplasmic remnants from broken cells.

The cytochemistry of cells exposed to different doses of miconazole, revealed important changes in the topographic distribution of phosphatases. Furthermore, a strong effect has been observed on the behavior of oxidative and peroxidative enzymes. This is in complete agreement with the quantitative data obtained biochemically. A decreased activity of these enzymes was seen after treatment with fungistatic doses, whereas a complete disappearance was observed after exposure to fungicidal doses.

A hypothesis was put forward on the possible involvement of the latter enzymes in the mechanism of action of this drug.

J. Histochem. & Cytochem: In Press.
"Cytochemical localization of NADH oxidase in Candida albicans". M. Borgers, S. De Nollin, F. Thone and H. Van Belle.

SUMMARY

The application of a recently published technique to localize NADH oxidase activity is described in glutaraldehyde-fixed C. albicans. The reaction product appears as a finely granular precipitate on the mitochondrial cristae and on the central vacuolar membrane and, if present, on the vacuolar contents. Fixation should be kept to a minimum and prolonged incubation times up to 2 h are necessary to show these reactive sites. The reaction appears to be strongly substrate-dependent and not affected by cyanide.

Exposure of C. albicans cells to the anti-mycotic miconazole resulted in a strong increase in NADH oxidase activity. The hypothesis is put forward that this enzyme, together with peroxidative and catalatic enzymes, may be implicated in the mechanism by which miconazole exerts its lethal effect on C. albicans.

XXVII. Institut f. Mikrobiologie, Technische Hochschule Darmstadt, 6100 Darmstadt, West Germany. Communicated by F. K. Zimmermann.

Wine yeasts: In collaboration with Zentralkellerei Badischer Winzergenossenschaften, 7814 Breisach A. K., Federal Republic of Germany, a systematic selection for efficient wine yeasts has been carried out since 1969. Heat pasteurized musts (90 sec at 85°C) are inoculated with 0.5 - 1.0% of a fully fermenting grape must (titer: 1.10^8 cells/ml). Fermentation in tanks with up to 120 000 l capacity is completed within less than 10 days. Alcohol yield is very high, acetaldehyde low (15 - 30 mg/l), volatile acid hardly increased above the level in the must (never more than 0.3 g/l), no foaming, low H_2S production, 20 - 30 mg of sulfurous acid formed, and perfect preservation of the must aroma. The highest alcohol level reached was 138 g/l in a 1975 Ruländer Beerenauslese within 10 days.

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

E. Minárik: The problem of sulphite and sulphide formation in in young wines. Kvasny prumysl 22, 1976 (in press).

Recent knowledge on suphite and sulphide formation by yeasts during the fermentation of grape must is summarized. The potential sources of hydrogen sulphide formation including sulphate, sulphite, cysteine and elemental sulphur are pointed out. Hydrogen sulphide formation from elemental sulphur proceeds by non-enzymatic reduction. Hydrogen sulphide is formed from sulphate and sulphite by yeasts as an end product of an enzymatic inorganic reduction process by which inorganic sulphur may turn into organic form. Hydrogen sulphide is formed in the course of the reduction pathway of sulphate either by release during the biosynthesis of methionine and/or cysteine, or as a result of a blocked reaction of biosynthesis. Hydrogen sulphide is formed from cysteine by desulphhydration. Practical aspects for wine making are briefly discussed.

E. Minárik: Some enological properties of Czechoslovak wine yeast strains. Kvasný prumysl (submitted).

Based on long-term studies on ecology, taxonomy, physiology and biochemistry of yeasts deposited in the Yeast Collection of the Research Institute for Viticulture and Enology in Bratislava, some very effective strains have been selected which are suited for large-scale fermentations of grape must and fruit juice. Advantages of pure yeast fermentations could be clearly confirmed. They consist in an immediate and unhindered start and course of fermentation, in the fermentation of the last sugar remnants of the wine and in forming minimal undesired fermentation by-products and off-odours. The necessity of the use of pure yeast starters may be recommended for heat-treated pomace fermentation, for refermentations, for sparkling wine fermentation, large-volume fermentations, at high or low fermentation temperatures, for strongly sulphited and racked musts,

and generally for fermenting fruit juices and at unfavourable fermentation conditions, e.g., in the presence of pesticide residues in musts, etc.

A. Navara - E. Minárik: Tolerance of some Saccharomyces sp. to sorbic acid. Kvasny prumysl (submitted).

A lot of complaints and polemics on reasons of yeast trouble in sweet wines stabilized by potassium sorbate are known. The activity of Saccharomyces sp. in the presence of sorbic acid in sweet wines has been studied. The strains Hlinik 1 (S. cerevisiae), Bratislava 1 (S. bayanus) and Fendant (S. bayanus) were used as test organisms. At low temperature (12°C) the inhibitory effect of sorbic acid on yeast activity is higher than at elevated temperature (24°C). Concentrations between 50 and 100 mg/l of sorbic acid are not sufficient to suppress activity of Saccharomyces sp. when sweet wines are stored at 24°C. At this temperature the strain Bratislava 1 is more sensitive than strain Hlinik 1. The psychrophilic strain Fendant is the most resistant strain against sorbic acid. Concentrations of sorbic acid up to 150 mg/l may cause increased tolerance of Saccharomyces sp. at 24°C in wines with residual sugar.

XXIX. Allied Breweries (Production) Limited, The Brewery, Station Street, Burton-on-Trent DE14 1BZ, England. Communicated by D. A. Lovett.

Below follow abstracts of two papers recently written by members of of the staff.

