YEAST

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Herman J. Phaff, Editor
University of California, Davis, California 95616

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From the Editor: We repeatedly receive checks from banks or subscription agencies in payment of subscriptions for the Yeast Newsletter without identification for whom the payment is made. It is obvious that our office, under these circumstances, cannot give credit to the individual arranging for payment. Your cooperation in this regard will be appreciated.

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

The following items are from the CBS:

1. The genera of the yeasts and the yeast-like fungi. J. A. von Arx, L. Rodrigues de Miranda, M. Th. Smith and D. Yarrow.

Summary. In the keys 78 genera of yeasts, yeast-like fungi and colourless algae are included. The yeasts with "asci" are classified in the order Endomycetales with the families Endomycetaceae, Ascoideaceae, Saccharomycodaceae, Saccharomycetaceae, Metschnikowiaceae and Schizosaccharomycetaceae. The yeasts related to the Ustilaginales (Ustomycota) and the Basidiomycetes with "ballistospores," teliospores (ustulospores) or basidium-like structures are arranged in the Sporobolomycetales with the families Sporobolomycetaceae and Filobasidiaceae. The imperfect yeasts comprising asexual states of Endomycetales and Sporobolomycetales are enumerated in the Torulopsidales. The genera are listed with references to the literature, synonyms and type species. The number of species recognized in "The Yeasts" (Lodder, 1970) is mentioned and any new species or other changes published since 1976 are listed.

(Reprints can be ordered from the Centraalbureau voor Schimmelcultures, Oosterstraat 1, Baarn, The Netherlands. The price is 15.- Dutch guilders.)

2. Kloeckera apis st. nov.

The imperfect state of Hanseniaspora guilliermondii Pijper.
Maudy Th. Smith[1], F. P. Simione, Jr.[2] and Sally A. Meyer[2].

[1]Yeast Division of the Centraalbureau voor Schimmelcultures, Laboratory of Microbiology, University of Technology, Delft, The Netherlands, and

[2]American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA.

Summary. K. apiculata var. apis Lavie nom. nud was found to be the imperfect state of H. guilliermondii Pijper by a high degree of DNA reassociation. The name is validated and raised to species rank, K. apis (Lavie ex Smith, Simione and Meyer) Smith, Simione and Meyer. K. apis and H. guilliermondii can be distinguished from H. uvarum and H. valbyensis by low DNA reassociation and by their ability to grow at 37C.

A manuscript regarding this topic has been accepted for publication in Antonie van Leeuwenhoek.

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

The strains listed have been added to the ATCC since December 31, 1976. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

Cryptococcus neoformans ATCC 34543, ATCC 34544	F. Staib Robert Koch Institut, Berlin
Endomycopsis lipolytica ATCC 20390, Released patent Production of 2-oxo-glutaric acid	P. Maldonado Institut Francais du Petrole Paris
Endomycopsis lipolytica ATCC 20460, Released patent Production of isocitric acid	P. Maldonado Institut Francais du Petrole Paris
Endomycopsis lipolytica ATCC 20461, Released patent Production of isotric acid	P. Maldonado Institut Francais du Petrole Paris
Hanseniaspora uvarum ATCC 34535, ATCC 34536, ATCC 34537 & ATCC 34538	Sally A. Meyer ATCC Rockville, Md.
Kluyveromyces lactis ATCC 34609, ATCC 34610 Mannan mutants	Richard Douglas for C. E. Ballou Univ. of California Berkeley, CA
Saccharomyces uvarum ATCC 34510, fdp mutant	K. W. Vande Poll Van't Hoff Lab., Utrecht
Saccharomyces rouxii ATCC 34517, ATCC 34518 osmophilic mutants	David Kerridge for T. Y. Koh Univ. of Cambridge Cambridge
Trichosporon jirovecii ATCC 34499	A. Kockova-Kratochvilova Slovak Acad. Sci. Bratislava

III. Northern Regional Research Laboratory, U.S.D.A., 1815 North University Street, Peoria, Illinois, 61604. Communicated by C. P. Kurtzman.

The following are abstracts of recently published papers from this laboratory.

Kurtzman, C. P. and M. J. Smiley. 1976. Heterothallism in Pichia kudriavzevii and Pichia terricola. Antonie van Leeuwenhoek 42:355-363.

ABSTRACT

Pichia kudriavzevi and P. terricola were found to be heterothallic, but not interfertile with one another; nor did they mate with P. membranaefaciens, P. scutulata, Candida lambica, C. diversa, C. ingens, C. silvae, C. valida, C. vini, C. norvegensis, or Torulopsis inconspicua. Limited conjugation occurred between mating types of P. kudriavzevii and C. krusei and conjugation and sporulation occurred in mixtures with C. sorbosa. The data indicate

C. krusei and C. sorbosa to be the same species and to represent imperfect forms of P. kudriavzevii. P. kudriavzevii is heterozygous for L-sorbose assimilation.

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Baptist, J. N. and C. P. Kurtzman. 1976. Comparative enzyme patterns in Cryptococcus laurentii and its taxonomic varieties. Mycologia 68:1195-1203.

ABSTRACT

Cryptococcus laurentii and its varieties flavescens and magnus were compared using starch gel electrophoresis of six different enzymes. Enzyme patterns of sexually reactive C. laurentii cultures differed from nonmating strains and from the varieties magnus and flavescens. Additionally, the variety magnus was shown to be composed of two distinct taxa. On the basis of these and other data, two new species are proposed from strains comprising the variety magnus. Cryptococcus laurentii also was compared with Tremella aurantia and T. encephala.

New taxa:

Cryptococcus magnus (Lodder et Kreger-van Rij) Baptist et Kurtzman, comb. nov.
Basionym: Cryptococcus laurentii (Kuff.) Skinner var. magnus Lodder et Kreger-van Rij

Cryptococcus heveanensis (Groenewege) Baptist et Kurtzman, comb. nov.
Basionym: Torula heveanensis (Groenewege)
Synonyms: Candida heveanensis (Groenewege) Diddens et Lodder
Torulopsis heveanensis (Groenewege) Mager et Aschner

IV. National Institutes of Health, NIAID, Laboratory of Clinical Investigation, Bethesda, Maryland, 20014. Communicated by K. J. Kwon-Chung.

The following papers have been published or are in press.

"A new species of Filobasidiella, the sexual state of Cryptococcus neoformans B and C serotypes." K. J. Kwon-Chung. Mycologia. 68:942-946, 1976.

SUMMARY

The perfect state of Cryptococcus neoformans B and C serotypes is described as Filobasidiella bacillispora. The new species differs from Filobasidium neoformans by the formation of narrow, smooth rod-shaped basidiospores.

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"Epidemiologic differences among serotypes of Cryptococcus neoformans." John E. Bennett, K. J. Kwon-Chung and Dexter H. Howard. Amer. J. Epi. (in press).

