Y E A S T

A News Letter for Persons Interested in Yeast

Official Publication of the
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June 1976 Volume XXV, Number 1

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I. Centraalbureau voor Schimmelcultures (Netherlands), Delft, Julianalaan 67a. Communicated by D. Yarrow.

Below follows a list of new species received by the CBS since my last contribution to the newsletter.

Bullera piricola CBS 6754 (Type), CBS 6784, CBS 6785. F. Stadelmann, A. v. Leeuwenhoek 41: 575-582 (1976).

Candida podzolica CBS 6819 (Type) I. P. Babjeva and I. S. Reshetova, Microbiologiya 44:333-338 (1975).

Pichia lindneri CBS 6502 (Type), W. Henniger and S. Windisch, Arch. Microbiol. 105:47-48 (1975). This species appears to be the perfect state of Torulopsis methanolovescens Oki and Kounu.

Sterigmatomyces ophidis CBS 6821 (Type), W. Henniger and S. Windisch, Arch. Microbiol. 105:49-50 (1975).

Sterigmatomyces nectairii CBS 6405 (Type).

Sterigmatomyces penicillatus CBS 5492 (Type), L. Rodrigues de Miranda, A. v. Leeuwenhoek 41:193-199 (1975).

Torulopsis sonorensis CBS 6792 (Type), CBS 6793. M. W. Miller et al., Int. J. Syst. Bact. 26:88-91 (1976).

Torulopsis spandovensis CBS 6875 (Type), W. Henniger and S. Windisch, Arch. Microbiol. 107:205-206 (1976).

Trichosporon jirovecii CBS 6864 = CCY 30-7-1. P. Fragner, Ceska. Mycol. 23:160-162 (1969).

Cladosporium fermentans (= Pichia burtonii) CBS 6874 (Type). S. Goto, J. Agric. Chem. Soc. Japan 49:377-381 (1975).

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

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

Candida albicans
ATCC 32354, ATCC 32449, ATCC 32470
ATCC 32552

Candida boidinii
ATCC 32195

Candida brassicae
ATCC 32196

Candida guilliermondii var. guilliermondii
ATCC 32542

Cryptococcus neoformans var. gatti
ATCC 32269

Cryptococcus terreus
ATCC 32422

Debaryomyces hansenii
ATCC 32458

Filobasidiella sp.
ATCC 32608, ATCC 32609

Candida krusei
ATCC 32672

Candida lambica
ATCC 32543

Candida parapsilosis
ATCC 32544

Candida requinyii
ATCC 32545

Candida tropicalis
ATCC 32546, ATCC 32547

Candida vartiovaarai
ATCC 32346

Cryptococcus albidus
ATCC 32420

Cryptococcus albidus var. albidus
ATCC 32553

Cryptococcus albidus var. diffluens
ATCC 32422

Cryptococcus neoformans
ATCC 32264, ATCC 32265, ATCC 32266, ATCC 32267, ATCC 32268, ATCC 32308, ATCC 32309, ATCC 32310, ATCC 32551, ATCC 32719, ATCC 32733, ATCC 32734, ATCC 32735, ATCC 32736, ATCC 32737, ATCC 32738, ATCC 32739, ATCC 32740 ATCC 32741, ATCC 32742

Pichia lindnerii
ATCC 32418

Pichia naganishii
ATCC 32816

Pichia norvegensis
ATCC 32296, ATCC 32297, ATCC 32298

Pichia scolyti
ATCC 32419

Pichia veronae
ATCC 32764, ATCC 32770

Filobasidium capsuligenum
ATCC 32289, ATCC 32290

Hanseniaspora uvarum
ATCC 32369

Hanseniaspora valbyensis
ATCC 32370

Hansenula ciferrii
ATCC 32291, ATCC 32292, ATCC 32293

Kloeckera apiculata
ATCC 32548

Leucosporidium capsuligenum
ATCC 32721

Lipomyces anomalus
ATCC 32435

Lipomyces lipofer
ATCC 32371

Lipomyces tetrasporus
ATCC 32372, ATCC 32373

Metschnikowia bicuspidata var. chathamia
ATCC 32294, ATCC 32295

Pachysolen tannophilus
ATCC 32691

Pichia lindnerii
ATCC 32658

Saccharomyces sake
ATCC 32694, ATCC 32695, ATCC 32696, ATCC 32697, ATCC 32698, ATCC 32699, ATCC 32700, ATCC 32701, ATCC 32702, ATCC 32703

Saccharomyces uvarum
ATCC 32634

Saccharomycopsis fibuligera
ATCC 32693

Saccharomycopsis lipolytica
ATCC 32338, ATCC 32339, ATCC 32340, ATCC 32341, ATCC 32342, ATCC 32343

Rhodotorula aurantiaca
ATCC 32759, ATCC 32760, ATCC 32761, ATCC 32765, ATCC 32766

Rhodotorula glutinis var. rufusa
ATCC 32767

Rhodotorula graminis
ATCC 32768

Rhodotorula minuta var. texensis
ATCC 32769

Rhodotorula pilimanae
ATCC 32762

Rhodotorula rubra
ATCC 32549, ATCC 32763

Saccharomyces aceti
ATCC 32299, ATCC 32300

Saccharomyces cerevisiae
ATCC 32527, ATCC 32685, ATCC 32686, ATCC 32687, ATCC 32688, ATCC 32689, ATCC 32690, ATCC 32747, ATCC 32748, ATCC 32749, ATCC 32810

Sporobolomyces salmonicolor var. novo
ATCC 32311

Sterigmatomyces aphidis
ATCC 32657

Sterigmatomyces halophilus
ATCC 32823, ATCC 32824

Sterigmatomyces polyborus
ATCC 32821, ATCC 32822

Torulopsis candida
ATCC 32550

Torulopsis glabrata
ATCC 32312, ATCC 32554

Torulopsis navarrensis
ATCC 32692

Trichosporon cutaneum
ATCC 32345

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

The following is an abstract of a paper presented before the annual meeting of the American Soc. of Microbiology in Atlantic City, May 1976.

DNA Base composition and DNA Reassociation of Hanseniaspora and Kloeckera species. Ruth E. Brown and Sally A. Meyer. American Type Culture Collection, Rockville, MD.

Twenty-four strains representing eight species of Hanseniaspora and its imperfect counterpart, Kloeckera, were examined for DNA base composition and DNA reassociation. These physiologically similar species showed a broad range of GC (ca. 27-40%), with the type strains of Hanseniaspora valbyensis (27.3%) an obvious standout at the low end of the range. DNA reassociation experiments substantiated species separation of H'spora valbyensis, H'spora uvarum (34.4%GC) and H'spora occidentalis (34.4%GC). H'spora valbyensis type strain showed no significant reassociation with the H'spora guilliermondii strain of H'spora valbyensis or with strains of the four different species of Kloeckera, including strains of Kl. apiculata. H'spora uvarum, on the other hand, revealed a significant degree of hybridization with the three strains of Kl. apiculata studied, demonstrating a perfect-imperfect relationship.

IV. Centraalbureau voor Schimmelcultures, Yeast Division, Julianalaan 67a Delft, The Netherlands. Communicated by Maudy Th. Smith.

The following is in press:

Maudy, Th. Smith, J. P. van der Walt and Ella Johannsen: "The genus Stephanoascus gen. nov. (Ascoideaceae)". In Antonie van Leeuwenhoek 1976.

SUMMARY

The imperfect species currently cited as Candida ciferrii was found to constitute the haploid mating types of an undescribed, filamentous, heterothallic ascomycete. This pefect state has been transferred to the new genus Stephanoascus. The diagnosis of the genus and the description of the species Stephanoascus ciferrii are given.

V. Georgia State University, Department of Biology, Atlanta, Georgia 30303. Communicated by D. G. Ahearn.

The following papers have been published or are in press.

"Physiological and DNA Characterization of Candida maltosa, a Hydrocarbon-Utilizing Yeast." S. A. Meyer, K. Anderson, R. E. Brown, M. Th. Smith, D. Yarrow, G. Mitchell, and D. G. Ahearn. Arch. Microbiol. 104; 225-231. 1975.

"Sporulation in Pichia spartinae." C. P. Kurtzman and D. G. Ahearn. Mycologia. (in press).

"The Effect of Heptachlor on Hexadecane Utilization by Selected Fungi." G. N. Smith, A. W. Bourquin, S. A. Crow and D. G. Ahearn. Dev. Ind. Microbiol. 17:331-336. 1976.

Abstract

Various concentrations of heptachlor dissolved in hexadecane were added to cultures of fungi grown in yeast nitrogen base prepared with synthetic sea water and deionized water. Candida maltosa and C. lipolytica showed the greatest utilization of hexadecane (20-91%) in the presence or absence of heptachlor. Isolates of Pichia spartinae, Cladosporium sp., Cephalosporium sp. and Penicillium sp. also utilized the hydrocarbon but to a lesser extent. Species of Kluyveromyces failed to grow with hexadecane as a carbon source. As compared to low concentrations, high concentrations of heptachlor appeared to have a slight stimulating effect on utilization of hexadecane by C. maltosa, but no effect with C. lipolytica.

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"Evaluation of commercial systems for the identification of clinical yeast isolates." P. I. Bowman and D. G. Ahearn. Jour. Clin. Micro.. (submitted).

Abstract

The API, Micro-Drop and Uni-Yeast Tek systems for the presumptive identification of common clinical yeast isolates were compared with the OF and a conventional procedure. With 229 coded isolates, the identification accuracies were API 90, MD 83, OF 82 and UYT 99%. The API system required the greater technical capability. The MD materials were prone to malfunction. OF media if incubated beyond 14 days gave an accuracy of 87%, but this offered no advantage to the conventional procedure. The UYT system was the easiest to use.

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

1. The following are recent publications from our laboratory.

Lysis of Yeast Cell Walls
Lytic β-(1→3)-Glucanases from Bacillus circulans WL-12

Frank Rombouts and Herman J. Phaff, Eur. J. Biochem. 63 121-130 (1976).

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Lysis of Yeast Cell Walls
Lytic β-(1→6)-Glucanase from Bacillus circulans WL-12

Frank Rombouts and Herman J. Phaff, Eur. J. Biochem. 63 109-120 (1976).

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A Rapid Method for Monitoring the Activity of Certain Carbohydrases
M. A. Lachance and H. J. Phaff, Analytical Biochemistry 67, 661-663 (1975).

A method for localizing activity peaks in eluates from chromatographic columns is described for hydrolases normally assayed by reducing-sugar determinations. The technique combines rapidity and small amounts of reagents. Results obtained with yeast exo-β-(1→3)-glucanase are shown.

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An Analysis of the Yeast Flora Associated with Cactiphilic Drosophila and Their Host Plants in the Sonoran Desert and Its Relation to Temperate and Tropical Association.

William B. Heed and William T. Starmer, Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721 USA.

Mary Miranda, Martin W, Miller and Herman J. Phaff, Department of Food Science and Technology, University of California, Davis, California 95616, USA.

Ecology (1976) 57: pp. 151-160

Abstract

A survey was made in the Sonoran Desert of yeasts living in the decaying arms of five species of cereus cacti and the four species of Drosophila that utilize them as host plants. The most common yeasts among 132 isolates from the cacti and 187 isolates from the flies, respectively were: Pichia membranaefaciens (45% and 67%), Candida ingens (17% and 4%), Torulopsis sonorensis (12% and 11%), and Cryptococcus cereanus (8% and 7%). Eighty-eight percent of the 66 initial isolates of P, membranaefaciens from Drosophila pachea and its host, senita cactus, assimilated D-xylose while only 12% of the remaining 257 initial isolates did so. Nineteen of the 20 isolates of T. sonorensis from flies were found in Drosophila mohavensis and 12 of the 14 isolates of Cryptotoccus cereanus came from D. pachea. The highest mean number of yeast species per cactus was 2.77 + 0.68 in organpipe cactus and per fly was 1.63 + 0.53 in D. pachea. The flies usually carried fewer yeast species than were found in the host plant but D. pachea had almost the same mean and variance as its host, senita cactus, which had 1.64 + 0.40. Yeast species diversity and average niche overlap have the following rank order among habitats and localities: temperate trees > temperate flies > tropical flies > desert cacti > desert flies. Habitat diversity and average niche width show: tropical flies > temperate flies and desert cacti > desert flies and temperate trees. The physiological properties of the desert yeasts are most similar to those of the tropical yeast. HOwever, desert yeasts have similarities with yeasts of temperate trees. Both have low fermentative ability and high assimilation ability of several alcohols and acids. the genus Pichia is by far the most common yeast genus associated with Drosophila in all habitats analyzed (36% of 1,426 isolates).

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H. J. Phaff, M. W. Miller, and Mary Miranda. Pichia scutulata, a new species from tree exudates. Int. J. Syst. Bacteriology. July issue 1976 (in press).

Summary

A novel representative of the yeast genus Pichia has been recovered 11 times during 1968, 1971, and 1972. We regard this organism as belonging to a new species, Pichia scutulata, with two varieties: Pichia scutulata var. scutulata, the type variety, and Pichia scutulata var. exigua. Strains of both varieties were found in tree exudates but were geographically separated. P. scutulata var. scutulata was isolated from slime exudates and flux-wetted soil of Myoporum trees on the island of Hawaii (6 strains) while P. scutulata var. exigua was found in fluxes or insect borings of various trees in the State of Washington, USA, and in the Province of British Columbia, Canada (5 strains). P. scutulata var. exigua differs from P. scutulata var. scutulata by its slower fermentation rate, weak ability to utilize glycerol, and higher maximum temperature for growth. The type strain of Pichia scutulata var. scutulata is UCD-FST 71-102 (= ATCC 32651 = CBS 6644) and of P. scutulata var. exigua, UCD-FST 68-979B1 (= ATCC 24185 = CBS 6836).