YEAST LIPID COMPOSITION AND THE CONTROL OF VIABILITY IN FERMENTATIONS OF CONCENTRATED BREWERS' WORT
A. Day, T. J. B. Webb, P. A. Martin

Paper presented to 5th International Fermentation Symposium, Berlin, 1976 and published in the Abstracts from this Symposium, p. 468.

Yeast fermentation performance was found to decline as the concentration of the wort increased and this may be associated with limitation in yeast growth caused by oxygen deficiency. Since oxygen is required for the biosynthesis of lipids, a study has been made of these lipids in relation to oxygen metabolism and cell viability in concentrated wort fermentations. It was found that more than 80% of the total fatty acid residues of the yeast consisted of $C_{16} + C_{18}$ saturated plus unsaturated acids. The amount and proportion of sterols and the ratio of saturated: unsaturated fatty acids varied according to the oxygen supply; increased oxygen supply increased the proportion of unsaturated fatty acid residues and improved crop viability.

GROWTH OF SACCHAROMYCES CEREVISIAE AND SACCHAROMYCES UVARUM IN A TEMPERATURE GRADIENT INCUBATOR
R. M. Walsh, P. A. Martin

Paper to be published shortly in the Journal of the Institute of Brewing.

A temperature gradient incubator has been used to determine the effect of temperature on the growth of strains of Saccharomyces cerevisiae and Saccharomyces uvarum. The maximum and optimum temperatures for growth were determined. The T_{max} values for Sacch. cerevisiae were in the range 37.5°C-39.8°C and the T_{opt} values were in the range 30.0°C-35.0°C. Strains of Sacch. uvarum formed two distinct groups with T_{max} values of 31.6°C-34.0°C or 38.2°C-40.0°C and T_{opt} values of 26.8°C-30.4°C or 30.0°C-34.6°C. It is proposed that the species name Sacch. carlsbergensis be reintroduced for the first of these groups which includes all the brewing strains of Sacch. uvarum tested. Measurements of the generation times for one brewing strain of Sacch. cerevisiae and one brewing strain of Sacch uvarum (Group A) over the temperature range 6.0°C-22.0°C have shown that there are significant differences between the yeasts at the lower end of the temperature range and that the relationship between generation time (GT) and temperature (T) for both yeasts closely follows the mathematical expression: $\text{Log } (GT) = a + b(T) + c(T^2)$.

XXX. Technische Universität Berlin, Fachbereich 13 - Lebensmitteltechnologie und Biotechnologie, Lehrstuhl für Microbiologie, 1 Berlin 65. Communicated by S. Windisch.

Below follow abstracts of several publications from our laboratory.

Studies in order to increase the yield of alcohol by fermentation.
I. The influence of sugar concentration and temperature.

S. Windisch, M. Stobbe and G. Koppensteiner

Branntweinwirtschaft 114 (8), 183-184, 1974

SUMMARY

Alcohol formation and fermentation velocity of many distillery yeasts decrease only above sugar concentrations of 17.5% sucrose. Periodic stepwise addition of the sugar in lower concentrations is superior to addition of all the sugar at once. The changes in osmotic pressure during the fermentation were followed by cryoscopic measurements. It has to be concluded that good distiller's yeasts should have a higher alcohol resistance. In experiments using various fermentation temperatures it was demonstrated that no significant differences with respect to alcohol formation and fermentation velocity were observed between 24 and 37C with the strains investigated; however, the higher temperatures caused a shorter lag period.

Studies in order to increase the yield of alcohol by fermentation.
II. About the influence of adding different substances and of varying technical conditions.

Mitt. Rebe u. Wein 24, 239-250, 1974

SUMMARY

S. Windisch and M. Stobbe

We have tested 16 different substances singly or in various combinations and concentrations to find out if they can stimulate the alcoholic fermentation. The substances tested were: Tronozym, silicone fluid, Tween-80, erucic acid, soy flour, 4 dried algal powders, 1 algal extract, $K_2Cr_2O_7$, Cr_2O_3, β-phenethyl alcohol and 3 kinds of activated carbon. Not all of the substances studied were stimulative, but some considerably accelerated the fermentation or increased the yield of alcohol. The efficacy of some substances is based on their surface action (silicone fluid, Tween-80) whereas Tronozym works as a nutritional agent. The algal powders and the soy flour increased the alcohol yield especially when higher concentrations of sugar were used. Moreover the algal powders improved the resistance of the yeast cells against the alcohol. This fact cannot yet be explained. In fermentation experiments the concentration of the molasses used could be increased without lowering the alcohol yield or extending the time of fermentation from the normal dilution step 1:6 to 1:5. There was neither any loss by a longer fermentation time nor any loss of yield, if the mashes were slowly stirred and if the inoculum rate of normally 4-5% in the distillery was increased to the three- to fourfold amount. Addition of 1% algal powder reduced that amount of seed yeast to less than 50%.

W. Henninger and S. Windisch have described 4 new species of yeasts or yeast-like fungi:

Pichia lindneri sp. n., a new methanol assimilating yeast from soil. Arch. Microbiol. 105, 47-48, 1975.

A new yeast of Sterigmatomyces, S. aphidis sp.n. Arch. Microbiol. 105, 49-50, 1975.

Torulopsis spandovensis sp.n., a new yeast from beer. Arch. Microbiol. 107, 205-206, 1976.

Kluyveromyces blattae sp.n., a new multispored yeast from Blatta orientalis. Arch. Microbiol. 109, 153-156, 1976.

Ability of non-osmotolerant yeast hybrids to raise hard-biscuit and short cake doughs.