ABSTRACT

In the USA, the most prevalent serotype of Cryptococcus neoformans is serotype A. This serotype constituted 203 of 272 isolates from the environment. Serotype B or C isolates were infrequent causes of infection, except in Southern California, and were not isolated at all from environmental sources. In Southern California the absence of serotypes B and C in 67 soil and pigeon dropping isolates was striking, considering that 25 of 49 isolates from infections were serotypes B or C. The site in nature where serotypes B and C exist is currently unknown but differs from that of serotypes A and D.

Serotype D may be unusually prevalent in both environmental and patient isolates from Denmark and Italy. Of 24 isolates from those countries, 21 were serotype D.

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"Perfect state of Cryptococcus uniguttulatus." K. J. Kwon-Chung. Int. J. Sys. Bact. (in press).

SUMMARY

The sexual state of Cryptococcus uniguttulatus has been described as Filobasidium uniguttulatum in the Filobasidiaceae. The new species is characterized by slender basidia with 4 to 10 sessile reniform to oval basidiospores produced in a terminal cluster. The strains of F. uniguttulatum are heterothallic. The morphological and physiological characteristics of F. uniguttulatum have been compared with those of two previously described species in the genus Filobasidium.

V. University of Lyon, 43 Bd du 11 Nov. 1918-69621, Villeurbanne, France. Communicated by M. C. Pignal.

The following article has appeared recently:

J. B. Ficol and G. Billon-Grand. Nitrite reductase des Saccharomyces (groupe Torulaspora) et des Debaryomyces. Implications systématiques. Mycopathologia 60:109-113. 1977.

The following article has been submitted to Mycopathologia:

G. Billon-Grand. Recherches d' enzymes intracellulaires dans le genre Schizosaccharomyces. Implications systématiques.

J. B. Fiol, F. Jacob, R. Montrocher and M. C. Pignal et S. Poncet will be participating in September 1977 at the 5th International Specialized Symposium on Yeasts at Keszthely (Hungary) and the following papers will be presented:

- R. Montrocher. Immunoelectrophoretic investigations in the genus Candida: taxonomic implications.

- M. C. Pignal, D. Lachaise and D. Bernillon. Yeast flora associated with Drosophilids of savanna in tropical Africa.

- S. Poncet. Is the genus Torulaspora Lindner emend. Van der Walt and Johannsen a natural group? Application of factor analysis.

VI. Microbiology Research Group, South African Council for Scientific and Industrial Research, P. O. Box 395, Pretoria 0001, South Africa. Communicated by J. P. van der Walt.

We have submitted a paper to Antonie van Leeuwenhoek which has been accepted and is to appear in vol 43.

PLOIDY, ASCUS FORMATION AND RECOMBINATION IN Torulaspora (Debaryomyces) hansenii

J. P. van der Walt, Maureen B. Taylor and N. V. D. W. Liebenberg

SUMMARY

X-ray inactivation studies on the type of Torulaspora hansenii carried out to determine ploidy, provided proof that the species has a haplontic life-cycle, a fact which hitherto has only been presumed.

Observations on the genesis of the ascus by light microscopy and transmission electron microscopy, provide no evidence for what some earlier workers in this field have presumed to be heterogamous conjugation between a mother-cell and its bud. They do, however, show that asci bearing obliquely-attached, vestigial bud-like appendages arise from some cells to form single, non-abstricting and frequently recurving protuberances which enlarge. These could conceivably be responsible for the impression that abstricted buds are connected to mother-cells by bent copulatory tubes.

The formation during sporulation of elongated protuberances and the presence of a medial, electron-dense line within the electron-translucent layer of the walls of ascospores fixed with OsO_4 preclude the possibility of using these features to differentiate between the genera Torulaspora and Debaryomyces.

Furthermore, recombinant studies, which involved the use of auxotrophic mutants, indicated that during sporulation the fusion of independent cells accounted for only 0.03-0.6 percent of the asci formed.

The conclusion was reached that somatogamous autogamy must be the main agency of diploidization and that the species is largely inbreeding.

VII. Dept. of Medical Microbiology, School of Medicine, University of California, Davis, CA, 95616. Communicated by Michael S. Collins.

The following is the abstract of a report to be presented at the Fourth International Conference on Mycoses in Brasilia, D. F., Brazil, June 6-8, 1977.

TAXONOMIC IMPLICATIONS OF POLYMYXIN B SENSITIVITY IN YEAST

Michael S. Collins, Carmen Bettencourt
and Demosthenes Pappagianis

A replica plating agar dilution method was employed to test the sensitivity of medically important yeasts to the peptide antibiotic, polymyxin B (PxB). All yeasts tested that are of apparent hemibasidiomycetous origin as characterized by high GC ratios, urea hydrolysis, type of bud formation, cell wall structure, etc. were inhibited by ≤25.0 µg PxB/ml, while nearly all yeasts considered to be of ascomycetous origin were resistant to 25.0 µg PxB/ml. Thus (no. of strains in parenthesis) Cryptococcus (=Filobasidiella) neoformans (5), Trichosporon cutaneum (3) and Rhodotorula rubra (3) were sensitive to PxB, while Candida albicans (3), C. guilliermondii (3), C. krusei (1), C. parapsilosis (5), C. pseudotropicalis (2), C. stellatoidea (3), C. tropicalis (4 of 6), Geotrichum candidum (1), Torulopsis glabrata (4) and Trichosporon capitatum (2) were resistant.

To determine if PxB sensitivity is a general property of hemibasidiomycetous yeasts, 149 species belonging to various taxa were examined. All yeasts belonging to the Hemibasidiomycetes and the basidiomycetous-like family Sporobolomycetaceae were inhibited by 25 µg PxB/ml. Of the ascomycetous yeasts, 33% were inhibited. The distribution of PxB sensitivity in this group ranged from 14% in the Endomycetaceae to 80% in the Spermophthoraceae. In the complex family Saccharomycetaceae, 100% of the Schizosaccharomycoideae and Lipomycetoideae, 80% of the Nadsonioideae and 28% of the Saccharomycoideae were inhibited. In the Deuteromycotina family, Cryptococcales, all species in the three basidiomycetous-like genera, Oosporidium, Phaffia and Rhodotorula were inhibited. In the complex genera Candida, Cryptococcus, Torulopsis and Trichosporon >90% of the species tested considered to be of basidiomycetous origin, e.g., C. curvata, Cr. albidus, T. fujisanensis and Tr. cutaenum and others, were sensitive, while most yeasts of apparent ascomycetous origin were resistant to PxB.

It was observed that addition of 0.01 to 0.25 minimum inhibitory concentration (MIC) of PxB to broth cultures of medically important yeasts reduced the MIC of the experimental antibiotic, scopafungin (Upjohn), by 4 to 60-fold. Synergism between PxB and scopafungin was seen with all yeasts tested. Cr. neoformans and Tr. cutaneum required as little as 0.1 µg PxB and 0.2 µg scopafungin/ml for complete suppression of growth, while yeasts of apparent ascomycetous origin, Candida sp., T. glabrata and Tr. capitatum required from 4 to 60x these concentrations.

