Y E A S T

A Newsletter for Persons Interested in Yeast

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

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

Herman J. Phaff
Editor

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

1. Below follows the abstract of Dr. Kay Schmeding's Ph.D. dissertation entitled, "Filobasidiella neoformans, the perfect state of Cryptococcus neoformans." The work was carried out at the ATCC as a part of the authentication program supported by the NSF Grant DEB-75-06286 to S. C. Jong. The degree was granted by the Graduate School of Arts and Sciences of the George Washington University, Washington, D.C.

ABSTRACT

Ninety-seven strains of yeast described as Cryptococcus neoformans, C. neoformans var. gattii, and C. bacillisporus were examined for 44 physiological characters and the results were analyzed numerically. One phenon emerged at the 86% level of similarity when strains were clustered according to their M-similarity values.

Forty-eight of the 97 strains of yeast produced the basidiomycetous perfect state either alone or when paired with a strain of compatible mating-type. Thirty-six strains were α mating-type, four strains were a mating-type and eight strains were self-fertile. Filobasidiella neoformans serovars A and D were interfertile with compatible mating-types of F. bacillispora serovars B and C. Cryptococcus neoformans var. gattii was interfertile with compatible mating-types of F. neoformans and F. bacillispora. F. bacillispora strains which utilized creatinine and L-malic acid were interfertile with compatible mating-types of F. neoformans which did not utilize creatinine and L-malic acid.

The similarity in the life cycles of serovars and biovars eliminates the need for recognizing the names Cryptococcus neoformans var. gattii, C. bacillisporus, and Filobasidiella bacillispora. It is proposed that C. neoformans var. gattii and C. bacillisporus be regarded as later, facultative synonyms of C. neoformans and that F. bacillispora be regarded as a later, facultative synonym of F. neoformans.

Single basidiospores were isolated from one self-fertile strain, ATCC 34868, and from two pairs of compatible mated strains, ATCC 28958 and ATCC 32735, and ATCC 34875 and ATCC 34631; α and a mating-type monosporous colonies evolved from the single basidiospores produced by the self-fertile strain and the mated strains. Each monosporous colony was interfertile with monosporous colonies in the same series, with monosporous colonies in another series, with parental colonies, and with additional fertile strains of compatible mating-type. Biallelic bipolar incompatibility was confirmed.

Mating strains produced dikaryotic hyphae, complete clamps, and basidia with basipetal chains of spores; self-fertile strains produced monokaryotic hyphae, incomplete clamps, and basidia with basipetal chains of spores. Four self-fertile strains produced dikaryotic hyphae and complete clamps when paired with a mating-type strains. One self-fertile strain produced dikaryotic hyphae and complete clamps when paired with α mating-type strains.

* * *

2. The strains listed below have been added to the ATCC since May 22, 1980. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

Saccharomyces bayanus ATCC 42506 investigation of sulfate assimilation	W. Dott Meckenheimer Allee 168 D-5300 Bonn West Germany
Saccharomyces cerevisiae var. ellipsoideus ATCC 42507 - 42508 investigation of sulfate assimilation	"
Saccharomyces cerevisiae ATCC 42905	S. M. Parmerter CPC International Argo, Illinois
Saccharomyces cerevisiae ATCC 42510 - 42511 succinic acid production	Dr. Kuczyaski Johannes Gutenberg Univ. Germany
Saccharomyces cerevisiae ATCC 42512 single spore isolate	R. E. Kunkee University of California Davis, CA
Metschnikowia bicuspidata ATCC 42519 - 42520 taxonomy	D. M. Spencer Goldsmith's College University of London England
Torulopsis bovina ATCC 42531 investigation of purine and sulfur metabolism	N. Polasa Osmania University Hyderabad - 7 (A.P.) India
Saccharomyces cerevisiae ATCC 42532 assay of α-factor	D. B. Finkelstein University of Texas Dallas, Texas
Saccharomyces cerevisiae ATCC 42544 - 42545 genetic strains	F. Boulay Centre de Recherche Foundamentale 38041 Grenoble Cedex France
Saccharomyces cerevisiae ATCC 42546 genetic studies	L. H. Johnston The Nat'l Inst. for Medical Researcl Mill Hill, London NW7 1AA England
Rhodotorula matritense ATCC 42552 type culture	C. Ramirez Joaquin Costa 32 Madrid, Spain

Saccharomyces cerevisiae ATCC 42563 - 42564 genetic strains	G. Johnston Dept. Microbiology Dalhousie University Canada
Saccharomyces cerevisiae ATCC 42595 - 42600 genetic studies	D. Mowshowitz Columbia University New York, N.Y.
Saccharomyces cerevisiae ATCC 42607 genetic studies	M. Frankenberg-Schwager 6000 Frankfurt West Germany
Saccharomyces cerevisiae ATCC 42673 - 42677 genetic studies	T. D. Petes University of Chicago Chicago, Illinois
Candida paratropicalis ATCC 42678 type culture	J. Baker Analytab Products Plainview, N.Y.
Saccharomyces cerevisiae ATCC 42679 - 42682 genetic strains	T. D. Petes University of Chicago Chicago, Illinois
Saccharomyces cerevisiae ATCC 42711 genetic studies	D. Siebert University Freiburg Germany
Candida acutus ATCC 42713 type culture	S. Goto Yomonishi University Japan
Candida placentae ATCC 42714 type culture	"
Candida mamillae ATCC 42715 type culture	"
Saccharomyces cerevisiae ATCC 42716 genetic studies	C. P. Hillenburg University Dusseldorf Germany
Candida lusitaniae ATCC 42720 isolated from leukemia patient	D. Pappagianis University of California Davis, CA
Saccharomyces cerevisiae ATCC 42747 - 42752 cytogenetic	N. Gunge Mitsubishi Chem. Indust. Yokohama 227 Japan

Saccharomyces cerevisiae ATCC 42800 genetic studies	T. M. Nisbet University Durham England
Torulopsis magnoliae ATCC 42811 ecological	M. Gilliam USDA Tucson, AZ
Rhodotorula rubra ATCC 42830 isolated from bottled pears	G. Szakacs University Tech. Sciences Budapest, Hungary
Saccharomyces cerevisiae ATCC 42879 - 42880 multiple drug resistant strains	G. H. Rank University Saskatchewan Canada
Cryptococcus albidus var. albidus ATCC 42920 soil yeast from Kenya	F. Fatichenti 07100 Sassari Italy
Cryptococcus albidus var. aerius ATCC 42921 soil yeast from Kenya	"
Cryptococcus laurentii var. laurentii ATCC 42922 soil yeast from Kenya	"
Kloeckera apiculata ATCC 42923 soil yeast from Kenya	"
Rhodotorula glutinus var. glutinus ATCC 42924 soil yeast from Kenya	"
Trichosporon pullulans ATCC 42925 soil yeast from Kenya	"
Saccharomyces cerevisiae ATCC 42926 - 42949 wine yeasts	R. E. Kunkee University of California Davis, CA
Saccharomyces cerevisiae ATCC 42950 - 42951 genetic studies	S. G. Oliver The Canterbury University Kent CT2 7N5 England

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

The following articles have been published recently:

Golubev, W.I., A.R. Manukian, A.B. Tsiomenko and V.M. Ushakov, 1980. Preparation of yeast capsular material. Prikl. Biochim. Mikrobiol. (Appl. Biochem. Microbiol.), 16, N 4, 591-596.

Parameters of the treatment of yeast cell suspensions in an ultrasonic homogenizer MSE-150 were selected in such a way as to assure cell intactness as well as preparation of undegraded and uncontaminated capsular material.

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Blagodatskaya, V.M., W.I. Golubev, N.G. Yanayeva and R.Sh. Trotsenko, 1980. A comparative study of Candida zeylanoides and Candida vinaria. Mikrobiologia, 49, N 2, 298-301.

C. zeylanoides and C. vinaria were found to differ in the assimilation of nine carbon sources, vitamin requirements, resistance to elevated NaCl concentrations, and the ability to utilize hydrocarbons. On the basis of their cell wall ultrastructure as observed by TEM C. vinaria appears to be related to the ascomycetous yeasts while C. zeylanoides to basidiomycetous ones.

* * *

Golubev, W.I., 1980. Taxonomy and identification of yeast fungi of the genus Cryptococcus. Pushchino. 90 pp.

In the opinion of the author the genus Cryptococcus should be restricted to asporogenous yeasts with basidiomycetous affinity. Species of this genus are defined as non-fermentive, urease-positive yeasts that produce extracellular xylose-containing and amylose-like (in acidic media only) polysaccarides. They can utilize pentoses, glucuronic acid and as a rule inositol as carbon sources for growth. Pseudomycelium, true mycelium may be formed, but no arthrospores. In accordance with this amended diagnosis, the species Candida amylolenta, C. curvata, C. humicola, C. marina and C. podzolica are transferred to the genus Cryptococcus and the following species are excluded from this genus: Cr. cereanus, Cr. infirmo-miniatus, Cr. lactativorus, Cr. macerans and Cr. melibiosum. Cr. neoformans (Sanfelice) Kwon-Chung is considered as the type species of the genus Cryptococcus.

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Blagodatskaya, V.M., Utkina, L.I., and I.S. Utkin, 1980. The yeasts of the genus Candida Berkhout (Systematics, identification). Pushchino. 125 pp.

In computer-produced keys 134 species are included. Using minimal number of tests these keys ensure maximal reliability of species identification.

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

1. The following paper has been accepted for publication:

Y. Yamada, Hisano Takinami-Nakamura, Y. Tahara and Maudy Th. Smith 1980. The coenzyme Q system in the classification of the ascosporogenous yeast genus Dekkera and the asporogenous yeast genus Brettanomyces. Antonie van Leeuwenhoek, Journal of Microbiology (in press).

ABSTRACT

Fourteen strains of the genera Dekkera and Brettanomyces were examined for the Co-Q system. Without exception they contained the Q-9 system. The results are discussed from the taxonomic point of view.

* * *

2. Submitted for publication in Antonie van Leeuwenhoek:

L. Rodrigues de Miranda, Wilma H. Batenburg-v.d. Vegte. Cryptococcus mollis Kutzing, type species of the genus Cryptococcus; investigation of the type material.

IV. University of Miami, School of Marine and Atmospheric Science, 4600 Rickenbacker Causeway, Miami, Florida 33149. Communicated by J.W. Fell.

Request for strains of Leucosporidium (Candida) scottii

We've been investigating the sexual incompatibility system in this yeast, which is a multiple allelic bifactorial system and we find, what appears to be, more than 2 alleles at both the A and B factors. So far, we've identified 5 A and 3 B factors. We'd like to determine if there are more A and B factors and if there are any specific substrates or geographical distributions for the factors. We'd appreciate any available strains of L. scottii, including homothallic strains.

Recent and pending publications

1) Fell, J. and A. Tallman. 1980. Mating between strains of the yeasts Aessosporon salmonicolor and Sporobolomyces spp. International Journal of Systematic Bacteriology 30(1): 206-207.

2) Yarrow, D. and J. Fell. 1980. Validation of the name of the yeast Sporobolomyces holsaticus Windisch. Mycotaxon 12(1) in press.

3) Fell, J. and A. Tallman. 1980. Rhodosporidium paludigenum sp. nov., a basidiomycetous yeast from intertidal waters of S. Florida. International Journal of Systematic Bacteriology (October Issue). In press.

4) Fell, J. and A. Tallman. 1981. Heterothallism in the basidiomycetous yeast genus Sporidiobolus Nyland. Current Microbiology. Accepted.

V. Department of Microbiology, Faculté de Pharmacie, Avenue Charles Flahault, 34060 MONTPELLIER Cedex, FRANCE. Communicated by J.-M. Bastide

Below follow abstracts of recent papers from our laboratory.

1. J.-M. Bastide, M. Mallie, M. Richard. Recent progress in immunological techniques for the diagnosis of systemic mycoses. Dermatologica, 159, suppl. 1, 70-86 (1979).

ABSTRACT

The new immunological techniques used for the diagnosis of systemic mycoses are very specific. Purified antigens are now available and new methods allow the detection of various classes of specific antibodies. Precipitation tests, such as bidimensional electrophoresis and electroimmunodiffusion, are compared with passive agglutination tests; radioimmunoassays and enzyme immunoassays are analyzed. The value and utilization of cellular immunity and various other tests are discussed.

* * *

2. M. Mallie, M. Richard, J.C. Lebecq and J.-M Bastide. Study of various media used to easily identify pathogenic yeasts. Bull. Soc. Mycol. Méd., 8, n° 2 (1979), p. 145-152.

SUMMARY

Three media used to readily identify yeasts have been tested. Yeasts of the genus Candida, Saccharomyces, Torulopsis and Hansenula were cultivated. These media contain either T.T.C., or bismuth sulfite, or phosphomolybdic acid, reduction of which gave a coloration of the culture and/or the medium. The authors tested 62 species representing 108 strains. This methodology allowed to identify rapidly such species as: C. albicans, C. krusei, C. stellatoidea. The classical methods of identification have to be applied to confirm the diagnosis.

* * *

J.-M. Bastide, D. Scheiber, E. Hadibi, S. Jouvert and M. Bastide. The cell-wall enzymolysis tests: taxonomic study of genera Candida and Torulopsis. Advances in Protoplast Research, p. 221-227, Proceed. Vth Internat. Protoplast Symposium, July 9, 1979, Szeged (Hungary), Publishing House of the Hungarian Academy of Sciences.

E. Gueho, S. Jouvert, M. Bastide. Taxonomic position of genus Geotrichum. Bull. Soc. Mycol. Méd., 8, n° 1 (1979), p. 95-98.

E. Gueho, S. Jouvert, M. Bastide. Taxonomic study of Candida curvata and Candida marina. Bull. Soc. Mycol. Méd., 9, n° 2 (1980), 181-184.

Using GC % evaluation, scanning electron microscopy and cell-wall enzymolysis tests, the authors presented taxonomic modifications of the genera Candida, Torulopsis, and Geotrichum. They considered that Candida curvata and Candida marina may be related to the genus Cryptococcus.

VI. Institute of Medical Microbiology, Bartholin Building. University of Aarhus, Denmark. Communicated by A. Stenderup.

1. From June 16-20, 1980 the Alfred Benzon Symposium 16 took place in Copenhagen. The theme was: Molecular Genetics in Yeast. 35 speakers from all over the world were invited. The lectures, including discussions, will be published in June 1981 by Munksgaard, Copenhagen: Editors: D. von Wettstein, J. Friis, M. Kielland-Brandt and A. Stenderup.

2. The Danish Society for Mycopathology held its annual meeting in Copenhagen on October 4 and 5. Main subject: Chemotherapy of fungal infections. The next meeting will take place in Cambridge, April 1981 by invitation from the British Society for Mycopathology.

3. From our laboratory the following recent papers on Cryptococci have been published:

   Cryptococcose. Mykosen 23: (2), 68-74, 1979.
   Cryptococcal meningitis. Scand. J. Infect. Dis. 12: 155-157, 1980.

4. Our laboratory has just analyzed the distribution of yeast in 5000 human specimens in which yeast infections were suspected. - The series will be published in "Das Arztliche Laboratorium" in July 1981.

VII. Juntendo University, School of Medicine, 1-1, Hongo 2chome Bunkyo-ku, Tokyo, 113 Japan. Communicated by Ryushi T. Nozawa.

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

Ryushi T. Nozawa, Reiko Sekiguchi, and Takeshi Yokota. 1980. Stimulation by Conditioned Medium of L-929 Fibroblasts, E. coli Lipopolysaccharide, and Muramyl Dipeptide of Candidacidal Activity of Mouse Macrophages. Cellular Immunology 53, 116-124.

A simple, quantitative assay method for microbicidal activity of phagocytic cells was devised using normal mouse peritoneal macrophages as effector cells and Candida parapsilosis as target cells. The macrophages were seeded in 96-multiwell tissue culture plates and infected with serially diluted candida cells. Outgrowth of candida cells in each well was estimated after a 48-hr. incubation period. The maximum number of microbes killed on macrophage monolayers was then determined. The conditioned medium of L-929 cells (L-CM) influenced the fungicidal activity of the macrophages a great deal. An addition of L-CM, to 20% of the culture medium, stimulated the killing activity more than 128-fold, compared with no addition of L-CM. In the medium containing the L-CM, macrophages spread very well on the plastic with several dendritic processes, whereas cells spread poorly and gradually cytolysed in the medium lacking L-CM. It was found that muramyl dipeptide at 100 μg/ml and E. coli lipopolysaccharide at 1-10 μg/ml stimulated the activity 4 to 16 times. An application of this method to destroying other kinds of microbes, measuring the activity of other phagocytes, and screening immunomodulators was discussed.

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

The following two abstracts represent recent projects from our laboratory.

1. D.K. Sandhu and M.K. Waraich. Ecology of Yeasts Associated with Pollinating Bees, Nectary Glands of Flowers and Fermented Foods.

Abstract of paper accepted for poster presentation at the Fifth International Symposium on Yeasts, July 20-25, 1980, London, Canada.

The prevalence of yeasts was studied in 652 samples, including 271 of the honey stomach of pollinating bees, 244 of nectary glands of flowers and 137 of fermented foods. All the samples were positive, yielding yeast strains belonging to 16 genera and 55 species; the common ones among these being Hansenula polymorpha, Endomycopsis fibuligera, H. ciferrii, Candida lipolytica, Pichia polymorpha and Torulopsis candida in order of frequency. The effects of temperature, pH, salt, and sugar concentration were studied to know the optima and range of these factors for the growth of yeasts. There was little variation in the response of different strains of the same species isolated from the same substrate. However, when strains of the same species isolated from different substrates were compared, variation with regard to all the above factors was observed.

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2. D.K. Sandhu and M.K. Waraich. Conversion of Cheese Whey into Single Cell Protein.

Abstract of paper presented at the Sixth International Conference on Global Impacts of Applied Microbiology, August 30th-6th September, 1980, Lagos, Nigeria.