IV. International Specialized Symposium on Yeasts "Yeast for industrial use", June 28 - July 3, 1976 Berlin Abstracts Y.13.

S. Windisch, S. Kowalski and I. Zander

ABSTRACT

Osmotolerant yeasts may spoil marchpane (marzipan). They ferment strongly under lack of oxygen. Attempts to put this CO_2 production to use were successful, some osmotolerant yeasts did indeed raise dry cookie doughs. Because sporulation was poor or lacking, genetic work did not succeed. The search turned therefore over to non-osmotolerant high quality strains of brewers, bakers and distillers yeasts. New hybrids were constructed in order to obtain non-osmotolerant strains

capable to raise heavy doughs. The CO_2 production of a large number of such strains and hybrids has now been tested with an Epsom Fermentometer in three different kinds of dough: hard-biscuit (B), short cake (S) (2), and flour dough (F). Our new strains differ from the bakers yeast in that they 1) are able to produce large amounts of CO_2 under adverse conditions (e.g., heavy dough), 2) produce more CO_2 under our experimental conditions than bakers yeasts even in flour dough, and 3) are capable of raising at least two different types of dough substantially, whereas bakers yeasts can only raise flour dough. Our results show that non-osmotolerant yeasts and yeast hybrids can raise heavy as well as light doughs astonishingly well without pre-treatment.

XXXI. Naarden International, P. B. 2, Naarden-Bussum (The Netherlands). Communicated by F. E. M. J. Sand.

Below follow summaries of recent studies on yeasts encountered in the soft drinks industries.

1. Yeasts isolated from Proportioning Pumps employed in Soft Drink Plants. F. E. M. J. Sand, G. A. Kolfschoten and A. M. Van Grinsven. Brauwissenschaft 29 (10) 294-298.

Ten proportioning pumps applied in eight soft drinks bottling plants in the Netherlands were sampled for yeasts. The 99 strains isolated include 14 species belonging to the genera Candida, Hansenula, Lodderomyces, Pichia, Saccharomyces, Saccharomycopsis and Torulopsis. Representative strains were submitted to routine growth tests on solidified soft drink media containing 0, 75 and 100 ppm of anti-microbially active benzoic acid at pH 3.0. The results are discussed and compared to those obtained by the examination of spoilt soft drinks.

2. Investigation of Yeast Strains isolated from Scandinavian Soft Drinks. F. E. M. J. Sand and A. M. van Grinsven. Brauwissenschaft 29 (11) 000-000.

The 59 strains isolated from 68 samples of carbonated beverages and fruit syrups bottled by 20 Scandinavian plants represent 15 species belonging to the genera Brettanomyces, Candida, Saccharomyces and Torulopsis. Most frequent was Br. naardenensis. All species have also been encountered in beverages manufactured outside Scandinavia. Hence they may be considered as being specific for soft drinks.

3. Comparison between the Yeast Flora of Middle Eastern and Western European Soft Drinks. F. E. M. J. Sand and A. M. van Grinsven. Antonie van Leeuwenhoek (in press).

Samples of a carbonated orange drink, raw materials and intermediate products originating from 6 Iraqi bottling plants were examined. 69 Drinks, 4 flavoured syrups and 19 simple syrups contained yeasts, whereas all samples from one plant and all samples of beverage base were free from viable yeasts. From the orange drink 2 species were isolated viz. Saccharomyces montanus and Torulopsis stellata. The following species were present in simple syrup: Hansenula anomala, Sacch. bisporus var. mellis, T. candida and T. stellata. Sacch. bisporus var. mellis was also isolated from flavoured syrup. Representative strains were submitted

to routine growth tests at reduced oxygen tension, at reduced water activity and on solid soft drink media containing various amounts of benzoic acid at pH 3.0. The results are discussed and compared to those obtained in European soft drinks.

4. Yeasts isolated from Korean Soft Drinks. F. E. M. J. Sand and A. M. van Grinsven. The Journal of Applied Bacteriology (in press). Microbiological examination of a series of soft drinks bottled by 12 plants in the Republic of Korea during 1974 yielded 132 yeast cultures, most of which belonged to Brettanomyces naardenensis. The following species were also found: Candida parapsilosis, C. pelliculosa, Hansenula anomala, Rhodotorula glutinis, Saccharomyces florentinus, Sacch. microellipsodes, Sacch. montanus, Torulopsis stellata and Candida spp. Representative strains were submitted to routine growth tests on solidified soft drink media containing various amounts of anti-microbially active benzoic acid at pH 3.0. the results are discussed and compared with those obtained in European soft drinks.

5. A case of fermentation of fountain syrup. F. E. M. J. Sand and A. M. van Grinsven. Brauwissenschaft (in press).

A Dutch made fountain syrup (pH 2.8, 60° Brix, 330 mg/kg benzoic acid) was attacked by Saccharomyces bailii.

6. Yeasts isolated from sugar syrups employed for the preparation of soft drinks. F. E. M. J. Sand and A. M. van Grinsven (in preparation).

129 Yeast strains were isolated from sugar syrups used by 21 - mostly Dutch - plants for the preparation of soft drinks. The following species were encountered: C. capsuligena, C. guilliermondii, C. intermedia, C. lusitaniae, C. parapsilosis, C. pelliculosa, C. sake, Cit. matritensis, Deb. castellii, H. anomala, Lod. elongisporus, Rh. glutinis, Sacch. cerevisiae, Sacch. bailii, Sacch. bisporus, Sacch. delbrueckii, Sacch. microellipsodes, Sacch. rouxii, Sacch. rosei, Sacch. oviformis, Sacch. uvarum, T. apicola, T. candida, T. glabrata, T. globosa, T. mogii and T. stellata.