The distribution of PxB sensitivity among yeast groups may prove useful in yeast taxonomy. Also, PxB may be of potential value in antifungal therapy, especially when combined with other antimycotic drugs.

VIII. Communicated by L. R. Hedrick, 14225 SW 150th Avenue, Portland, Oregon 97223.

CANDIDA FLUVIOTILIS SP. NOV. AND OTHER YEASTS FROM AQUATIC ENVIRONMENTS

Antonie Van Leeuwenhoek 42:(No. 3) 329-332 (1976)

SUMMARY

Candida fluviotilis appears to be more closely related to Candida shehatae Buckley et van Uden (1967) than to other nitrate-negative Candida species. However, C. fluviotilis differs from C. shahatae in: pseudohyphal cell morphology, fermentation of galactose, and the assimilation of L-sorbose, L-arabinose and D-ribose. C. fluviotilis is one of the few described

species of yeasts that is negative for the assimilation of L-sorbose but positive for the utilization of glucose, galactose, sucrose, maltose and lactose.

Strains L3 and L7, also from river water, have the physiological characteristics of Pichia spartinae (Ahearn, Yarrow and Meyers, 1970) but they do not conjugate with each other or with mating types of this Pichia.

Strain L8 (from river water) has characteristics which agree with those for Candida steatolytica (Yarrow, 1969), but L8 does not split fat.

Strain 124-70, from Alaska glaciers, has the characteristics of Cryptococcus albidus var. albidus, except that it is negative for the utilization of inositol in over 20 tests (these were confirmed in at least three other laboratories), but positive (weak or slow) in two or three other tests.

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

The following papers have been published or are in press:

M. A. Lachance, M. Miranda, M. W. Miller, and H. J. Phaff (1976). Dehiscence and active spore release in pathogenic strains of Metschnikowia bicuspidata var. australis: possible predatory implication. Canad. J. Microbiol. 22, 1756-1761.

H. J. Phaff (1977). Enzymatic yeast cell wall degradation (a review). Advances in Chemistry (in press).

T. G. Villa, M. A. Lachance and H. J. Phaff (1977). On the structure of the β-(1→3)-glucan component of the cell wall of baker's yeast. FEMS Letters (in press).

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Dr. William T. Starmer has spent one year in our laboratory for a study in-depth of the yeast flora associated with necrotic cacti and Drosophila in the Sonoran desert. Dr. Starmer will assume the position of Assistant Professor in the Biology Department of Syracuse University, New York in September.

SUMMARY

We have recently completed an extensive survey of the yeast flora inhabiting decaying stems of 9 species of cereoid cacti, which support cactophilic Drosophila. The taxonomy of the yeasts and ecological relationships among the host plants, yeasts and Drosophila of the mainland portion of the Sonoran desert have been reported (Phaff et al. 1974; Miller et al. 1976; Heed et al. 1976 and Starmer et al. 1976). Subsequently the collection range was extended to include the peninsula of Baja California, Mexico, so that it now encompasses most of the North American Sonoran desert. Although 29 distinct species of yeast were recovered from 267 samples of the 9 host plants, only the more frequently isolated yeast species and substrates are listed in Table I. This table shows the degree of host specificity of the yeasts (niche widths) and the relative extent to which the yeast

species share habitats (niche overlap). Our recent study of the collection from Baja California has revealed that the most frequent isolate, previously identified as Pichia membranaefaciens, is actually composed of four or more species of Pichia. These species all have metabolic patterns similar to those of P. membranaefaciens and C. valida, but each contains different mol% G + C in their respective genome DNAs: P. cactophila n. sp. (36.5%), P. heedii n. sp. (32.5%), Pichia sp. D (33.0%), Pichia sp. A (30.0-31.0%) and Pichia kluyveri var. nonfermentans (27.9%). The mol% G + C of P. membranaefaciens and C. valida have been found to be approximately 44-45%. It is possible that the origins of the desert adapted species of Pichia are tropical and have different ancestors than the P. membranaefaciens which have been mainly recovered and studied from temperate regions. Thus the similarity in physiology of the cactus yeasts and P. membranaefaciens is possibly due to evolutionary convergence. We have expanded the number of carbon compounds commonly used for yeast classification, which has allowed us to distinguish two of the species from P. membranaefaciens while host specificity and mol% G + C separates the other species. The distinguishing characteristics for the cactus yeast species can be found in Table II.

Isolates of Cryptococcus cereanus have been found to exhibit a significant range (39.0-49.5%) in GC% of their DNAs. This physiologically and morphologically similar group is sufficiently different from typical Cryptococcus species to be considered a new genus. It is presently under investigation to determine the extent of speciation. We have also isolated similar forms from the slime fluxes of Douglas fir and Chestnut oak. Candida ingens and C. tenuis are possibly the only yeasts, which we have found in substantial numbers, that are not cactus specific. These species have been previously isolated from industrial sources and bark beetles, respectively.

The niche widths of the yeast species are comparable to niche widths for yeasts which inhabit temperate trees and indicates the high degree of specialization toward which these forms have evolved. The magnitude of the yeast niche overlaps for the habitats might be explained by the physico-chemical properties and physical size of the hosts, the geographic distribution of the cacti and/or Drosophila specificities for the cacti. The similarity of yeast distributions among cacti show the overall evolutionary pressure on the yeast and is reflected in the phylogeny of the cacti. That is, M. gummosus, L. thurberi and R. alamosensis belong to the tribe Stenocereinae and are characterized by the yeast species T. sonorensis, Pichia sp. A, Pichia sp. D, Torulopsis sp. and C. tenuis, while P. pringlei, L. schottii and C. gigantea are in the Tribe Pachycereinae and have representative yeasts, P. heedii, Candida sp. and P. delftensis-like.

Taken all together the majority of the yeasts recovered from cacti are members of the genus Pichia, while the imperfect species Torulopsis sonorensis, Candida ingens, and Candida tenuis would also be members of Pichia if their perfect forms were known. The only exceptions to this are Cryptococcus cereanus and Torulopsis sp.

Heed, W. B., W. T. Starmer, M. Miranda, M. W. Miller and H. J. Phaff. 1976. Ecology 57(1):151-160.

Miller, M. W., H. J. Phaff, M. Miranda, W. B. Heed and W. T. Starmer. 1976. Intern. J. of Syst. Bact. 26:88-91.
Phaff, H. J., M. W. Miller, M. Miranda, W. B. Heed and W. T. Starmer. 1974. Intern. J. of Syst. Bact. 24(4):486-490.
Starmer, W. T., W. B. Heed, M. Miranda, M. W. Miller and H. J. Phaff. 1976. Microbial Ecology 3:11-30.

TABLE I.