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2. Genome comparison in yeast systematics: polynucleotide sequence relatedness among members of the ascogenous genera Debaryomyces, Pichia, Saccharomyces and Schwanniomyces.

C. W. Price and H. J. Phaff

The taxonomic positions of members of four ascogenous yeast genera (Debaryomyces, Pichia, Schwanniomyces and those former Torulaspora species now classified as Saccharomyces Group III) was elucidated by molecular means. Most of these yeasts are haploid and exhibit a characteristic life cycle involving mother-daughter cell conjugation or occasional conjugation between independent cells. Taken together, these yeasts represent about ten percent of all known ascogenous species.

The phylogenetic relatedness of these organisms was determined by comparison of their whole cell DNA, involving characterization by base composition and base sequence relatedness. The molecular methodology included isopycnic buoyant-density gradient centrifugation in cesium chloride and liquid phase DNA reassociation reactions in which the degree of duplex formation was assayed by the hydroxylapatite batch method. Particular attention was devoted to minimizing the contribution of the minor DNA species in renaturation reactions.

Many yeasts currently classified as separate spcies, primarily by physiological characteristics, should by the criterion of high base sequence similarity be considered members of a single species. The opposite case of yeasts seemingly identical by conventional criteria, which nonetheless shared few common base sequences, was also frequently observed. A number of conclusions can be drawn from the data obtained:

(1) As in prokaryotic systematics, accurate DNA base composition values proved to be valuable exclusionary criteria in yeast classification.

(2) The sensitivity of DNA base sequence comparison is such that these studies are most useful for the delineation of natural yeast species. With proper reassocation criteria, those strains which have in common between about 70 and 100 per cent of their nuclear base sequences may reasonably be considered to comprise a species. Seldom were values obtained ranging between 25 and 70 per cent. Thus the natural relationships among species with less than 25 per cent base sequence complimentarity can best be studied by methods capable of accurately quantifying more distant relationships (e.g., comparison of homologous proteins or DNA-RNA reannealing studies).

(3) Glucose fermentation per se (particularly slow fermentation) and the oxidative utilization patterns of various sugars (particularly disaccharides, oligosaccharides and polysaccharides) do not necessarily reflect phylogenetic relationships at the species level. However, the utilization of n-alkanes and other unusual compounds correlated rather well with our DNA studies. This suggests that an expanded investigation of the nutritional versatility of yeasts may provide practical means to distinguish yeast species using tests which reflect underlying filiation.

(4) Differences in ascospore surface fine structures revealed by scanning electron microscopy proved to be useful taxonomic criteria,

functioning in an exclusionary manner analogous to the use of base composition values. The type of ubiquinone pigment of a yeast also seems to be a reasonable classificatory criterion.

(5) Systematics based on serological analysis or PMR spectra, which indirectly reveal the structure of the mannan component of the yeast cell wall, did not agree with our molecular methods with sufficient consistency to encourage the use of serology or PMR in any but a supportive role.

(6) The Adansonian classifications proposed by Campbell and others are in many instances at variance with the DNA-derived relationships. The lack of reasonable congruence in yeast systematics between numerical taxonomy and molecular techniques is not the rule in well-characterized bacterial systems. These inconsistencies undoubtedly arise from an undue emphasis in the numerical classifications of yeast on only a few phenotypic characters, usually those features involving carbohydrate metabolism, rather than the large number of broad phenotypic and physiological characters employed by bacterial systematists.

Details of this study, including the strains investigated, molecular results and taxonomic conclusions, are currently being prepared for publication.

3. A project carried out by Mr. André Lachance is currently underway to study the phylogeny of certain yeast groups using exo-β-(1→3)-glucanase as a marker. The structural changes are detected by determining the molecular weight of this enzyme for each strain studied and by measuring the immunological distance to reference strains, by the micro-couplement fixation technique. Relationships between different ascomycetous genera and between the species of Kluyveromyces are emphasized in this study.

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

Abstracts of the following four publications are currently in press.

Kurtzman, C. P. and N.J.W. Kreger-van Rij. 1976. Ultrastructure of ascospores from Debaryomyces melissophilus, a new taxonomic combination. Mycologia, March-April issue.

Abstract

Scanning electron microscopy showed numerous wartlike protrusions on the surface of spores from D. melissophilus. When viewed by transmission electron microscopy, the spores have a thin outer electron-dense layer and the protrusions arise from the thick inner layer. The inner layer is electron-light with slightly denser protrusions. Conjugation in D. melissophilus occurs both by mother cell-bud fusion and between separate cells.

Kurtzman, C. P. and D. G. Ahearn. 1976. Sporulation in Pichia spartinae. Mycologia, May-June issue.

Abstract

Freshly isolated strains of Pichia spartinae from oyster grass in Barataria Bay, Louisiana, formed hat-shaped ascospores. Single spore isolations showed the species to be heterothallic and appropriate matings with the now asporogenous type strain gave hat-shaped spores. Round aborted spores are commonly formed, and this seems to account for the spheroidal spore shape reported in the original description of P. spartinae.

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Ulloa, M. and C. P. Kurtzman. 1975. Occurrence of Candida parapsilosis, C. tropicalis, and Saccharomyces cerevisiae in pozol from Tabasco, Mexico. Bol. Soc. Mex. Mic. 9:7-12.

Abstract

Pozol is a fermented maize dough that, diluted in water, is drunk raw as a staple food by the Indian and mestizo populations in several southeastern states of Mexico. Various bacteria, yeasts, and filamentous fungi have been isolated from pozol. Most recently C. parapsilosis, C. tropicalis, and S. cerevisiae were identified in this product.

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Seymour, F. R., M. E. Slodki, R. D. Plattner, and R. M. Stodola. 1976. Methylation and acetolysis of extracellular D-mannans from yeast. Carbohydrate Res. In Press.

Abstract

Methylation-fragmentation analyses were conducted on a series of extracellular, yeast α-D-linked mannans representing six different structural types. D-Mannans of low degree of branching were produced by Hansenula capsulata strains and by species related to H. holstii. The former consisted primarily of (1→2)- and (1→6)-linked D-mannosyl residues; the latter, of (1→2)- and (1→3)-linked D-mannosyl residues. Although the remaining structural types were highly branched, each gave distinct methylationpatterns indicative of (1→6)-linked backbones to which are appended non(1→6)-linked side-chains. Acetolysis studies were correlated with the methylation analyses, and the correlation demonstrated that each branched polymer possesses side chains of heterogeneous length.

VIII. Clinical Mycology Section, N.I.A.I.D., National Institutes of Health, Bethesda, Maryland 20014. Communicated by K.J. Kwon-Chung.

Ultrastructure of Septal Complex in Filobasidiella neoformans

(Cryptococcus neoformans)

K. J. Kwon-Chung and T. J. Popkin, J. Bacteriol. 126:524-528 (1976).

ABSTRACT

Electron microscopy of F. neoformans, the perfect state of Cryptococcus neoformans, revealed basidiomycete doliporesepta between hyphal cells and also between clamp connections and adjacent cells. The pore-occluding material was a heterogeneous flattened plate with dark margin and a lighter center, as seen in the species of Filobasidium. Representative basidiomycete parenthesomes were lacking, and endoplasmic reticulum was seen in the dolipore region.

IX. Department of Biology, National University Chonnam, South Korea 500. Communicated by M. S. Park.

The distribution and population densities of yeasts and the epiflora of seaweeds in inshore waters of Mok-po, Korea. By M. S. Park and S. B. Chun.

Abstract

Nine species representing 48 isolates were isolated from the inshore waters of Mok-po Korea. The population densities of yeasts in inshore waters and their epiflora on seaweeds were estimated over two months in this area. Rhodotorula, Torulopsis and Debaryomyces that are widespread in estuaries were of common occurrence in this water body. The highest counts represented Torulopsis candida which predominated in the temperate estuarine zone. The distribution of seaweeds seems to be correlated with the population densities of yeasts. Two species of marine algae harbored yeasts during May and July 1975. Among seaweeds isolates Rhodotorula glutinis var. glutinis was most common.

A dominant colonization of these strains on Chlorophycea and Phaeophyta and a role of water temperature for the growth of yeasts are discussed.

X. Alko, box 350. SF-00101 Helsinki 10, Finland. Communicated by H. Suomalainen.

Some Aspects of the Structure and Function of the Yeast Plasma Membrane

by Heikki Suomalainen and Timo Nurminen

A lecture presented at Canadian Labatt Lectureships in the Department of Biochemistry, University of Western Ontario, London; in the Division of Biological Sciences of the National Research Council of Canada, Ottawa, and in the biochemistry Division, University of Windsor, November 1975.

Submitted for publication in Journal of the Institute of Brewing.

The permeation of many compounds into the yeast cell is largely regulated by the plasma membrane according to their lipid solubility and degree of dissociation. Thus the permeation rate of the fatty acids and α-keto acids is proportional to their relative lipid solubility. The highly dissociated and poorly lipid-soluble di- and tricarboxylic acids and halogenated acetic acids permeate only very slowly; the impermeable α-ketoglutaric acid becomes easily permeable when made lipid-soluble by esterification. The lipid composition of the plasma membrane can thus be of decisive importance in regulating the movement of different compounds into and out of the yeast cell. Lipid analyses revealed that anaerobiosis clearly affected the neutral lipid composition of the plasma membrane. The aerobic membrane contained more unsaturated fatty acids, mainly palmitoleic and oleic acids, more total sterol, much more ergosterol and much less squalene. The main sterol in the aerobic membrane, ergosterol, was mainly in the free form, whereas zymosterol and other minor sterols were predominantly esterified. In contrast, the anaerobic membrane contained small amounts of biosynthetic sterol precursors of ergosterol, and was clearly richer in saturated fatty acids having a greater variation in chain length. Both plasma membranes contained a considerable amount of triacylglycerols.

The Capacity of Baker's Yeast to Assimilate Carbon Dioxide

by Erkki Oura and Sampsa Haarasilta

Proceedings of the Second National Meeting on Biophysics and Biotechnology in Finland, 12.-13.2. 1976, Espoo, Finland.

The assimilation of CO_2 by Saccharomyces-yeast cultivated on a synthetic medium with a feed of $^{14}CO_2$ into the growth suspension was determined. As predicted by theoretical calculations the amount of assimilated CO_2 varied with differing growth conditions. In yeast grown anaerobically on glucose 8 % of the cellular carbon originated from CO_2; when the medium was supplemented with asparate the corresponding value was about 2 %. The CO_2 assimilation by yeasts cultivated aerobically on glucose, glycerol, pyruvate or ethanol was between these limit-values. The values for anaerobically grown yeasts and for yeasts grown aerobically on glucose and glycerol were practically the same as predicted. From the distribution of label in cellular components the participation of the TCA cycle and glyoxylate path could be calculated. The unexpected high value for ^{14}C observed in yeast grown on ethanol is explained by a CO_2 exchange reaction catalyzed by phosphoenolpyruvate carboxykinase.

Qualitative Requirements and Utilization of Nutrients: Yeast

by Erkki Oura and Heikki Suomalainen

Submitted for publication in Handbook of Nutrition and Food, VI, ed. by M. Rechceigl, CRC Press, Cleveland, Ohio.

This is a review of qualitative requirements and utilization of nutrients by different yeast species presented mostly in tabular form. Requirements of minerals, and vitamins, fermentation of carbohydrates, oxidative utilization of carbohydrates and other carbon compounds, and utilization of inorganic and organic compounds as nitrogen sources are included.

The Formation of Glycerol and Succinic Acid During Fermentations by Yeast

by Erkki Oura

Abstract of paper to be presented at the Fourth International Specialized Symposium on Yeasts, Berlin (West), 28.6. - 3.7.76

During yeast fermentations glycerol and succinate are produced in quantities corresponding to 3-6 % of the original amount of sugar. The formation of glycerol is associated with the over production of $NADH_2$ during fermentation; the most important reason for the excess $NADH_2$ is the formation of succinate. If this could be lowered or completely eliminated, the consumption of sugar in glycerol metabolism would be reduced and the yield of ethanol correspondingly increased.

The following publications have appeared since the last communications. The abstracts of reports have been given in Yeast News Letter 24:2, 76,77, 78,79, 1976.

Grba, S. & Oura, E. and Suomalainen, H., On the formation of glycogen and trehalose in baker's yeast. Eur. J. Appl. Microbiol. 2, 29-37, 1975.

Haarasilta, S. and Oura, E., Effect of aeration on the activity of gluconeogenetic enzymes in Saccharomyces cerevisiae growing under glucose limitation. Arch. Microbiol. 106, 271-273, 1975.

Londesborough, J. and Varimo, K., On the enzymatic composition of yeast nuclei and their membranes. 4th International Symposium on Yeast and Other Protoplasts, Nottingham 1975, Abstracts, p. 59.

Nurminen, T. & Taskinen, L. and Suomalainen, H., Distribution of membranes, especially of plasma-membrane fragments, during zonal centrifugations of homogenates from glucose-repressed Saccharomyces cerevisiae. Biochem. J. 154, 751-763, 1976.

Varimo, M. and Oura, E., The role of oxaloacetate as feed-back inhibitor of isocitrate lyase in baker's yeast. Acta Chem. Scand. B 29:9, 982-984, 1975.

XI. The University of Sydney, N.S.W. 2006, Australia, Department of Biochemistry. Communicated by Audrey M. Bersten.

The following is a summary of a paper presented by Audrey M. Bersten and Nicolle H. Packer at the Australian Biochemical Society's Annual Meeting held in May 1976. (Proceedings of the Australian Biochemical Society Vol. 9, 1976).