XXXII. International and National Meetings

A. Minutes of the Meeting of the Executive Board of the IAMS Commission on Yeasts and Yeast-Like Microorganisms at the Fourth International Specialized Symposium on Yeasts, Berlin (West), June 29, 1976. Communicated by U. B. Sleytr, Secretary of the Commission.

Present were:
B. Bachman, Poland
A. Fiechter, Switzerland
P. Galzy, France
K. Jarl, Sweden
H. Klaushofer, Austria, Chairman
L. Rodrigues de Miranda, The Netherlands
S. Nagai, Japan
U. Sleytr, Austria, Secretary
G. Stewart, Canada
H. Suomalainen, Finland
H. Verachtert, Belgium
T. Wiken, The Netherlands
S. Windisch, German Federal Republic

At the meeting of the Executive Board the following was decided:

1). To accept the offer of Prof. P. Galzy, France, to organize a specialized yeast symposium on "Genetics and Metabolism" in September 1978.

2). To accept the offer of Dr. G. Stewart, Canada, to organize a specialized symposium on "Yeasts for Industrial Use" in London, Ontario, in July or August 1980, simultaneously with the "Sixth International Fermentation Symposium".

3). To consider the proposed statute for the commission on yeast and yeast-like microorganisms at one of the next yeast symposia (in Hungary or Canada).

4). Dr. G. Stewart and Dr. M. Heslot were nominated as new members of the Commission as representatives of Canada and France, respectively.

Proposal for a Statute of the International Commission for Yeasts

Czechoslovak Yeast Specialists
Bratislava, 1976 05 28

Article 1: History. In the year 1967 the Council for Yeast Research, composed of prominent specialists in the field of yeasts was established in Bratislava. In 1971 the Council was transferred into the International Association of Microbiological Societies (IAMS).

Article 2: Name. The name of the organization shall be the International Commission for Yeasts and Yeast-like Microorganisms (ICY).

Article 3: Structure. The International Commission for Yeasts shall be an organization within the IAMS. For this reason the ICY is subject to the statute of IAMS and shall establish only specialized by-laws.

Article 4: Affiliation. Any regional yeast commission organized within a National Microbiological Society irrespective of size or location may seek affiliation with ICY.

Article 5: Headquarters of ICY. Headquarters of ICY shall be the office of the Chairman and Secretary of ICY elected for a term of five years.

Article 6: Election of the Chairman and Secretary. The Chairman and Secretary of ICY, for a term of five years, shall be the Chairman and Secretary of the Organizing Committee of the last General Symposium.

Article 7: Membership. Membership of ICY shall be of two types: affiliated and sustaining. Affiliated membership shall be open to National Yeast Commissions established within the National Microbiological Societies. Sustaining membership shall be open to any individual from those regions, where the Microbiological Society was not established or where the Yeast Commission within the Micro-

biological Society does not exist; an affiliated member is represented by one or more individuals, sustaining members shall have all the rights and privileges of ICY. Membership shall not be on national basis nor shall there be any restriction of the number of members from one nation or institution.

Article 8: Objectives. The general objectives of ICY shall be:

a). To establish an effective liaison between persons and organizations concerned with investigations of yeast and yeast-like organisms and between them and practical users of results of investigations (inclusive yeast culture collections).

b). The ICY shall organize the international trend of investigations (i.e., questions to be solved) and undertake the relations between individual investigators (ICY shall give the list of some questions, which need to be solved completely by various specialists, and shall be the mediator between individual investigators).

c). ICY shall coordinate in some important questions, investigation of which shall be supported financially by individual organizators (industry, UNESCO, etc).

d). ICY shall establish a taxonomic subcommission for the determination of new species (genera). The discovery of new species (genera) be patented in many countries (the diplom for discovery) and therefore it is necessary that the membership in this subcommission is represented by taxonomists from those countries where such patent laws exist and great collections have been built.

e). ICY shall publish a journal for communication. This journal shall be the "Yeast-News Letter" edited by Dr. H. J. Phaff with an international editorial board.

f). ICY shall organize conferences and symposia on topics and problems of common interest. Each five years a General Symposium and if possible any year in the meantime a Specialized Symposium shall be held. Members of ICY shall be informed about regional conferences of yeasts.

Article 9: Activities. The activities of the ICY shall be conducted through the General Assembly, composed of all members of ICY and the Executive Board of ICY (EBICY).

Article 10: Executive Board. The Executive Board of ICY shall consist of up to four members from one country. The membership of EBICY shall reflect the various interests of the membership of the ICY.

Article 11: Officers of the EBICY. The officers of the EBICY shall be a Chairman, a Vice-Chairman, and a Secretary. The officers of the EBICY shall be automatically the Chairman and Secretary of the Organizing Committee of the last General Assembly and General Symposium in one five year term, the Vice-Chairman is automatically the retiring Chairman of the previous General Symposium. The word "term" refers to the time elapsing between successive symposia of the General Assembly

(see Article 5). The officers of the EBICY shall retire at the next General Assembly (after a five year term). The retiring Chairman shall be the Honorary Chairman and shall remain a member of EBICY permanently; in addition the retiring Chairman of the Council (see Article 1) shall be Honorary Chairman of the ICY.