	M. gummosus	L. thurberi	R. alamosensis	P. pringlei	L. schottii	C. gigantea	TOTAL	Niche Width
Number of plants sampled	100	30	14	27	62	16	249	
P. cactophila n. sp.	72	21	14	16	7	8	138	0.725
Cr. cereanus	2	5	4	2	9	4	26	0.689
C. ingens	7	11	8	1	16	4	47	0.666
T. sonorensis	40	16	4	5	3	1	69	0.666
Cr. albidus	3	1	0	1	1	0	6	0.590
P. kluyveri var. nonfermentans nov. var.	11	3	0	0	0	2	16	0.475
Pichia sp. D	19	1	1	0	1	0	22	0.469
C. tenuis	3	7	1	1	0	0	12	0.458
P. heedii n. sp.	1	0	0	1	39	9	50	0.370
Candida sp.	0	0	0	5	2	2	9	0.395
P. delftensis like	1	0	0	2	2	4	9	0.381
Pichia sp. A	7	2	0	0	0	0	9	0.301
Torulopsis sp.	10	1	0	0	0	0	11	0.235
Uniques	2	3	3	4	2	2	16	0.000
TOTAL	178	71	35	38	82	36	440	
Niche overlaps	0.796	0.866	0.742	0.857	0.713	0.883		

TABLE II. Distinguishing characteristics of Pichia species from cacti in comparison with Pichia membranaefaciens.

	D-xylose	Glycerol	Glucono-δ-lactone	citric acid	Glucosamine	Spores/ascus	mol% (G + C)	Principal host
P. cactophila	-	+latent	-	+rarely-	+	2	36.5	most cacti
P. heedii	+	+latent	-	+rarely-	-	4	32.5	L. schottii & C. gigantea
Pichia sp. D	-	+strong	+	-	-	4	33.0	M. gummosus
Pichia sp. A	-	-rarely+	-	-	-	4	30-31	M. gummosus & L. thurberi
P. kluyveri var. nonfermentans	-	+	-	-	-	4	27.9	M. gummosis & L. thurberi
P. membranae-faciens	+or-	+or-	-	+or-	-	2-4	44-45	widespread (but not found in cacti)

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

Cell Surface Topography of Candida and Leucosporidium Yeasts as Revealed by Scanning Electron Microscopy

Kenneth Watson and Helen Arthur
J. Bacteriol. 130, April 1977

The cell surface topography of the following yeast strains was examined by scanning electron microscopy: Candida slooffii, C. lipolytica, Leucosporidium frigidum and L. nivalis. Multipolar and lateral budding were observed in the Candida yeasts in contrast to bipolar budding in the Leucosporidium species. The cell surface topography and the morphology of the bud and birth scars in these yeasts differed markedly. Apart from the bud and birth scars, the cells of C. slooffii showed a relatively smooth topography. The bud scars were seen as a circular ridge of wall material surrounding a markedly convex scar plug. Birth scars were raised, rounded structures, which appeared to distend upon cell growth. In contrast, bud scars of C. lipolytica were platelike, lacked a distinct annulus of wall material, and were much less protuberant than those of C. slooffii. Birth scars were a more permanent feature of these cells. The topography of Leucosporidium yeasts was characterized by the presence of numerous protrusions on the cell surface. In some cases, the entire cell surface was covered by these protrusions. There appeared to be some correlation between the age of the cell and the extent of surface

protrusions and degree of surface convolution. The bud scars in these yeasts were seen as a circular, broken collar of material surrounding a slightly convex scar plug. Birth scars were not unlike those seen in C. lipolytica. If the varied appearances of birth and bud scars are considered together with scanning electron micrographs showing bud-parent junctions before bud release, three different modes of budding are suggested.

The following publications have appeared since the last communications. The abstracts of these papers have been given in Yeast Newsletter XXV, 2, 25, 26.

Arthur, H. and Watson, K. Thermal Adaptation in Yeast: Growth Temperatures, Membrane Lipid and Cytochrome Composition of Psychrophilic, Mesophilic and Thermophilic Yeasts. J. Bacteriol. 128, 56-68 (1976).

Watson, K., Arthur, H., Watson, P. and Shipton, W. A. Leucosporidium Yeasts: Obligate Psychrophiles which Alter Membrane-lipid and Cytochrome Composition with Temperature. J. Gen. Microbiol. 97, 11-18 (1976).

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

The following is a summary of work just completed on the yeast Trigonopsis variabilis, in our laboratory.

Title of a paper submitted for publication in the Journal of General Microbiology, "Effect of Tween 80 on the Morphology of Trigonopsis variabilis."

Nicolle H. Packer and Audrey M. Bersten

Tween 80 (polyoxyethylene sorbitan mono oleate) when given as a supplement in basal ammonium sulphate, glucose medium induced the development of populations of T. variabilis, almost entirely of triangular-shaped cells. The induction was completely inhibited by short chain alcohols at specific concentrations, and not utilized as carbon sources. The chemical characteristic of Tween 80 which produced the effect was the oleate esterified to sorbitan, since only Tween 80 among the Tween detergents and only Span 80 (sorbitan mono oleate) among the span detergents induced the formation of triangular cells, and since Brij (polyoxyethylene-10-oleyl ether) and 0.1% w/v sodium oleate were ineffective. The effect of Tween 80 was constant from early logarithmic to stationary phase. The significant effect of Tween 80 on the morphology of T. variabilis and the simple means of its inhibition by alcohols provides a convenient and more reproducible system, than has previously been available for further investigation into the morphogenesis of this yeast.

XII. Department of Biology, York University, Faculty of Science, 4700 Keele Street, Downsview, Ontario, M3J 1P3, Canada. Communicated by R. H. Haynes.

Below follow two abstracts of papers that will be presented by Dr. Friederike Eckardt and myself at the Second International Conference on Environmental Mutagens, University of Edinburgh July 11-15, 1977.

UV-INDUCED MUTAGENESIS IN YEAST

We have measured the frequency of UV-induced reversions (locus and suppressor) for the ochre alleles ade2-1 and lys2-1, and the frequency of forward mutations (ade2 adex double auxotrophs) in a haploid excision-deficient (rad2-20) strain of Saccharomyces cerevisiae; similar measurements were made in RAD wild-type strains. For low UV doses both the forward and reversion systems exhibit linear induction kinetics. As dose increases a different response is observed: in the selective reversion system a transition to higher order induction kinetics occurs, whereas in the non-selective forward system the mutation frequency passes through a maximum and then declines. This contrast in induction kinetics is most readily explained by assuming that mutation induction is basically a linear, Poisson process and that departures from linearity may be associated with events during mutation expression and/or cell multiplication which differentially affect the probability of clone formation by mutant and non-mutant cells.