A chemostat culture was used to investigate the morphogenesis of the yeast Trigonopsis variabilis under various growth conditions in order to resolve the effects produced in batch culture by the change in growth rate which accompanies an alteration in growth temperature. Cells were grown in minimal medium with excess methionine (18mM) as nitrogen source and with glucose (0.2%) as the limiting substrate. The growth temperature was increased from 25°C to 30°C while maintaining a constant dilution rate (corresponding to a constant growth rate) of $0.075hr^{-1}$ or $0.05hr^{-1}$. At 25°C ($0.075hr^{-1}$, pH 5.6) the cell population was maintained almost completely in the triangular morphology. Increasing the growth temperature to 30°C at the same growth rate resulted in an entire population of ellipsoidal cells after three generation times. The faster growth rate of $0.07hr^{-1}$ was found to be a necessary condition for the development of the triangular cells at 25°C, as was a saturation level of oxygen in the medium.

Analysis of the total phospholipids of cells grown in a glucose-limiting chemostat culture in medium containing methionine as nitrogen source at 25°C (98% triangular cells) and 30°C (100% ellipsoidal cells) showed an increased synthesis of $C_{16:1}$ with a higher proportion of $C_{18:2}$ to $C_{18:1}$ at 25°C compared with that at 30°C. The triangular cells also contained twice as much sterol (mg/g dry wt) as the ellipsoidal cells.

The lower temperature of 25°C is seen to be a necessary but not sufficient condition for triangular cell formation with methionine as a sole source of nitrogen. The changes in lipid composition observed in the phospholipid and free sterol of the whole cell infer a difference in lipid composition of the plasma membrane at the two temperatures and thus a possible correlation with the morphological differentiation of the cell.

XII. Biological Adaptation Branch, Ames Research Center, NASA Moffett Field, California 94034. Communicated by T. Satyanarayana.

ABSTRACT

Arch. Biochem. Biophs. (in press)

A procedure for the purification of a stable acetyl-CoA synthetase (ACS) from aerobic cells of Saccharomyces cerevisiae is presented. The steps include differential centrifugation, solubilization of the bound enzyme from the crude mitochondrial fraction, ammonium sulfate fractionation, crystallization to constant specific activity from ammonium sulfate solutions followed by Bio-Gel A-1.5 m column chromatography. The resulting enzyme preparation is homogeneous as judged by chromatography on Bio-Gel

columns, QAE-Sephadex A-50 anion exchange columns, analytical ultracentrifugal studies, and polyacrylamide gel electrophoresis.

Sedimentation velocity runs revealed a single symmetric peak with an $s_{20,w}$ value of 10.6. The molecular weight of the native enzyme as determined by gel filtration and analytical ultracentrifugation, is 250,000 ± 500. In SDS polyacrylamide gel electrophoresis, the molecular weight of the single polypeptide chain is 83,000 ± 500. The purified enzyme is inhibited by palmityl-CoA with a Hill interaction coefficient, n, of 2.88. These studies indicate that the ACS of aerobic S. cerevisiae is composed of three subunits of identical or nearly identical size.

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

Based on the historical experiments by Harden-Young, Nilsson and Meyerhof on dried cells of yeasts, we have succeeded in producing various cytidine coenzymes. Using the energy (ATP) produced through glycolysis of dried cells of yeasts, we could phosphorylate CMP to CTP and convert it to CDP-choline (yield was almost 100%).

Recently, we have been trying to analyse the control mechanism of the fermentative production of cytidine coenzymes, about which A. Kimura is going to present two papers at the "4th Specialized Symposium on Yeasts, and "Fifth International Fermentation Symposium," both of which will be held in Berlin on June 28-July 3, 1976.

The following papers have recently been published from our laboratory.

1. Akira Kimura, Kazuhiko Hirose, Yashuro Kariya, and Susumu Nagai: Phosphorylation of Mononucleotides and Formation of Cytidine 5'-Diphosphate-Choline and Sugar Nucleotides by Respiration-Deficient Mutants of Yeasts. J. Bacteriol., 125, 744 (1976).

2. Akira Kimura, Minoru Hashimoto, and Haruhiko Mori: The Fermentative Formation of CDP-Choline by Haploid Cells of Yeasts and the Change of the Temperature-sensitivity of a Mutant of a Yeast, Saccharomyces rouxii. Agr. Biol. Chem., 40, 93 (1976).

3. Akira Kimura and Makoto Morita: Fermentative Formation of CDP-CHoline by Intact Cells of a Yeast, Saccharomyces carlsbergensis (IFO 0641) Treated with a Detergent, Triton X-100. Agr. Biol. Chem., 39, 1469 (1975).

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

The following papers have been published in 1975-76 or are in the press:

1. N. van Uden and A. Madeira-Lopes, Dependence of the maximum temperature for growth of Saccharomyces cerevisiae on nutrient concentration. Arch. Microbiol. 104, 23, 1975.

2. A. Madeira-Lopes, Inheritance of the maximum temperature for growth in Saccharomyces cerevisiae, Ciênc. Biol. (Portugal) 1, 89, 1975.

3. J.M. Cardoso-Duarte, M.J. Marinho and N. van Uden, Flow microcalorimetry of the chemostat, in Continuous Culture of Microorganisms, Plenum Publishing Corporation, New York (in the press).

4. N. van Uden and M.M. Vidal-Leiria, Thermodynamic compensation in microbial death. Studies with yeasts. Arch. Microbiol. (in the press).

5. N. van Uden and A. Madeira-Lopes, Yield and maintenance relations of yeast growth in the chemostat at superoptimal temperatures. Biotechnol. Bioeng. (in the press).

Current research interest are centered in the following areas:

- effects of antibiotics on yeast growth as a function of the temperature
- kinetics and energetics of yeast growth on starch
- cellulases of thermophilic microorganisms
- cyanide resistant respiration in Saccharomyces cerevisiae
- inositol catabolism in yeasts.

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

The following paper is in press and will appear in Carlsberg Research Communication 41, 1976 (formerly: Compt. Rend. Trav. Lab. Carlsberg): (see also C.R.L.C. 38, 351, 1971

"The cell cycle in heat- and selection synchronized Schizosaccharomyces pombe", by

Birte Kramhøft. Susanne B. Missen and Erik Zeuthen.

Abstract: Schizosaccharomyces pombe, growing in a complex medium at 32°C, was treated with 6 or 7 heat shocks (30 min. 41°C) spaced one normal cell generation apart (110 min. 32°C). This treatment results in synchronization of cell division, nuclear division, and DNA synthesis.

Between successive shocks increases in the activities of ATCase and OTCase are stepwise. However, steps are not seen at constant temperature, neither in free running heat synchronized, nor in selection synchronized cells. The enzyme steps we have studied therefore seem to be directly induced by the temperature shocks, and they are dissociable from the pattern of classic cell cycle events, which we find almost identical in heat and in selection synchronized cells.

XVI. Department of Bacteriology, University of California, Davis, California 95616. Communicated by M. P. Starr.

Below follow abstracts of two papers currently in press in Phytochemistry. They deal with the characterization of the carotenoid pigment astaxanthin which is the principal pigment of the recently described yeast Phaffia rhodozyma (Int. J. Syst. Bacteriol. 26, 286-291, 1976).

CAROTENOIDS OF PHAFFIA PHODOZYMA, A RED-PIGMENTED FERMENTING YEAST

Arthur G. Andrewes, Herman J. Phaff and Mortimer P. Starr*

Abstract The red-pigmented fermenting yeast Phaffia rhodozyma contains astaxanthin as the principal carotenoid pigment. Echinenone, 3-hydroxyechinenone and phoenicoxanthin were also isolated and identified: isocryptoxanthin and canthaxanthin were absent. Evidence is presented for a new carotenoid, 3-hydroxy-3'4'-didehydro-βψ-caroten-4-one. A possible biosynthetic scheme for the formation of astaxanthin in P. rhodozyma is suggested.

(3R,3'R)-ASTAXANTHIN FROM THE YEAST PHAFFIA RHODOZYMA

Arthur G. Andrewes and Mortimer P. Starr

Abstract Astaxanthin isolated from the yeast Phaffia rhodozyma has the 3R,3'R-configuration, opposite to that of astaxanthin from other sources which have been so far investigated. This is the first example of a naturally occurring carotenoid biosynthesized in different optical forms. A possible explanation is advanced.

XVII. L.N.R.S. Laboratoire D'Enzymologie, 91190 GIF-SUR-YVETTE, France. Communicated by J. Schwencke.

Below follows the summary of our paper: "The transport of S-Adenosyl-L-methionine in isolated yeast vacuoles and spheroplasts" by J. Schwencke and H. Robichon-Szulmajster which has been recently accepted for publication in the Journal of European Biochemistry.

Summary

1) The properties of S-Adenosyl-L-methionine accumulating system for both vacuoles and spheroplasts are described. Yeast vacuoles were obtained by a modified metabolic lysis procedure from spheroplasts of Saccharomyces cerevisiae.

2) Isolated vacuoles accumulate S-Adenosyl-L-methionine by means of a highly specific transport system as indicated by competition experiments with structural analogs of S-Adenosyl-L-methionine. The S-Adenosyl-L-methionine transport system shows saturation kinetics with an apparent Km of 68 μM in vacuoles and 11 μM in spheroplasts.

3) S-Adenosyl-L-methionine accumulation into vacuoles does not require glucose, phosphoenol pyruvic acid, ATP, ADP nor any other tri- or di-phosphorylated nucleotides. It is insensitive to azide and 2.4-dinitrophenol which strongly inhibit the glucose-dependent accumulation of S-Adenosyl-L-methionine in spheroplasts.

4) The transport of S-Adenosyl-L-methionine into vacuoles is optimal at pH 7.4 and is insensitive to nystatin while the uptake of S-Adenosyl-L-methionine into spheroplasts is optimal at pH 5.0 and is strongly sensitive to nystatin. On this basis it has thus been possible to measure both the intracytoplasmic and the intravacuolar pool of S-Adenosyl-L-methionine.

5) Our results indicate the existence of a highly specific S-Adenosyl-L-methionine transport system in the vacuolar membrane which is clearly different from the one present in the plasma membrane of yeast cells.

XVIII. Universiteit van Amsterdam, Laboratorium voor Microbiologie, Plantage Muidergracht 14, Amsterdam, Nederland. Communicated by H. Aiking.

In our laboratory the research on yeast concentrates on the physiological role that potassium might have in yeast, and the relation of that role to that in bacteria-which is clearly different. The first results of this research topic will appear shortly in Arch. Microbiol. 108, 117-124 (1976) under the title "Growth and Physiology of Candida utilis NCYC 321 in Potassium-Limited Chemostat Culture" by H. Aiking and D. W. Tempest.

Abstract

When grown in a defined simple salts medium, plus vitamins, Candida utilis displayed an absolute requirement for potassium. But the potassium content of this yeast was exceedingly variable and, with aerobic chemostat cultures (grown at a dilution rate of 0.1 h^{-1}; 30°C; pH 5.5) was low (<0.2%, w/w) when they were potassium-limited and high (>2%, w/w) when glucose-limited. With potassium-limited cultures, the cell-bound potassium content also varied markedly with growth rate, though hardly at all with glucose-limited cultures; magnesium- and phosphate-limited cultures gave intermediate responses.

Changes in cell-bound potassium content correlated only weakly with changes in the cellular contents of magnesium, phosphate and RNA, but strongly with changes in both the $Y_{glucose}$ and Y_O values, indicating an involvement of potassium in the generation of energy by oxidative phosphorylation reactions and/or the utilization of this energy for growth processes.

Studies with isolated mitochondria revealed that potassium-limited organisms had a changed content of cytochrome b relative to cytochrome a, and lacked coupling at either site 2 or site 3 of the respiratory chain.

These results are discussed in relation to the reported functions of potassium in the growth of microorganisms, and the organizational differences between prokaryotic and eukaryotic cells.

XIX. Janssen Pharmaceutica - Research Laboratories - Turnhoutsebaan 30 B-2340 Beerse - Belgium. Communicated by S De Nollin.

Below appears the abstract of a recently published paper.

Scanning electron microscopy of Candida albicans after in vitro treatment with miconazole. Sonja De Nollin and M. Borgers. Antimicrobial agents and Chemotherapy, 7 (5), 704-711 (1975). An abstract of this article can be found in the Yeast News Letter XXIV, June 1975, p. 31.

Enzyme cytochemistry of Candida albicans. Sonja De Nollin, F. Thone and M. Borgers. The Journal of Histochemistry and Cytochemistry, 23 (10), 758-765 (1975).

The application of a new preparation method for demonstrating the activities of hydrolytic and oxidative enzymes in Candida albicans is reported. The problem of inadequate penetration of fixatives into yeast cells has been solved by sectioning solidified pellets of the cells in the presence of glutaraldehyde, a procedure that yields a fairly well preserved ultrastructure and sufficient enzyme activities. The subcellular distribution of most specific and nonspeficic phosphatases and of peroxidases is at variance with that found in mammalian cells. The activities towards β-glycerophosphate, p-nitrophenyl-phosphate, adenosine triphosphate, adenosine monophosphate, thiamine pyro-phosphate and glucose 6-phosphate are almost exclusively confined to the central vacuolar apparatus. Oxidative and peroxidative activities are demonstrated only in mitochondria. Specific marker enzymes for endoplasmic reticulum, plasmalemma, Golgi apparatus and peroxisomes in C. albicans were not found. The possible function of the various subcellular organelles in relation to their enzymatic content is discussed.

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

1) Beginning December 22, 1975 A. Martini has been appointed head of the Istituto de Microbiologia Agraria e Tecnica of the University of Perugia, Italy.

2) We received a 3-year grant from the Italian Research Council for reviewing and reorganizing our collection in which more than 2,000 wine yeast strains collected over a 50 years period from different European and Extra-european areas are maintained. After a selection for favorable technological characters, we intend to reduce its size to 4-500 strains.