Article 12: Duties of the EBICY and its officers. the EBICY shall preside over the work of ICY, execute requests of the General Assembly and generally promote the objectives of ICY as defined in Article 8 hereof. The Chairman shall preside at meetings of the General Assembly of ICY and at meetings of EBICY. The Vice Chairman shall, in the absence of Chairman, perform the duties and exercise the powers of the Chairman. The Secretary shall record the minutes of meetings of the General Assembly and of the EBICY. He shall be responsible generally for the maintenance of effective liaison between the EBICY and affiliated organizations and individual members of ICY. He shall prepare reports of EBICY for presentation to the General Assembly and its meetings.

Article 13: Meetings. Ordinary meetings of the General Assembly (GAICY) shall be held at the General Symposium in five year intervals if necessary. Extraordinary meetings may be held only by EBICY. Meetings of EBICY shall be held at the Specialized and General Symposia. By this point, the place of meetings are determined. Members unable to attend the meeting of the General Assembly appoint a proxy by writing to the Secretary. Two thirds of the members of EBICY shall constitute a quorum for the meetings of the board.

Article 14: Sponsoring organizations. It should be underlined that those countries where Microbiological Societies were established and are affiliated members of IAMS, pay membership fees. Therefore membership in this organization (ICY) shall be free of charge. The Chairman and Secretary to be elected shall have all facilities and economic base in their own country enabling them to organize the General symposium. The representatives of sponsoring organizations shall be invited to the General Assembly and Symposium.

Article 15: Applications of membership and notices of registration. Application for membership or notices of registration shall be made in writing to the Secretary of ICY. Applications for membership shall be provided for those proving permanent investigative activity in the field of yeast research. Membership may be terminated by the General Assembly on the recommendation of the EBICY.

Article 16: Rights and privileges of membership. All members shall enjoy:

a). Full participation in the affairs of the ICY except where otherwise stated in statutes.

b). Information on the various symposia organized by ICY.

c). Receipt of copies of the journal "Yeast-News Letter" against the subscription rate, sent to the editor.

Article 17: Obligations of membership. No member shall use his connection with the ICY to further interests of his or any other

organization except as is provided for in the statutes. The ICY shall not be responsible for the utterance or acts of its individual members.

Article 18: Amendments to the statutes. Any proposals for amendments to the statutes may be made in writing to the secretary of the ICY at least six months prior to a meeting of the General Assembly of the ICY.

This proposal was elaborated by: Kockova-Kratochvilova A.,
Beran K.,
Minarik E.

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B. Ctr. Res. Inst., Suntory Ltd., 475 Hirose, Shimamoto-cho, Mishima-gun, Osaka 618, Japan. Communicated by Isamu Takano.

The Ninth Annual Meeting of Yeast Genetics Conference-Japan

The Ninth Annual Meeting of the Yeast Genetics Conference-Japan was held on September 30 to October 2, 1976 at Kinro-fukushi Kaikan, Tokyo, Japan. Around eighty yeast researchers met and the following topics were presented and discussed.

Session 1: Mutation and Radiation Effects (Chairman - S. Nakai).
1. S. Nakai and I. Machida (Div. Genet., Natl. Inst. Rad. Sci., Anakawa-cho, Chiba 280) - Genetic pathways of rad genes controlling radiation induced recombination and repair.

2. T. Saeki (Div. Genet., Natl. Inst. Rad. Sci.) -Liquid-holding recovery from gamma-ray-induced lethal damage in rad mutants of yeast.

3. T. Ito (Inst. Phys., Col. Gen. Edu., Univ. Tokyo, Komaba, Meguro-ku, Tokyo 153) - Conformation change in stationary chromatin after low dose of UV.

4. K. Kobayashi and T. Ito (Inst. Phys., Col. Gen. Edu., Univ. Tokyo) -In vivo photodynamic activities of various sensitizers.

5. A. Fukui (Biophy. Lab., Dept. Phys., Rikkyo Univ., Nishiikebukuro, Toshima-ku, Tokyo 171) - Determination of the number of photoreactionating enzyme molecules in yeast UV^{S}_{1} by single flash photolysis.

Session 2: Recombination, Fine Structure and Mapping (Chairman - T. Takahashi).
6. T. Takahashi (Suita Lab., Brew. Sci. Res. Inst., Deguchicho, Suita 564) - Gene conversion and crossing over.

7. S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., Yamadakami, Suita 565) - Genetic mapping by three factor analysis using tetrad data.

Session 3: Gene Regulation (Chairman - Y. Oshima).
8. T. Oshima, K. Arima and I. Takano (Ctr. Res. Inst., Suntory Ltd., Hirose, Shimamoto-cho, Mishima-gun, Osaka 618) - Genetical and biochemical studies of glucoamylase in Saccharomyces yeast; Relation between mating type and the enzyme activity.

9. S. Inoue, K. Matsumoto, A. Toh-e and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., Yamadakami, Suita 565) - Fine structure mapping of the regulatory genes of positive control.

Session 4: Cytoplasmic Inheritance and Drug Resistance (Chairmen - I. Mifuchi and N. Gunge).

10. N. Gunge (Ctr. Res. Lab., Mitsubishi Chem. Indust. Ltd., 1000 Kamoshida, Midori-ku, Yokohama 227) - Gene dosage effect in yeast mitochondrial genetics.

11. K. Suda, M. Takagi, M. Maekawa, K. Mori (Biol. Lab., Nara Univ. Educ., Nara 630) and A. Uchida (Biol. Div., Col. Gen. Educ., Kobe Univ.) - Recombination of mitochondrial genes with isogenic yeast strains.

12. K. Suda, M. Imamura and S. Nagai (Dept. Biol., Fac. Sci., Nara Women's Univ. Nara 630) - Genetics of Blasticidin S resistance in Saccharomyces (II).