In the reversion systems the induction of suppressors parallels that of locus mutants and the ratio of locus to suppressor mutations varies from 3:1 to 10:1. However, this ratio is both dose and strain dependent, and in rad2 strains there appears to be a selection for suppressors at high doses. In the forward systems, the ratio of pure clones to sectors is 10:1 at all doses in RAD wild-type, whereas in rad2 this ratio is approximately 1:1 at low doses. However, in preliminary tests with the excision-deficient strain rad1-1 we have found that sectors outnumber pure clones at low doses in a ratio of about five to one; thus the rad1 locus may control heteroduplex repair, a processes that is thought to be involved in pure mutant clone formation. (Work supported by the National Research Council of Canada.)

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ANALYSIS OF MUTATION INDUCTION KINETICS IN YEAST

Mathematical analysis of the dose-dependence of mutation induction is helpful in formulating mechanistic models of mutagenesis and is essential for making realistic extrapolations of induced mutation frequencies into the low dose range relevant to risk estimation for environmental mutagens. Mutation and killing, as well as other radiation or chemically-induced genetic effects such as recombination or gene conversion, are normally assayed on an 'all-or-none' basis. Thus, single-event Poisson statistics is applicable to the analysis of such dose-response relations. For mutation, we can show that,

$$M(x) = \{1 - \exp(-H_m)\} \exp(1-\delta)H_k$$

where $M(x)$ is the induced frequency, H_m and H_k are the expected number of mutational and lethal hits at dose x; and $\delta = H_{km}/H_k$, where H_{km} is the expected number of lethal hits in cells that have suffered also a mutational hit. In the simplest cases δ is a constant (close or equal to unity) and the H's are directly proportional to dose; in other cases, especially those involving dose-dependent repair processes, δ and the H's can all be complex functions of x. The induction curves are linear at low doses if H_m contains a linear term; this is precluded if a mutational hit arises from the interaction of two or more physical

hits, the overlap of two repair events, the induction of a strictly non-constitutive error-prone repair process, or the inhibition of an error-free process potentially capable of repairing all mutagenic lesions. If H_m contains a linear term, then high dose departures from linearity could be associated with additional non-linear terms in H_m or values of δ different from one.

In haploid yeast, M(x) is linear at low doses and the observed positive and negative departures from linearity are most plausibly explained by assuming that the probability of clone formation by the mutants is either greater or less than that of the non-mutant cells, i.e., if δ is slightly less or greater than unity, respectively. (Work supported by the National Research Council of Canada.)

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

1. The genetics of multiple forms of alcohol dehydrogenase (ADH) in Saccharomyces cerevisiae. Recessive mutants, specifically deficient in one of the known ADH isozymes (ADHI, ADHII, m-ADH) were isolated by the allylalcohol selection technique. Genetic analysis showed that two unlinked genes (ADR1, ADR2) were necessary for the formation of the glucose-repressible ADHII. The mitochondrial ADH activity (m-ADH) is controlled by a nuclear gene, ADM. In adc1 mutants the constitutive ADHI activity was strongly reduced or completely absent. This mutant type did not grow anaerobically and was found to be lethal in the respiratory deficient state. By means of naturally occurring, electrophoretic variants of ADHI and of intragenic adc1 revertants it was concluded that ADC1 is the structural gene coding for the ADHI subunit. Quite the same situation was found for ADR2, the structural gene for the ADHII subunit. The tetrameric structure of ADHII (as well as of ADHI) was indicated by hybrid enzyme formation in ADHII-slow x fast heterozygous diploids. Starting with an ADHI, m-ADH deficient strain (wild type with respect to the ADHII genes), three allelic mutants were selected in which ADHII synthesis was insensitive to strong glucose repression. The mutational sites (designation: $ADR3^c$) were proven to be cis-dominant and tightly linked to the ADHII structural gene. $ADR3^c$ was interpreted as a promotor or operator site adjacent to ADR2. (Appeared in: Mutat. Res. 29, 315-326 (1975), Mol. gen. Genet. 138, 157-164 (1975), Mol. gen. Genet. 145, 327-333 (1976).

2. Yeast mutants deficient in the constitutive ADHI (adc1) were used for the isolation of mutants with various defects of the intermediary carbon metabolism, and of mutants defective in carbon catabolite derepression. Mutants were recognized by their inability to grow on YEP-glycerol and/or on ethanol synthetic complete medium. They were either defective in isocitrate lyase (icl1), succinate dehydrogenase (sdh1), or malate dehydrogenase (mdh1, mdh2). Three gene loci could be identified by mutants pleiotropically defective in many or all of the enzymes tested. In ccr1 mutants, derepression of isocitrate lyase, fructose-1,6-biphosphatase, ADHII and possibly of the cytoplasmic MDH is prevented, whereas the mitochondrial TCA-cycle enzymes, succinate dehydrogenase and malate dehydrogenase, are not significantly affected. CCR2 and CCR3 have quite similar action spectra. Both genes are obviously necessary for derepression of all enzymes tested. It could be shown that ccr1, ccr2 and ccr3 mutants are not respiratory deficient. (To appear in Molec. gen. Genet., 1977.)

3. Totally ADH-less yeast strains have recently been proven useful for the selection of mutants in which glucose repression of mitochondrial enzymes is abolished. The selection is based on the fact that growth of ADH cells on high glucose medium depends on the repressed levels of respiration and oxidative phosphorylation. Thus, ADH cells hardly can grow on 8% glucose medium. On that medium, revertants appeared after UV mutagenesis, which were obviously not reverted in one of the ADH genes but were affected in glucose repression of respiration and respiratory enzymes. The mutants obtained so far showed high (derepressed) levels of malate dehydrogenase and succinate dehydrogenase activities, when grown in the presence of 8% glucose. This glucose-resistant trait was dominant in most cases when the respective mutants were crossed to a wild type strain (glucose-repressible and ADH^+). These mutants seem to be useful to elucidate the genetic mechanisms involved in glucose repression of the oxidative metabolism in yeast.

XIV. University of Oregon. Institute of Molecular Biology, Eugene, Oregon, 97403. Communicated by Jeffrey N. Strathern.

Below follows the abstract of my dissertation for the degree of Doctor of Philosophy in the Department of Biology under the guidance of Professor Ira Herskowitz. June 1977. Title: Regulation of Cell Type in Saccharomyces cerevisiae.

The a (MATa) and α (MATα) alleles of the mating type locus of Saccharomyces cerevisiae regulate the haplophase-diplophase cycle by controlling the functions involved in the specific mating reaction between a and α cells and the a/α functions involved in meiosis and sporulation. MATa and MATα are co-dominant, indicating that both code for one or more active products. In this dissertation the mechanism of control by MATa and MATα of a, α, and a/α specific functions has been investigated using mutations of MATa and MATα and suppressors of those mutations. It is demonstrated that MATα contains two or more complementation groups. It is proposed that one of the MATα products positively regulates α-specific functions and another product negatively regulates a-specific functions. Evidence is presented which indicates that a-specific mating functions are only under negative control by MATα. In other words in α cells, α-specific functions are turned on and a-specific functions are turned off. In a cells, α-specific functions are not turned on and α-specific functions are expressed because they are not turned off.