Thirteen yeast species belonging to 9 genera were screened for the production of single cell protein (SCP) using cheese whey as the substrate. Cheese whey supplemented with minerals and yeast extract proved to be the best medium for yield, lactose utilization, biomass production and conversion efficiency. β-Galactosidase production was studied in Brettanomyces anomalus, Kluyveromyces fragilis, Trichosporon cutaneum and Wingea robertsii. The last proved to be the best species combining high yield with a short incubation time.

IX. Department of Biology, Indiana University, Bloomington, Indiana 47405, U.S.A. Communicated by Donald G. Gilbert.

The following paper was recently published:

D.G. Gilbert, 1980. Dispersal of yeasts and bacteria by Drosophila in a temperate forest. Oecologia (Berl.) 46:135-137.

SUMMARY

The dispersal of yeasts, bacteria and molds by Drosophila and Hymenoptera was examined in a deciduous woods in Bloomington, Indiana. Drosophila were found to be significant vectors of yeasts and bacteria, while Hymenoptera also transmitted molds. Analyses of their microbe contents indicated sap feeding Drosophila carried mainly yeasts, fungus feeding Drosophila carried primarily bacteria and ants carried primarily molds.

X. Department of Microbiology, Queen Elizabeth College, University of London, Campden Hill Road, London W8 7AH, England. Communicated by I. Salmon and R.K. Poole

Below follows an abstract of a paper we shall be presenting orally at the 90th Ordinary Meeting of the Society for General Microbiology to be held at Queen Elizabeth College, University of London, 16-18 December 1980.

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

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

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

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

1. Salmon, I. and Poole, R.K. (1978) Bulletin of the British Mycological Society 12, 121.

2. Salmon, I. and Poole, R.K. (1980) Society for General Microbiology Quarterly 7, 85.

3. Galdiero, F. (1973) Experientia 29, 496.

XI. Mitsubishi-Kasei Institute of Life Sciences, 11, Minamiooya, Machida-shi, Tokyo, Japan. Communicated by Norio Gunge

The following is a summary of our recent work on the new yeast plasmids isolated from Kluyveromyces lactis, which will appear in J. Bacteriology.

ABSTRACT

The linear DNA plasmids, designated pGKℓ-1 and pGLℓ-2, were isolated from a yeast Kluyveromyces lactis IFO 1267, which have molecular weights of 5.4×10^6 and 8.4×10^6 daltons, respectively. Both plasmids possessed

the same density of 1.687 g/cm^3, lighter than the densities of mitochondrial (1.692 g/cm^3) and nuclear (1.699 g/cm^3) DNAs. A restriction map with EcoRI, HindIII, PstI and BamHI was constructed on pGKℓ-1. pGKℓ-2 was cleaved with EcoRI into seven fragments and with BamHI into two fragments, respectively. K. lactis IFO 1267 killed both Saccharomyces cerevisiae sensitive and killer strains and certain strains of Sacch. italicus, K. lactis, K. thermotolerans and K. vanudenii. All K. lactis strains lacking the pGKl plasmids were non-killers. A hybrid was constructed between K. lactis IFO 1267 and a non-killer K. lactis strain lacking the plasmids and subjected to tetrad analysis after sporulation. The killer character was extrachromosomally transmitted in all tetrads in association with the pGKl plasmids. Double stranded RNA killer plasmid could not be detected from any K. lactis killer strains. It is thus highly probable that the killer character is mediated by the linear DNA plasmids. A single chromosomal gene was found which is responsible for the resistance to the K. lactis killer.

XII. Donner Laboratory, University of California, Berkeley, California, 94720. Communicated by R. Mortimer and D. Schild

A. Recent Publications

1. A.J. Kingsman, L. Clarke, R.K. Mortimer and J. Carbon, 1979. Replication in Saccharomyces cerevisiae of plasmid pBR313 carrying DNA from the yeast trp1 region. Gene 7: 141-152.

Plasmid pBR313 carrying a 1.4 kb EcoRI fragment from the yeast TRP1$_+$ region (designated pLC544) is capable of transforming yeast trp1 mutants to Trp$^+$ at high frequency (10^3 - 10^4 transformants/μg DNA). Transformation can be achieved either by using purified plasmid DNA or by fusion of yeast spheroplast with partially lysed Escherichia coli [pLC544] protoplast preparations. The Trp$^+$ yeast transformants are highly unstable, segregating Trp$^-$ cells at frequencies of 0.18 per cell generation (haploids) and 0.056 per cell per generation (diploids) in media containing tryptophan. Plasmid pLC544 replicates autonomously in the nucleus of yeast cells and segregation of Trp$^-$ cells is associated with the complete loss of plasmid sequences. In genetic crosses, pLC544 is randomly assorted during meiosis and is carried unchanged through the mating process into haploid recombinants.

2. F. Hilger and R. Mortimer, 1980. Genetic mapping of arg1 and arg8 in Saccharomyces cerevisiae by trisomic analysis combined with interallelic complementation. J. Bacteriol. 141: 270-274.

Through use of multiple disomic strains, the genes arg1 and arg8 were excluded from all of chromosomes I to XVII except (i) XV and (ii) and XV, respectively. Further aneuploid analyses showed that these two genes were on the same chromosome. By tetrad analysis, arg1 was shown to be linked to SUP3 on the left arm of chromosome XV (parental ditype: nonparental ditype: tetratype = 74:6:139) and arg8 was shown to be loosely linked to arg1 (parental ditype: nonparental ditype: tetratype 72:17:220) on the same arm. The sequence of the genes of this chromosome arm is centromere-SUP-arg1-arg8. Because arg1 had previously been used to define an 18th chromosome, these results reestablish the minimum chromosome number in Saccharomyces cerevisiae as 17.

3. R.K. Mortimer and D. Schild, 1980. Genetic map of Saccharomyces cerevisiae. Microbiol. Rev., December, 1980.

This article includes a compilation of most of the published, as well as a large amount of unpublished, yeast mapping data. These data have been analyzed using the mapping program developed by Richard Snow (Genetics 92: 231-245, 1979). The resultant map (Fig. 1) describes the location of 317 genes on 17 chromosomes and 3 fragments.

4. R.K. Mortimer and D. Schild, 1981. Genetic mapping in Saccharomyces cerevisiae, in "Molecular Biology of the Yeast Saccharomyces", in press, ed. J.M. Strathern, E.W. Jones, and J.R. Broach, Cold Spring Harbor Laboratory Publication.

This chapter reviews all the procedures used to locate genetic markers in yeast. An appendix to the book includes a glossary of gene symbols, a list of mapped genes, and the genetic map.

5. D. Schild, H.M. Anathaswamy, and R.K. Mortimer. An endomitotic effect of a cell cycle mutation in Saccharomyces cerevisiae. Genetics, submitted 1980.

A recessive temperature-sensitive mutation of Saccharomyces cerevisiae has been shown to cause an increase in ploidy in both haploids and diploids. Genetic analysis revealed that the strain carrying the mutation was an aa diploid, although MNNG mutagenesis had been done on an a haploid strain. When the mutant strain was crossed by an αα diploid and the resultant tetraploid sporulated, some of the meiotic progeny of this tetraploid were themselves tetraploid, as shown by both genetic analysis and DNA measurements, instead of the diploid products normally expected of tetraploid meiosis. The ability of these tetraploids to continue to produce tetraploid meiotic progeny was followed for four generations. Homothallism was excluded as a cause of the increase in ploidy; visual pedigree analysis of spore clones to about the 32 cell stage failed to reveal any zygotes and haploids which diploidized retained their mating type. An extra round of meiotic DNA synthesis was also considered and excluded. It was found that tetraploidization was independent of sporulation temperature but was dependent on the temperature of germination and the growth of the spores. Increase in ploidy occurred when the spores were germinated and grown at 30° but not at 23°. Two cycles of sporulation and growth at 23° resulted in haploids, which were shown to diploidize within 24 hr when grown at 30°. Visual observation of the haploid cells incubated at 36° revealed a cell-division-cycle phenotype characteristic of mutations that affect nuclear division; complementation analysis demonstrated that the mutation, cdc31-2, is allelic to cdc31-1, a mutation isolated by Hartwell and colleagues and characterized as causing a temperature-sensitive arrest during late-nuclear division. The segregation of cdc31-2 in heterozygous diploids was 2:2 and characteristic of a non-centromere linked gene.

B. Work in Progress

a. Chromosome loss in diploids homozygous for rad mutations. D. Schild, R. Contopoulou, and R. Mortimer.

We have observed that diploids homozygous for rad52 or rad57 have greatly elevated frequencies of chromosome loss. Exposure of such cells to X-rays makes this effect even more pronounced, such that survivors of X-ray treatment frequently are near haploid. This method of haploidization has been used to determine the chromosomal location of previously unmapped genes.

b. Clonging of RAD genes. D. Schild, R. Contopoulou, I. Lopez-Calderone, and R. Mortimer.

We have cloned RAD52 and are currently attempting to clone other genes in the RAD50-RAD57 series. One of the objects of these studies is to determine the products and regulation of these genes.

c. Double-strand break repair. M. Budd.

rad54-3 strains are sensitive to X-rays at 36°C but not at 23°C. Such strains have been shown to be capable of repairing double-strand breaks at 23°C but not 36°C.

d. Analysis of alkane non-utilizing mutants of Saccharomycopsis lipolytica. J. Bassel.

Twenty hydrocarbon non-utilizing, fatty acid utilizing mutants were isolated following UV irradiation. Complementation and recombination studies indicate that these mutants represent 19 different genes.

e. Development of strains for detecting chromosome loss, recombination, and mutation. Martha Lee Dixon.

Two basic strains have been developed: one for detecting chromosome loss and one for detecting mitotic crossing over, gene conversion, base pair reversion, frameshift reversion, and mitochondrial mutation. This system is being tested currently with a variety of "aneugens," recombinogens and mutagens.

XIII. Ruhr-Universität Bochum, Institut für Physiologische Chemie D-4630 Bochum 1, Postfach 10 21 48, West Germany. Communicated by W. Dunze.

R. Betz and W. Duntze. Primary Structure of a-Factor Peptides from Saccharomyces cerevisiae

We have isolated a-factor, the specific mating hormone of mating type a cells from culture filtrates of wild type X2180-1A cells. Three peptides exhibiting a-factor activity are reproducibly found in the cultures. These peptides designated as a1, a2 and a3 co-purify throughout all steps of the purification procedure but can be separated by thin layer chromatography. a2 possesses the highest specific biological activity in the standard morphogenetic assay while a1 and a3 differ only slightly in their activities. Amino acid analyses show that the a-factor peptides have very similar compositions each consisting of 11 amino acids. In standard amino acid analyses a1 and a2 yield identical amino acid compositions. a3 differs from a2 only by the exchange of a leucine for a valine residue. From data obtained by enzymatic digestions the following tentative amino acid sequences have been assigned to the a-factor peptides:

a1: Tyr - Ile - Ile - Lys - Gly - Val - Phe - Trp - Ala - Asx - Pro

a2: Tyr - Ile - Ile - Lys - Gly - Val - Phe - Trp - Ala - Asx - Pro

a3: Tyr - Ile - Ile - Lys - Gly - Leu - Phe - Trp - Ala - Asx - Pro

Asx appears to be an aspartyl residue which may be substituted at the β-carboxyl group in different ways in the three peptides. The primary structures of these peptides show no apparent homologies with the structure of α-factor.

XIV. Odense University, Department of Molecular Biology, Campusvej 55 - DK 5230 Odense M, Danmark. Communicated by Jørgen Friis.

Below follow abstracts of two recently published papers.

Kay Gulløv and Jørgen Friis. Chromosomal Proteins in Saccharomyces cerevisiae I. Number and Properties of Individual Proteins. Current Genetics 2:69-74 (1980).

SUMMARY

Proteins were isolated from purified yeast chromatin and subjected to two-dimensional electrophoresis. The cellular and the chromosomal content of the major nonhistone proteins was measured. Two polypeptides of molecular weights 55,000 and 53,000, identified as α and β tubulin, and a polypeptide of molecular weight 63,000, associated with the nuclear DNA to a very high degree, account for nearly 50% of the nonhistone proteins present in chromatin. Only one tenth of the RNA polymerase subunit with the molecular weight of 23,000 was associated with nuclear DNA following chromatin purification in metrizamide gradients.

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Kay Gulløv and Jørgen Friis. Chromosomal Proteins in Saccharomyces cerevisiae. II. Chromosome Dosage Effect on the Cellular and Nuclear Content of Nonhistone Proteins. Current Genetics 2:75-78 (1980).

SUMMARY

The effect of varying the chromosomal dosage in aneuploids for chromosome I or VII on the synthesis of individual nonhistone proteins was revealed. The nuclear content of most nonhistone proteins seemed to be little impaired in a strain with tetrasomy for chromosome VII as compared with a strain which is disomic for chromosome VII. On the contrary, tetrasomy for chromosome I as compared with disomy for this chromosome seems to impair both the cellular and the nuclear content of a number of chromosomal proteins, including the tubulin subunits.

XV. Universidade Federal de Rio de Janeiro, Departamento de Bioquimica, Instituto de Quimica, 21941 Rio de Janeiro RJ, Brasil. Communicated by Anita Panek.

We have developed a permeabilization technique by freezing and thawing for the "in situ" determination of α-glucosidase, δ-aminolevulinic acid synthase and δ-aminolevulinic acid dehydrase. The method includes a protein determination after solubilization of permeabilized cells. The papers are being submitted to Analytical Biochemistry.

Below follow abstracts of some papers we have recently submitted for publication. Co-author James R. Mattoon is from the Department of Biology, University of Colorado, Colorado Springs, Colorado 80907, U.S.A.

1. Dulce E. de Oliveira, Elisabete G.C. Rodrigues, James R. Mattoon and Anita D. Panek. Relationships between trehalose metabolism and maltose utilization in Saccharomyces cerevisiae II. Effect of constitutive MAL genes.

ABSTRACT

A pattern of active accumulation of trehalose during growth on glucose medium, TAC(+) phenotype, is controlled by a polymeric series of maltose fermentation (MAL) genes. An essential requirement for expression of the TAC(+) phenotype is that the MAL gene be in the constitutive state, MAL^c. Mutation of a constitutive MAL allele to a maltose-inducible or nonfermenting (mal) state, alters the pattern of trehalose metabolism so that little or no trehalose accumulation occurs during growth on glucose medium. The TAC(+) phenotype is obtained in MAL^c strains whether or not α-glucosidase formation is sensitive or resistant to carbon catabolite repression. However, trehalose accumulation is sensitive to glucose levels even in MAL^c strains in which α-glucosidase formation is insensitive to catabolite repression. The effects of constitutive MAL genes on trehalose accumulation cannot be accounted for by an increase in trehalose-6 phosphate synthase or a decrease in trehalase as determined in vitro. A mechanism is proposed in which the gene-product of a MAL gene serves as a common positive regulator for expression of four genes coding, respectively, for maltose permease, maltase, α-methylglucosidase and a component of the trehalose accumulation system.

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2. Gloria R.B. Padrao, Dulce R. Malamud, Anita D. Panek and James R. Mattoon. Regulation of Energy Metabolism in Yeast. Inheritance of a Pleiotropic Mutation Causing Defects in Metabolism of Energy Reserves, Ethanol Utilization and Formation of Cytochrome $a.a_3$.

ABSTRACT

The recessive, nuclear gene mutation glc1, which causes glycogen deficiency in Saccharomyces cerevisiae, is highly pleiotropic. Studies of the inheritance of glc1 revealed two classes of phenotypic characteristics: I. Traits invariably associated with the mutant gene and II. Traits whose expressions require the presence of glc1 and one or more additional genes. Class I traits include glycogen deficiency and the loss of capacity to accumulate trehalose in nonproliferating conditions. Traits in the second class include a decreased rate of growth on ethanol medium, a deficiency in cytochrome $a.a_3$ and an enhanced accumulation of pigment, probably a metalloporphyrin. Constructed strains containing both glc1

and the constitutive maltose fermentation gene $MAL4^c$ can accumulate trehalose but not glycogen during growth on glucose. However, accumulated trehalose is degraded when cells are exposed to nonproliferating conditions. It is proposed that the glc1 mutation affects a regulatory system, probably involving a protein kinase and/or protein phosphatase, which regulates glycogen synthase and trehalase. Independent regulation of trehalose synthesis by a system controlled by $MAL4^c$ is indicated.

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3. E.G. Oestreicher, M.S. Operti, D.E. Oliveira, A.B. Freitas-Valle, J.R. Mattoon and A.D. Panek. Biochemical Genetics of Trehalose Metabolism in Yeast. Evidence for Alternative Pathways of Trehalose Synthesis.

ABSTRACT

A specific deficiency in UDPG - linked trehalose-6-phosphate synthase activity in the yeast, Saccharomyces cerevisiae has been associated with a single nuclear gene, sst1. Strains bearing this abnormal allele lacked the capacity to accumulate trehalose during growth on glucose or galactose medium or when incubated with glucose in nonproliferating conditions. However, sst1 strains still exhibited trehalose accumulation during growth on maltose medium, provided they contained a gene for maltose fermentation (MAL gene). Introduction of a constitutive MAL^c gene into a sst1 strain rendered the strain capable of accumulating trehalose during growth on glucose medium, but did not restore the normal capacity to convert glucose to trehalose in nonproliferating conditions. Different systems, I and II of trehalose accumulation are proposed. System I would require the UDPG-linked synthase whereas system II, which is normally specific for maltose, would utilize a different enzyme. It is unlikely that system II produces trehalose by transglucosylation, since it converted glucose to trehalose in MAL^c sst1 strains. The results indicate that maltose specifically induces the production of the MAL gene-product, which, in turn, would stimulate the formation (or activation) of system II.

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4. D.R. Malamud, G.R.B. Padrao, L.M. Borralho, M. Arrese, A.D. Panek and J.R. Mattoon. Regulation on Porphyrin Biosynthesis in Yeast. Use of δ-Aminolevulinic Acid in Characterizing in vivo Effects of Mutation.