Below follow summaries of our recent studies on yeasts:

3) Taxonomic Implications of the Quantitative Amino Acid Composition of the Whole Yeast Cell." A. Martini and Ann Elizabeth Vaughan Martini. Accepted, Annali di Microbiologia, 1975.

Summary

Amino acid composition of the whole cell of 10 different yeast species has been studied in relation to its possible use as a taxonomic tool. Amino acid patterns vary with culture age even though data from mid-exponential phase of growth appear stable and reproducible. Interpreting the results on the basis of numerical taxonomy of Sneath showed that amino acid composition of the whole yeast cell is an individual and unique characteristic of the species.

4) Biosintesi dell'acido indol-3-acetico dal triptofano ad opera dei lieviti (Biosynthesis of indol-3-acetic acid from tryptophan by yeasts)." F. Tafuri, A. Martini, L. Scarponi and M. Businelli. In press, Agrochimica, 1976.

Summary

A screening survey showed that 96 yeast strains out of 147 studied are able to form extracellular indole-3-acetic acid (IAA) on a culture medium enriched with tryptophan, while this property is present in only 46 strains on the same minimum medium not enriched. Gas-chromatographic determinations of the IAA produced showed that its amount is directly related to the initial tryptophan content of the medium and to the amount consumed by the yeast as well. Microbiological and agricultural-chemical implications of these results are discussed.

5) A New Approach to the STudy of the Yeast Flora Associated with Fruit Surfaces." A. Martini and F. Federici. In press, Giornale Botanico Italiano, 1976.

Summary

The yeast flora associated with the surface of strawberries has been studied on the assumption that individual cells are firmly embedded in gummy fruit secretions. New techniques based on vigorous shaking or sonication of samples allowed the recovery of many yeast species not normally obtained previously with the traditional isolation procedures.

6) Protein Content and Amino Acid Composition of the Yeasts Kluyveromyces fragilis, Saccharomyces cerevisiae and Candida utilis." A. Martini, Vaughan Martini Ann E. and M. W. Miller. In press, Annali della Facolta di Agraria di Perugia, 1975.

Summary

The protein and total amino acid contents of cells from three yeast species were determined. General amino acid profiles did not vary significantly from one organism to another. It is confirmed that methionine and tryptophan are the most limiting amino acids. Candida utilis produces less cellular protein than Kluyveromyces fragilis and Saccharomyces cerevisiae.

7) "Protein Content and Amino Acid Composition of the Yeasts Saccharomyces uvarum, Schwanniomyces castellii, Saccharomycodes ludwigii, Pichia membranaefaciens and Lipomyces starkeyii. A. Martini, Vaughan Martini Ann E. and M. W. Miller. In press, Annali della Facoltà di Agraria di

Perugia, 1975.

Summary

Protein content and amino acid composition were studied in five yeast species belonging to five different genera in relation to their possible use as SCP producers. Saccharomyces uvarum and Schwanniomyces castellii proved to be very similar to Candida utilis. The remaining species showed low protein contents.

XXI. University of Puget Sound, Tacoma, Washington. Communicated by John G. Kleyn.

Publications in press:

1. Kleyn, J. 1976. Relationship of Yeast Type (Fermentative or Oxidative) to the Biological Activity of Aminopterin. Proc. Am. Soc. for Microbiol. Atlantic City, May 1976.

2. Kleyn, J. 1976. Dearrangement of Yeast Folic Acid Metabolism and Practical Consequences Thereof in Fermentation and Packaging. Proc. Am. Soc. of Brewing Chemists, Milwaukee, May 1976. In press.

3. Kleyn, J. 1976. The effect of various environmental factors on the yeast antifolate acitivty of aminopterin (in press).

4. Kleyn, J. 1976. Yeast Bioassay Method for the Analysis of Aminopterin in Urine and Plasma. In press.

5. Kleyn, J. 1976. Comparative Antifolate Activity of Aminopterin-Like Folic Acid Analogues. In press.

A goodly portion of this research was conducted at the Delft Technical University, Dept. of Microbiology, Delft, Netherlands. I would like to express my sincere thanks to Professor Torsten Wiken for having provided space and encouragement for conduct of this research, to various members on the staff of the department for help in various aspects of this research, in particular Mrs. Batenburg (electron microscopy), Messrs. Diederick (media preparation), Nieuwdorp (electron microscopy), Schuur (phase microscopy and preparation of graphical material), and Yarrow (cultures), and to the Research Council of the Delft Technical University for some of the necessary funding.

XXII. Leningrad Chemical Pharmaceutical Institute. Ul. Prof. Popova 14. Leningrad 22, USSR. Communicated by N. P. Elinov.

The following is a summary of recent work in our laboratory:

V. P. Komov, N. P. Elinov, T. F. Tachmanina, A. N. Shutko, G. A. Vitovskaya, "Interaction of ^{14}C-mannan of Rhodotorula rubra with cells of blood and thymus of rats." The authors have established interaction of ^{14}C-mannan with erythrocytes and thymocytes while following the distribution of it in higher organisms.

The polysaccharide, practically, was not metabolized in the blood for 20 days. When ^{14}C-mannan was incubated with a suspension of erythrocytes, 46.8% of it had become associated with the red cells. The character of interaction was unequal since 10.0% of the polysaccharide was detected in washes of erythrocyctes, 11.2%- in washes of erythrocyte membranes, and 0.33%- in membranes of cells after several washings with saline; ^{14}C-mannan (1,85%) was connected with hemoglobin and 5.2% with catalase.

The accumulation of ^{14}C-mannan in the rat thymus has a temporal character. The quantity of the preparation in the thymus was maximal 24 hours after injection. Then it decreased and a second increase in mannan content occurred up to the 20th day.

The authors have analysed the dynamics of accumulation of polysaccharide in cytoplasmic and nuclear fractions of cells after incubation of ^{14}C-mannan with a suspension of living thymocytes.

It turned out that the maximum binding of mannan in the cytoplasm was after 45 min (3.5×10^6 molecules/cell) and in nuclei after 30 min (3.5×10^5 molecules/nucleus). Then the elimination was determined which was most pronounced at 80 min (4.1×10^5 molecules/cell and 2.0×10^5 molecules/nucleus); the elimination was replaced with an increase in incorporation level of ^{14}C-mannan (5.3×10^6 molecules cell and 3.0×10^5 molecules/nucleus).

Thus the ability was shown of β-structural mannan of Rhodotorula rubra to penetrate into erythrocytes and thymocytes of rats. Thymocytes eliminate some of the polysaccharide and this explains the phase character of including ^{14}C-mannan in the thymus.

XXIII. Department of Genetics Institut National Agronomique 16, Rue Claude Bernard, 75005 Paris, France. Communicated by H. Heslot.

Summary of two recently published papers:

1. Mutants of Saccharomycopsis lipolytica defective in lysine catabolism.

C. Gaillardin, P. Fournier, G. Sylvestre and H. Heslot. J. Bacteriology, 125, 1976, n° 1, 48-57.

Wild-type strains of Saccharomycopsis lipolytica are able to use lysine as a carbon or a nitrogen source, but not as a unique source for both. Mutants were selected that could not use lysine either as a nitrogen or as a carbon source. Some of them, however, utilized N-6-acetyllysine or 5-aminovaleric acid. Many of the mutants appeared to be blocked in both utilizations, suggesting a unique pathway for lysine degradation (eigher as a carbon or as a nitrogen source). Genetic characterization of these mutants was achieved by complementation and recombination tests.

2. A kinetic study of homocitrate synthetase activity in the yeast Saccharomycopsis lipolytica.

C. Gaillardin, L. Poirier and H. Heslot.
Biochim. Biophys. Acta 422, 1976, 390-406.

A rapid method for estimating the activity of the first enzyme of lysine biosynthesis in yeasts (acetyl-coenzyme A:2-ketoglutarate C-acetyl transferase, EC.4.1.3.21) is described.

In the wild type strain, the fixation of one substrate, S-acetyl coenzyme A, shows sigmoidal saturation kinetics. The initial rate experiments indicate that the reaction obeys an ordered mechanism, 2-ketoglutaric acid binding before S-acetyl coenzyme A.

The activity is completely inhibited in vitro by lysine and by some lysine analogs, which all show cooperative binding and have an heterotropic effect on 2-ketoglutaric binding sites. A second class of effectors is found, including 2-aminoadipic acid, pipecolic acid and dipicolinic acid, which all affect the cooperativity of S-acetyl coenzyme A binding sites.

Two types of mutation which modify these inhibition patterns without affecting the catalytic activity are described. One results in a desensitization towards lysine and lysine analogs only. The other entirely abolishes the susceptibility towards the second type of inhibitors, without affecting the susceptibility to lysine.

No variations of the specific activity could be detected in the wild type strain at all; mutants showing an increased or a reduced activity were isolated.

Our results do not support the existence of isoenzymes at the level of homocitrate synthetase in this yeast.

XXIV. Institut für Biochemie der Universität Erlangen-Nürnberg, Egerlandstr. 7, 8520 Erlagen, West Germany. Communicated by E. Schweizer.

Temperature-sensitive Nuclear Petite Mutants of Saccharomyces Cerevisiae

G. Burkl, W. Demmer, U. Holzner and E. Schweizer

270 temperature-sensitive Saccharomyces cerevisiae mutants have been isolated. They grow on nonfermentable carbon sources like lactate, glycerol or ethanol only at 22°C, but not at 36°C. By complementation analysis, these mutants could be attributed to 106 different complementation groups. In general, these mutants are respiratory deficient when grown at non-permissive temperature. Interestingly, however, among 66 complementation groups studied, the mutants of about 16 groups still exhibit wild type-like respiration rates even at non-permissive temperature. These mutants are considered as potentially uncoupled or phosphorylation-deficient strains. Mutants of about 20 other groups are almost completely converted into cytoplasmic petites when grown for 3-5 generations at 36°C. Most of these secondary cytoplasmic mutants still contain mitochondrial DNA as indicated by the CsCl or $Cs_2SO_4/HgCl_2$ density gradient centrifugation profiles obtained with total cellular DNA.

Control of Fatty Acid Synthetase Levels by Exogeneous Long Chain Fatty Acids in the Yeasts Candida lipolytica and Saccharomyces cerevisiae

K. -H. Meyer and E. Schweizer

Endogenous fatty acid biosynthesis in the two yeast species, Saccharomyces cerevisiae and Candida lipolytica is completely repressed by the addition of long chain fatty acids to the growth medium. In Candida lipolytica, this repression is accompanied by a corresponding loss of fatty acid synthetase activity in the cell homogenate, when the cells were grown on fatty acids as the sole carbon source. The activity of the Saccharomyces cerevisiae fatty acid synthetase, however, remains unaffected by the addition of fatty acids to a glucose-containing growth medium. From fatty acid-grown Candida lipolytica cells no fatty acid synthetase complex can be isolated, nor is there any immunologically cross-reacting fatty acid synthetase protein detectable in the crude cell extract. FRom this it is concluded that Candida lipolytica, but not Saccharomyces cerevisiae, is able to adapt to the growth on fatty acids either by repression of fatty acid synthetase biosynthesis or by a fatty acid-induced proteolytic degradation of the multienzyme complex. Similarly, the fatty acid synthetase complex disappears rapidly from stationary phase Candida lipolytica cells even after growth in fatty acid-free medium. Finally, it was found that the fatty acid synthetase complexes from Saccharomyces cerevisiae and Candida lipolytica, though very similar in size and subunit composition, were immunologically different and had no common antigenic determinants.

End Group Analysis of Yeast Fatty Acid Synthetase

H. Schwietz, G. Dietlein and E. Schweizer

The purified yeast fatty acid synthetase complex has been subjected to amino and carboxyl terminal amino acid end group analysis. Amino end groups were studied by Edman degradation and by dansylation of the sodium dodecyl sulfate- or urea-denatured complex. No N-terminal amino acid could be identified by either method. C-terminal amino acids were investigated by tritium labeling and by digestion of the complex with carboxypeptidases A and B. By both methods, the two amino acids valine and lysine were consistently identified as the C-termini of two different polypeptide chains. After separation of the fatty acid synthetase subunits A and B. by sodium dodecyl sulfate polyacrylamide gel electrophoresis lysine was identified as the C-terminus of subunit A and valine as that of subunit B. The results are interpreted as additional evidence that the yeast fatty acid synthetase complex is basically composed of two nonidentical and multifunctional polypeptide chains.

XXV. University of Pittsburgh, Department of Life Sciences, Pittsburgh, Penn. 15261. Communicated by T. G. Cooper.

Below follow a number of summaries of as yet unpublished work from our laboratory.

Biochemistry and Genetics of Allantoin Uptake in Saccharomyces cerevisiae. R. Sumrada and T. G. Copper. Allantoin uptake in Saccharomyces occurs by a low K_m (ca 20 μM) transport system that is absolutely dependent upon energy that is generated in the cytoplasm. Uptake does not occur in the absence of glucose or in the presence of DNP, CCCP, fluoride or arsenate, but occurs normally in the presence of cyanide. The rate of accumulation is maximal at pH 5.2 and appears to be unidirectional. In contrast to the urea transport system, preloaded, radioactive allantoin is not lost from cells that are resuspended in allantoin-free buffer. However, treatment with nystatin does release the accumulated radioactivity. Production of the uptake system appears to be constitutive, but is repressed by growth of the cells in medium containing readily utilized amino acids. We have isolated mutant strains that will not utilize allantoin as sole nitrogen source. These strains will utilize allantoate and possess normal amounts of all of the allantoin degradative enzymes. The locus containing these mutations (we suggest designating it dal 4) is located on linkage group IX midway between the dal 1 and dal 2 loci. Currently we are determining whether, as would be predicted from their phenotype, these mutant strains are unable to accumulate allantoin.