Further analysis of a suppressor of matα1 mutations (ssp515 of Hicks, 1975) shows that it suppresses the mating defect of four independently isolated matα mutations which belong to at least two complementation groups. In addition, ssp515 relieves the normal requirement for both MATa and MATα for sporulation. The ssp515 allele is recessive and does not suppress known amber or ochre nonsense mutations. Models for the role of SSP^+ in the regulation of cell type are discussed.

The mating type locus of yeast is of particular interest because some strains of yeast (homothallic) have a mechanism for interconverting the MATa and MATα alleles at high frequency. The nature of interconversion was studied by observing changes in sensitivity to the mating pheromone α-factor, changes in the mating specificity or changes in the budding pattern of cells. It has been found (Hicks and Herskowitz, 1976b) that conversion of mating type occurs in particular pairs of cells during the growth of a clone of cells. That work has been extended to include observation of

conversion of mating type in a/a and α/α homothallic diploids. Further, extension of haploid pedigrees of homothallic cells demonstrates that the ability to give rise to a pair of cells with switched mating type is asymmetrically distributed at each division. Cells which have budded previously may be capable of generating two switched cells. In contrast, cells budding for the first time always generate cells which have not switched mating type. The observed switching in pairs coupled with the pattern of switching in α/α and a/a diploids leads to the proposal that homothallic conversion affects both strands of the DNA and occurs after the point in the cell cycle where mating can occur, but prior to the replication of the mating type locus.

Homothallic interconversion of mating type occurs in a/a and α/α diploids but not in a/α diploids. Therefore, some part of the homothallic system must be under the control of MATa and MATα. Results reported here identify mutations of MATa (mata* of Kassir and Simchen, 1976) and MATα (matα10-73) which are defective in the regulation of homothallism seen in a/α diploids.

As has been observed earlier with other defective MATα alleles (Hicks and Herskowitz, 1977), matα10-73 and mata* can both be converted to functional MATa and MATα by the homothallic system. These observations suggest that yeast carry silent MATa and MATα genes which can be activated to provide the defective product.

MATa and MATα are viewed as non-homologous blocs of DNA. Homothallic interconversion is viewed as replacing the resident DNA with an active copy of silent MATa or MATα information carried elsewhere on the genome. The pattern of involvement of HMA and HMα in homothallism and their map position relative to chromosomal aberrations associated with some heterothallic conversions of mating type suggest HMA and HMα as the silent MATα and MATa information, respectively.

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

Below follows the abstract of a paper which has been submitted for publication to the Europ. J. Pharmacol.

Yeast as a model for the study of DNA-damage from alkylating agents I. Formation and fate of cross-links induced by poly-functional anticancer drugs. R. Fleer and M. Brendel.

ABSTRACT

This report describes a method to detect interstrand cross-links in DNA of Saccharomyces cerevisiae by means of Eaton press homogenization and isopycnic ultracentrifugation and shows the results of its application to a yeast strain defective in excision repair treated with alkylating antitumor drugs.

1. Efficient separation of single- and double-stranded DNA requires low cell density and addition of glycerol during homogenization. A significant amount of renaturable DNA, even at strict conditions of denaturation, was detected in untreated control DNA when using homogenates pressed with glycerol containing higher molecular weight (mol wt) DNA but could not be detected with low mol wt DNA.

2. Formation of interstrand cross-links after treatment of yeast cells with biological doses of the polyfunctional alkylating agents Mechlorethamine (nitrogen mustard), Triaziquone and Chlorambucil could be demonstrated. The sensitivity of this method mainly depends on the mol wt of isolated DNA and on the growth phase of cells to be treated.

3. There exists a correlation between cross-linking reaction, time of application and concentration of alkylating agent, resp., within a certain dose range. Higher doses lead to DNA fragmentation and loss in detectability of cross-links.

4. The cross-linking reaction still increases after the end of application though excessive alkylating agent is removed. After having reached a maximum the fraction of renaturable (cross-linked) DNA decreases with prolonged incubation. The speed of this "after-reaction" depends on temperature. 48 h after the end of treatment most cross-links have disappeared when after-reaction is performed at 36°C. The absence of simultaneous DNA fragmentation and the observed restitution of denaturable DNA of high MWT suggest the influence of enzymatic repair processes though the first step of excision repair is genetically blocked in the strain used.

It is concluded that DNA cross-linking plays an important role in the cytostatic effect not only in procaryotes. Furthermore it is suggested that the simple eucaryotic system used in this report may be a valuable tool in detailed studies of DNA lesions and their enzymatic repair after alkylation by antitumor drugs.

XVI. Mikrobiol. Institute, Federal Institute of Technology, 8092 Zürich, Switzerland. Communicated by R. Hütter.

Die allgemeine Kontrolle der Aminosäure-Biosynthese bei Saccharomyces cerevisiae.
Niederberger, P. A.
Dissertation Nr. 5882, ETH-Zürich 1977, 91 pp.

Summary

A regulatory system, called the "general control of amino acid biosynthesis" was investigated in Saccharomyces cerevisiae. Limitation for single amino acids led to the derepression of tryptophan-, histidine- and arginine-biosynthetic enzymes and a simultaneous retardation of growth. The limitation was accomplished by the use of 5-methyl-tryptophan or by bradytrophic mutants in the leucine-, lysine-, arginine-, serine- and histidine pathways. The derepression continued for 3-7 hours before plateaux of enzyme activities 1.5-5 times higher than in wild type were reached. For tryptophan and histidine enzymes no repression by their cognate amino acids could be observed, whereas arginine enzymes were found to be repressible.

This general regulatory system can be altered by mutation. Two classes were characterized: ndr-mutants, which do not derepress amino acid biosynthetic enzymes upon starvation for an amino acid, and cdr-mutants, in which those enzymes are constitutively derepressed even on a complex medium.

Limitation of the leucine bradytrophic mutant RH 657 for leucine led to a strong derepression of the leucyl-tRNA synthetase, whereas other aminoacyl-tRNA synthetases were weakly or not at all derepressed. The introduction of an ndr-mutation into this bradytrophic strain led to lower levels of all aminoacyl-tRNA synthetases. Upon starvation for leucine, their activities decreased even more.

Aminoacylation of several tRNA's tested, particularly of leucine-tRNA, was not significantly affected under any of the above mentioned conditions. An active involvement of the aminoacyl-tRNA synthetases, probably in conjunction with the cognate tRNA's, in the general regulation system is proposed.

The growth behaviour of mutants with a defective general regulation was studied. Upon limitation for any one of several amino acids, non-derepressing strains showed a growth rate which was approximately 30% lower than that of the wild type. Furthermore, upon shifts from amino acid deficient to supplement medium and vice versa, those mutants showed increased lag periods compared to wild type.

Under several amino acid limitation conditions, the appearance of guanosine tetraphosphate (ppGpp) could not be detected.