ABSTRACT

Saccharomyces cerevisiae mutants bearing mutations at the cyc4 locus are partially deficient in cytochrome synthesis. Although the mutation is not in the structural gene for δ-aminolevulinic acid (Alv) synthase, the mutants are deficient in Alv synthesis in vivo, as indicated by abnormally low intracellular Alv concentrations. The cyc4 mutation causes cells to grow very slowly in minimal glucose medium, but not in yeast extract-pepton-glucose medium. A simple nutritional defect caused by the cyc4 mutation is not involved because cytochrome deficiency is enhanced by growing cyc4 cells in yeast extract-peptone medium. A regulatory role

for CYC4 is indicated. Evidence for negative feedback control of Alv synthase by heme is provided by the observation of enhanced intracellular Alv accumulation in yeast mutants partially deficient in decarboxylation of uroporphyrinogen and coproporphyrinogen, respectively.

XVI. Institut für Allgemeine Biochemie der Universität Wien. Währinger Strasse 38, A-1090, Vienna, Austria. Communicated by Michael Breitenbach.

Below follow the summaries of two papers I have recently written and submitted to the Journal of Bacteriology and the Spores VIII book, respectively. In the coming year our research will be centered on mitochondrial gene products in yeast differentiation and on the mitochondrial ribosomes and (possibly) RNA polymerases. My present co-workers are Renee Schroeder, Eva Lachkovics and Klaus Hartmuth.

1. Renee Schroeder and Michael Breitenbach. The Metabolism of Myo-Inositol During Sporulation of Myo-Inositol Requiring Yeast.

ABSTRACT

We investigated the sporulation properties of a series of diploid yeast strains homozygous for inositol auxotrophic markers. The strains required different amounts of inositol for the completion of sporulation, reflecting their different leakiness. Shift experiments revealed two phases of inositol requirement during sporulation which coincide with the two phases of lipid synthesis found by earlier workers. Phase I was at the beginning and during premeiotic DNA-synthesis, phase II immediately preceded the appearance of mature asci. 90% of the inositol taken up by sporulating cells was incorporated into inositol phospholipids. By two-dimensional thin-layer chromatography, 8 compounds could be resolved, one of which was sporulation-specific. The majority of inositol phospholipids was, however, identical to those found in vegetatively growing cells.

In the absence of inositol the cells did not sporulate but after a certain time were unable to return to vegetative growth. These non-sporulating cells did, however, incorporate acetate into lipids and double their DNA content in the premeiotic phase. We believe that it is this lack of coordination of biosynthetic events which causes inositol-less death on sporulation media without inositol.

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2. Renee Schroeder and Michael Breitenbach. The role of the mitochondrial genome in yeast differentiation "Spores VIII" H.L. Levinson, A.L. Sonenshein, D.J. Tipper, eds. (Eighth International Spore Conference October 9-12, 1980, Marine Biological Laboratory, Woods Hole, Massachusetts 02543).

ABSTRACT

In a recent publication (A. Hartig and M. Breitenbach. Curr. Genetics 1, 97-102 (1980)) we have shown that sporulation is possible in a class of respiratory-deficient mitochondrial mutants mapping in the central region of the oxi3 gene. We want to report here the isolation, characterization

and mapping of a mitochondrial point mutation (V-17) which is respiratory-competent but germination-deficient. Respiration and DNA synthesis during sporulation is quite normal, as is the morphology of the asci. The spores are, however, unable to germinate or to mate, as could be shown by micromanipulation and by experiments with pure spore preparations. It could be shown by cytoduction, by deletion mapping with a series of isogenic rho$^-$-strains and by restriction mapping that mutation V-17 is located on the mitochondrial genome between the genes cob and oli2. Experiments with the isogenic wild type show that there exist differentiation-specific products of mitochondrial transcription and translation.

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

Below follows summaries of manuscripts which have recently appeared or are in press.

Kenneth Watson. Homeoviscous Adaptation in Psychrophilic, Mesophilic and Thermophilic Yeasts. In: Membrane Fluidity: Biophysical Techniques and Cellular Regulation Ed. by M. Kates and A. Kuksis, Humana Press, 349-363, 1980.

Thermophilic and psychrophilic yeasts were differentiated on the basis of their ability, in the case of thermophiles, and inability, in the case of psychrophiles, to (a) grow under stricktly anaerobic conditions and (b) produce stable respiratory-deficient mutants either spontaneously or on treatment with acriflavine or ethidium bromide. A possible correlation between these properties and the polyunsaturated fatty acid composition of the membrane phospholipids was proposed. There were marked changes in the fatty acyl composition of membrane phospholipids isolated from the thermophilic yeast, Torulopsis bovina, when monitored at different stages of growth under aerobic and anaerobic conditions. During aerobic induction of anaerobically grown cells, the direct desaturation of palmitic acid residues on phosphatidylcholine to palmitoleic acid appeared to be a mechanism whereby cells adjusted their membrane fluidity.

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Kenneth Watson, Helen Arthur and Mark Blakey. Biochemical Correlations Among the Thermophilic Enteric Yeasts Torulopsis bovina, Torulopsis pintolopesii, Saccharomyces telluris and Candida slooffii. Journal of Bacteriology 143, 693-702 (1980).

Spontaneous and drug-induced respiration-deficient mutants were isolated from the thermophilic enteric yeasts Torulopsis bovina and Saccharomyces telluris. The biochemical properties of these yeasts were compared with those of two naturally occurring respiration-deficient thermophilic yeasts T. pintolopesii and Candida slooffii. Succinate dehydrogenase was not detected in mitochondrial-enriched fractions from C. slooffii but was present in all other species. Cytochrome c oxidase, succinate oxidase, and reduced nicotinamide adenine dinucleotide oxidase were not detected in C. slooffii, T. pintolopesii and the respiration-deficient mutants. Low-temperature cytochrome spectra revealed the presence of cytochromes aa_3, b, c_1 and c

in T. bovina and S. telluris; cytochromes b, c_1 and c in C. slooffii and T. pintolopesii; and cytochromes c_1 and c in the spontaneous respiration-deficient mutants. Palmitoleic and oleic acids were the major fatty acids in all the species. It was noteworthy that T. pintolopesii was rich in lauric and myristic acids. CsCl equilibrium centrifugation experiments showed the presence in all the yeasts of a light buoyant-density (1.6785 to 1.6837-g/cm^3) DNA band which was identified as mitochondrial DNA by its selective elimination on treatment of cells with ethidium bromide. The latter result indicated that the spontaneous respiration-deficient mutants were similar to cytoplasmic petite mutants of S. cerevisiae. Although classical assimilation and fermentation tests indicated that the spontaneous respiration-deficient mutants were strains of T. pintolopesii, it was concluded, on the basis of marked physiological and biochemical differences, that this was not the case.

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Cheryl A.J. Thorne and Kenneth Watson. The Fatty-acyl Composition of Phospholipids from Psychrophilic, Mesophilic and Thermophilic Torulopsis Species is Dependent on the Growth Phase. FEMS Microbiology Letters, in press.

Three main conclusions were drawn from these studies. Firstly, there are characteristic variations, with growth, in the fatty-acyl residues of phosphatidylcholine, phosphatidylethanolamine, phosphtidylinositol and phosphatidylserine from Torulopsis species classified as psychrophilic, mesophilic and thermophilic. Secondly, these changes, when represented graphically, show distinct reciprocal relationships, the nature of which is dependent on the phospholipid species and the temperature domain of the yeast. Finally, individual phospholipids have different but intrinsic fatty-acyl residues, the nature of which is also subject to the thermal domain of the yeast.

XVIII. Centro de Investigaciones en Fisiologia Celular, Universidad National Autonoma de Mexico, Department of Microbiology, Apartado postal 70-600, Mexico 20, D.F. Communicated by Antonio Peña

Aurora Brunner, Nancy Carrasco and Antonio Peña.
Correlation between resistance to ethidium bromide and changes in monovalent cation uptake in yeast. Submitted for publication

SUMMARY

A mutant of Kluyveromyces lactis resistant to ethidium bromide was studied and found to have an impairment to transport the dye. As described for other mutants of this kind, the fluorescence changes of the dye that are observed when the cells transport it were not observed in the mutant strain.

Simultaneous to this difficulty to take up the mutagen, the cells showed a diminished ability to take up monovalent cations, as compared to the wild type strains.

The defect of the mutant strain does not seem to reside in the capacity to pump out protons, which also indicates that it has no alterations of the general energy conversion systems. This view is also supported by

the fact that the growth yields are similar in both the mutant and the wild type strains.

Both ethidium and K^+ failed to stimulate respiration of the mutant yeast when present in the medium, as compared to the wild type strains.

The mutant strain shows a normal cation content, which indicates that the impairment to take up monovalent cations, although much decreased, may still be enough to maintain a normal content of cations within the cells.

According to the investigation carried out, the mutant cells seem to be normal, except for the fact that they are unable to transport both ethidium and K^+ from the medium. The data support the hypothesis that ethidium bromide and K^+ may be transported by the same system in yeast.

XIX. Carlsberg Foundation Biological Institute, DK-2200 Copenhagen, N. 16, Tagensvej, Denmark. Communicated by Birte Kramhøft

The following papers have recently been submitted for publication or are in press.

1. Kirsten Hamburger and Birte Kramhøft: The effect of chloramphenicol on respiration, fermentation and growth in Schizosaccharomyces pombe. Journal of General Microbiology, in press.

SUMMARY

Schizosaccharomyces pombe grows and multiplies for many generations in the presence of chloramphenicol (CAP, 2 mg/ml). CAP-grown cells exhibit nearly fully suppressed respiration, while fermentation occurs at the same rate as in untreated controls. The growth rate of the cultures is reduced in proportion to the diminished energy production, but the content of protein and bulk RNA remain as in untreated cells.

The effects of CAP are reversible. It is argued that CAP in S. pombe specifically inhibits mitochondrial protein synthesis without interfering with cytoplasmic protein synthesis.

CAP-treated cultures can be division synchronized either by a selection technique or by a controlled temperature regimen and by the synchronous divisions studied after removal of CAP.

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2. Helge A. Anderson and Birte Kramhøft: Inhibition of RNA synthesis in yeast protoplasts by a peptide factor from Tetrahymena cells. Cell Biology International Reports, in press.

Protoplasts of Schizosaccharomyces pombe, grown on a rich nutrient medium were treated with a peptide factor isolated from cultures of the protozoan Tetrahymena pyriformis. The peptide factor is known to inhibit RNA synthesis in Tetrahymena. It has now been shown that the peptide factor also inhibits RNA synthesis in yeast protoplasts without affecting protein synthesis.

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3. Graeme M. Walker: Cell cycle specificity of certain antimicrotubular drugs in the fission yeast Schizosaccharomyces pombe. Journal of General Microbiology, submitted for publication.

SUMMARY

Of the seven antimicrotubular drugs used in this study, nocadazole, mebendazole and trifluralin failed to inhibit cell division in S. pombe while at the following concentrations, carbendazim, thiabendazole, chloropropham (50 µg/ml) and amiprophos methyl (200 µg/ml) all completely arrested cell division. This inhibition was associated with striking morphological changes in which carbendazim-treated cells became elongated and pseudohyphal; amiprophos methyl-treated cells appeared as small V-shaped pairs. Suspected blockage of defined cell cycle stages was confirmed by pulse induction experiments which revealed that cells could be synchronized into division using drug exposure times of one generation. Further experiments with selection synchronized cultures showed that different drugs possessed different transition points; carbendazim and thiabendazole were effective in blocking at late cell cycle stages just prior to division, amiprophos methyl affected a very early stage and chloropropham affected an intermediate stage. The results suggest that these drugs exert cell cycle specificity in S. pombe either by impairing microtubule assembly mechanisms (as with carbendazim and thiabendazole) or by inhibiting synthesis of tubulin subunits (as with amiprophos methyl). It is concluded that these drugs could prove useful in future studies of microtubule biogenesis during the cell cycle in yeast.

XX. Kyoto University, Laboratory of Applied Microbiology, The Research Institute for Food Science, Uji, Kyoto 611, Japan. Communicated by Akira Kimura.

Below follow abstracts of four recent papers from our laboratory.

1. Kimura, A., Okuda, M., and Fukuda, H. Separation and comparison of the Hexokinases of Aerobic and Anaerobic Cells of the Yeast Hansenula jadinii. J. Appl. Biochem. 1, 127-138 (1979).

Aerobically grown cells (A cells) of Hansenula jadinii could neither metabolize glucose nor phosphorylate nucleotides in the presence of a concentration of phosphate (300 mM) required for the fermentative production of nucleotides. On the other hand, cells (D cells) grown in an atmosphere low in oxygen could metabolize glucose and phosphorylate nucleotides under such conditions. A and D cells were found to differ in their complement of hexokinase isozymes, A cells containing two activities (1 and 2), and D cells containing three (1', 2', 3'). Both activities in the A cells and two of those in the D cells were inhibited at high phosphate concentration, while the third one in the D cells was not. Other properties of the hexokinases are also reported.

2. H. Fukuda and A. Kimura. Transfer of Mitochondria into protoplasts of Saccharomyces cerevisiae by mini-protoplast fusion. FEBS Letters 113, 58 (1980).

To avoid the troublesome isolation of mitochondria, we prepared intact mitochondria in the form of mini-protoplasts, which can be

obtained by the treatment of log-phase cells; they have only cytoplasmic components but not nuclei. The mitochondria of S. cerevisiae were transferred interspecifically to ρ° strains and made into $\rho+$.

3. A. Kimura, Y. Tatsutomi, H. Fukuda and H. Morioka. Effect of acriflavine on the hexokinase isozyme pattern of a yeast, Hansenula jadinii. Biochim. Biophys. Acta. 629, 217-224 (1980).

Dried cells of a yeast, Hansenula jadinii, that had been cultured aerobically with acriflavine, contained three hexokinase isozymes and metabolized glucose at 0.6 M to produce ATP to phosphorylate nucleotides in the presence of a high concentration of phosphate. Dried cells cultured aeorbically without acriflavine contained two hexokinase isozymes and could not metabolize glucose under the same conditions. Two of the isozymes of the yeast cultured with acriflavine were similar to isozymes of the yeast cultured without acriflavin. However, the third isozyme was resistant to a high phosphate concentration and caused regeneration of ATP through glycolysis and phosphorylation of nucleotides.

4. A. Kimura, Y. Tatsutomi, R. Matsuno, A. Tanaka and H. Fukuda. Some properties of an Immobilized Glycolysis System of Yeast Fermentative Phosphorylation of Nucleotides. Eur. J. Appl. Microbiol. Biotechnol. (in press) (1980).

Immobilized dried yeast cells, which contain glycolytic and some other enzymes, required NAD but not ATP for the (e.g., choline kinase, pyrophosphorylase) fermentative production of CDP-choline, when washed and reused. The immobilized system was more resistant to heat than dried cells, which had previously been used for the same purpose. However, when too many cells were immobilized, leakage of the enzymes from the resin lattice was observed during repeated use. To prevent this leakage, the ratio of cells to resin should be considered.

XXI. Chelsea College, University of London, Manresa Rd. London, SW3 6LX, England. Communicated by Anthony E. Beezer.

1. Below follows the abstract of the Ph.D. dissertation of B.F. Perry, completed in October, 1980.

Flow Microcalorimetric studies of some commercial yeasts: storage, metabolism and growth.

ABSTRACT

Classical knowledge relating to Saccharomyces cerevisiae has been derived using different techniques and yields little information on the relative performance of such strains under identical propagation and test conditions. Standardized, quantitative tests of a wide variety of strains may enable more accurate definition of any strain variation.

Traditional methods which tend to be insensitive, require supervision and sampling, are time consuming, rely for strain characterization on quantitative measures of the ability to ferment a sugar, the rates of fermentation, fermentation patterns, etc.

Liquid nitrogen stored yeast inocula have been shown to be useful in testing procedures. Thus, the utility of this technique for storage of yeasts of industrial interest, metabolic performance relative to fresh cultures, together with a limited study of mutation rates following such storage has been investigated.

Microcalorimetry has been investigated as a method of strain characterization. Necessarily this involved study of the fundamental aspects of microcalorimetrically monitored yeast growth and metabolism. The relevance of these findings to microbial microcalorimetry in general and to microcalorimetric identification of microorganisms in particular is described. The conclusions drawn are (i) that microbial identification by microcalorimetry is not possible and (ii) that great caution must be exercised in interpreting metabolic data derived from microcalorimetric studies.

It has been shown that with rigid control over experimental design it is possible to (within the limits of survey covered by this thesis) characterize yeasts for industrial utility and to establish criteria for microcalorimetric microbial incubations. With liquid nitrogen stored inocula and a standardized microcalorimetric procedure it was possible to determine the quality of complex substrates such as molasses. Microcalorimetric strain characterization and evaluation of molasses are of potential industrial importance.

Present Address: Brian Perry, LKB-Produkter AB, Fermentation/Calorimetry, Box 305, S-161 26 Bromma, Sweden.

2. The following paper has been published:

B.F. Perry, A.E. Beezer and R.J. Miles. Flow Microcalorimetric Studies of Yeast Growth; Fundamental Aspects. Journal of Applied Bacteriology, 1979, 47, 527-537.

3. The following chapter is in press:

B.F. Perry. Microcalorimetry in Antibiotic Bioassay. In: Thermal Techniques in Clinical Analysis, ed. N. Jespersen, Elsevier.

4. Abstract of the Ph.D. dissertation by B.Z. Chowdhry, University of London, 1980.

Study of the Interaction of Yeast Cells with Polyene Antibiotics.

Studies of the interaction of the polyene antibiotics nystatin, lucensomycin, pimaracin, candicidin, filipin and amphotericin B with Saccharomyces cerevisiae NCYC 239 yeast cells recovered from liquid nitrogen storage by isothermal heat conduction flow microcalorimetry are described. Flow microcalorimetry is shown to be suitable for the quantitative assay and measurement of biopotency of polyene antibiotics using:

1. late stationary phase yeast cells respiring in phthalate buffer (pH 4.5) containing 10 mM glucose or

2. midexponential phase yeast cells growing in semidefined media under anaerobic conditions at 303K.