Execution Times of Macromolecular Synthetic Processes Involved in the Induction of Allophanate Hydrolase at 15 C. J. Bossinger and T. G. Cooper. We have observed that transcription, involved in production of allophanate hydrolase, is completed 2.5 minutes after addition of inducer at 15 C. The rna1 gene product must be functional up until 10 minutes, protein synthesis initiates at 20 minutes and is terminated by 24 minutes. Two minutes later active enzyme appears. These results confirm our earlier observations and eliminate any uncertainty that might have clouded identification of the time within the lag period that is occupied by RNA synthesis.

Requirement of RNA Synthesis for Induction of Allophanate Hydrolase and Arginase in Saccharomyces cerevisiae. J. Bossinger and T. G. Cooper. The induction of allophanate hydrolase and arginase in Saccharomyces requires at least two processes. The first is synthesis of an allophanate hydrolase or arginase-specific synthetic capacity (presumably a specific messenger RNA) and the second is expression of this enzyme forming potential. We have demonstrated the expression process to be protein synthesis by its sensitivity to protein synthesis inhibitors and its loss in temperature sensitive mutant strains defective in either translational initiation or elongation. The first process has been tentatively identified as RNA synthesis because of its inhibition by the RNA polymerase inhibitor, lomofungin. However, to be convincing, such a requirement for RNA synthesis must be supported by another method of demonstration which does not involve the use of metabolic inhibitors with their inherent ambiguities. Such a method has become available with the isolation of mutant strains containing temperature sensitive RNA polymerases (J. Bacteriol. 125:25). In these strains, induction of allophanate hydrolase and arginase occurs normally at the permissive temperature. If, however, mutant strains are placed in medium at the non-permissive temperature prior to addition of inducer, no enzyme production occurs. This confirms the requirement of RNA synthesis in the production of both enzymes.

Accumulation and Decay of Arginase Synthetic Capacity in Saccharomyces cerevisiae J. Bossinger and T. G. Cooper.

Our previous work has shown that: (1) a very small amount of time (3 minutes) elapses between addition of inducer and appearnce of active allophanate hydrolase (AH), and (2) the synthetic capacity to produce AH decays 7 times faster than that of most other cellular proteins. To ascertain whether AH is unique in these respects, a second enzyme, arginase (ARG), was studied in a similar manner. Our results demonstrate that: (1) 4 minutes elapse between addition of inducer (homoarginine) and appearance of active ARG; (2) induction requires a functional rna1 gene product; (3) production of ARG-specific synthetic capacity occurs in the absence of protein synthesis but requires the presence of inducer; (4) termination of induction by inducer removal, addition of the RNA polymerase inhibitor, lomofugin, or shifting to 35°C a culture of a strain which contains a temperature sensitive mutation in the rna1 gene, results in loss of ARG-specific synthetic capacity with half lives of 5, 4, and 3 minutes respectively. These results indicate that the relatively rapid turnover of AH-specific synthetic capacity is not unique to this enzyme but may be a general property characteristic of other inducible, degradative enzyme systems as well.

Basic Amino Acid Inhibition of Growth in Saccharomyces cerevisiae. R. Sumrada and T. G. Cooper.

Growth of Saccharomyces cerevisiae on poor nitrogen sources such as allantoin or proline is totally inhibited by addition of lysine to the medium. The same result is observed with ornithine if its degradation to glutamic semialdehyde is prevented. Inhibition of growth is accompanied by a pronounced increase in cell size and a 5-fold increase in the ratio of budded to unbudded cells. These morphological changes are expected if DNA synthesis is inhibited. Therefore, cells growing with proline as sole nitrogen source in the presence and absence of 2.5 mM lysine were tested for their ability to carry out DNA, RNA, and protein synthesis. Lysine addition resulted in almost complete loss of ability to incorporate uracil into alkali resistant, trichloroacetic acid precipitable material. RNA and protein synthesis, although decreased, were much less sensitive to the effects of lysine addition. Mutant strains resistant to this effect have been isolated and should prove useful in elucidating the physiological basis for the basic amino acid inhibition of macromolecular synthesis and growth.

XXVI. National Research Council, Ottawa, Canada K1A-OR6. Communicated by Byron F. Johnston.

1. The following chapter should appear later in 1976.

A Model for Controlled Autolysis During Differential Morphogenesis of Fission Yeast

Byron F. Johnson, C. B. Calleja and Bong Y. Yoo

(Department of Biology, University of New Brunswick

Fredericton, New Brunswick)

In: Eucaryotic Microbes as Model Developmental Systems

Eds: D. H. O'Day and P. A. Horgen Marcel Dekker, 1976

Summary: A variety of morphogenetic activities such as cell division, copulation, conjugation, and sporulation are considered within the framework of a model for coordinated autolytic/synthetic activities originally proposed for extensile growth.

2. Alterations in Mitochondrial DNA of Yeast which Accompany Genetically and Environmentally Controlled Changes in ρ^- Mutability

C. V. Lusena and A. P. James, Molec. gen. Genet. 144, 199-125 (1976)

Summary: Alterations in the physical characteristics of mitochondrial DNA accompanied increased spontaneous mutability to cytoplasmic respiratory-deficiency in yeast. Two systems were used to modify mutation rates, one physiological, the other genetic. Cells in log phase were shown to be more mutable than cells in stationary phase, and glucoserepressed cells were shown to be more mutable than unrepressed cells. A nuclear gene which acts as a mitochondrial mutator was found to increase spontaneous mutation rate by a factor of ten. An increase in endogenous formation of G + Crich fragments of mt-DNA accompanied a physiological state conducive to higher mutability, and it is proposed that increased in vivo digestion of A + T-rich regions is involved in these alterations. Greater nuclease(s) activity accompanied the presence of the mutator gene, and it is proposed that this gene is concerned with the regulation of nuclease activity or with repair mechanisms.

XXVII. Central Research Laboratory Mitsubishi Chemical Industries Limited, 1000 Kamoshida-cho, Midori-ku, Yokohama 227, Japan. Communicated by Norio Gunge.

The following is a summary of work done recently in this laboratory.

The effects of strain-ploidy on the transmission and recombination of the mitochondrial drug resistance genes C, E and O for chloramphenicol, erythromycin and oligomycin, respectively, were studied. In ω^- haploid x ω^- diploid homosexual crosses, the ploidy effect on the marker transmission was of a similar magnitude at the different loci, and the C, E and O alleles from the diploid parent were preferentially co-transmitted. The recombination frequency was decreased as compared with ω^- haploid x ω^- haploid control crosses. In ω^+ haploid x ω^- diploid heterosexual crosses, on the contrary, the diploid effect was expressed in different ways depending upon the loci; e.g. there was a preferential transmission in favor of the alleles from ω^+ parent at the C locus and of the alleles from the ω^- parent at the o locus while a near equal transmission of the parental alleles was seen at the E locus. The recombination frequency

was increased as compared with the control crosses of ω^+ haploid x ω^- haploid.

The effects of ploidy-elevation were quantitatively interpreted in terms of the theoretical calculations of the marker transmission and recombination which were obtained on the assumption that the mitochondrial genomes from haploid and diploid parents were introduced into the zygotes in a ratio of 1:2 and subjected to random parings and recombination. The theoretical calculations predicted that the crosses of ω^+ diploid to ω^- and ω^+ haploids would show a preferential transmission in favor of diploid derived alleles, with the different magnitude for the C, E and O loci in the heterosexual crosses and with the same magnitude in the homosexual crosses. These crosses were predicted to give lower frequency of recombination than their respective control crosses of ω^+ haploid to ω^- and ω^+ haploids.

The recombination polarity (ratio of reciprocal recombinants) was not affected by the elevation of strain-ploidy, confirming that the ploidy is under the control of the ω gene.

Details of the study will be published shortly in Molecular and general Genetics.

XXVIII. Department of Genetics, University of California, Berkeley, California 94720. Communicated by Amar J. S. Klar.

The Action of Homothallism Genes in Saccharomyces Cerevisiae Diploids

Amar J. S. Klar and S. Fogel

The following is a summary of recent work in our laboratory.

A procedure based on ultraviolet induced mitotic recombination (homozygosity for cryptopleurine resistance) allowed us to identify and recover colonies sectored for the mating-type locus (a/a and α/α) in Saccharomyces cerevisiae. Using this technique we could study the action of homothallism genes in vegetatively growing diploid cells. Taken collectively our results show that homothallism genes can act as well during regular vegetative growth cycles as during the early divisions after spore germination. Homothallism genes can switch a/a or α/α vegetative diploid cells to a/α diploids or to a/a/α/α tetraploids during a given mitotic division cycle. We found that both a/a and α/α sectors generated from a strain with genotype HO/HO hmα/hmα hma/HMa switched to a/α diploids or a/a/α/α tetraploids. This finding supports Naumov and Tolstorukov's suggestion (1973, Gentika 9:82-91) that the hma allele provides for the same function as the HMα locus, namely, an $\alpha \rightarrow$ a mating-type switch. Thus, HO gene is dominant to ho allele, hma and HMa loci are co-dominant.

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

Below follows the abstract of an invited lecture that Dr. Eckhardt will be presenting in the Symposium on Mutagenesis at the Annual Meeting of the Genetics Society of Canada on May 26, 1976.

FORMAL THEORY OF UV-INDUCED MUTAGENESIS, Friederike Eckardt and R. H. Haynes.

Formal mathematical analysis of the dose-dependence of mutation induction can be useful in formulating molecular models of mutagenesis, and is essential for the low dose extrapolations required in assessing the genetic risks posed by environmental mutagens. In assays with microorganisms, every surviving cell in the population is scored either as a mutant or a non-mutant, and so 'single event' Poisson statistics enables us to write mutation frequency in the form

$$\text{Mutants/Survivors} = [1 - \exp(-mx)] \exp(1-\delta)kx$$

where x is dose, k and m are the cross-sections for killing and mutation and $\delta = k_m/k$ is the ratio of mutant to non-mutant cell sensitivity to killing. This equation indicates that mutation induction is linear for biolgically accessible doses if k and m are constant and $\delta = 1$. However, both 'positive' and ;negative' departures from linearity can occur with increasing dose if δ is not equal to unity and/or m is not a constant. In Saccharomyces cerevisiae we have measured UV-induced mutation frequencies in haploid RAD wild-type and rad 2 (excision defective) strains isogenic for the auxotrophic markers ade 2-1 and lys 2-1 both of which are supersuppresible ochre alleles. These strains allow one to score reversions (locus and supersuppressors) under selective conditions, and forward mutations in 5 ade genes (scoring for the double auxotrophs ade 2 ade x) under non-selective conditions. We found that for all reversion systems mutation induction increases linearly in the range of low killing but rises to dose-squared and even higher order kinetics as killing increases. On the other hand, for the forward systems, although mutation induction increases linearily for low cell killing, it reaches a maximum and eventually declines as killing increases. In excision defective strains these departures from linearity most likely arise from a differential survival probability for mutant and non-mutant cells ($\delta \neq$); however, in RAD wild-type strains we cannot rule out the possibility of dose-dependent mutability (m ≠ constant) as a factor also contributing to the non-linearity. (Work supported by the National Research Council of Canada and the Deutsche Forschungsgemeinschaft).

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The Influence of Repair Processes on Radiobiological Survival Curves Robert H. Haynes. in 'Cell Survival after Low Doses of Radiation: theoretical and clinical implications' (T. Alper, ed), John Wiley and Sons, Ltd., London and New York, 1975.

The operation of processes capable of repairing or bypassing some fraction of the initial, potentially lethal damage produced by radiations in cells is one of the important factors controlling the shape of survival curves. In particular, it has been shown mathematically that an initial shoulder must exist on survival curves for cells whose repair efficiency is itself inactivated with increasing dose (Haynes 1964a, 1966). It is possible to fit accurately most survival data with equations obtained on this basis. However, in itself this is hardly surprising since almost any continuous, monotonically decreasing function of dose with two or three adjustable parameters would be expected to provide a decent empirical fit to survival data. Mechanistic models are deemed significant to the extent that the parameters in them can be fixed by independent genetic or biochemical measurements. The parameters in the model described here are the radiosensitivity of repair deficient mutant strains of the organism in question and the radiosensitivity of the repair process as measured biochemically. R. Wheatcroft recently measured cellular efficiency for excision repair of UV-induced pyrimidine dimers in a wild-type strain of haploid yeast over a wide range of UV doses. This provides a direct estimate of the UV sensitivity of the excision process itself. We also know the radiosensitivity of various excisionless mutants. We have found that the wild-type and mutant survival data, coupled with the excision data, are quantitatively coherent, in that the wild-type survival curve can be predicted with reasonable accuracy from the excision and mutant data alone. This also indicates that essentially all of the difference in survival between wild-type and excision deficient strains can be accounted for quantitatively on the basis of excision repair of pyrimidine dimers.

Haynes, R. H. 1964a. Physical Processes in Radiation Biology, ed. L. Augenstein, R. Mason and B. Rosenberg New York: Academic Press) pp. 51-72.

Haynes, R. H. 1966. Radiat. Res. suppl. 6, 1-29.

XXX. From the Departments of Biology, Goldsmith's College, University of London, and Thames Polytechnic, London. Communicated by J.F.T. Spencer.

Genetic Characterization of Non-Sporulating Industrial Yeast Strains.

The genetic behavior of some non-sporulating brewers and distillers yeasts is being investigated. The strains were hybridized with haploid a and α and diploid aa and $\alpha\alpha$ tester strains and those hybrids which then sporulated were further investigated. Segregation of the markers in the test strains was variable, apparently depending on the nature of the prototrophic parent in the cross. In some crosses the markers were mostly expressed in the clones from the original hybrid, while in others the diploid clones thus obtained were nearly all prototrophic themselves, the auxotrophic markers not being expressed until the latter clones were themselves sporulated and the asci dissected.