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

We are presently engaged in a program to investigate the genetic components of carbon catabolite repression and catabolite inactivation. Mutants with defects in these regulatory circuits have been isolated. The procedure involved the use of strains with MAL2-8^c (largely constitutive but still glucose repressible maltase synthesis) and SUC3 (a gene for formation of constitutive but glucose repressible invertase). Fully repressed cells were plated on media with raffinose (a very poor substrate for invertase) and 150 ppm 2-deoxyglucose (a repressing analog of glucose). Only cells with non-repressible invertase should grow on such a medium since fructose released from raffinose should counteract the various detrimental effects of 2-deoxyglucose. Over 100 mutants with glucose non-repressible invertase and maltase were isolated. No mutant with only non-repressible invertase was found. 61 Mutants were allele-tested and could be located in three unlinked or widely separated genes. Mutants with reduced (1/6-1/3) hexokinase activity fell into one gene; mutants with increased hexokinase activity (up to 2-fold) were located in a centromere linked gene and were all inhibited by maltose even in the presence of other sugars. Another type had no abnormal hexokinase levels. All mutants were shown to be of Mendelian nature by tetrad analysis. The lack of repression also partly affected malate dehydrogenase total activity, whereas isocitrate lyase was still fully repressed.

Mutants with reduced hexokinase were studied in more detail by K. D. Entian. Repression showed a paradoxical pattern: high glucose concentration was not repressing at all, but there was repression increasing with decreasing glucose concentrations. Maltose and fructose also showed some degree of repression. However, carbon catabolite inactivation was alleviated for all sugars. The nature of the reduced hexokinase activity was difficult to assess since there are a number of enzymes involved in the phosphorylation of hexoses. Heat inactivation and also starch gel electrophoresis (carried out by R. Kern) showed that there was at least a change in the quantitative isozyme pattern. Glucose uptake was measured following the disappearance of glucose from a growth medium. The mutant showed a reduced capacity of uptake which was maximal at 2% and slightly declined at higher concentrations. The growth rates on various sugars were measured. They were found to be reduced on glucose, fructose and maltose, and the growth rate on 8% glucose was even lower than on glycerol. Growth rates on glycerol and galactose were the same for mutant and wild type. Intracellular levels of glucose-6-phosphate, fructose-6-phosphate and fructose-1, 6-bisphosphate were determined in cells growing on various sugars. There were no clear differences between mutant and wild type. However, mutant cells growing on maltose had 14.0 µmoles glucose/g dry weight, the wild type only 3.12. Intracellular ATP and hexose phosphates were also measured in cells that had been grown on ethanol medium; ATP was normal whereas the metabolites were very low. Upon addition to this medium of glucose, ATP slightly increased in wild type but sharply dropped in the mutant during the first 10 min and then increased again. The metabolites behaved largely identical in mutant and wild type. Maltose also led to an inactivation of susceptible enzymes in wild type but not in the mutant. However, intracellular glucose in the mutant reached a sharp peak of 85 µmoles/g dry weight but only of 23 in wild type. Galactose did not inactivate in wild type even though intracellular glucose-6-phosphate reached levels which were arrived at with maltose at the time when inactivation started. This suggested that inactivation cannot be triggered alone by high intracellular levels of glucose, glucose-6-phosphate, fructose-6-phosphate and fructose-1,6-bisphosphate alone. It is tempting to speculate that it is one or several hexokinases which together with their substrates and/or reaction products are initiating inactivation. The mechanism of repression seems to be more complex and possibly different from inactivation. The striking observation was that the inactivation of fructose-1,6-bisphosphatase and isocitrate lyase was abolished in the mutant but repression still active.

Further studies into the mechanism of catabolite inactivation were possible by a discovery of M. Grossmann. We have reported in a recent publication (Zimmermann et al., Molec. gen. Genet. 151, 95-103, 1977) on two genes involved in the derepression process. Gene CAT1 is available in two mutant states: cat1-1 with no derepression of fructose-1,6-bisphosphatase and isocitrate lyase, whereas the dominant allele $CAT1\text{-}2^d$ accelerates it as does recessive allele cat2-1 of gene CAT2. $CAT1\text{-}2^d$ prevents catabolite inactivation whereas cat1-1 has up to 5-fold higher levels of protease B, the protease responsible for inactivation. At present it is not possible to present the mechanism of action of these mutants but a first step towards the elucidation of the genetic basis of catabolite inactivation has been done.

Wine Yeasts: A systematic selection of wine yeast in collaboration with Zentralkellerei Badischer Winzergenossenschaften, 7814 Breisach a. K, has yielded a yeast with constant properties. At the end of each fermentation season, yeast was re-isolated from fermentation tanks and re-tested. The isolates of the 1976 campaign were quite uniform and sporulated. Spore viability was quite good and the resulting progenies were placed on sporulation media. Sporulation was found in all sporal clones indicating the homothallic nature of the yeast.

XVIII. Department of Biochemistry, School of Biological Sciences, Madurai University, Madurai 625021, India. Communicated by J. Jayaraman.

The following are the salient features of the theses to be submitted by K. Chandrashekaran and T. Somasundaram of this group interested in the study of mitochondriogenesis in yeast:

a) The mechanism of glucose repression has been looked into in detail and it appears that mitochondria are the primary target of attack inhibiting mitochondrial protein synthesis in vivo. Addition of cyclic AMP relieves this inhibition, as has been demonstrated with the use of immunoprecipitates of purified cytochrome oxidase.

b) Concurrent studies with synchronous cultures also support the above contention. Using Ethydium Bromide-induced mutagenesis of mit DNA as a criterion, it is suggested that the conformation of mit DNA itself is altered during glucose repression.

c) Several workers have shown the accumulation of cytoplasmic products (needed for mitochondrial assembly) when mitochondrial protein synthesis is inhibited by chloramphenicol or vice versa. Our experiments with synchronous cultures of yeast demonstrate that at least in the case of ATPase, this phenomenon may be part of the normal cell cycle events. The significance of cytoplasm continuing to produce ATPase under conditions where there is no mitochondriogenesis is as yet not understood.

d) In the last report, Yeast Newsletter (June 1975) we reported on the technique to temporally sequence the appearance of mitochondrial products using yeast spheroplasts. Using the technique, we have now shown the following sequence: Subunits I and II of cytochrome oxidase, membrane factor of ATPase, Transhydrogenase and Subunit III of cytochrome oxidase. Currently attempts are being made to extend this study to QH_2 - cyt c reductase and the lipid ubiquinone and cardiolipin.

XIX. Queens College of The City University of New York, Department of Biology, Flushing, New York 11367. Communicated by Corinne A. Michels.

Research in our laboratory concerns the mechanism of catabolite regulation in yeast. The following is an abstract of recent work whose goal is to obtain a non-metabolized analogue of glucose capable of producing glucose repression.