The use of flow microcalorimetry for the quantitative assay of polyene antibiotics using respiring yeast cells, which have been recovered from liquid nitrogen storage, has the advantages of high reproducibility (± 3%) speed (< 1 hour), simplicity of experimentation, possibility of automation and applicability (with present instrumentation) in the concentration range 10^{-5} - 10^{-6}M.

The bioactivity of polyene antibiotics towards yeast cells differs between respiring yeast cells and midexponential phase yeast cells. Both these orders differ from the published bioactivity order obtained by minimum inhibitory concentration measurements.

Measurements of the effect of pH, metal ions (calcium and magnesium) and sterols (cholesterol and ergosterol) on the bioactivity of the polyene antibiotic nystatin towards respiring yeast cells by flow microcalorimetry are also reported.

Measurements of the efflux of:

1. total amino acids as well as the amino acids glutamic acid, lysine and serine and

2. potassium ions,

by automatic amino acid analysis and atomic absorption spectrophotometry respectively, from respiring yeast cells recovered from liquid nitrogen storage under anaerobic conditions between 273K and 313K by nystatin are given.

The efflux of the above species from yeast cells by the polyene antibiotic nystatin are shown to follow first order kinetics. The release of total amino acids and potassium ions from yeast cells can be used for the quantitative analysis of the polyene antibiotic nystatin.

5. Abstract of the Ph.D. dissertation by Parveen Bala Sharma, University of London, 1980.

Biophysical Studies of the Interaction of Polyene Antibiotics with Yeast Cells.

Polyene antifungal antibiotics (complex compounds of microbial origin) present numerous problems in analysis and hence in the understanding of their precise mechanism of action. Several theories (including a pore theory) have been proposed for their mechanism of action from model membrane studies. Extension of these theories to natural membrane systems gives rise to numerous discrepencies which need attention. Many reports exist on studies of the mechanism of polyene interaction with sensitive organisms. These studies involved many variations of the polyene/microorganism/sterol system and this multiplicity makes it difficult to compare and draw useful correlations from these studies. Moreover, a study which results in quantitative description of a sequence of events following exposure of sensitive cells to a polyene antibiotic is absent.

The present study was, therefore, an attempt to gain some knowledge about a proposed sequence of events following antibiotic presentation to the yeast cell. The studies were performed using a single 'standardized' microorganism and a single, clinically important polyene, nystatin. The studies involved

(i) electrophoretic study of yeast/nystatin system; (ii) nystatin adsorption, penetration through the cell wall and interaction with membrane component(s); (iii) kinetic studies of (a) leakage of three cytoplasmic constituents (varying molecular weights) and (b) cell death.

The kinetic study of nystatin uptake by yeast cells is shown to be diphasic and to yield a Langmurian type of adsorption isotherm. The results of the studies (adsorption isotherms, electrophoretic mobilities, kinetic parameters, activation energies) are used as the basis for suggesting that after a rapid initial uptake onto the cell wall slow diffusion takes place to the cell membrane. The apparent first order leakage processes (similar rate constants and activation energies) can be accounted for by membrane permeability changes rather than pore creation.

The thesis concludes with a resume of suggestions for future work.

XXII. National Aeronautics and Space Administration, Ames Research Center, Moffett Field, CA 94035. Communicated by T. Satyanarayana.

Below follows the summary of one of my recent papers.

T. Satyanarayana[a], Charles H. Chervenka[b] and Harold P. Klein. Subunit Specificity of the Two Acetyl-CoA Synthetases of Yeast as Revealed by an Immunological Approach. Biochimica et Biophysica Acta, 614, 601-606, (1980).

[a]Extraterrestrial Research Division, NASA-Ames Research Center, Moffett Field, CA 94035 and

[b]Beckman Instruments, Inc., Spinco Division, Palo Alto, CA 94304 (U.S.A.)

SUMMARY

1. In the present paper, the two acetyl-CoA synthetases (acetate: Coenzyme A ligase (AMP-forming), EC 6.2.1.1) elaborated under aerobic or nonaerobic conditions are further differentiated by an immunological approach.

2. The subunit of the aerobic isozyme was prepared and found to be homogeneous by disc gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) and by ultracentrifugal studies. An $s_{20,w}$ of 3.6 and an apparent molecular weight of 80,500 $\pm$ 500 were calculated for this subunit.

3. The subunit was precipitated by antibody prepared against the aerobic enzyme. Antibody prepared against the subunit also reacted in precipitin tests with the subunit, but not with the native enzyme. The latter antibody nevertheless inhibited the native enzyme but not the nonaerobic isozyme.

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

Below follows a list of our work published since June 1980.

Erkki Oura, Sampsa Haarasilta and John Londesborough. Carbon Dioxide Fixation by Baker's Yeast in a Variety of Growth Conditions. Journal of General Microbiology 118: 51-58 (1980)

Fixation of CO_2 by Saccharomyces cerevisiae growing under $^{14}CO_2$ in a chemostat was investigated. Under anaerobic conditions, CO_2 provided 6.5 + 1% of the total carbon of yeast grown on glucose and 1.6% of the total carbon of yeast grown on glucose plus excess aspartate. Under aerobic conditions, 2.6% of the yeast carbon was derived from exogenous CO_2 during growth on glucose or glycerol, and 3.3% during growth on pyruvate or ethanol.

The distribution of the fixed carbon among chemical components of the yeast, including some individual amino acids, was determined. Equilibration of CO_2 across the cell membrane was probably not quite complete. Under anaerobic conditions, the similar molar radioactivities of aspartate and glutamate indicated that oxaloacetate was not metabolically compartmented. The unequal labelling of aspartate and glutamate during aerobic growth was consistent with operation of the glyoxylate bypass and/or compartmentation of oxaloacetate. Increased CO_2 fixation and labelling of carbohydrate during growth on pyruvate or ethanol are ascribed to the activity of phosphoenolpyruvate carboxykinase.

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John Londesborough. The Cyclic AMP Phosphodiesterases of Baker's Yeast. Abstract of paper presented at the fourth International Conference on Cyclic Nucleotides, July 22-26, 1980, Brussels, Belgium.

The in situ activities of the Mg-independent high-Km PDE and the Mg-dependent low-Km PDE were measured at 2 mM EDTA or 5mM $MgCl_2$ in cells permeabilised by treatment with cytochrome c. Between 5mM and 20 µM cAMP, the large decreases in V and Km as the pH changed from 8.2 to 4.5 agreed with the behaviour of high-Km PDE of 94% purity. Below 1 µM cAMP, the Mg-dependent rate had a Km of 0.2 µM at pH 8.0, as does crude low-Km PDE in vitro. But between 1 and 20 µM cAMP, both activities were greater than predicted from in vitro observations. The results suggest the presence in situ of Mg-dependent activity with a Km about 2 µM, possibly due to low-Km PDE reversibly-bound to microsomes. This low Km PDE has been purified 2000-fold to a spec.act. of 0.4 $\mu mol.min^{-1}$/mg protein at 0.25 µM cAMP, pH 8.0, 30°C. The partially purified enzyme has a mol.wt. of 45000, Km of 0.14 µM, and can still bind to microsomes.

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Elke Parkkinen. Temperature-Induced Increase of Carboxylesterase E_{1A} Activity in Intact Yeast Cells. Abstract of paper presented at the 13th Meeting (The Federation of European Biological Societies), August 24-29, 1980, Jerusalem, Israel.

The carboxylesterase (EC 3.1.1.1.) activity of intact baker's yeast increased markedly when the yeast was stored at 35°C for up to 2 days.

By isolation and purification of the different carboxylesterases of yeast stored for 0 to 5 days at 35°C we found that E_{1A}, the esterase with the highest molecular weight (230 - 270 000) (1), increases its activity toward p-nitrophenyl acetate 3.5 times during the first 2 days storage. Thereafter, it decreased and on day 5 the activity was the same as of fresh yeast. The other four esterases (E_{1B}, E_2, E_3, E_4) (1) lost

70 to 90% of their activity during the first 3 days storage at 35°C. The properties of the esterase E_{1A} were examined in more detail.

For comparison we determined also the changes in activities of other hydrolases during storage of the yeast at 35°C. Intact yeast showed very low proteinase A, proteinase B and carboxylpeptidase Y activity till day 4. All three proteinase activities of stored yeast measured after disintegration and incubation at pH 5 for 20 h (to activate the proteinases) decreased rapidly in the first 3 days of storage but then increased again. Thus proteinases were not induced by short storage at higher temperature. The mechanism of induction of esterase by incubation at 35°C is at present not understood and is now under investigation; this may later help in understanding the physiological role of the esterases in the yeast cell.

(1) Parkkinen, E., Multiple forms of carboxylesterases in baker's yeast (1980) Cellular & Molecular Biology, 25: 001-008

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Kaija Varimo. Essential Arginine in Yeast Adenylate Cyclase. Abstract of paper presented at the 13th Meeting (The Federation of European Biological Societies), August 24-29, 1980, Jerusalem, Israel.

Previous studies on Lubrol-solubilized adenylate cyclase of baker's yeast have shown a rapid inactivation by 2,3-butanedione, 1,2-cyclohexanedione and phenylglyoxal, which all react specifically with arginine at pH 6.8-8.8 (1). A powerful protection against inactivation shown by a substrate analog, adenylylimidodiphosphate (AMP-PNP), gave additional evidence for the presence of an essential arginine, which probably participates in the binding of the negatively charged substrate either at the catlytic site or at some regulatory site.

The adenylate cyclase has now been further purified using precipitation with polyethylene glycol 6000 and hydrophobic chromatography on Dodecyl-Sepharose. These procedures removed a large part of the contaminating ATPase activity from the preparation. Studies are now reported of the protection of such preparations by ATP and ADP against inactivation by arginine-blocking reagents. For 20 mM butanedione in borate this protection is complete at 9 mM Mn-ATP.

(1) Varimo, K., Londesborough, J. (1979) FEBS Letters 106: 153-156

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John Londesborough and Tiina-Maaria Lukkari. The pH and Temperature Dependence of the Activity of the High-Km Cyclic Nucleotide Phosphodiesterase of Baker's Yeast. The Journal of Biological Chemistry (in press).

The activity of the high-Km cyclic nucleotide phosphodiesterase of baker's yeast was studied over a wide range of temperature, ionic strength and $MgCl_2$ concentration. Km and Vmax were insensitive to changes in the $MgCl_2$ concentration between 1 and 30 mM, implying that this enzyme (which does not require free divalent metal ions) does not discriminate between free cyclic AMP^- and the Mg.cyclic AMP^+ complex. Vmax decreased below pH 6.8 because of protonation of a group in the enzyme-substrate complex that was tentatively identified as imidazole. Vmax/Km decrease

above pH 6.8 because of ionisation of an unidentified group in the free enzyme. In the physiological pH range (6.4-7.5) Vmax/Km and, therefore, the rate of reaction at very low cyclic AMP concentration were nearly independent of temperature. Under physiological conditions, the Km (e.g., Km = 20 μM at pH 6.9 and 12°C) approaches the upper limit of in vivo cyclic AMP concentrations in yeast, and at normal in vivo cyclic AMP concentrations the pH optimum is within or below the physiological range of pH in yeast.

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The following publications have appeared since the last communications. The abstracts of reports have been given in Yeast News Letter 28 (1979): 2, 79, 80.

Erkki Oura, Estimation of the suitability of molasses as yeast substrate, Proceedings of: Problems with Molasses in the Yeast Industry, Helsinki 1979, pp. 77-80.

Elke Parkkinen, Multiple forms of carboxylesterases in baker's yeast, Cellular and Molecular Biology 26 (1980), 147-154.

XXIV. Department of Food Science and Technology, Faculty of Agriculture, Nagoya University, Nagoya 464, Japan. Communicated by Makoto Shoda.

Below follow abstracts of two recent papers from this laboratory.

Makoto Shoda and Shigezo Udaka. Preferential Utilization of Phenol Rather than Glucose by Trichosporon cutaneum Possessing a Partially Constitutive Catechol 1,2-Oxygenase. Appl. Environm. Microbiol. 39:1129-1133, 1980.

A phenol-utilizing yeast, Trichosporon cutaneum POB 14, which has a partially constitutive activity of catechol 1,2-oxygenase, utilized phenol in preference to glucose in a medium containing both phenol (200 mg/liter) and glucose (0.15%) as carbon sources. The glucose consumption was not observed until the concentration of phenol decreased to around 10 mg/liter. This phenomenon was confirmed by [U-^{14}C]glucose uptake experiments. The intracellular activities of hexokinase (EC 2.7.1.1) and catechol 1,2-oxygenase (EC 1.13.1.1) changed inversely when phenol was added during growth in the glucose medium.

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Makoto Shoda, Kiminari Maruta and Shigezo Udaka. Isolation and Properties of Phenol-Utilizing Microorganisms with Special Reference to Catechol 1,2-Oxygenase. Agric. Biol. Chem., 44(8), 1841-1846, 1980.

Phenol-utilizing yeasts were isolated from soil. The relationship was examined between distribution of phenol uptake rate using intact cells and distribution of the activities of catechol 1,2-oxygenase which is one of the key enzymes in phenol metabolism. Two of the isolates showed catechol 1,2-oxygenase activity even when grown in glucose medium, though the enzyme activity was about 1% of the full activity induced by phenol. Partially constitutive mutants for catechol 1,2-oxygenase were obtained by mutagenesis of an inducible strain. The level of mutant enzyme activity

was close to that of the isolated constitutive strain. One isolate, Trichosporon cutaneum, preferentially utilized phenol to glucose in medium containing both phenol (200 ppm) and glucose (0.1%), until the concentration of phenol decreased to 10-20 ppm.

XXV. Waksman Institute of Microbiology, Rutgers University, P.O. Box 759, Piscataway, New Jersey 08854. Communicated by J. O. Lampen.

Three papers resulting from the joint efforts of my laboratory and that of Dr. Jan S. Tkacz have been published or submitted for publication during the past year. Abstracts are given below.

Dr. Luis Rodriguez from the Department of Microbiology, University of Salamanca, Spain, spent two years with our groups and returned to Salamanca at the end of August. Dr. Takemitsu Mizunaga of the Department of Agricultural Chemistry, University of Tokyo, left in the Fall of 1979 after a similar two-year period. Both worked on various aspects of the biochemistry and biosynthesis of invertase.

1. H. Russell Onishi, Jan S. Tkacz, and J. Oliver Lampen. Glycoprotein Nature of Yeast Alkaline Phosphatase, Formation of Active Enzyme in the Presence of Tunicamycin. The Journal of Biological Chemistry, 254, 11943-11952, 1979.

The nonspecific alkaline phosphatase of yeast (Saccharomyces strain 1710) has been purified by ion exchange, hydrophobic, and affinity chromatography. This vacuolar enzyme has a molecular weight of 130,000 and is composed of subunits (probably of 66,000 molecular weight). It also has a small quantity of covalently associated carbohydrate; hydrolysis yielded mannose and glucosamine. The endo-β-N-acetylglucosaminidase of Streptomyces plicatus released carbohydrate indicating that the latter was attached to protein through an N-acetylglucosaminylasparaginyl bond.

Synthesis of active alkaline phosphatase by yeast protoplasts is not depressed by tunicamycin, an inhibitor of dolichol-mediated protein glycosylation. Unlike the enzyme normally produced, the alkaline phosphatase which is formed in the presence of the antibiotic does not interact with concanavalin A and, therefore, is deficient in or lacking carbohydrate. We infer that there is no regulatory link in yeast between the glycosylation of a protein and its synthesis. The fact that other Asn-GlcNAc-type glycoprotein enzymes of yeast such as acid phosphatase are not produced in their active forms by tunicamycin-treated protoplasts may mean that, as unglycosylated proteins, they cannot be correctly folded or processed.

Protoplasts derepressed for phosphatase production contained substantial amounts of a second alkaline phosphatase which differed from the purified enzyme in substrate specificity, sensitivity to calcium, and reactivity with concanavalin A.

2. Takemitsu Mizunaga, Jan S. Tkacz, Luis Rodriguez, Richard A. Hackel, and J. Oliver Lampen: Temperature-Sensitive Forms of Large and Small Invertase in a Mutant Derived from a SUC1 Strain of Saccharomyces cerevisiae. Mol. Cell. Biol. (submitted)

SUMMARY

Mutagenesis of the sucrose-fermenting (SUC1) Saccharomyces cerevisiae strain 4059-358D yielded an invertase-negative mutant (D10). Subsequent mutagenic treatment of D10 gave a sucrose-fermenting revertant (D10-ER1) that contained the same amount of large (mannoprotein) invertase as strain 4059-358D but only trace amounts of the smaller intracellular nonglycosylated enzyme. Limited genetic evidence indicated that the mutations in D10 and D10-ER1 are allelic to the SUC1 gene. The large invertases from D10-ER1 and 4059-358D were purified and compared. The two enzymes have similar specific activity and Km for sucrose, cross-react immunologically, and show the same subunit molecular weight after removal of the carbohydrate with endo-beta-N-acetylglucosaminidase H. They differ in that the large enzyme from the revertant is rapidly inactivated at 55°C whereas that from the parent is relatively stable at 65°C. The small invertase in extracts of D10-ER1 is also heat-sensitive when compared with the small enzyme from the original parent strain. The low level of small invertase in mutant D10-ER1 may reflect increased intracellular degradation of this heat-labile form. In several crosses of D10-ER1 with strains carrying the SUC1 or SUC3 genes, the temperature sensitivity of the large and small invertases and the low cellular level of small invertase appeared to cosegregate. These findings are evidence that SUC1 is a structural gene for invertase and that both large and small forms are encoded by a single gene. A detailed genetic analysis is presented in a companion paper.

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3. Luis Rodriguez, J. Oliver Lampen and Vivian L. Mackay: The SUC1 Gene of Saccharomyces: A Structural Gene For the Large (Glycoprotein) and the Small (Carbohydrate-Free) Forms of Invertase. Mol. Cell. Biol. (submitted).