The percentage of slow-growing clones, as estimated from the numbers of minute colonies found during the dissections, varied from 20 to 70%. A further study is being made of these clones to determine the nature of

the defect leading to slow growth. The possibility that the slow growth rate and failure to express some of the markers is due to the presence of supersuppressors is being investigated.

The spore viability ranged from good to poor, with the lowest viability occurring in the hybrids with haploid tester strains. This is consistent with the brewing strain used as parent being diploid or near-diploid, giving a triploid as a result of the original cross.

Sporulation of the clones obtained from the original hybrid with diploid tester strains was of interest, being very variable. In one cross, only about 15% of the resulting clones, apparently diploid, subsequently sporulated, while in others, more than 90% sporulated. In the latter hybrids, the auxotrophic markers were only expressed in the haploids obtained by sporulation and dissection of these clones.

The mating types of the various clones, insofar as these could be determined (some clones being prototrophic and some being non-mating diploids) were a, α, o(non-mater) and a/α (bisexual). The bisexuals have not yet been characterized. However, some may be the same as the bisexual diploid isolated by Haber. Others may be previously unknown haploid bisexuals or may be formed by self-diploidization normally but carrying a gene giving bisexual behavior as described by Haber.

A more detailed study of the mating behavior of the bisexual strains is in progress. In addition, new methods of hybridizing non-sporulating industrial yeast strains is under development.

XXXI. Arbeitsgruppe Mikrobengenetik, Fachbereich Biologie, J W. Goethe Universitat, 6 Frankfurt am Main, Robert-Mayer-Str. 7-9, Federal Republic of Germany. Communicated by M. Brendel.

Since the last issue of the Yeast News Letter the following papers have been accepted or submitted for publication:

DNA-specific labelling by deoxyribonucleoside 5'-monophosphates in Saccharomyces cerevisiae. M. Brendel, W. W. Fath, R. Toper. In: Radiation and Cellular Control Mechanisms in Simple Eucaryotic Systems, Springer Verlag, in press.

Summary. Growth of 5'-dTMP low-requiring stains is inhibited by exogenous 5'-dGMP ad 5'-GMP at concentrations higher than 5 x 10^{-4} M. Synthesis of nucleic acids ceases and cells remain fixed in their respective place in the cell cycle. At concentrations lower than 10^{-5}M. deoxyribonucleoside 5'-monophosphates may be employed for radioactive labelling, the label being preferentailly used for DNA synthesis. Affinity to DNA of the 5'-dTMP is in the order of 5'-dAMP> 5'-dGMP> 5'-dCMP> 5'-dUMP. DNA-specific label is achieved with 5'-dAMP when the medium is supplemented with adenine and deoxyadenosine.

Incorporation of methyl ^{3}H-5'-dTMP into the DNA of dipolid yeast cells as tracer of LHR after X-ray irradiation. G. Holtz, M. Brendel. In: Radiation and Cellular Control Mechanisms in Simple Eucaryotic Systems, Springer Verlag, in press.

Summary: The liquid holding phenomenon was examined in diploid yeast strain 211 Bg-which is auxotrophic for 5'-dTMP. For maximal LHR this strain needs 10 mmole/ml glucose in the LHR-buffer.

Repari of X-ray damage in the DNA as meausred by incoporation of labelled 5'-dTMP occurs during the first three hours of the LHR-treament. After the third hour of the LHR-treatment the number of colony forming units increases significantly.

Incorporation of 5'-dTMP into the DNA during the first three hours of LHR is not caused by normal DNA replication of some cells. Comparing the incorporation of 5'-dTMP due to repair to that due to normal replication, it was concluded that 2 to 9 nucleotides per DNA damage are required for repair under LHR-conditions in diploid yeast.

Isolation and properties of yeast mutants with highly efficient thymidylate utilization. W. W. Fath, N. Brendel. Z. Naturforschg., submitted.

Summary. A screening procudure is presented which allows the isolation of yeast mutants (typ tlr) with highly efficient utilization of exogenous 5'-dTMP (> 50%). Data are given concerning the phenomenon of 5'-dTMP utilization in general: (i) The ability of S. cerevisiae to incorporate exogenous 5'-dTMP was found to already be a wild type feature of this yeast, i.e. apparently not to be due to any mutation such as typ, tup, tmp per or tum. COnsequently these mutations are interpreted as amplifiers of a pre-given wild type potency. So far eight stages of 5'-dTMP utilization were detected as classified by the optimal 5'-dTMP requirement, with 5'-dTMP biosynthesis blocked, of the corresponding mutant strains isolated. All of them fit well into a mathematical series of the type "2^{n} x 1.5 (m = 0,1,2...,11)., where the product term for n = 11 represents the 5'-dTMP requirement (µg/ml) of the best 5'-dTMP utilizing wild type strains found. (ii) Amplification of the 5'-dTMP utilizing potency obviously is due to any genetically determined alteration of the yeast 5'-dTMP uptaking principle itself or of physiological processes accompanying the monophosphate's uptake. (iii) The functioning of 5'-dTMP uptake requires acidic (= or L pH 6) conditions in the yeast cells outer environment. (iv) Some yeast typ and typ tlr mutants were found to exhibit a more or less pronounced sensitivity toward exogenously offered 5'-dTMP. The response of a sensitive strain towards inhibitory concentrations of the nucleotide apparently is co-conditioned by the presence or absence of thymidylate biosynthesis. With 5'-dTMP biosynthesis blocked the 5'-dTMP mediated inhibition is a permanent one and finally leads to the death of a cell. With a functioning thymidylate biosynthesis, in contrast, the inhibition is only temporary. (v) Yeast typ or typ tlr strains were observed to dephosphorylate exogenous 5'-dTMPto thymidine due to a phosphatase activity which cannot be eliminated at pH7+70 mM P_i conditions in

the growth medium. This 5'-dTMP cleavage obviously occurs outside the cell and does not seem to be correlated either to the monophosphate's uptake or to the phenomenon of 5'-dTMP sensitivity. The destruction of 5'-dTMP does not disturb (5'-dTMP) DNA-specific labelling.

A simple method for the isolation and characterization of thymidylate uptaking mutants in Saccharomyces cerevisiae. M. Brendel, Molec. gen. Genet., submitted.

Summary. A thermoconditional thymidylate auxotrophy conferred by mutant gene temp-10Y^{ts} is employed for the selection of 5'-dTMP uptaking mutants. At the nonpermissive temperature yeast cells phenotypically wild type for thymidylate uptake can grow for only 3 to 4 generations in the presence of 10^{-2} M 5'-dTMP. Thymidylate utilizing mutants (tum mutants) were isolated which can grow in the presence of 12 to 24 µg 5'-dTMP/ml. Genetical analysis revealed one of these strains to be a double mutant, tum1 tum2. For normal growth haploid thymidylate auxotrophic strains require approximately 360 µg 5'-dTMP when tum1 and 24 µg 5'-dTMP/ml when tum2 is present, respectively. Cells prototrophic for thymidylate (TMP) harboring tum1 tum2 will also take up 5'-dTMP and incorporate it specifically into their DNA. Thymidylate utilization in such strains is independent of functional mitochondria, as similar incorporation of labelled 5'-dTMP is found in isogenic strains with rho$^+$, rho$^-$ and rho° status. Optimal stimulation of the 5'-dTMP uptaking principle in haploid TMP strains is found at 4 µg 5'-dTMP/ml when tum1 and tum2 are present.

XXXII. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, Bldg 4, Room 116, Bethesda, Maryland 20014. Communicated by Reed B. Wickner.

Below follow abstracts of two of our recent papers. Also recently published on the same subject:

Wickner, R. B. "Mutants of the killer plasmid of Saccharomyces cerevisiae dependent on chromosomal diploidy for expression and maintenance. Genetics 82:273-285 (1976).

Wickner, R. B. and M. J. Leibowitz. Two chromosomal genes required for killing expression in killer strains of Saccharomyces cerevisiae. Genetics 82:429-442 (1976).

Wickner, R. B. and M. J. Leibowitz "Chromosomal Genes Essential for Replication of a Double-stranded RNA Plasmid of Saccharomyces cerevisiae: The Killer Character of Yeast." J. Mol. Biol., in press.

SUMMARY

Strains of Saccharomyces cerevisiae carrying a small double-stranded RNA species (the killer plasmid) secrete a toxin which is lethal only to strains not carrying this plasmid.

We have isolated mutants in eight chromosomal genes essential for replication or maintenance of the killer plasmid, called mak1 through mak8. Seven of these genes have been mapped. Mak4 and mak5 are on chromosome II; mak1 and mak8 are on chromosome XV; mak3 and mak6 are on chromosome XVI; and mak7 is on chromosome VIII. We have not yet located mak2. Two other chromosomal genes, m and pets, have been previously shown to be required for replication or maintenance of the killer plasmid.

One allele of mak1 results in temperature sensitivity for host growth. Two independent pets isolates also result in the petite phenotype, as well as temperature sensitivty for growth.

Wild-type killer strains have been reported to carry two species of double-stranded RNA of 2.5×10^6 and 1.4×10^6 molecular weight (designed L and M, respectively); wild-type nonkillers carried only L. We estimate the size of the L and M species at 3.0×10^6 and 1.7×10^6 daltons, respectively. We have also detected a third species of double-stranded RNA of molecular weight 3.8×10^6 (XL) present in all killer and nonkiller strains examined.

Mutation of any of mak1 through mak8 results in loss of the killer-associated species of double-stranded RNA (M; 1.7×10^6 daltons). These mutants retain both the L species (3.0×10^6 daltons) and the XL species (3.8×10^6 daltons) of double-stranded RNA, and have acquired two new minor RNA species.

Leibowitz, M. J. and R. B. Wickner "A chromosomal Gene Required for Killer Plasmid Expression, Mating, and Spore Maturation in Saccharomyces cerevisiae." Proc. Natl. Acad. Sci. U.S.A., June 1976.

ABSTRACT

"Killer" strains of Saccharomyces cerevisiae are those which harbor a double-stranded RNA plasmid and secrete a toxin which kills only strains not carrying this plasmid (sensitives). Two chromosomal genes (kex1 and kex2) are required for the secretion of toxin by plasmid-carrying strains. The kex2 gene, which maps at a site distinct from the mating-type locus, is also required for normal mating by α strains and meiotic sporulation in all strains. Strains which are α mating-type and kex2 fail to secrete the pheromone α-factor or to respond to the a-factor II pheromone which causes a morphological change, but they do respond to a-factor I which causes G_1 arrest in α cells. Strains which are a mating-type and kex2 show no defect in mating, pheromone secretion, or response to α-factor. Diploids which are homozygous for the kex2 mutation, unlike wild-type or heterozygous diploids, fail to undergo sporulation, with the defect occurring in the final spore maturation stage. These same defects in the sexual cycle are present in all kex2 mutants independent of the presence of the "killer" plasmid.

XXXIII. Department of Fermentation Technology, Osaka University, Yamadakami, Suita-shi, Osaka 565, Japan. Communicated by Y. Oshima.

The following are recent publications from our laboratory.

A Constitutive Mutation, phoT, of the Repressible Acid Phosphatase Synthesis with Inability to Transport Inorganic Phosphate in Sacchaormyces cerevisiae. Molec. gen Genet. 136, 255-259 (1975). Yoshinami Ueda and Yasuji Oshima.

Two New Genes Controlling the Constitutive Acid Phosphatase Synthesis in Saccharomyces cerevisiae. Molec. gen Genet. 141, 81-83 (1975). Akio Toh-e, Sei-ichiro Kakimoto and Yasuji Oshima.

Genes Coding for the Structure of the Acid Phosphatases in Saccharomyces cerevisiae. Molec. gen. Genet. 143, 65-70 (1975). Akio Toh-e, Sei-ichiro Kakimoto and Uasuji Oshima.

Rare Occurrence of the Tetratype Tetrads in Saccharomycodes ludwigii. Journal of Bacteriology, Feb. 1976. p. 461-466, Vol. 125, No. 2. Toyohiko Yamazaki, Yuwao Ohara and Yasuji Oshima.

Isolation and Characterization of Recessive, Constitutive Mutations for Repressible Acid Phosphatase Synthesis in Saccharomyces cerevisiae. Journal of Bacteriology, June 1975, p. 911-922, Vol. 122, No. 3. Yoshinami Ueda, Akio Toh-e, and Yasuji Oshima.

XXXIV. Department of Genetics, Haryana Agricultural University, Hissar-125004 (India). Communicated by J. Mohan.

The following is a summary of our current research.

Macromolecular synthesis in temperature-sensitive mutants of Saccharomcyes cerevisiae.

Jag Mohan, R. K. Bansal and S. N. Kakar.

Temperature-sensitive mutants were isolated from two strains of yeast (a - lys 1-7 and α-S 288 C) using ultraviolet light and gamma rays. These mutants were classified into four groups depending upon their growth response to different temperatures i.e. (i) high temperature sensitive which did not grow at 42°C (ts), (ii), low temperature (cold) sensitive which did not grow at 12°C (cs), (iii) high temperature dependent which grew better at 42°C (hd) and (iv) low temperature dependent which grew better at 12°C (ld). The hd and ld mutants arose exclusively with gamma rays. The restrictive temperature of 42°C was found to be better than 36°C as it gave completely non-leaky mutants. Twenty one ts mutants obtained by gamma rays which complemented each other, were employed for biochemical studies. Synthesis of alkaline phosphatase during a 4 hour incubation at the permissive (25°C) and restrictive temperature (42°C) was studied in these mutants. While nearly all mutants synthesized similar amounts of alkaline phosphatase at permissive temperature several mutants were defective in this character at restrictive temperature. The activity of alkaline phosphatase increased approximately four-fold in the parent strains but in the mutants it averaged only two-fold. Synthesis of RNA and protein was studied in four mutants (a 2, a 11, α

104 and α 108) employing radioactive precursors during a 4 hour incubation at the restrictive temperature. Except for α 104, where RNA synthesis was decreased to about 70 percent of the parent strain, the other three mutants exhibited normal rates of RNA synthesis. Protein synthesis on the other hand ranged from 30 to 70 per cent of the parent strains in all four mutants. It would, therefore, appear that defective synthesis of alkaline phosphatase in our mutants might be primarily due to some defect in the translation machinery. This is also indicated by the fact that all our mutants complemented with eleven known mutants defective in RNA metabolism isolated by Hartwell and his colleagues.