13. Y. Iwamoto and I. Mifuchi (Dept. Microbiol., Shizuoka Col. Pharm., Kojika, Shizuoka 420) - The appearance of Antimycin resistant variants of Candida utilis.

14. I. Mifuchi, M. Fukunaga, T. Morita (Dept. Microbiol., Shizuoka Col. Pharm.) and K. Wakabayashi (Dept. Biochem., Fac. Med., Univ. Tokyo) - The loss of the drug resistance mitochondrial genetic markers in yeast by 4-nitroquinoline 1-oxide.

15. J. Ishiguro and Y. Arakatsu (Dept. Biol., Fac. Sci., Konan Univ., Okamoto, Kobe 658) - Ribosomal proteins from cycloheximide-sensitive and -insensitive yeasts.

16. K. Wakabayashi (Dept. Biochem., Fac. Med., Univ. Tokyo, Hongo 1-7-3, Bunkyo-ku, Tokyo 113) - Fragment analysis of yeast mitochondrial DNA by restriction endonucleases.

17. F. Miyamoto (Fac. Educ., Univ. Wakayama, Masago-cho, Wakayama 640) - Effect of glucose on the RD induction of yeast by p-nitrophenol.

18. S. Nagai, N. Kane, S. Ochi (Dept. Biol., Fac. Sci., Nara Women's Univ., Nara 630), K. Kawai (Fac. Sci., Osaka Univ.) and T. Yamazaki (Dept. Ferment. Technol., Yamanashi Univ.) - Further characterization of respiratory mutants in Saccharomyces ludwigii.

Session 5: Biochemistry and Metabolism (Chairman - M. Hayashibe).
19. H. C. Bhandai and M. Hayashibe (Dept. Biol., Fac. Sci., Osaka City Univ., Sumiyoshi-ku, Osaka 558) - Utilization of hexoses in fission yeast; Schizosaccharomyces pombe: Purification and properties of glucose and mannose binding proteins.

20. I. Nakamura, Y. Nishikawa, Y. Omura, N. Isobe, T. Kamihara and S. Fukui (Lab. Indust. Biochem., Fac. Engi., Kyoto Univ., Yoshidahonmachi, Sakyo-ku, Kyoto 606) - Action of vitamine B_1 and B_6 of yeast: Effect of δ-aminolevulinate on the B_1-induced respiratory deficiency and alternation in lipid metabolism.

21. C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ., Sumiyoshi-ku, Osaka 558) - Secretion of exo-β-glucanase in Saccharomyces cerevisiae.

Session 6: Structure and Function of Cell Organelles (Chairmen - T. Hirano and M. Osumi).

22. N. Kawakami, H. Mondo (Dept. Ferment. Technol., Fac. Engi., Hiroshima Univ., Senda-cho, Hiroshima 730) and H. Kawakami (Dept. Nutri., Suzugamine Women's Col.) - Fusion of protoplasts in Saccharomyces cerevisiae.

23. T. Hirano (Tokyo Metropol. Isotope Res. Inst., Fukazawa, Setagaya-ku, Tokyo 158) - The relationships between glycogenesis and cytoplasmic granules in yeast cells.

24. M. Osumi (Dept. Biol., Japan Women's Univ., Mejirodai, Bunkyo-ku, Tokyo 112) - Ultrastructure of yeast cells on the observation by frozen-thin sectioning.

Session 7: Sexuality and Life Cycle (Chairmen - N. Yanagishima and I. Takano).

25. N. Yanagishima (Biol. Inst., Fac. Sci., Nagoya Univ., Chikusa-ku, Nagoya 464) - Regulation of sexual agglutinability in Saccharomyces cerevisiae.

26. A. Sakurai, S. Tamura (Inst. Phys. Chem. Res., 2-1-1 Hirosawa, Wako-shi, Saitama 351), N. Yanagishima (Biol. Inst., Fac. Sci., Nagoya Univ.) and C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.) - Isolation and structure of a peptidyl factors, α-substance I_A, inducing sexual agglutinability in Saccharomyces cerevisiae.

27. M. Tsuboi (Dept. Biol., Fac. Sci., Osaka City Univ., Sumiyoshi-ku, Osaka 558) - Ribonucleases during sporulation of Saccharomyces cerevisiae.

28. M. Hayashibe (Dept. Biol., Fac. Sci., Osaka City Univ.) - Spore formation in Schizosaccharomyces japonicus.

29. C. Shimoda, K. Nishi and M. Hayashibe (Dept. Biol., Fac. Sci., Osaka City Univ.) - Germination and outgrowth of ascospores in Schizosaccharomyces pombe.

30. I. Takano and T. Oshima (Ctr. Res. Inst., Suntory Ltd., Hirose, Shimamoto-cho, Mishima-gun, Osaka 618) - Conversion of mating types and polyploidization of diploid cells by the action of homothallism genes in Saccharomyces.

31. S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., Yamadakami, Suita 565) - On the function of homothallism genes.

32. T. Takahashi (Suita Lab. Brew. Sci. Res. Inst.) - Mutation affecting on the diploidization at germination of homothallic ascospore. - Dominance of homothallism.

33. H. Mori (Noda Inst., Sci. Res., 399 Noda, Noda 278) - Ploidy, mating type and salt tolerance of Saccharomyces rouxii, IFO strains.

34. T. Yamazaki (Dept. Ferment. Technol., Yamanashi Univ., Takeda, Kofu 400) - Nuclear events in the single-spore cultures of Saccharomycodes ludwigii.