ABSTRACT

Saccharomyces cerevisiae revertant strain D10-ER1 has been shown to contain thermosensitive forms of the large (glycoprotein) and small (carbohydrate-free) invertases and a very low level of the small enzyme, along with a wild-type level of the large form (T. Mizunaga et al., 1980, Molec. Cell. Biol. 000: 000-000). These characteristics cosegregated in crosses of the revertant strain with wild-type sucrose-fermenting (SUC1) or non-fermenting (suc0) strains. In addition, there is tight linkage between sucrose and maltose fermentation in revertant D10-ER1 (characteristic of the SUC1 and MAL1 genes) and loose linkage between the mutant SUC gene and the ade3 gene, which is known to be on the same arm of chromosome VII as SUC1. From this we infer that a single reversion event is responsible for the several changes observed in D10-ER1, and that this mutation maps within or very close to the SUC1 gene present in the ancestor strain 4059-358D.

The revertant SUC1 allele in D10-ER1 (termed SUC1-R1) was expressed independently of the wild-type SUC1 gene when both were present in diploid cells. Diploids carrying only the wild type or the mutant genes synthesized invertases with the characteristics of the parental Suc^+ haploids. The possibility that a modifier gene was responsible for the alterations in the invertases of revertant D10-ER1 was ruled out by appropriate crosses. We conclude that SUC1 is a structural gene that codes for both

the large and the small forms of invertase and suggest that SUC2 through SUC5 are structural genes as well.

XXVI. Albert Ludwigs Universität, Biochemisches Institut, D-7800 Freiburg I. Br., Hermann-Herder-Str. 7, West Germany. Communicated by D.H. Wolf.

Below follows a summary of recent developments in our laboratory concerning the field of proteolysis in yeast. The work is submitted for publication. Part of this work is in press in D.H. Wolf (1980), Proceedings of the Third International Fungal Spore Symposium (G. Turian and H.R. Hohl, eds.) Academic Press, London - New York. It was furthermore presented at the "10th International Conference of Yeast Genetics and Molecular Biology."

Tilman Achstetter, Claudia Ehmann and Dieter H. Wolf. New Proteolytic Enzymes in Yeast.

The fundamental role proteolytic enzymes play in cellular regulation has been realized in recent years. In the yeast Saccharomyces cerevisiae seven proteolytic enzymes have been found - two endoproteinases, two carboxypeptidases, three aminopeptidases and one dipeptidase (for review see 1). From in vitro studies proteinase B, an enzyme located in the vacuole, and the only known endoproteinase active at neutral pH, was considered for several specific intracellular regulatory processes such as activation of chitin synthetase, a vital process during yeast budding, and the inactivation of a variety of enzymes unnecessary under certain growth conditions (for review see 1). However, in vivo experiments, using proteinase B mutants, made involvement of the vacuolar proteinase B in these specific processes unlikely (for review see 1). Therefore, we searched in order to find new proteolytic activities, which because of action around the neutral pH range might be responsible for proteolytic processes outside the vacuole. Chromogenic peptide substrates designed for the relatively specific serum proteinases were applied during this search. As a prerequisite for these studies, a triple mutant devoid of proteinase B, carboxypeptidase Y and carboxypeptidase S was constructed, ensuring lack of activity of known enzymes in the cell against the new substrates used.

Separation of proteins of a 100,000 x g supernate of mutant crude extract by molecular weight using sephadex chromatography and test against four different substrates led to the detection of a variety of new proteinases. Partial characterization of their sensitivity to various inhibitors indicate eleven activities. Two activities, called proteinase M and proteinase P were found in the sedimentable membranous fraction of mutant extracts.

(1) Wolf, D.H. (1980). Advances in Microbial Physiology (A.H. Rose and J.G. Morris, eds.) Vol. 21, pp. 267-338, Academic Press, New York - London

XXVII. The University of Kansas Medical Center, College of Health Sciences and Hospital, Department of Biochemistry, Rainbow Boulevard at 39th, Kansas City, Kansas 66103. Communicated by Wilfred N. Arnold.

Dr. Charles R. Harrington, who recently completed doctoral studies at the University of Glasgow has joined my laboratory as a postdoctoral associate.

"Yeast cell envelopes - biochemistry, biophysics and ultrastructure", a 2-volume work edited by W.N. Arnold, is in the hands of the publisher, CRC Press, and we hope for a Spring or early Summer of 1981 appearance date.

Recent titles:

W.N. Arnold, A.T. Pringle and R.G. Garrison, "Amphotericin B-induced changes in K^+ content, viability, and ultrastructure of yeast phase Histoplasma capsulatum." J. Bacteriol. 141: 350 (1980).

W.N. Arnold and A.T. Pringle, "Scanning electron microscopy of cells and protoplasts of Saccharomyces rouxii." Current Microbiol. 3: 283 (1980).

W.N. Arnold and R.G. Garrison, "Kinetic limitations on the trapping of nascent phosphate for cytochemical localization of yeast acid phosphatase." Current Microbiol. in press.

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

The following are abstracts of recently published or orally presented papers from work carried out in The Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh, Scotland and partly in The Biological Institute of the Carlsberg Foundation, Copenhagen, Denmark.

1. Graeme M. Walker, James C. Thompson, J. Colin Slaughter and John H. Duffus. Perturbation of enzyme activity in cells of the fission yeast Schizosaccharomyces pombe subjected to continuous-flow centrifugation. Journal of General Microbiology 119, 543-546 (1980).

ABSTRACT

Fluctuations in glutamine synthetase activity were observed when exponentially dividing cells of Schizosaccharomyces pombe were synchronized by continuous-flow centrifugation. These fluctuations were not seen in a control culture in which cells were allowed to grow asynchronously following filtration. However, when an asynchronous control culture was prepared by allowing cells to pass quickly through the continuous-action rotor without size selection, marked fluctuations in glutamine synthetase activity occurred. It is concluded that when the continuous-flow size selection method of cell synchronization is used, suitable control experiments are essential before any conclusions can be drawn about how enzyme synthesis is regulated during the cell cycle.

2. Graeme M. Walker, James C. Thompson, J. Colin Slaughter and John H. Duffus. Correlation of glutamine synthetase activity with cell magnesium concentration during cell division in yeast synchronized induction. Journal of General Microbiology, in press.

ABSTRACT

Synchronization of cell division in the fission yeast, Schizosaccharomyces pombe and the budding yeast, Kluyveromyces fragilis was achieved by induction using the DNA synthesis inhibitor, 2'-deoxyadenosine and by a magnesium-exhaustion technique. The activity of glutamine synthetase in these synchronized cultures oscillated. Variations in the intracellular magnesium

concentration were also observed, and peaks in magnesium concentration correlated with peaks in enzyme activity. We suggest that the enzyme from yeast is unstable, and its activity is regulated *in vivo* by changes in the intracellular concentration of magnesium.

3. Graeme M. Walker and John H. Duffus. The regulation of cell division in yeast by magnesium. Paper presented at the Vth International Symposium on Yeasts, London, Canada, July 1980.

ABSTRACT

Cells of the fission yeast, *Schizosaccharomyces pombe* and the budding yeast, *Kluyveromyces fragilis*, fail to divide in Mg-free minimal media but can be induced to divide synchronously on restoration of Mg to "exhausted" cultures. Mg-depleted *S. pombe* cells appear abnormally long, lack cell plates and possess elongated nuclei. This, together with evidence of a late cell cycle arrest by the ionophore A23187, suggests that specific Mg limitation blocks cells late in the G2 phase of the cell cycle. Moreover, analysis of cell magnesium in cultures synchronized by various techniques reveals that there is a fairly steady fall in Mg concentration as cells grow, terminating in a rapid influx of Mg just before division. These results lead to the hypothesis that intracellular Mg concentration is the transducer of size and consequently time related control of the cell cycle.

4. Graeme M. Walker, John G. Comerford and John H. Duffus. Magnesium and control of yeast cell division. Paper presented at the Vth European Cell Cycle Workshop, Salamanca, Spain, September 1980.

ABSTRACT

Cell division in budding and fission yeast can be synchronized by ionophores, chelating agents and magnesium deprivation. A hypothesis linking cell magnesium levels to control of cell division has been formulated. Studies on cell cycle mutants of *Schizosaccharomyces pombe*, blocked at different stages of cell division, have been undertaken to test how far they conform with the hypothesis.

XXIX. National Research Council, Division of Biological Sciences, Ottawa, Canada K1A 0R6. Communicated by Byron F. Johnson.

Below follow four abstracts of papers just published or in press.

1. P.Y. Wang, B.F. Johnson and H. Schneider. Fermentation of D-xylose by Yeasts using Glucose Isomerase in the Medium to Convert D-xylose to D-xylulose. Biotech. Letters 3:273-278, 1980.

SUMMARY

A method to obtain the fermentative conversion by yeasts of D-xylose to ethanol is described. The method depends on a combination of two factors; (1) the ability of glucose isomerase to isomerise D-xylose to D-xylulose and (2) the ability of a number of yeasts to ferment D-xylulose.

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2. G.B. Calleja[2], B.F. Johnson[2] and B.Y. Yoo[3]. Macromolecular changes and commitment to sporulation in the fission yeast Schizosaccharomyces pombe. Plant and Cell Physiol. 21:1980 (in press).

[2]Molecular Genetics Section, Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6.

[3]Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 5A3

Homothallic cultures of Schizosaccharomyces pombe in stationary phase may be induced to flocculate by aeration. Flocculation is followed by copulation, conjugation, zygote formation, meiosis and sporulation. This developmental sequence was monitored for respiratory activity, changes in protein, RNA and DNA, catabolite repression, and commitment to sporulation. Respiratory activity, apparently a prerequisite to induction, increased 5-fold prior to maximum flocculation and remained at that level up to the end of the sequence. Protein and RNA content increased prior to conjugation but gradually decreased shortly thereafter. A round of premeiotic DNA synthesis occurred after copulation, presumably during conjugation. The developmental sequence was repressible by glucose. Cyclic AMP at low concentrations stimulated sporulation somewhat, but the stimulatory effect was not sufficient to offset repression due to glucose. Commitment to sporulation was determined by adding glucose at various times during the developmental sequence and then observing refractoriness of the events to catabolite repression. Cells not committed were repressed by glucose and reverted to mitotic cell cycles. Committed cells proceeded to sporulate in the presence of exogenous glucose. Commitment to sporulation appears to occur soon after premeiotic DNA synthesis.

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3. G.B. Calleja, M. Zuker, Byron F. Johnson and B.Y. Yoo[1]. Analyses of Fission Scars as Permanent Records of Cell Division in Schizosaccharomyces pombe. J. theor. Biol. 84:523-544 (1980).

[1]Department of Biology, University of New Brunswick, Fredericton, N.B., Canada E3B 5A3

Cell division in fission yeast is recorded on the walls of the progeny as fission scars and fuscannels. Theoretical analyses of scars in a population of cells allow us to deduce the total number of scars, the average number of scars per cell and the distribution of scars in the population. The number of scars in the population is twice the number of cells; the average number of scars per cell is two. The predicted distribution of scars in the population is as follows: one-scar class, 33.33%; two-scar class, 43.50%; three-scar class, 15.96%; four-scar class, 5.08%; five-scar class, 1.52%; six-scar class, 0.44%; seven-scar class, 0.12%. The other scar classes are very rare. The predicted distribution fits the observations quite well. In the analyses, we assume that the cell lays down its septum with equal probability to the left or to the right of an end-most scar (which at fission time approximates the middle of the cell). We show the validity of this assumption and describe the possible modes of segregation of the scars in one generation. Given a cell with a certain number of scars, we deduce in terms of scar number its most likely progeny as well as its

most likely progenitor. Finally we rationalize the rarity of the multi-scar cell, demonstrate the improbability of its further aging, and describe its mechanism for rejuvenation.

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4. G.B. Calleja, B.F. Johnson and B.Y. Yoo. The Cell Wall as Sex Organelle in Fission Yeast. In:Sexual Interactions in Eukaryotic Microbes, D.H. O'Day and P. Horgen (eds), Academic Press, 1981.

ABSTRACT

The life cycle of the homothallic fission yeast, Schizosaccharomyces pombe, is simple enough to serve as a manipulable model for morphogenesis, differentiation, and development of a eukaryote. The fission yeast cell in the presence of an energy source becomes ready for sexual interaction when it becomes mitochondrially sufficient and when released from catabolite repression, which otherwise imprisons it in the mitotic cycle. Cells become induced to competence to form social structures called flocs. Induction brings about changes in the properties of the cell wall, which is the sex organelle. The changes require cytoplasmic synthesis of proteins, including the putative protein molecules that must be covalently attached to the cell wall. Among the numerous cells in complementary contact in the floc, covalent linkages between two walls are forged. This activity is presumed to be catalyzed by wall-ligating enzymes. Mural activity involving both synthetic and lytic enzymes brings about the deformation of copulating ends to form the conjugation tube between two cells. Soon after mural fusion, erosion takes place until the two-layer crosswall between the two cells is gone, thereby making two cells functionally and morphologically one.

XXX. Microbiology and Fermentation Section, Brewing Research Foundation, Nutfield, Redhill, Surrey, U.K. Communicated by Roy S. Tubb

The following are abstracts of papers from this laboratory which are in press or submitted for publication:

1. Thurston, P.A., Taylor, R. & Ahvenainen, J. Effects of Linoleic Acid Supplements on the Synthesis by Yeast of Lipids and Acetate Esters. J. Inst. Brew. (in press).

SUMMARY

The concentrations of acetate esters in beer were reduced by up to 85% by addition of linoleic acid to the fermentation or by pitching with yeast previously enriched with this unsaturated fatty acid. Linoleic acid was rapidly incorporated into yeast lipids and was effective in reducing the rate of ethyl acetate formation within 2 hours. Addition of linoleic acid altered the pattern of synthesis of fatty acids by yeast, causing a shift from medium toward long chain acids. Secondly, the amount of squalene in yeast was reduced by up to 70% whereas that of lanosterol was increased threefold. Since total yeast lipid synthesis was reduced by up to 40%, we conclude that less acetylCoA is synthesized in the presence of linoleic acid. Further, high concentrations of linoleic

acid decreased the proportion of acetylCoA consumed by the synthesis of acetate esters. Therefore, linoleic acid may directly decrease acetate ester synthesis in addition to its effect via reduction of acetylCoA availability.

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2. Quain, D.E., Thurston, P.A. & Tubb, R.S. The Structural and Storage Carbohydrates of Saccharomyces cerevisiae: Changes during Fermentation of Wort and a Role for Glycogen Catabolism in Lipid Biosynthesis. J. Inst. Brew. (in press).

SUMMARY

Detailed analysis of the structural and storage carbohydrates of Saccharomyces cerevisiae (NCYC 240) during wort fermentation showed that there were no significant changes in the amounts of trehalose or alkali-soluble glycogen. However, glucan and mannan individually increased from ca 4% of the yeast dry weight at pitching to ca 6% during the first 3-17h of fermentation, but then declined to the former level. In the first 2h of fermentation, prior to yeast multiplication, acid-soluble glycogen was rapidly dissimilated from ca 40% to ca 6% of the yeast dry weight. During this period of oxygen uptake, wort sugars were not removed by the yeast. Glycogen, therefore, was the sole source of metabolic energy for lipid resynthesis and hexose transport appeared to require the formation of a competent membrane. During the latter phase of fermentation when the yeast was not growing but expending energy for maintenance of cellular functions, glycogen reserves were slowly depleted; after a period of prolonged anaerobic storage, the content of glycogen fell well below that which was initially present in the pitching yeast.

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3. Quain, D.E. The Determination of Glycogen in Yeasts. Anal. Biochem. (submitted for publication).

SUMMARY

Four methods for the determination of glycogen in yeast have been compared in five strains of Saccharomyces cerevisiae over a range of glycogen contents. A new method has been developed which is specific, precise and more exhaustive than previously published procedures. After extraction with sodium carbonate and perchloric acid, the glycogen was hydrolysed with amyloglucosidase (E.C.3.2.1.3) to glucose, which was estimated enzymically. The greater extraction of glycogen using this method cannot be explained by acid hydrolysis of glucans prior to treatment with amyloglucosidase. Further, the older data using the non-specific methods of W.E. Trevelyan and J.S. Harrison (1956, Biochem. J. 63, 23-33) may now be equated with values obtained using a specific method for glycogen determination.

4. Searle, B.A. & Tubb, R.S. Regulation of Amyloglucosidase Production by Saccharomyces diastaticus. J. Inst. Brew. (in press).

SUMMARY

In wort fermentations, production of extracellular amyloglucosidase (AMG) by Saccharomyces diastaticus was greatest, and initiated earliest, when the requirement of yeast for oxygen or unsaturated lipid was incompletely satisfied. During such fermentations, maltotriose disappeared before maltose; glucose, the product of AMG action, accumulated and the wort was inadequately attenuated. In all circumstances examined, commencement of dextrin breakdown coincided with initiation of AMG excretion. Prior to excretion, active enzyme was associated with yeast cells but was located externally to the plasma-membrane.

Dextrin or starch was not required to induce AMG production which is initiated, we conclude, when the carbon and energy-source becomes growth-limiting. Sacch. diastaticus strains hydrolysed only a small proportion of wort dextrins and AMG produced in beer was unable to release glucose from pullulan. For production of low carbohydrate beer, strains which (i) synthesise significant amounts of a 'debranching' enzyme and (ii) are catabolite-derepressed for AMG production would be advantageous.

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5. Brown, A.J.P., Goodey, A.R. & Tubb, R.S. Interstrain Transfer of the 2μm Plasmid of Saccharomyces by Cytoduction. J. Inst. Brew. (in press).