XXXV. Allied Breweries (Production) Limited, The Brewery, Station Street, Burton-on-Trent DE14 1BZ, England. Communicated by P. A. Martin.

Below follow summaries of papers which have been recently published by members of the Process Research Department.

DETECTION OF BACTERIA IN YEAST USING A FICOLL GRADIENT. J. Harrison, T.J.B. Webb, P. A. Martin. J. Inst. Brew., 1975, 81, 439.

It has been found that a Ficoll 400 density gradient can be successfully used for the separation and preliminary identification of low numbers of bacteria in yeast by a differential sedimentation method. When 1 ml of a yeast-bacteria mixture is layered on top of 9 ml 20% aqueous Ficoll 400 solution in a centrifuge tube and the tube centrifuged the yeast cells form a plug and the bulk of the bacterial cells remain in the upper layer. A method combining incubation in a nutrient medium and simultaneous separation in a Ficoll gradient allows the direct microscopic detection of 10 viable bacteria/10^6 yeast cells after overnight incubation.

FACTORS AFFECTING THE SURVIVAL OF LYOPHILIZED BREWERY YEAST STRAINS. J. F. Hall, T.J.B. Webb, J. Inst. Brew., 1975, 81, 471-5.

Some of the factors that contribute to the loss of viability of brewery yeast strains during lyophilization (freeze drying) have been investigated. A lyophilization technique for the maintenance of brewery yeast strains with higher viabilities than those previously reported has been developed. Three lyophilized strains of ale yeast still had a survival rate of 60% after periods of storage of up to three years, while a lager yeast strain maintained a viability of approximately 50% during storage for eighteen months.

XXXVI. Labatt Breweries of Canada Limited 150 Simcoe Street, London, Ontario N6A, 4M3, Canada. Communicated by G. G. Stewart.

The following is a summary of progress of a research project underway in the Beverage Science Dept. of Labatt Breweries to elucidate the biochemical and genetic factors that influence yeast flocculation.

1. Inducer peptides containing a high proportion of the acidic amino acids aspartic and glutamic, present in wort, are necessary in the

growth medium before some strains of Saccharomyces cerevisiae are able to flocculate. The degree of specificity of such peptides does not appear to be high because peptide material from sources other than cereal extracts, for example, gelatin and peptone and even the L- and D-isomers of aspartic and glutamic acids, when present in excess in the growth medium, are all capable of inducing flocculation in some Sacch. cerevisiae strains.

2. No strain of Sacch. carlsbergensis (uvarum) has been found that requires the inducer peptide for flocculation.

3. Electron microscopy has revealed that flocculent cells have a fimbriate or "hairy" surface whereas non-flocculent cells are smooth.

4. SDS poly-acrylamide gel electrophoresis of basic de-flocculation extracts from flocculent cultures and similar extracts from non-flocculent cultures has revealed a number of protein/peptide components with molecular weights from 14,000 to 68,000. Extracts of the flocculent cultures of strains requiring an inducer peptide in the growth medium prior to flocculation, posses an intense peptide component (mol. wt. approx. 48,000), whereas this component is nearly absent in extracts of non-flocculent cultures of the same strain. De-flocculation extracts of strains flocculent in all complete media and extracts of strains non-flocculent in all complete media possess similar peptide/protein banding patterns to one another but lack the 48,000 mol. wt. peptide component.

5. Flocculent cells adsorb Ca^{++} ions more firmly than non-flocculent cells ie., the Ca^{++} ions adsorbed by the non-flocculent cells are easily removed by washing.

6. Attempts to correlate the flocculation state of cultures with the binding of the positively charged dye alcian blue has indicated a greater protein involvement in flocculation of those strains that are only flocculent after growth in a medium containing inducer material than in strains flocculent after growth in all complete media. In all likelihood, carboxyl is the primary anionic group involved in the flocculation of the former group whereas in the latter group phosphate serves a similar function.

7. Environmental conditions such as wort gravity and incubation temperature influence the flocculation of some, but not all, yeast strains studied, whereas the flocculation of all strains studied is influenced by wort adjunct level, yeast pitching rate and wort oxygen level.

8. As genetic studies on brewer's yeast strains are fraught with difficulties due to their frequent triploid, polyploid or aneuploid nature, low degree of sporulation and their low spore viability, initial genetic studies on flocculation have centered upon a flocculent haploid strain (coded 169). The flocculation of 169 has been found to be dominant and controlled at a single gene locus. The flocculation character of both hybrids and haploids derived from such hybrids, appears to be influenced by the repression or de-repression status of the culture.

9. Mapping studies of this flocculence gene have revealed that it is linked to *ade* 1 and therefore located on Chromosome I. Consequently, this is a different gene to the three flocculation genes studied in other laboratories because they have found such genes to be unlinked to *ade* 1. The flocculation gene being studied in this laboratory has been designated *FLO* 4.

10. *FLO* 4 has been found to be located 32/33 cM from the Chromosome I centromere and 37 cM from *ade* 1 (ie., *FLO* 4 is on the opposite side of the centromere to *ade* 1).

XXXVII. Department of Environmental Biology, Microbiology Division, University of Guelph, Guelph, Ontario, Canada. Communicated by J. D. Cunningham.

During the past 2-3 years, investigations have been conducted on yeasts and applied fermentations as related to the Brewery, Distillery, and Single Cell Protein. Brief summaries for each area are presented accordingly. Individuals who have contributed to this research include Ms. S. Lymburner, Ms. D. Kanelakos, Mr. A. M. Gould, and Mr. D. Ames.

(1) Brewing (a) Accelerated Fermentations with Lager Yeasts

Selected lager yeasts were investigated with respect to their fermentative and physiological acitivity. Temperature adaptations and fermentation accelerations were subsequently investigated without changing the quality of the fermented product. Based on studies to date, it has been possible to optimize and complete a lager fermentation within three days by introducing incremental temperature programming of a selected Saccharomyces carlsbergensis strain subjected to CO_2 purging or sparging. Gas liquid chromatographic studies for higher alcohols, etc., diacetyl determinations and taste evaluations have revealed that it is possible to produce an acceptable lager beer within a shorter period of time under controlled conditions.

(b) Specific Physiological Characteristics of Brewery Yeasts

Methodologies were established for screening yeasts for production purposes. Characteristics of flocculation were investigated with respect to synergism and stability. Combinations of certain flocculent and nonflocculent yeasts revealed marked differences in the ability to flocculate under experimental conditions. Immunofluorescence was studied in order to differentiate between ale and lager yeasts, with some modification of the techniques reported in literature. The major difference in the use of fluorescent antibody stain is the staining of the desired production yeast whereas the wild yeasts and undesirable yeast contaminants remained unstained, thus permitting differentiation. Adaptability or practicability of this technique to identifying yeast contamination of in-plant production yeasts is still under investigation.

(2) Distillery. Efficiency Evaluation of Yeasts in Rum Manufacture

High temperature investigations in the fermentation of molasses by selected yeasts established certain conditions that permit a more efficient fermentation control and alcohol production. Factorial experiments indicated that preferentially temperatures of 30° and 35°C and ammonium sulfate at 0.1 to 0.2% levels favoured more rapid conversion (72 hrs) of molasses with increased alcohol yields than under the conventional methods of rum manufacture.

(3) Single Cell Protein. Microbial Utilization of Whey

Whey as a high B.O.D. effluent from the cheese manufacturing industry, constitutes production in excess of 2.6 billion pounds annually. The effect of various agitation and aeration systems on yeast fermentations were investigated, employing substrate supplementations with corn steep liquor. Biomass production with yields up to 25 g/liter in 18 hours were obtained with selected strains of Kluyveromyces (Saccharomyces) fragilis and the entire biomass recovered by centrifugation and washed and spray-dried. Nutritional evaluation of the yeast as single cell protein for animal feeding trials are in progress in addition to studies on the production of lactase and recoverable alcohol during the early stages of fermentation.

XXVIII. Miller Brewing Company, Milwaukee, Wisconsin 53201. Communicated by James F. Rice.

Inhibition of Beer Volatiles Formation by CO_2 Pressure. James F. Rice, Etzer Chicoye, J. Raymond Helbert and John Garver.

Total volatiles formation and yeast growth were shown to be inversely related to the degree of CO_2 counterpressure applied during 100 liter pilot fermentations, while the rate of fermentation remained relatively unaffected. Thus, 8 psig (22.7 psia) CO_2 counterpressure in a 22° fermentation repressed both total yeast growth and total volatiles concentration to the levels present in a 15° fermentation with no CO_2 counterpressure. Individual volatile compounds were not uniformly repressed, however.

The effects of temperature, CO_2 counterpressure and agitation upon the dissolved CO_2 concentration during active fermentation were quantified. It is shown that the degree of supersaturation (supersaturation coefficient), which is the ratio of the dissolved CO_2 concentration during active fermentation to the CO_2 saturation level, is constant over the wide ranges of temperature and CO_2 counterpressure examined, at constant agitation level. The supersaturation coefficient varies inversely and in a linear manner with agitation (expressed as rpm). At sufficiently high rpm, no CO_2 supersaturation exists during active fermentation, thus the supersaturation coefficient is 1.0.

Two Verdant Types of S uvarum as Determined by Cytochrome Absorption Spectra. E. J. Kot and J. Raymond Helbert.

Wallerstein Laboratories' Nutrient (WLN) agar has been described as useful for differentiating normal colonies of Saccharomyces uvarum from mutant colonies and for subdividing the latter into petites and verdants.

In our hands, the petites all formed small, white colonies with dark green centers on this medium. The verdants, however, formed colonies with very different shades of green. A study was undertaken to determine what other differences, if any, existed among the verdants. Cytochrome absorption spectra of verdant isolates, grown aerobically in a low-glucose broth at 25°C, revealed two cytochrome patterns. The moderate green verdant possessed a normal cytochrome absorption spectrum, while the dark green verdant lacked cytochromes aa_3, b, and c_1. In addition, the dark green verdant accumulated a substance absorrbing at about 575 nm. This substance is believed to be the cytochrome precursor, protoporyphyrin IX. Oxygen uptake studies also indicated that the moderate green verdants were respiratory-sufficient, while the dark green verdants were respiratory-deficient.

Although the moderate green verdant did not differ from the normal control in respect to respiration, a metabolic difference was found. Carbohydrate profiles of incompletely fermented worts showed that the moderate green verdant utilized maltotriose more rapidly than the normal control.

These results demonstrate that there are at least two types of verdants which may be isolated on WLN agar. The moderate green type is respiratory-sufficient, although its carbohydrate utilization differs from that of the strain from which it was derived. The dark green type is respiratory-deficient and accumulates protoporyphyrin IX.

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

E. Minari, - P. Regala: Influence of some herbicides on the composition of the yeast flora of fermenting musts. Mitteilungen Klosterneuburg 25, 1975: 357-370.

In a series of assays carried out in the course of eleven years the influence of different herbicides on the yeast flora of grapes and fermenting musts in large-scale field- and laboratory tests was investigated. Triazin- and mixed triazin-herbicides used in usual concentrations showed no or unimportant influence on yeast activity in must even after many years application. Herbicides on the basis of urea did not influence the composition of the yeast flora either. Herbicides on the basis of chlorothiamide showed strong inhibition of sporogenous yeast species only when unburied in the soil. Similar behavior may be expected in combination of this unburied herbicide with other preparations, e.g. Semparol. Herbicides on the basis of dichlobenil do not seem to show any inhibition of sporogenous yeast growth. General aspects of the by-action of herbicides on the course of spontaneous fermentation of grape juice are discussed.

E. Minarik: Reduction of sulfate to sulfite and its taxonomic significance for the classification of yeasts. In: A. Veres (ed.): Progrès de la Recherche Viti-vinicole 7, 279-298, Maison d Edition de l Academic Slovaque des Sciences, Bratislava 1975 (in French).

The ability of yeasts to reduce sulfate to sulfite only and not as far as hydrogen sulfide in fermenting grape must as is usually the

case, has proven to be strain specific. It was observed in strains of various species of the genus Saccharomyces, e.g. S. cerevisiae, S. carlsbergensis, S. pastorianus etc. For this reason this character may be regarded as insignificant for yeast taxonomy. The quantity of sulfite formed and its accumulation in fermenting must depends also on the concentration of sulfate in must prior to fermentation, but only in SO_2-forming strains. Technological aspects for the wine production are briefly discussed.

XL. The Australian Wine Research Institute, Private Bag, Glen Osmond, South Australia, 5064. Communicated by B. C. Rankine.

"For many years the study of yeast cultures for winemaking has been part of the writer's activities at The Australian Wine Research Institute, and the emphasis is now on establishing the best means of using the selected yeasts under practical conditions, an aspect of particular importance in winemaking.

Most of Australia's wine is made with the aid of selected yeasts, and some elaborate propagation equipment is in use. In some cases the fermenting pure starter culture is aerated, giving cell counts considerably higher than the 100-150 million cells per ml. obtained under anaerobic conditions.

Some winemakers are now counting the cells in their inoculum so that the grape juice is inoculated with between 5 and 10 million yeast cells per ml. final concentration. This inoculum has been found adequate to dominate the naturally-occurring microflora in grape juice from sound grapes.

Advantages resulting from the use of selected yeast cultures in winemaking have been found to be:

1. Rapid onset of fermentation enabling maximum use of fermentor capacity.

2. Even and complete fermentation leaving no residual fermentable sugar, and not placing an undue load on refrigeration due to very rapid and tumultuous fermentation.