Session 8: Reports from International Meetings (Chairman - T. Ito).
35. S. Nagai (Dept. Biol., Fac. Sci., Nara Women's Univ.) - Reports on the 4th International Specialized Symposium on Yeast held in Berlin June 28 - July 3, 1976. - Topics in the 8th International Conference on Yeast Genetics and Molecular Biology held at Schliersee near Munich, August 29 - September 3, 1976.

36. K. Wakabayashi (Dept. Biochem., Fac. Med., Univ. Tokyo) - Topics in the Interdisciplinary Conference on the Genetics and Biogenesis of Chloroplasts and Mitochondria.

Yeast researchers interested in these papers are welcome to contact the author. The next annual meeting of the yeast genetics conference-Japan will be held in Kyoto or Osaka in September, 1977.

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C. Kobo-Kenkyukai Inst. Ferment. Osaka Jusohommachi 2-17-85, Yodogawa, Osaka 532, Japan. Communicated by I. Banno.

presents information concerning the first and the second general Yeast Symposium in Japan.

1. The first general Yeast Symposium, Japan, organized by the Kobosaibo-Kenkyukai was held on May 28 and 29, 1974 at E-zai Hall, Tokyo. Around 270 yeast specialists met, and subjects on the theme of Metabolism and Physiology were presented and discussed. The proceedings of this meeting (in Japanese) were published by the executive committee of Kobosaibo-Kenkyukai (Secretary; Dr. M. Osumi, Dept. Biol., Japan Women's Univ., Mejirodai, Tokyo 112).

2. The second general Yeast Symposium, Japan, organized by Kobo-Kenkyukai was held on May 27 and 28, 1976 at Takeda Chem. Co., Training Center, Suita City, Osaka. Around 200 yeast scientists met, and the following subjects on the theme of Adaptation and Regulation were presented and discussed. The proceedings of this meeting (in Japanese) will be edited by the executive committee of Kobo-Kenkyukai (Dr. T. Hasegawa, Inst. Ferm., Juso-honmachi, Osaka 532) and published through University of Tokyo Press (Hongo, Tokyo 113), next March.

Scientific program:

1). T. Yamamoto, I. Okumura, K. Kondo, K. Itaya, Y. Miyake (Fac. Sci., Osaka City Univ., Osaka 558): Induction, locality, and mechanism of catalitic reaction series of enzymes involved in metabolism of uric acid of Candida utilis.

2). R. Hayashi, T. Hata (Res. Inst. Food Sci., Kyoto Univ., Uji, Kyoto 611): Yeast proteases and their biological functions.

3). T. Saheki, H. Betz*, H. Matern*, Y. Matsuda**, T. Katsunuma, H. Holzer* (Dept. Biochem., School Med., Tokai Univ., Isebara City, Kanagawaken 259-11, *Biochem. Inst., Univ. Freiburg, **Inst. Enz. Res., Sch. Med., Tokushima Univ., Tokushima): Proteinases and specific inhibitors in yeast.

4). A. Tohe, Y. Oshima (Dept. Ferm. Tech., Fac. Eng., Osaka Univ., Osaka 565): Genetic regulation of acid phosphatase synthesis in yeast.

5). K. Abe, S. Fukui (Inst. Appl. Microbiol., Univ. Tokyo, Tokyo 113): Cell morphology and nucleus behavior during early stages of mating process in Rhodosporidium toruloides.

6). K. Hieda (Biophys. Lab., Fac. Sci., Rikkyo Univ., Tokyo 171): Genetic effect of dehydration on Saccharomyces cerevisiae.

7). T. Murayama, H. Tohoyama, N. Naiki* (Biol. Inst., Ehime Univ., Matsuyama 790, *Lab. Biol., Fac. General Education, Gifu Univ., Gifu): Resistance of yeast to heavy metals.

8). T. Yoshida (Lab. Appl. Microbiol., Fac. Agr., Hokkaido Univ., Sapporo 060): Ecological studies on yeasts of Soya mashes.

9). S. Hayashida, M. Hongo (Lab. Appl. Microbiol., Fac. Agr., Kyushu Univ., Fukuoka 812): Effects of environmental factors on alcohol-tolerance of yeast.

10). T. Imamura, M. Kawamoto, Y. Takaoka (Tatsuma Honke Shuzo Co., Ltd., Nishinomiya City 662); Studies on killer yeast in Saké brewing.

11). H. Tanaka*, E. Takenouchi, K. Soda (Lab. Microbiol. Biochem., Inst. Chem. Res., Kyoto Univ., Uji, Kyoto 611, *Lab. Biochem., Kyoto College of Pharmacy): Production of lysine by lysine analogue-resistant mutants of Candida pelliculosa and its mechanism.

12). A. Kimura (Dept. Food Sci. Tech., Fac. Agr., Kyoto Univ., Kyoto 606): Structure and function of yeast cells in fermentative formation of cytidine coenzymes.

13). T. Tabuchi (Inst. Appl. Biochem., Univ. Tsukuba, Ibarakiken 300-31): Organic acid fermentation and metabolism of even- and odd-carbon n-alkanes by yeasts.

14). A. Tanaka, M. Mishina, S. Fukui (Lab. Ind. Biochem., Fac. Eng., Kyoto Univ., Kyoto 606): Fatty acid metabolism in hydrocarbon-assimilating yeasts.

15). T. Kamiryo, M. Mishina, S. Parthasarathy, S. Numa (Dept. Med. Chem., Fac. Med., Kyoto Univ., Kyoto 606): Regulation of fatty acid biosynthesis in yeast.