SUMMARY

Respiratory-sufficient, [rho$^+$], cytoductants were obtained from matings where the respiratory-deficient, [rho$^-$], parent lacked 2μm DNA. In these crosses, the cytoduction frequency (no. cytoductants x 100/no. cytoductants plus no. diploids) was ca.20% or less, even though the [rho$^+$] parent was defective in nuclear fusion. This apparent suppression of the kar defect was specific to matings where the kar nucleus was derived from a [rho$^+$] cytoplasm, but was not a consequence of the lack of 2μm DNA in the recipient strains. Of 16 cytoductants examined from such a cross, only 3 contained 2μm DNA. This confirmed an anomolous mode of cytoplasmic inheritance for the plasmid but demonstrated that the frequency of transmission during cytoduction can be somewhat lower than has been reported previously. The plasmid, in those [cir$^+$] cytoductants which received it, was stably maintained over more than 120 cell doublings. From a preliminary comparison of [cir$^+$] and [cir^0] cytoductants, no evidence for a plasmid-specific phenotype was obtained.

6. Goodey, A.R., Brown, A.J.P. & Tubb, R.S. Transfer of Nuclear Genes during Cytoduction in Saccharomyces. J. Inst. Brew. (in press).

SUMMARY

Transfer of a nuclear gene STA 3 was obtained as a rare-event when forcibly selected for during cytoduction. A stable hybrid which inherited STA 3 in this way also received the markers MAT a and HIS 4 from chromosome III. Genetic analyses showed that the transferred nuclear genes remained cromosomally located and were not inherited cytoplasmically; the hybrid, therefore, was disomic at least for chromosome III. The technique described here can be used to construct yeast hybrids which received only a discrete portion of nuclear material from one parent.

7. Tubb, R.S., Brown, A.J.P., Searle, B.A. & Goodey, A.R. Development of New Techniques for the Genetic Manipulation of Brewing Yeasts. Proc. Vth International Symposium on Yeasts, London, Ontario 1980 (submitted for publication).

SUMMARY

Traditional procedures for hybridising strains of Saccharomyces have met with little success in producing novel or improved strains for brewing. Here, hybrids were obtained by 'rare-matings' between a non-sporulating asexual lager yeast and dextrin-fermenting (Sacch. diastaticus) haploids. These hybrids fermented brewers' wort at rates similar to that of the brewing strain parent; in addition, they produced exocellular amyloglucosidase and were able, therefore, by hydrolysis of malto-dextrins, to produce low-carbohydrate beers. However, such beers possessed an objectionable 'herbal phenolic' aroma, which was characteristic of the non-brewing parent. Significant concentrations of 4-vinyl guaiacol were detected in phenolic beers; although this compound may not have been solely responsible for the off-flavour, its presence was symptomatic of the defect. We suggest that by first 'breeding out' this undesirable dominant character in the haploid parent, it should be possible to construct hybrids which will produce palatable beer.

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8. Thurston, P.A. & Tubb, R.S. Screening Yeast Strains for their Ability to Produce Phenolic Off-Flavours: A Simple Method for Determining Phenols in Wort and Beer. J. Inst. Brew. (in press).

SUMMARY

Amounts of several phenolic compounds in wort and beer were measured by gas-chromatography following extraction with a small volume of chloroform. The procedure described is not specific for phenols and the limit of sensitivity is 0.05 - 0.1 µg 'phenol'/ml. However, the precision is reasonable and this relatively rapid method has proved useful for screening yeast strains for their ability to produce phenolic off-flavours. Phenolic-tasting beers contained significant amounts of 4-vinyl guaiacol; whilst this compound may not be the sole cause of the undesirable flavour, its production is symptomatic of the unsuitability of a yeast strain for brewing.

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

The following is the summary of a paper submitted for publication in WEIN-WISSENSCHAFT (GFR) in 1980:

Ľ. Drobnica, E. Šturdík, E. Minárik and P. Rágala. Influence of vine protection agents on wine yeasts.

The inhibitory action of 22 vine protection agents on Saccharomyces cerevisiae, Sherry strain has been characterized. Only those preparations containing active components capable of reacting with thiol-groups of the yeast cell, e.g., Delan flussig, Euparen, Kafalon, Orthocide 50 and Phaltan 50, reveal a notable anti-yeast activity. Trough thiol-enzyme inactivation

basic energy providing processes (fermentation and respiration) are fundamentally inhibited. This leads to the inhibition of biosynthetic processes and thus to a total inhibition of yeast growth. Anti-yeast activity examination methods described in the paper may be useful in preliminary studies on possible inhibitory actions of new vine protection agents on yeast in the course of grape juice fermentation.

* * *

The following is the summary of a paper accepted for publication in Mitteilungen Klosterneuburg (Austria).

E. Minárik. Saccharomyces bailii - cause of turbidity in sweet bottled wines (in German).

Ecological investigations on yeasts and yeast-like microorganisms in young wines prior to first racking and in bottled wines in two wine regions of Czechoslovakia has been carried out. In the microflora of wines species of the genus Saccharomyces prevailed. Apart from the obligate S. cerevisiae and S. oviformis the thermo- and osmotolerant S. bailii var. bailii, resistant also to officially authorized food preservatives, represents a potential danger of causing wine turbidity, mainly in bottled wines containing residual sugar. Some physiological characteristics of S. bailii var. bailii are given.

XXXII. Centre Technique Experimental de l'Institut Technique de la Vigne et du Vin, 12 rue Etienne Pallu 37033 - TOURS CEDEX (France). Communicated by C. Cuinier.

1. The following is an abstract of a recent article:

Cuinier, C. and Lacoste, J. "Essai d'utilisation de levures sèches actives en Touraine. Controle de l'efficacité du levurage". Connaissance de la Vigne et du Vin 14, 1, 53-64 (1980).

Four preparations of active dry yeasts have been studied: number of viable cells, SO_2 formation, foam production. Compared to spontaneous fermentation, these yeasts have been used for white wine making. Tasting has not revealed any preference among the wines. But oenological differences have appeared. At the species level, identification of isolated yeasts during the five fermentations does not permit to conclude in favour of yeast-adding efficiency.

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2. A list of recently published papers follows:

Cuinier, C. (1978). Evolution de la microflore au cours de la vinification des vins de Chinon. Vignes et Vins n° 269, 29-41.

Cuinier, C., Leveau, J.Y. (1979). L'identification des levures des vignobles et des vins. Méthode rapide a l'aide de la galerie API 20 C. Vignes et Vins n° 283, 44-49.

Cuinier, C. (1979). Influence des fongicides sur les levures de la vigne et du vin. Vignes et Vins n° 285, 42-48.

XXXIII. Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2WI. Communicated by J.D. Cunningham.

Research Areas on Yeasts

1. Single Cell Protein (S.C.P.) Studies
2. Waste Utilization for Ethanol as an Energy Fuel

1. Single Cell Protein: Yeast-Whey Biomass

Whey supplementations and rapid conversion by selected strains of Kluyveromyces fragilis were accomplished within a 12 hour period under controlled fermentation. Protein improvement with ranges from 53 to 58% protein values were obtained from yeast-whey fermentations. Decreased efficiencies were observed if concentrated wheys were fermented, and it was therefore postulated that this would exhibit the 'reverse Pasteur' or Crabtree effect.

Three protein fractions (with and without methionine supplementation) were evaluated with lab animals using the protein efficiency ratio (PER) and the net protein ratio (NPR). Yeast diets alone produced significantly lower PER and NPR, however, methionine additions improved these diets in addition to the yeast-whey and whey diets when compared to casein.

Investigations on the enrichment of white and whole wheat flour breads with the whey-yeast (S.C.P.) and commercial Candida yeasts have since been undertaken in order to determine organoleptic acceptance and evaluation and objective measurement of the physical characteristics of the dough and baked loaves.

2. Ethanol Production from Industrial Wastes

Whey and waste sulphite liquor have been initially investigated for the feasibility of producing ethanol as an energy fuel alternative. Yeast fermentations within 12 to 24 hours have been conducted under batch conditions employing whey and whey permeates with or without supplementation and subsequent recovery of ethanol. Yields up to 2.4 (anhydrous alcohol: gal/bus) may be feasible when whey/starch streams or corn starch supplementations are introduced. Other agro-industrial waste streams are under investigation.

Waste sulphite liquor (S.S.L.) derived from softwoods and hardwoods have been considered for ethanol production. Screening for yeasts capable of sulfur- and ammonium hydroxide-tolerances in the industrial processes are under investigation in addition to the utilization of fermentable sugars and pentoses especially since the latter carbohydrates constitute a relatively high level in the S.S.L.

XXXIV. Chaire de Génétique et Microbiologie, E.N.S.A.M. - I.N.R.A., 34060 Montpellier Cedex, France. Communicated by P. Galzy.

The following are recent papers from our laboratory:

G. Moulin, Helène Boze, P. Galzy
Inhibition of alcoholic fermentation by substrates and ethanol.
Biotechnol. Bioeng. 1980. In press.

C. Moulin, Maguy Guillaume, P. Galzy
Etude de la production d'alcool sur lactoserum.
Ind. Alim. Agric. 97, n° 5, 471-474, 1980.

G. Moulin, K. Oteng-Gyang, P. Galzy
Influence of amylase excretion on biomass production by amylolytic yeast.
Acta Microbiologica. Hungarica 27, 155-159, 1980.

J. P. Guiraud, C. Devouge, P. Galzy
Selection of yeast strains with a view to the production of proteins on inulin.
Biotechnol. Letters, 1, 461-464, 1979.

Isabelle Beluche, J. P. Guiraud, P. Galzy
Inulinase activity of Debaryomyces cantarellii.
Folia Microbiol. 25, 32-39, 1980.

J. P. Guiraud, C. Viard-Gaudin, P. Galzy
Etude de l'inulinase de Candida salmanticensis Van Uden et Buckley.
Agric. Biol. Chem. Jap. 44, 1245-1252, 1980.

XXXV. The Institute of Enology & Viticulture, Yamanashi University, 400 Kofu, Japan. Communicated by S. Goto.

S. Goto. Changes in the Wild Yeast Flora of Sulfited Grape Musts. Submitted to J. of Enology and Viticulture, Yamanashi University.

ABSTRACT

Progressive changes in the wild yeast flora at the initial stage of incubation of grape musts added with various concentrations of SO_2 were studied qualitatively and quantitatively. Initial total counts of yeasts in the original grape must were 6.5×10^5 cells/ml and increased rapidly within a short period of time and, after 32 hours, fermentation was observed. As early as 4 hours after incubation, total counts of yeasts and the proportion of the five yeast groups (Apiculate, Saccharomyces, Torulopsis, Film, and Others) were affected by the initial levels of free-SO_2 added. Total counts of yeasts in the must containing 46.7 ppm of free-SO_2 decreased gradually and reached a minimum after 48 hours. After this period, however, total counts of yeasts increased gradually, and this was due to the increase of the Saccharomyces group. Total counts in the musts containing 97.5 and 228 ppm of SO_2 decreased rapidly to less than 10 cells/ml after 24 and 48 hours. The apiculate yeast group and part of the film and other yeast groups decreased rapidly in the initial stage of incubation of sulfited grape musts, whereas the Saccharomyces and Torulopsis yeast groups decreased gradually. Yeasts remaining in the sulfited musts were resistant to SO_2.

XXXVI. Departement de Biologie - U.E.R. Scientifique de Luminy, Case 901-70, route Leon-Lechamp - 13288 Marseille Cedex 2, France. Communicated by E. Azoulay.

Abstracts 1, 2 and 3 represent posters presented at the VIth International Fermentation Symposium, London, Ontario, Canada, 7, 20-25, 1980.

1. Protein enrichment of cassava by Candida tropicalis: continuous culture process.

J. Janssens and J-M. Lebeault, University of Technology of Compiegne. 60200 Compiegne - France - E. Azoulay, E.R. 143 - CNRS, 13288 Marseille - France

A strain of Candida tropicalis utilizing starch has been isolated. By using this yeast a fermentation process has been developed for protein enrichment of cassava.

The process operating conditions have been optimized for increasing yeast productivity in continuous culture. The maximum productivity achieved was 3.5 g l^{-1} h^{-1} of biomass at a dilution rate of 0.26 h^{-1}.

Depending on residence time, protein content of enriched cassava may be adjusted between 14 and 40%, compared with the starting material (3% in protein).

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2. Growth of C. tropicalis on starch and cassava powder for obtaining high-protein feed. E. Azoulay, J. Janssens, A. Raphael and J.C. Bertrand.

Candida tropicalis, strain CBS 6948 grows on soluble starch and cassava powder without requiring that these substrates be previously hydrolysed.

The cultivation of the yeast on cassava powder leads directly to a balanced feed. The growth of this microorganism requires the assimilation of the polysaccharides contained in this powder and the resultant protein content increase of the product is due to the presence of the synthesized yeast biomass. The enrichment of cassava powder results, after fermentation with C. tropicalis CBS 6948, in an increase of 18 to 20% of the protein content. If we refer to the levels of lysine and methionine, the essential amino acids composition is relatively balanced.

The growth of this yeast is possible on cassava concentrations ranging from 2 to 100 gr per liter. The maximum specific growth rate obtained was 0.4 hr^{-1} and the maximum productivity of batch culture attainable was estimated to be about 0.038 g cell/l/hr. The yield coefficient was determined as 0.55 g cell per g metabolized cassava starch. In continuous culture, C. tropicalis CBS 6948 grew best in the pH range of 4.0 to 5.0 but for work in non aseptic conditions, a pH of 4.0 was optimal. With pH 4.0, the maximum cell concentration was attained at 35°C. The maximum biomass productivity obtained was 3.6 g/l/hr at a dilution rate 0.26 hr^{-1}. The dilution rate for the production of Single Cell Protein, corresponding to the maximum productivity was 0.25 hr^{-1}.

C. tropicalis possesses the inducible enzymes needed to hydrolyse starch. It probably grows at the expense of 1-4 polysaccharidic chains of the amylase type, amylopectin representing the fraction of the starch which is not assimilated. The fact that no extra enzymes are required to hydrolyse starch, results in a particularly elegant way of improving the nutritious value of amylaceous products, through a single step fermentation process.

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3. Study of hydroxylase and its components in Candida tropicalis grown on alkane. Bertrand, J.C., M. Gilewicz, M. Zacek, D. Mansuy and E. Azoulay.

The primary oxidation of alkanes by Candida tropicalis is ensured by the hydroxylase, a polyenzymatic system composed of a cytochrome P-450 and a NADPH-cytochrome c (P-450) reductase. The study of the properties of NADPH-cytochrome c (P-450) reductase was made on the enzyme solubilized either by osmotic wash (MW : 67 000 daltons) or treatment with sodium cholate (MW : 76 000 daltons). These two methods give enzymes having comparable properties regarding the various electron acceptors (cytochrome c, dichloroindophenol, menadione, ferricyanide), inhibitors (chloromercuribenzoate, $HgCl_2$), ionic strength, FMN and FAD content (1 mol each of FMN and FAD per mol of enzyme), but only the sodium cholate-solubilized enzyme permit lauric acid hydroxylation in a reconstituted system using partially purified cytochrome P-450.

The hydroxylase system is specifically synthesized during growth on certain hydrocarbons (straight-chain alkanes having at least 10 carbon atoms, alkenes and higher alcohols) and its level varies greatly with the growth phase.

In vitro, the specificity is even narrower: a good activity is obtained only with fatty acids which are hydroxylated in the ω position resulting in the corresponding diacid. Alkanes are little or not hydroxylated. This could be due to an alteration of the structure of cytochrome P-450 during the preparation of the microsomal membranes. This hypothesis is confirmed by the fact that type 1 spectral alterations with alkanes have never been observed with cytochrome P-450 of Candida tropicalis. With ligands the spectral properties of this cytochrome do not differ from that obtained with the cytochrome of mammals or with that of Saccharomyces cerevisiae (characteristic type II and reverse type 1 spectra).

In the case of Candida tropicalis, the location of this hydroxylase will be determined and discussed.

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4 Edgard Azoulay, Francoise Jouanneau, Jean-Claude Bertrand, Alain Raphael, Jacques Janssens and Jean Michel Lebeault. Fermentation Methods for Protein Enrichment of Cassava and Corn with Candida tropicalis. Applied and Environmental Microbiology, 39:41-47, 1980.

Candida tropicalis grows on soluble starch, corn and cassava powders without requiring that these substrates be previously hydrolyzed. C. tropicalis possesses the enzyme needed to hydrolyze starch, namely, an α-amylase. That property has been used to develop a fermentation process whereby C. tropicalis can be grown directly on corn or cassava powders so that the resultant mixture of biomass and residual corn or cassava contains about 20% protein, which represents a balanced diet for either animal fodder or human food. The fact that no extra enzymes are required to hydrolyze starch results in a particularly efficient way of improving the nutritional value of amylaceous products, through a single-step fermentation process.

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5. J.C. Bertrand, M. Gilewicz, H. Bazin and E. Azoulay. Purified Detergent-Solubilized NADPH-Cytochrome C (P-450) Reductase from Candida Tropicalis Grown on Alkanes. Biochemical and Biophysical Research Communications, 94(3): 889-893, 1980.

SUMMARY

A microsomal NADPH-cytochrome c reductase from yeast was purified by column chromatography on Phenyl-Sepharose CL-4B, DEAE cellulose, hydroxylapatite and Sephadex G-150 in the presence of sodium cholate and Mulgofen BC-720, a non ionic detergent. On SDS-polyacrylamide gel electrophoresis, the purified enzyme gives a single band of 76,000 MW. FMN and FAD were present in approximately equal amounts. Contrary to the enzyme obtained by osmotic wash, the cholate-solubilized reductase is able to transfer the electrons from NADPH to cytochrome P-450 and may thus be considered as a NADPH-cytochrome c (P-450) reductase.

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6. Daniel Mansuy, Maryse Carlier, Jean-Claude Bertrand, and Edgard Azoulay. Spectral Characterization of Cytochrome P-450 of a Strain of Candida tropicalis Grown on Tetradecane. Eur. J. Biochem. 109, 103-108 (1980).