3. Absence of undesirable off-odors and flavors, such as hydrogen sulphide, yeastiness and other by-products of fermentation.

4. Prevention of excessive foaming enabling fermentors to be filled to greater capacity.

5. Efficient initiation of the secondary alcoholic fermentation in production of sparkling wines, using yeasts selected for this purpose."

XLI. Department of Microbiology, Haryana Agricultural University, Hissar-125 004, India. Communicated by R. K. Bansal.

Development of High Ethanol Producing Strains of Yeast S. cerevisiae var. ellipsoideus for the Fermentation of Sugar Cane Molasses.

Three strains of yeast (Saccharomyces cerevisiae var. ellipsoideus) namely S-288C (Haploid) and 522 and 523 (Diploids) were employed to isolate mutants which can produce more alcohol in molasses medium. In succinate synthetic medium (single strength) with 25 per cent initial sugar concentration strains 522 and 523 produced a maximum of 10.2 and 10 per cent alcohol, respectively, while strain S-288C produced 7.6 per cent after 48 hr incubation. In double strength succinate synthetic medium under identical conditions strains 522 and 523 produced even higher concentrations of alcohol (11.25 per cent) than strain S-288C (5.5 per cent). In molasses medium a maximum of 6.8 per cent alcohol was produced by strain S-288C while strains 522 and 523 produced 5.6 and 6.5 per cent, respectively. Out of 20 glucose resistant mutants only one produced more alcohol (7.2 per cent in comparison to wild type (6.8 per cent). Out of 14 respiration-deficient mutants none gave higher yield than the parent strain. By natural selection methods one mutant designated as strain 523 HAU was isolated by inoculating the parent strain 523 on molasses medium plates containing 25 per cent sugar and 10 per cent ethanol. This new isolate tolerated more alcohol and sugar and produced more alcohol. At an initial concentration of 25 and 30 per cent sugar in succinate synthetic medium the new isolate produced 12 per cent alcohol while the parent produced only 10 per cent. In molasses medium with 20 per cent sugar, the new isolate produced 7.5 per cent alcohol in contrast to the parent which could produce only 6.5 per cent. The fermentation time was considerably reduced when 20 per cent inoculum was used. Addition of ammonium sulphate did not influence the alcohol production by strain 523 HAU. An initial pH of 5 was found to be optimal for maximum alcohol production. When 523 HAU was compared with the yeast used in a distillery in a large scale experiment, strain 523 HAU produced 7.9 per cent alcohol as compared to 5.4 per cent by the industrial strain after 36 hr incubation.

XLII. International and National Meetings

1. The "5th Specialized International Symposium on Yeasts" will be held in Hungary, Keszthely, at the Lake Balaton, September 12-15, 1977. Scope: Systematics and related subjects (taxonomy, ecology, serology, immunology). The proposed topics are the following:

Taxonomy

1. The position of yeasts and yeast-like microorganisms in the system of fungi.

2. Natural groups of yeasts and yeast-like microorganisms.

3. The connection of yeast-taxonomy with the Botanical Code of Nomenclature.

4. The relation of the species concept to the taxonomical properties of yeasts and yeast-like microorganisms.

5. Certainty of identification, uncertainty of taxonomical properties.

6. Serological methods in taxonomy and identification.

7. Current taxonomic trends.

Ecology

1. Yeasts in soil, water and air

2. Yeast flora of plants and plant pathogenic yeasts

3. Yeast flora of animals and animal pathogenic yeasts

4. Yeast flora of the human body and human pathogenic yeasts.

5. Immunological connections of colonized higher organisms to their yeast flora

6. Flora of industrial fermentors, contaminations by yeasts.

7. Flora of yeast-industrial products (wine, beer etc.)

8. Yeast flora in food-industry and food products.

Inquiries may be addressed to the Chairman of the Organizing Committee: Dr. E. K. Novak, Department of Mycology, National Institute of Hygiene /H-1966 Budapest, P.O.B. 64. Hungary.

2. VIIIth Annual Conference of the Commission of Yeasts of the Czechoslovak Microbiological Society, Smolenice 11 - 13th February, 1976. Communicated by A. Kockova-Kratochvilova.

Section: Biochemistry

A. Kotyk: Various kinds of membrane kinetics of substance transport.

M. Opekarova: The transport of amino acids in Saccharomyces cerevisiae

A. Knotkova: The importance of the proton gradient in the transport of yeasts.

A. Vojtkova: Hydrolytic enzymes of hyphal fungi, splitting polysaccharide protein complexes in yeasts.

M. Valaskova: Studies of glycoprotein structure in Cryptococcus albidus.

P. Biely, Z. Kratky, S. Bauer: The interaction of extracellular mannan-protein in yeasts with phytohaemagglutinine Concanavalin

Z. Kratky, M. Vrsanska, P. Biely: The affinity of extracellular mannan-protein in yeast cell walls

V. Farkas: The characterization of mannosyltransferase in yeast mutants defective in mannan biosynthesis

S. Balint, V. Farkas, S. Bauer: The biosynthesis of yeast beta-glucan catalyzed by an isolated enzyme system.

J. Subik: Nuclear mutant of yeast Saccharomyces cerevisiae with a cell division related to the mitochondrial function.

G. Takaczova, J. Subik, M. Psenak: Cytological activity of antimicrobially active N-oxides.

J. Kolarov: Adenine nucleotide transport system in mitochondrial membrane of yeasts genetically and physiologically modified.

B. Kralova, V. Sicho: The influence of selected antimetabolites against enzymes acting in the final phase of the thiamine biosynthesis.

Z. Hunkova: A complex of knowledge about the toxic affect of fatty acids on yeast cells.

Section: Cytology, genetics, immunology and pathogenicity

A. Tomsikova: The effect of some antimycotics on pathogenic and nonpathogenic yeasts.

M. Stollarova: The occurrence of yeasts on various kind of fruits

E. Slavikova: Yeasts as etiologic agent of mass lethal disease of horned cattle

A. Svoboda: The importance of cell wall for the conjugation of yeasts.

M. Havelkova: The regeneration of yeast cell wall in protoplasts and spheroplasts of Nadsonia elongata

L. Silhankova: Regeneration of the glucose metabolism in yeasts and its genetic control

B. Skarka: An experience with the preparation of mutants of Saccharomyces cerevisiae.

Povazaj: Factors affecting the copulation and metabolism of strains of Candida lipolytica

Section: Technology

A. Kockova-Kratochvilova: The genus Debaryomyces and its practical importance.

A. Prokop and J. Votruba: The evaluation of the yeast growth on ethanol in the way of two-substrate Monod's relationship.

D. Halama: The possibilities of the influence of the metabolite production in yeast single cell culture

D. Longarerova: The significance of cultivation parameters in the utilization of n-alcanes by yeasts.

J. Farkas: The inhibitory effect of 5-nitrofurylacrylate on microorganisms in wine

E. Minarik, P. Ragala, A. Navara: The influence of some herbicides on the yeast flora in ciders

A. Navara, E. Minarik, G. Vojtekova: The adaptation of some strains of wine yeasts to potassium sorbate.

Z. Kohnova and K. Lesicka: World information centers in the field of natural sciences and their services.

Section: Minisymposium on yeast proteins organized by Prof. V. Krumphanzl

V. Krumphanzl: World development of industrial production of yeast proteins

A. Prokop: The use of hydrocarbons in the preparation of yeast biomass.

O. Volfova: The utilization of some alcohols and acids for the preparation of yeast biomass

Z. Fencl: The kind of preparation of yeast protein concentrates

M. Rut: Some kinds of preparation of yeast protein concentrates for human consumption.

J. Kejmar: Criteria for the evaluation of single cell proteins

L. Kuzela: Criteria for the use of yeast protein concentrates for human nutrition

J. Vavak: The utilization of yeast biomass in the fodder industry.

R. Bartak: The production of yeast proteins from acid hydrolyzate of animal excrements.

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3. "Robert Stanton (in his capacity as UNEP/UNESCO/ICRO Southeast Asian Regional representative) has informed us that, for political reasons, the Fifth Global Impacts of Applied Microbiology Conference has been postponed pending transfer to a new venue outside Malaysia. It will not be taking place in Kuala Lumpur from March 22-27 as previously announced. The associated workshops in Pennang and Serdang are also rescheduled to other territories.

Names and addresses of all who have written inquiring about participation in GIAM V have been recorded. This information will be passed to the new oganizer, who will be responding to all inquiries. Plans are well advanced for hosting at a new venue in the Region, probably in mid-1977. The review publication, which was to be the pre-conference volume, and for which the manuscripts had already been prepared, will be published later this year under the title:

"Global Impacts of Applied Microbiology: State of the Art 1976"

Inquiries about the above publication, or participation on the rescheduled GIAM, or other UNEP/UNESCO/ICRO activities in the Region may be sent to the address below:

Professor Robert Stanton
UNEP/UNESCO/ICRO Regional Representative
P. O. Box 666
Kuala Lumpur
West Malaysia

He also brings to our attention the holding of a private symposium, which he is organizing jointly with Dr. Ken Ruddle, Director, Natural Resource Systems Planning Project, Technology and Development Institute, east-West Center, Honolulu, Hawaii 96822, U.S.A., on SAGO at Kuching, Sarawak 2-5 May 1976. This symposium is being jointly sponsored by various industries interested in the industrial utilization of sago, a starch from palms, for fermentations, food and other starch-derived products. It grows in equatorial swamps and is an ecologist's dream crop in that it maintains a full canopy all the year round and can be cultivated without destroying the swamp. The plant and its products present many interesting problems for the applied and industrial microbiologist and it is likely to be a crop of which we shall hear much in the future, in view of increasing awareness of the need to study energy capture by plants and conserve our resources."

4. The Society for Industrial Microbiology will depart from its custom of meeting on a university or college campus to holds its 1976 meeting at The Wanderer Motel on Jekyll Island, Georgia.

August 16-20. Society for Industrial Microbiology, The Wanderer Motel, Jekyll Island, Georgia. Information - Mra. Ann Kulback, SIM c/o AIBS, 1401 Wilson Blvs., Arlington, VA. 22209.

The program will include Symposia and Roundtables: Industrial Sterilization and Regulatory Aspects, Convenor, F. E. Halleck; Industrial Applications of Thermophillic Microorganisms, Convenor, R. J. Belly; Microbial Transformation of Waste Materials into Useful Products, Convenor, Arthur Kaplan: Model Eco-System Approach to Biodegradation Studies, Convenor, W. Gledhill; and Food Microbiology, Convenor, Donald Murray.

A Fermentation Workshop is also being organized.

August 15 Society for Industrial Microbiology Fermentation Workshop, Microbial Process Development programs, The Wanderer Motel, Jekyll Island, Georgia. Professor D. Perlman, School of Pharmacy, University of Wisconsin, 425 M. Charter St., Madison, Wisconson 53706.

Emily M. Owen, chairman
1976 Publicity Committee
Society for Industrial Microbiology

5. Course in Microbial Breeding

The 5th Course of the International School of General Genetics was presented in Erice, Sicily from June 18-25, 1976. The school is a part of Ettore Majorana, the Centre for Scientific Culture, and is directed by G. Sermonti of the University of Perugia. The Course Director is A. L. Demain of M.I.T. The purpose of the course in MICROBIAL BREEDING is to integrate the knowledge recently gained in microbial genetics, microbial biochemistry, molecular biology and industrial microbiology toward practical goals. The course, directed toward research workers, featured lectures, discussions and exercises reviewing the basic concepts of secondary metabolism in nature and in fermentors. It covered advanced techniques and rationales in genetic and biochemical handling of prokaryotic and eukaryotic species employed in industrial fermentations. Topics included the natural role of antibiotics, new methods for discovering antibiotics, targets of antibiotics, resistance development, biochemical genetics of antibiotic biosyntheses, mutational modification of secondary metabolism, yield improvement, and plasmids, episomes and recombination in industrial microorganisms. Lectures included C. Ball (UK), K. Chater (UK), A. L. Demain (USA), H. Heslot (France), D. A. Hopwood (UK), R. Hutter (Switzerland), G. Lancini (Italy), J. Nuesch (Switzerland), E. Rosenberg (Israel), M.R.J. Salton (USA), G. Sremonti (Italy), A. M. Torriani (USA), and Z. Vanek (Czechoslovakia).

XLIII. Brief News Items

1. The following paper is in press in the Canadian Journal of Microbiology.

N.J.W. Kreger-van Rij and M. Veerhuis, Conjugation in Guilliermondella selenspora Nadson et Krassilnikov. N.J.W. Kreger-van Rij, Laboratorium voor Medische Microbiologie R.U. Oostersingel 59, Groningen, The Netherlands.

2. Dr. P. Brechot, Institut Pasteur, 25 Rue du Docteur Roux, 75024 Paris, Cedex 15, France has retired from active service. Dr. Brechot writes: "I am the General Secretary of the "Société Française de Mycologie Medicale" which publishes in the "Bulletin de la Société Française de Mycologie Médicale the papers by Members of this Society as well as articles from other mycologists. If any of the readers of the Yeast Newsletter is interested in the review, we can accept them as foreign members. The membership is 50 francs per year, for which they will receive the review.

3. In recent months our laboratory was visited by Professor I.S. Kulaev, Moscow; Prof. A. S. Antonov, Moscow; Dr. S. G. Kharatyan, Moscow. All were sponsored by the USA and USSR Academies of Sciences.

In July 1976, Dr. T. Starmer will join our group as a postdoctoral visitor for one year. Dr. Starmer is currently at the University Arizona, Tucson in the laboratory of Prof. William B. Heed. He will participate in our work on yeast ecology and taxonomy.

H. J. Phaff
University of California, Davis