Yeast researchers interested in these papers are welcome to contact the author. The 3rd general meeting will be held in Tokyo in May, 1978.

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The Society for Industrial Microbiology will hold its 28th Annual Meeting August 21-26, 1977 at Michigan State University East Lansing, Michigan concurrently with the American Institute of Biological Sciences. Registration with SIM will provide admission to all sessions.

The SIM program includes symposia on Microbiological Testing for Mutagenicity, Cosmetic Microbiology, Bacterial Vaccines, Genetic Engineering and Plant Cell Culture. Contributed papers on a wide variety of topics will be included.

For further information contact Mrs. Ann Kulback, Society for Industrial Microbiology, 1401 Wilson Boulevard, Arlington, VA, 22209.

XXXIII. Brief News Items

1. The article "Ultrastructure of the ascospores of some species of the Torulaspora group" by N. J. W. Kreger-van Rij and M. Veenhuis will appear in Antonie van Leeuwenhoek, last issue 1976.

   A second article from this laboratory "Conjugation in the yeast Guilliermondella selenospora Nadson et Krassilnikov" by N. J. W. Kreger-van Rij and M. Veenhuis has appeared in the Canadian Journal of Microbiology, 22, 960-966, 1976.

   N. J. W. Kreger-van Rij
   Laboratory for Medical Microbiology R.U.
   Oostersingel 59
   G oningen, Holland

2. I have accepted the post of Chairman of the Department of Biology at the University of Winnipeg, Winnipeg, Manitoba, Canada, from January 1, 1977. I am hoping to continue research on sterol mutants and the control of purine biosynthesis - administration and other commitments permitting!

   R. A. Woods
   The University of Sheffield, England
   Department of Genetics

3. Berliner, M. D., C. Ryan. 1976. Autofluorescence versus Immunofluorescence of Candida. Microbios 14:143-149.

I would like to ask the readers of the News Letter of any records they may have of yeast autofluorescence. The literature is very scanty and there is only one reference to yeast fluorescence to my knowledge.

Martha D. Berliner
Simmons College
300 The Fenway
Boston, MA 02115

4. The following is the title of a note from this laboratory: Loss of mitochondrial DNA in respiration-deficient mutant of yeast induced by 4-nitroquinoline 1-oxide. Tamotsu Morita, Masahito Fukunaga, and Ichiji Mifuchi. Gann, 66, 697-700, Dec. 1975.

I. Mifuchi, Dept. of Microbiology
Shizuoka College of Pharmacy
2-2-1, Oshika, Shizuoka-Shi, Japan

5. Section 915 of "Standard Methods for the Examination of Water and Waste Water" covers the Fungi. Included in the current (14th) edition is a section of that section which mentions yeasts.

Readers of the Yeast News Letter who see this book may be interested in suggesting additional approaches which might be used in the study of yeasts in the aquatic environment whether of public health interest or not. The Joint Task Group on Fungi, of which I am Chairman, would appreciate input from anyone for improvement of the chapter which will appear in the second to next (16th) edition. A manuscript for the next (15th) edition has been submitted and accepted.

Wm. Bridge Cooke
1135 Wilshire Ct.
Cincinnati, Ohio, 45230

6. From R. J. Pankhurst and J. A. Barnett

Subject: Identification of Yeasts

On page 23 of A New Key to the Yeasts (J. A. Barnett and R. J. Pankhurst, North-Holland, 1974), we described a system of identifying yeasts by means of punched cards. We wish to draw attention to the availability of this system, and to its merits over a conventional key.

1). Any set of tests may be used, just as they appeal to the user.

2) The tests may be done and evaluated in any order.

No computer is necessary for the identification of yeasts by the use of these cards. The punched card key is available with a sheet of instructions and a list of species. The cost, inclusive of package and posting, is either:

a. For the United Kingdom, 10 pounds first copy, further copies at 2 pounds each;

b. For overseas, 25 US dollars (or equivalent) for first copy, further copies 5 dollars each.

Enquiries and cheques to:

R. J. Pankhurst
203 Sheen Lane
London SW14 8LE, England

7. Committee D-19 on water of the American Society for Testing and Materials (ASTM) has established recently two task groups on water quality which include aspects of yeast distribution and ecology. Interested persons should contact the following Task Group Chairmen for details; we need input from workers with methods for field enumeration and laboratory identification, specific definitions of organisms, and data on occurrence and distribution of yeasts in natural waters.

Fungi in Aquatic Environments:

Dr. Alvin L. Rogers
Dept. of Botany and Plant Pathology
Michigan State University
East Lansing, MI 48823

Candida albicans in aquatic environments:

Dr. John D. Buck
University of Connecticut
Marine Research Laboratory
P. O. Box 278
Noank, CT 06340

8. THE SAD SAGA OF SACCHRO

I've a story to tell of a beast,
This particular beast is a yeast;
He could eat no more,
so became a spore,
To await a more yeastable feast.

Now the nitrogen level was great,
And there's glucose, ade, his, aspartate!
"I'll a haploid no more!"
Cried this spore with a roar,
"Bring the combs or the spray, bring a mate!"

So this alpha went seeking an "a",
For to get her to go all the way;
Saw a lovely petite,
His minus, but sweet,
So he wooed her without delay.

First they talked of her his disorder,
But his sensitivities soon floored her.
     He was quite an agressor
     (Contained a suppressor)
Before the hour he had scored her.

They lived as a diploid in bliss,
Reproducing without any his.
     But their bliss was abated
     When he sadly mutated.
His and miss, alas -- the abyss.

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