Several properties of the cytochrome P-450 induced in the yeast Candida tropicalis by growth on tetradecane have been studied by differential visible spectroscopy on microsomes. The spectral changes typical of this cytochrome have been obtained by subtraction of an unspecific spectral change, possibly due to the presence of other hemoproteins in microsomes, from the experimental difference spectra. Like the previously described cytochromes P-450 from yeast and mammalian liver, C. tropicalis cytochrome P-450 is in spin-state equilibrium at ambient temperature: about 30% of the originally low-spin cytochrome is converted to the high-spin state upon increasing the ionic strength of the medium, whereas 30% of the originally high-spin cytochrome is converted to the low-spin state upon addition of hydrophobic alcohols. C. tropicalis cytochrome P-450 readily binds nitrogenous ligands, isocyanides and phosphines in the ferric and ferrous state with spectral characteristics similar to those reported for other yeast or mammalian cytochromes P-450. It also reacts successively with cumylhydroperoxide and 1,3-benzodioxole to form a high-valent iron-oxo species and an iron-carbene metabolite complex. However, it fails to produce any spectral or spin-state change upon addition of hydrophobic non-coordinating compounds such as n-tetradecane, its substrate in vivo.

XXXVII. Meetings

1. Vth International Symposium on Yeasts (ISY), University of Western Ontario, London, Ontario.

The Vth International Symposium on Yeasts (ISY) was held in conjunction with the VIth International Fermentation Symposium (IFS) on the campus of the University of Western Ontario, July 20-25, 1980. Some 1700 delegates from 52 countries attended the joint symposium and due to the conjoint nature of the symposia it is difficult to estimate the exact number of delegates that attended the yeast symposium per se. However, as 160 papers were presented at the Vth ISY an estimate of 350 to 400 delegates would not seem unreasonable.

The ISY opened with four plenary lectures:

"Saccharomyces cerevisiae" as a model experimental eukaryote." A.H. Rose (Univ. Bath, U.K.)

"Why use yeast in studies of molecular biology and genetics?" F. Sherman (Univ. Rochester, U.S.A.)

"The species concept in yeast: physiologic, morphologic, genetic and ecologic parameters." H.J. Phaff (Univ. California, U.S.A.)

"The view through the microscope." C. Robinow (Univ. Western Ontario, Canada)

The rest of the week was taken up with symposia on:

1. Industrial and Agricultural Uses of Yeast. 2. Genetics of Yeast. 3. Sporulation and Conjugation. 4. Biochemistry of Yeasts. 5. Taxonomy and Ecology. 6. Yeast Cell Cycle.

Free communications in both oral and poster form were presented throughout the week. On the morning of the final day of the conference a special symposium was held in honour of Prof. H.J. Phaff at which papers were presented by his former students and associates.

Copies of the abstracts of the joint symposium can be obtained for ten dollars (Canadian) from:

Publication Sales and Distribution, National Research Council Canada, Ottawa, Canada, K1A 0R6, NRC 18371, ISBN 0-660-50590-8.

The papers presented at the ISY are being published by Pergamon Press of Canada, Ltd. and can be obtained from:

Pergamon of Canada Limited, 150 Consumers Road, Suite 104, Willowdale, Ontario, M2J 1P9, Canada.

International Commission for Yeasts (ICY)

During the Vth International Symposium on Yeasts a meeting of the International Commission for Yeasts (ICY) was held and attended by 22 commission members. The following is a summary of the business conducted.

In accordance with tradition, Dr. G.G. Stewart (Chairman, Vth ISY) assumed the chairmanship of the ICY from Prof. H. Klaushofer who had held the position since 1974. The commission passed unanimously the resolution thanking Prof. Klaushofer for his sterling efforts over the past six years.

Due to retirement, Prof. C. Robinow (Canada) and Dr. R.B. Gilliland (Ireland) resigned from the commission. The commission formally thanked them for their loyalty and support over the years. A number of scientists from a variety of countries became new members of the commission. These are reflected in the current membership of the commission listed below

ICY Commission Members (1980)

Australia: B.C. Rankine; Austria: H. Klaushofer (Vice-Chairman), U. Sleytr;

Belgium: H. Verachtert; Brazil: A. Panek;

BRD: S. Windisch; Bulgaria: P.V. Venkov;

Canada: B.F. Johnson (new member), G.G. Stewart (chairman), I. Russell (secretary);

Czechoslovakia: A. Kockova-Kratochvilova, A. Kotyk, E. Minarik;

Denmark: A. Stenderup; DDR: H. Koch, P. Lietz, W. Nordheim;

Egypt: A.S. ElNawawy; England: A.H. Rose, J.F.T. Spencer;

Finland: H. Suomalainen; France: J. Bastide, P. Galzy, H. Heslot;

Hungary: E. Novak; India: T.V. Subbaiah;

Ireland: A.J. Forage (proposed as new member to replace R.B. Gilliland);

Israel: C. Shalitin; Japan: Y. Fukazawa, K. Iwata, S. Nagai;

Netherlands: J.C. Hoogerheide, L. Rodrigues de Miranda; Poland: J. Jakubowska, B. Bachman;

Portugal: L. do Carmo-Sousa; Scotland: I. Dawes, E.O. Morris;

South Africa: J.P. van der Walt; Spain: J. Gancedo, R. Santandreu;

Sweden: K. Jarl, T. Wiken; Switzerland: A. Fiechter, P. Matile;

U.S.A.: C.P. Kurtzman (new member), H.J. Phaff, F. Sherman (new member);

U.S.S.R.: I. Babjeva, N. Elinov, M. Meissel, G. Shavlovsky;

Yugoslavia: V. Johanides.

Future yeast meetings sponsored by the ICY

7th Specialized Symposium on Yeast (ISSY), September 1981 - Valencia, Spain, "Yeast Cell Surface" (Prof. Rafael Santandreu; see Yeast Newsletter, June 1980).

8th Specialized Symposium on Yeast (ISSY), Late 1982 or early 1983 - Bombay or Bangalore, India, "Yeast Technology" (Dr. T.V. Subbaiah).

9th Specialized Symposium on Yeast (ISSY), 1983 - "Yeast Taxonomy and Ecology", Bratislava, Czechoslovakia (Dr. A. Kockova-Kratochvilova).

10th Specialized Symposium on Yeast (ISSY), 1984 or 1985 - "Molecular and Genetic Aspects of Yeast", Sofia, Bulgaria (Dr. P.V. Venkov).

6th International Symposium on Yeast (ISY), 1984 or 1985 - Bath, England or Montpellier, France.

It is hoped that the exact time and location of the symposia in 1984 and 1985 will be announced in the next issue of the Yeast Newsletter.

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2. The Thirteenth Annual Meeting of the Yeast Genetics Conference - Japan. Communicated by Kotaro Hieda, Rikkyo Univ., Department of Physics, Tokyo 171, Japan.

The Thirteenth Annual Meeting of the Yeast Genetics Conference-Japan was held from October 21 to 23, 1980, at Ginza Hall of Sapporo Breweries, Ltd., Tokyo, Japan. The following topics were presented and discussed.

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

Tadashi Hirano, Akira Tanaka* and Yoshio Sekiguchi* (Jikei Univ. Sch. Med.; *Tokyo Metropol. Inst. Med. Sci.). Fine structure of yeast protoplasts.

Takashi Sasaki and Norio Nishikawa (Cent. Res. Labs., Sapporo Breweries, Ltd.). Regeneration process of spheroplasts of Saccharomyces cerevisiae.

Kanji Takeo and Ei-ichi Nakai* (Res. Inst. Chemobiodynamics, Chiba Univ.; *Kyoto Univ.). The plasma membrane of Pityrosporum has natural markers to show how the cell grows.

Aiko Hirata and Kenji Tanaka* (Inst. Appl. Microbiol., Univ. Tokyo; *Nagoya Univ., Sch. Med.). Fine structure of ascospore formation in the fission yeast Schizosaccharomyces japonicus.

Takashi Toda, Masayuki Yamamoto and Mitsuhiro Yanagida (Fac. Sci., Kyoto Univ.). Pathway of chromosome structual change during mitosis of the fission yeast, Schizosaccharomyces pombe - analysis by selection synchrony and cdc mutants.

Yasushi Hiraoka and Mitsuhiro Yanagida (Fac. Sci., Kyoto Univ.). Isolation of nuclei from Schizosaccharomyces pombe and analysis of nuclear DNA distribution with fluorescent dyes.

Session 2: Radiation and Mutation (Chairpersons, K. Hieda, T. Ito and K. Ouchi).

Sumie Nishiyama-Watanabe (Col. Gen. Educ., Univ. Tokyo). Sensitivity of yeast strains S. cerevisiae to the photosensitization of thiopyronine and their ability of dye bleaching.

Takashi Ito (Col. Gen. Educ., Univ. Tokyo). Photosensitization of yeast cells by hematoporphyrin.

Takashi Ito and Atsushi Ito (Col. Gen. Educ., Univ. Tokyo). Studies on light induced non-DNA damage -membrane damage as tested by dye penetration.

Tetsuya Saeki (Div. Genetics, Natl. Inst. Radiol. Sci.). Timing of fixation of UV-induced recombination and gene conversion during mitosis in Saccharomyces cerevisiae.

Isamu Machida and Sayaka Nakai (Div. Genetics, Natl. Inst. Radiol. Sci.). Production of mutation, conversion and recombination by UV-irradiation during commitment to meiosis in Saccharomyces cerevisiae.

Yumiko Ishino, Bun-ichiro Ono and Sumio Shinoda (Dept. Pharm., Okayama Univ.). Nonsense mutations of canavanine resistance in the yeast Saccharomyces cerevisiae.

Masamichi Takagi, Masahide Kawamura and Kenji Yano (Fac. Agr., Univ. Tokyo). On mutants of Candida yeast with high growth rate on glycerol.

Yasuo Nakatomi and Kohji Uchida (Res. Labs., Oriental Yeast Co., Ltd). Heterogeneity of clones in spore cultures of baker's yeasts.

Session 3: Gene Regulation, Recombination and Mapping (Chairpersons K. Matsumoto, M. Yanagida and B. Ono).

Kozo Ouchi, Hitoshi Shimoi, Masahiko Shimoda and Hiroichi Akiyama (Natl. Res. Inst. Brewing). Genetic analysis of TTC (2, 3, 5-triphenytetrazolium chloride) reduction in Saccharomyces cerevisiae.

Hideo Tamaki (Doshisha Women's Col.). Genetic analysis of the hybrid between Saccharomyces diastaticus and S. rouxii by protoplast fusion.

Toyohiko Yamazaki (Dept. Ferment. Technol., Yamanashi Univ.). Linkage groups of Saccharomycodes ludwigii.

Kunihiro Matsumoto and Isao Uno* (Dept. Ind. Chem., Tottori Univ.; *Inst. Appl. Microbiol., Univ. Tokyo). The role of cyclic AMP in Saccharomyces cerevisiae.

Yujiro Arao, Yumiko Ishino, Bun-ichiro Ono, Sumio Shinoda (Dept. Pharm., Okayama Univ.). On the problem that there is a homo-cluster of $tRNA^{Ser}_{UCA}$ gene in Saccharomyces cerevisiae genome, or not.

Bun-ichiro Ono, Yumiko Ohno, Nozomi Moriga, Yumiko Ishino, Sumio Shinoda (Dept. Pharm., Okayama Univ.). Does the yeast Saccharomyces cerevisiae have ochre suppressor?

Masayuki Yamamoto (Fac. Sci., Kyoto Univ.). Analysis and mapping of the genes responsible for antimitotic drug resistance in Schizosaccharomyces pombe.

Yasuhisa Nogi and Toshio Fukasawa (Fac. Med., Keio Univ.). Isolation and characterization of temperature-sensitive mutants for gal3 gene in Saccharomyces cerevisiae. II.

Yoshinobu Kaneko, Akio Toh-e and Yasuji Oshima (Dept. Ferment. Technol., Osaka Univ.). Genetic characterization of the phoI mutant defect in repressible alkaline phosphatase and the pep4 mutant defect in proteases.

Jiro Akimaru, Akio Toh-e and Yasuji Oshima (Dept. Ferment. Tecnol., Osaka Univ.). pho system of yeast: characterization of PHOP mutation.

Session 4: Sporulation and Life Cycle (Chairpersons, S. Harashima and M. Tsuboi).

Michio Tsuboi (Dept. Biol., Osaka City Univ.). Sporulation and meiosis of protoplasts and protoplast-fusion products in Saccharomyces cerevisiae.

Chikashi Shimoda (Dept. Biol., Osaka City Univ.). Sporulation-specific proteins in fission yeast, Schizosaccharomyces pombe.

Shozi Hashida and Chikashi Shimoda (Dept. Biol., Osaka City Univ.). Isolation and genetic analysis of the mutant of fission yeast (Schizosaccharomyces pombe) which produces spores sensitive to organic solvents.

Susumu Okamoto and Tetsuo Iino (Fac. Sci., Univ. Tokyo). Formation of spindle pole body lacking the outer plaque in the hfd mutant of Saccharomyces cerevisiae.

Satoshi Harashima, Atsuko Takagi and Yasuji Oshima (Dept. Ferment. Technol., Osaka Univ.). Dominant sterile mutation in Saccharomyces cerevisiae.

Atsuko Takagi, Satoshi Harashima and Yasuji Oshima (Dept. Ferment. Technol., Osaka Univ.). Discrimination of isogenic diploid and tetraploid cells in Saccharomyces cerevisiae by using dye plate.

Session 5: Biochemistry (Chairpersons, K. Suda and M. Takagi).

Hidetoshi Iida and Ichiro Yahara (Tokyo Metropol. Inst. Med. Sci.). G_1-G_0 transition and it's regulation in yeast, Saccharomyces cerevisiae.

Teijiro Kamihara, Ichiro Nakamura, Naohiko Isobe, Shuichi Tawara and Saburo Fukui (Dept. Ind. Chem., Kyoto Univ.). Regulation of glycolysis and ethanol fermentation in yeasts.

Kazuaki Nakura, Masanori Joho and Tetsuo Murayama (Biol. Inst., Ehime Univ.). Effect of cadmium on the regeneration of ribosomes in cadmium resistant strain of yeast.

Masazumi Miyazaki (Inst. Molec. Biol., Nagoya Univ.). Purification and properties of elongation factors, EF-1 and EF-2, from yeast.

Takemitsu Mizunaga, Toshihiro Noguchi and Yoshiharu Maruyama (Fac. Agr., Univ. Tokyo). Effect of tunicamycin on yeast acid Phosphatase biosynthesis.

Session 6: Cytoplasmic Inheritance (Chairperson: C. Shimoda).

Fumiko Miyamoto (Fac. Educ., Wakayama Univ.). Induction of respiratory deficient mutants by ethidium bromide in aerobically and anaerobically cultivated Saccharomyces cerevisiae.

Susumu Nagai, Sayoko Ochi, K. Nishimura and Atsuko Takagi (Biol. Labs., Nara Women's Univ.). Comparison of suppressiveness in rho mutants obtained by mild treatments.

Kohta Suda, Motoharu Nishimura, Kaori Ikai, Keizo Tano and Akira Uchida* (Biol. Lab., Nara Univ. Educ.; *Biol. Div., Kobe Univ.). Methodology on suppressiveness of yeast.

Session 7: Plasmid and Cloning (I. Takano and N. Gunge).

Setsuzo Tada, R.B. Wickner*, Akio Toh-e, and Yasuji Oshima (Dept. Ferment. Technol., Osaka University; *N.I.H.). 2μmDNA-like plasmid isolated from Saccharomyces rouxii.

Akio Toh-e (Dept. Ferment. Technol., Osaka Univ.). Chromosomal gene needed for the maintenance of 2-μm DNA of yeast.

Norio Gunge, Atsuko Tamaru and Kenji Sakaguchi* (Cent. Res. Lab., Mitsubishi Chem. Ind.; *Mitsubishi-Kasei Inst. Life Sci.). Killing phenomenon of Kluyveromyces lactis harboring pGKl plasmids.

Satoshi Harashima, Rajinder S. Sidhu, Akio Toh-e and Yasuji Oshima (Dept. Ferment. Technol., Osaka Univ.). Cloning of yeast (S. cerevisiae) HIS5 gene.

Rajinder S. Sidhu, Akio Toh-e, Satoshi Harashima and Yasuji Oshima (Dept. Ferment. Technol., Osaka Univ.). Cloning of pho genes of yeast.

Session 8: Reports (Chairperson: S. Nagai)

Isamu Takano and Hikaru Kimura* (Suntory Inst. Biomed. Res.; *Kyoto Univ.). Report of the 6th International Fermentation Symposium and the 5th International Symposium on Yeasts.

Toshiaki Takahashi (Cent. Res. Labs., Asahi Breweries, Ltd.). Herschel L. Roman Symposium.

Norio Gunge and Kazuhiko Wakabayashi* (Cent. Res. Lab., Mitsubishi Chem. Ind.; *Univ. Yamanashi Med. Sch.). Report of the 10th International Conference on Yeast Genetics and Molecular Biology.

3. Tenth International Conference on Yeast Genetics and Molecular Biology: A Review. Communicated by Terrance G. Cooper, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260.

Louvain-la-Neuve was the site of the Tenth International Conference on Yeast Genetics and Molecular Biology. During 24 plenary presentations, 18 evening workshops and over 200 posters, members of the international community participated in an exhaustive exchange of information about Saccharomyces cerevisiae and a few related organisms. From the depth and breadth of information presented, one could conservatively conclude that S. cerevisiae has become a favorite model for studying the structure and operation of eucaryotic cells. To review, even briefly, all of the topics discussed would be impossible. Therefore, a limited number of topics has been chosen. They provide a flavor of the information exchanged and an assessment of new and potentially important findings.

The meeting was opened by presentation of an engaging hypothesis derived from studies of mitochondrial genetics and DNA sequencing. The gene encoding cytochrome b is composed of a mosaic of six exons and five introns. It was suggested that one of the introns encodes a protein which participates in the removal of intron sequences from the cytochrome b gene transcript, i.e., an

RNA splicing enzyme or maturase. This model was consistent with data presented by several investigators and supported by four important observations. Strains harboring missense mutations within the intron sequence (box 3) were unable to process the transcripts derived from the cytochrome b gene. Nonsense, but not missense mutations in the exon adjacent to the 5' end of the box 3 region generated a similar phenotype prompting a suggestion that the N-terminus of the presumptive maturase was located in that exon. Box 3 intron mutations could be suppressed by drugs such as paromomycin which cause translational misreading; exon mutations were not suppressible under these conditions. DNA sequencing of the box 3 region revealed a long open reading frame. Colinearity could also be established between positions of nonsense mutations situated in the box 3 intron and the sizes of protein fragments derived from mutant translation products. Although these data support an enticing hypothesis, it is important to note that no nuclease or ligase activities have yet been associated with the box 3 protein identified on SDS gels.

RNA splicing was also a focal point of studies concerning expression of tRNA genes. It was suggested that the splicing system responsible for processing primary transcripts from eight or nine tRNA genes was distinct from that performing this function for mRNA. Mutants unable to remove intron sequences from SUP4 tRNA primary transcripts were found to result from mutations distributed throughout a recombinational fine structure map of the SUP4 locus. From these data, it was concluded that the entire three-dimensional structure of the intron-containing tRNA precursor is likely to be important for recognition by the splicing enzyme system; this was hypothesized to provide a final opportunity for proof-reading before activation of the newly synthesized tRNA.

Production of the var1 polypeptide (a component of the small ribosomal subunit from mitochondria) may involve yet another example of RNA-RNA splicing. This possibility derives from the identification of a genetic element which in some way specifies the size of a transcript, but is not continguous with the DNA coding for it. The var1 polypeptide is found in polymorphic forms ranging from 40K to 44K daltons in size. A var1 determinant, shown to be responsible for the polymorphism, is situated between the ery and oli1 loci on the mitochondrial genome. However, complementation studies demonstrated that the var1 determinant acts in trans. DNA sequencing studies revealed that the determinant does not possess apparent coding capacity (it is AT rich with no open reading frames) and is far too small to code for the var1 transcript. Normal amounts of the var1 transcript are found in petites containing only repeated copies of the var1 determinants (these strains carry no other mitochondrial DNA) and hybridization experiments show a sequence homology of only 200 to 250 bp between the 1900 to 2000 bp var1 transcript and this petite mitochondrial DNA. These observations have been argued to suggest, by elimination, that the structural gene for the var1 protein is located in the nucleus; a directly testable hypothesis.

For investigators interested in the production of primary transcripts and the regulation of this process, the nature and location of the promoter occupied center stage. The search was conducted in one of two ways. Sequences upstream from the 5' terminus of a gene were deleted to various extents; specific transcription was then assessed in these deletion strains. Alternatively, transcription was assayed in strains possessing insertions at various points upstream from the beginning of transcription. The expectation was that a eucaryotic promoter would comprise about 35 bp, as found in procaryotes. The results emphasized once again that control of gene expression in eucaryotes

differs markedly from that in procaryotes. A deletion ending 140 bp upstream from the ATG of the his3 gene (transcription begins at position -45) resulted in loss of gene function as did a deletion ending 250 bp upstream from the ATG of the cyc1 gene. Insertion of a Ty1 sequence 161 bp upstream from the ATG of the his4 gene (transcription begins at position -63) similarly eliminated transcription. In the case of the gal gene cluster, transcription of the contiguou gal 1 and gal 10 genes diverges from a single region of approximately 700 bp. It is not yet known whether or not transcription of the two genes is initiated from the same point. From these results, it appears that a surprisingly large region might be required for effective promotion of transcription. However, it is important to note that deletions do not simply remove stretches of DNA. They also fuse the flanking sequences that remain. It may be important to ascertain the consequences of these fusions. Such studies have been initiated for the his3 gene. Also evident from the data presented was the fact that the structure of eucaryotic "promoters" remains unknown even though a significant amount of sequence data is already available.

In a related area, four laboratories reported the structural characteristics of cloned genes (adr2, car1, cyc2, dur1,2) derived from mutant strains in which production of an enzyme was rendered constitutive by a mutation situated close to the structural gene. Constitutivity was observed in MATa and MATα haploid strains and MATa/MATa and MATα/MATα diploid organisms, but it was lost in MATa/MATα diploids. In each case analysed there appeared to be an insertion in or near the mutated gene. In two instances the inserted DNA was shown to be related to Ty sequences. Pertinent to these observations is the fact that RNA hybridizing to Ty elements has been reported to be the most abundant transcript expressed in parallel with mating-type specific functions, i.e., such RNA is present in MATa and MATα cells, but is diminished in MATa/MATα diploid strains. While this raises the possibility that the constitutively expressed genes have been placed under the control of a new promoter as a result of the DNA rearrangement, transcripts derived from wild-type and constitutive ADR2 mutants associated with a Ty element have been compared on Northern blots and were found to be the same size. It is also important to point out that insertion of Ty sequences into the "control" region of a gene does not always result in its constitutive expression or association of expression with mating-type functions. Two mutants unable to express the his4 gene (his4-912 & his4-917) were found to harbor Ty elements upstream from the transcribed sequences. A His^- phenotype was observed both in haploid and homozygous MATa/MATα diploid versions of these mutants.

Association of Ty sequences with a major proportion of the chromosomal rearrangements analysed thus far prompted a search for other genetic elements associated with the influence of these mobile elements on genes in their proximity. This was done by selecting His^+ revertants of the his4-912 and his4-917 mutants just discussed. One class of mutants (spm2 & spm3) suppressed the effects of the his4-917 mutation, but were incapable of suppressing the his4-912 lesion. However, the reversion frequency of the latter mutation was increased 100 fold in Spm2- and Spm3- strains. Analysis of representative revertants revealed that recombination had occurred between the two directly repeated δ sequences flanking the inserted Ty element leaving behind a single copy of the δ sequence in place of the entire Ty element. Such revertants were found to be cold sensitive in a wild-type background, i.e., His^+ at 37°C, but His^- at 22°C. In $Spm2^-$ or $Spm3^-$ strains cold sensitivity of the revertants was suppressed. A second class of mutations

(spm1) suppressed the effects of both his4-912 and his4-917 mutations and cold sensitivity of the his4-912 revertants. In formal terms these observations share several characteristics in common with the two component spm control system of maize.

Operation of the mating-type locus remains a topic of broad interest. Northern analysis and R-looping studies have demonstrated that each of the mating-type alleles, MATa, MATα, codes for two messages that are transcribed divergently from opposite DNA strands. Surprisingly, the DNA sequences of the MAT locus and the corresponding silent gene are identical. These observations raise the provocative question: why are the mating-type genes expressed only when situated at the mating-type locus, but not at the silent loci? Genetic studies demonstrating that DNA from the silent loci can recombine with DNA at the mating-type locus have been interpreted as supporting a model of switching that involves a gene conversion type mechanism. The death of homothallic strains containing mutations in the rad52 locus argues that the product of this gene plays an important role in the switching process. However, mechanistic data presently available are insufficient to explain the pedigree data for mating-type switching in homothallic strains, namely that only a mother cell switches mating-type.

Two important gene products under mating-type control are the a and α-factor pheromones. Although the primary amino acid sequence of α-factor has been known for several years, similar information for a-factor has not been available until now. It was reported that a-factor has been purified 100,000 fold and appears to be an ondecapeptide. The sequence of the first five amino acids has been determined raising the possibility that the entire structure will be available shortly. However, it is already clear from composition studies that a-factor is likely to be structurally quite distinct from α-factor even though both pheromones seem to operate in a similar manner.

The investigation of sporulation has progressed substantially on several fronts. Characterization of DNA synthesis and the stage at which recombination occurs were important steps in defining landmark events in sporulation. The time occupied by premeiotic DNA synthesis was shown to be 1.5 to 2.0 hours. The average replicon size and rate of replication were estimated to be 84 kb and 2 kb per minute respectively; the latter two values are similar to those observed for mitosis. The time at which recombination occurs was estimated in two ways. One method involved the use of a reversible temperature-sensitive variant in which sporulation is arrested at pachytene when sporulating cells are shifted to the non-permissive temperature. Holding this variant at 36°C for increasing times resulted in a dramatic increase in recombination frequency (40-50 fold for post meiotic segregation, 10-20 fold for gene conversion and 2 fold for reciprocal recombination) thus pointing to pachytene as a likely stage for the occurrence of recombination. A second method depended on the existence of two strains possessing restriction site polymorphisms in the SUP4 region. By following the patterns of restriction fragments generated on digestion of DNA derived from cells going through sporulation it was possible to follow recombination directly at the molecular level. Recombinant molecules were observed between four and six hours after initiation of sporulation which correlates with the approximate time at which cells pass through the pachytene stage of meiosis.

A novel and highly useful system for the examination and manipulation of recombination-deficient mutants was presented. It has been difficult to

diagnose recombination deficiency in the past, because the reductional chromosome segregation associated with meiosis I requires recombination. Its absence leads to a high frequency of non-disjunction and spore inviability. The inability of putative recombination-deficient strains to sporulate has forced reliance on indirect assays of recombination. The newly developed system which circumvents these problems depends on the spo13 mutation. Strains harboring a lesion in this locus enter meiosis, undergo recombination and then, by-passing the reductional division of meiosis I, proceed directly to a single equational division characteristic of meiosis II. The latter division does not require recombination. Therefore, by coupling a putative recombination deficient mutation to spo13, it is possible to follow recombination and sporulation independently. By this procedure both spo11 and rad50 mutants were definitely shown to be deficient in meiotic recombination. The observation that a rad52, spo13 double mutant was unable to sporulate, whereas the rad50, rad52, spo13 triple mutant produced viable Rec^- spores raised the possibility that either the rad50 gene product acts prior to that of rad52 or alternatively that the two gene products interact in some manner.

Another useful characteristic of the spo13 mutation is that it permits the sporulation of haploid strains expressing a MATa/MATα phenotype (strains either disomic for chromosome III and heterozygous for the MAT locus or Mar^-). Therefore, it should greatly facilitate the isolation of recessive mutations causing a deficiency in recombination or those which fail to prevent expression of the silent mating-type genes, i.e., mutations generating phenotypes similar to those of the sir or mar class.

The characteristics of putative recombination deficient mutants generated much discussion among those interested in recombination mechanisms as did the suggestion that gene conversion was the basic signal of meiotic recombination and that the different types of segregation observed were a function of how the recombination-heteroduplex was corrected. The rad52 gene product was shown to be needed for a wide variety of recombination associated processes including: mating-type switching, meiotic recombination, γ-radiation repair and meiotic and mitotic gene conversion. However, sister strand exchange, plasmid integration into a chromosome and excision of integrated plasmids were found to occur normally in $Rad52^-$ strains. Mutants defective in the latter two processes were isolated by scoring mutagenized cells for inability to excise an integrated plasmid carrying the dominant trichodermin sensitivity allele (TCM). The recessive mutations harbored in these strains segregated 2+:2-, did not cause radiation sensitivity and did not affect sporulation. One more extensively studied mutant was found to be deficient in all forms of mitotic recombination assayed. Transformation of this strain with plasmids that required integration (Ars^-) was found to be 25-fold lower than in the wild-type. Also isolated was a class of mutants (cor) that exhibited elevated frequencies of meiotic recombination. Complementation studies identified at least four genetic loci among the mutants characterized. As a group the Cor^- mutants were not sensitive to X-ray or U.V. radiation and did not affect meiotic map distances or the frequency with which gene conversion was initiated. However, correction of heteroduplex intermediates generated during recombination was reported to be markedly reduced and the length of the correction tracts were appreciably shortened compared to wild-type when correction did occur.

The phenomenology and genetics associated with repair of DNA damaged by radiation or chemical mutagens have been quite complex. Approximately 100

mutants have been isolated and characterized. This, however, may represent an overestimate of the genetic elements participating in repair, because allelism tests have not been completed. The results of characterization studies in many laboratories have led to the conclusion that five repair systems seem to exist. One mutagenic (error-prone) and two non-mutagenic systems are involved in the repair of radiation damage. Work with anti-mutator mutants suggests the existence of two mutagenic repair systems. One of these systems is identical with the mutagenic repair system associated with radiation damage; however, the second system appears to be independent of radiation damage. A fifth system seems to be needed for the repair of damage generated by the DNA alkylating agent, methyl methane sulfonate (mms). This suggestion is based on the observation that some mms-sensitive mutants are insensitive to radiation. The repair of psoralen generated cross linked DNA requires both non-mutagenic repair systems associated with repair of radiation lesions (the rad3 and rad51 systems). A new assay system has also been developed which permits one to follow the repair of DNA crosslinks in 2 μ circular DNA. This system should prove useful for studying DNA repair at the molecular level.

Finally, two reports describing very interesting types of DNA were presented. First was the centromeric DNA (CEN3) from chromosome III which was isolated on a 1.6 kb segment of DNA located near the centromere-linked cdc10 locus. When present on a plasmid carrying a yeast chromosomal replicator (such as ars1), CEN3 DNA enabled the plasmid to segregate as a chromosome both mitotically and meiotically. In meiosis the plasmid segrated 2+:2- rather than the 4+:0- characteristic of other unintegrated plasmids. In addition, plasmids containing centromeric DNA were found to be strikingly more stable. plasmids containing centromeric DNA were found to be strikingly more stable. This stability would be a most desirable characteristic in transformation cloning vectors. Such vectors are now being constructed. The second interesting type of DNA was reported to exist as a naturally occurring plasmid in Kluyveromyces lactis. This organism appears to harbor two linear DNA plasmids of 5.4 and 8.4 x 10^6 daltons, respectively.

They represent the first non-circular DNA plasmids reported in yeast or any other organism. Strains possessing the two linear plasmids were shown to kill both S. cerevisiae killer and sensitive strains. Non-killing K. lactis strains both sensitive and resistant to being killed themselves were also found.

4. IVth International Conference on Culture Collections (July 20-24, 1981, Brno, Czechoslovakia)

Papers and/or posters are invited on the following topics:

1. Role of culture collections (both in fundamental and applied research, education, disease control, industrial production, etc.); 2. Problems of running of culture collections; 3. Strain selection and improvement for various purposes; 4. Preservation of cultures; 5. The use of computers in culture collections for data storage, retrieval, analysis and identification; 6. Biohazards in culture collections and their control; 7. Culture collections as depositories for patent cultures; 8. Packaging and shipping of cultures. Postal and quarantine regulations; 9. Posters on activity of the WFCC members; 10. Taxonomy and identification (posters only); and 11. Exhibition of media and laboratory equipments by manufactures.

Address to which correspondence should be mailed:

Dr. M. Kocur
Czechoslovak Collection of Microorganisms
J.E. Purkyne University
662 43 B r n o
Czechoslovakia

5. FEMS Symposium on Overproduction of Microbial Products. Hradec Kralove, Czechoslovakia, 9 - 14 August, 1981.

Correspondence

All correspondence should be addressed to: Dr. C. Novotny, Secretary of the FEMS Symposium on Overproduction of Microbial Products, Institute of Microbiology, Czechoslovak Academy of Sciences, Videnska 1083, 142 20 Praha 4, Czechoslovakia.

6. Fourth International Symposium on Genetics of Industrial Microorganisms. June 7 (Monday) - 11 (Friday), 1982, Kyoto, Japan.

Prof. Akira Kimura
Secretary General
Organizing Committee
Fourth International Symposium on Genetics of
Industrial Microorganisms (GIM 82)
c/o Kyoto International Conference Hall,
Takara-ike, Sakyo-ku, Kyoto, 606 JAPAN

XXXVIII. Brief News Items.

1. WANTED!! Does anyone in their yeast stocks have a strain of Saccharomyces cerevisiae with the isoleucine-valine mutant gene ilv4 alias iv-II alias iso-4? If so, I would be most grateful if you could send it to me.

Jens G. Litske Peterson
Carlsberg Laboratory, Department of
Physiology, Gl. Carlsberg Vej 10,
DK-2500 Copenhagen Valby, Denmark

2. The following three articles were published recently:

K. Werkmeister, F. Wieland, E. Schweizer. 1980. Coenzyme A: Fatty acid synthetase apoenzyme 4'-phosphopantetheine transferase in yeast. Biochem. Biophys. Res. Commun. 96:483-490.

M. Mishina, R. Roggenkamp, E. Schweitzer. 1980. Yeast mutants defective in acetyl-coenzyme A Carboxylase and Biotin: Apocarboxylase Ligase. Eur. J. Biochem. 111:79-87.

R. Roggenkamp, S. Numa, E. Schweizer. 1980. Fatty acid - requiring mutant of Saccharomyces cerevisiae defective in acetyl-CoA carboxylase. Proc. Natl. Acad. Sci. U.S.A. 77:1814-1817.

Eckhard Schweizer
Institut für Mikrobiologie und
Biochemie der Universität
Lehrstuhl für Biochemie
852 Erlangen
Egerlandstr. 7, West Germany

3. After leaving the Central Drug Research Institute, Lucknow, I am presently working as Chief Quality Control in Indian Drugs & Pharmaceuticals, Ltd., Virbhadra (Rishikesh) U.P. Pin:249202. NORTH INDIA.

Dr. T.R. Thyagarajan

4. The following paper was recently published:

R.T. Moore, 1980. Taxonomic proposals for the classification of marine yeasts and other yeast-like fungi including the smuts. Botanica Marina XXIII:361-373.

ns.

5. Our second book on SAGO entitled SAGO '79 which will be published by

Martinus Nijhoff
Lange Voorhout 9-11
P.O. Box 566
2501 CN The Hague
The Netherlands

This will consider tree-starches as the product of large-scale energy plantations of equatorial swamps, producing power-alcohol and renewable-resource feedstrock for the fermentation and chemical industries. Already a project is being planned in New Guinea based on the large area of semi wild sago forests in that territory.

Dr. W.R. Stanton

6. I. Nakamura, N. Isobe, T. Kamihara, S. Fukui. 1980. Effects of thiamine and pyridoxine on respiratory activity in Saccharomyces carlsbergensis Strain 4228. Arch. Microbiol. 127:47-51.