An evaluation of D-glucosamine as a gratuitous catabolite repressor of Saccharomyces carlsbergensis, A. Furst and C. A. Michels. Molecular General Genetics, in press.

ABSTRACT

Glucose represses mitochondrial biogenesis and the fermentation of maltose, galactose and sucrose in yeast. We have analyzed the effect of D-glucosamine on these functions in order to determine if it can produce a similar repression. It was found that glucosamine represses the rate of respiration (QO_2) but more rapidly than glucose and to a final level slightly higher than in glucose-treated cells. Derepression of the respiration rate following either glucose or glucosamine repression was similar. A two-hour lag was followed by a linear increase in QO_2 to the derepressed level. Both glucose and glucosamine repressed the level of cytochrome oxidase to the same level. Glucosamine was also found to repress maltose and galactose fermentation but not sucrose fermentation. The derepression of maltase synthesis was inhibited by glucosamine. The constitutive synthesis of maltase was repressed by the addition of glucosamine. Glucosamine was judged to produce a repressed state similar to glucose repression in many respects. [See also Genetics 84, 311, 1976 for a related article - Editor.]

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

An abstract of a recent publication is given below.

H. Schneider, F. Gmünder and A. Fiechter: n-Alkane Oxidation by Candida tropicalis. II. Induction and Repression of Catalase by Substrate-Shifts in a Chemostat. European J. App. Microbiol. 1977, in press.

The induction of the catalase enzyme by n-alkanes and its repression by glucose was investigated in chemostat experiments by shifting the substrate from glucose to hexadecane and back to glucose. Induction of catalase followed immediately after replacing glucose by n-alkanes. However, the activity of the catalase was not inhibited by shifting from n-alkanes to glucose. Therefore, glucose seems to control the synthesis of catalase, but not its enzymatic activity.

XXI. University of Ottawa, Department of Biology, Ottawa, Ontario, Canada K1N 6N5. Communicated by J. G. Kaplan.

About ten years ago, I began a small project involving molecular biology of lymphocytes; over the years, more and more of my time was directed to what was originally a sideline. Last year I finally came to the conclusion that I could no longer work in widely different fields and so, with much regret, took the decision to abandon my work dealing with the regulation of the pyrimidine pathway and of catalase of yeast.

The last papers dealing with this work have appeared.

Biochimie, 58, 1925, 1976. Studies of the regulation and reaction mechanism of the carbamyl phosphate synthetase and aspartate transcarbamylase of bakers' yeast. (with P. F. Lue and D. M. Aitken).

Molec. gen. Genet. 145, 259-271, 1976. Fine structure of the URA2 locus in Saccharomyces cerevisiae. II. Meiotic and mitotic mapping studies. (with M. Denis-Duphil).

The work in relation to the pyrimidine pathway continues in the laboratory of my two former collaborators and dear friends, Dr. Michèle Denis-Duphil of the Laboratoire de Génétique Physiologique, Gif-sur-Yvette, France, and Dr. Francois Lacroute of the Institut de Biologie Moléculaire et Cellulaire, Université de Strasbourg.

Work on the typical and atypical catalases which we purified from yeast:

J. Biol. Chem. 1973, 248, 2889. Purification and properties of yeast catalase. (with Tony Seah).

Can. J. Biochem. 1973, 51, 1551. Novel catalatic proteins of baker's yeast. (with Tony Seah and A. R. Bhatti).

is being continued in the laboratory of Dr. H. Ruis, University of Vienna.

I send my best wishes to you and to all my other friends in the yeast field all over the world.

XXII. Department of Biochemistry, Purdue University, West Lafayette, IN 47907. Communicated by G. B. Kohlhaw.

Our work addresses itself to the question in what way increased cellular complexity affects a given regulatory system. Comparing the regulation of leucine biosynthesis of enteric bacteria with that of Saccharomyces cerevisiae, we have recently detected a qualitative change in the properties of α-isopropylmalate synthase. While the yeast enzyme has retained a sensitivity to leucine, it possesses the additional feature of being specifically and reversibly inactivated by CoA (in the presence of Zn^{2+}). We have obtained evidence that the recognition by this enzyme of a "CoA signal" in addition to the leucine signal may reflect a novel regulatory mechanism and may be the reason for the enzyme's localization in the mitochondria. So far, a total of three enzymes have been found to be subject to CoA inactivation (α-isopropylmalate synthase, homocitrate synthase, HMG-CoA reductase). All of these are associated with the mitochondria.

The following papers relating to this and similar subjects have recently been published or are in press.

1. J. W. Tracy and G. B. Kohlhaw (1977), Evidence for Two Distinct CoA Binding Sites on Yeast α-Isopropylmalate Synthase, J. Biol. Chem., in press.

Abstract

α-Isopropylmalate synthase (E.C. 4.1.3.12) from Saccharomyces cerevisiae was purified to a purity of about 95%. The molecular weight of the enzyme is approximately 127,000, as determined by sedimentation equilibrium centrifugation and by inter-subunit cross-linking. Under denaturing conditions, one major species (95%) with molecular weight of about 65,000 is obtained. The dimeric structure of the enzyme is apparently unaffected by the presence of various ligands, including substrates, the feedback

inhibitor leucine, and the inactivating combination of CoA plus Zn^{2+}. Our previous observation that CoA, a product of the reaction, causes a very specific, Zn^{2+}-dependent, reversible inactivation of yeast α-isopropylmalate synthase was further analyzed. Evidence is presented for the existence of two distinct CoA sites on each enzyme subunit. The first site (product site) interacts with CoA and desulfo-CoA, both of which are competitive inhibitors with respect to acetyl-CoA, with apparent K_i values of 70 μM and 90 μM, respectively. Equilibrium dialysis experiments show that up to one product site per subunit can be saturated with CoA, and that the binding capacity changes in parallel with the specific activity of the enzyme. The dissociation constant for CoA binding at the product site is approximately 65 μM. The second site ("regulatory site") appears to be absolutely specific for CoA. Binding of CoA to this site occurs only when Zn^{2+} is present, is independent of the specific activity of the enzyme, and does not eliminate CoA binding at the product site. Thus, when Zn^{2+} is present, the number of CoA sites saturable per subunit increases by 1.0. A dissociation constant of about 35 μM can be calculated for the Zn^{2+}-dependent binding of CoA. The evolutionary establishment of a second CoA binding site is interpreted as further corroborating the idea that the CoA-mediated inactivation of α-isopropylmalate synthase has regulatory significance.

2. G. B. Kohlhaw and A. Tan-Wilson (1977), Carnitine Acetyltransferase, Candidate for the Transfer of Acetyl Groups Through the Mitochondrial Membrane of Yeast, J. Bacteriol. 129, 1159-1161.

Abstract

On the basis of its specific activity and its affinity for acetyl-CoA, carnitine acetyltransferase appears to be the most likely candidate for acetyl group transfer out of yeast mitochondria.

3. A. Tan-Wilson and G. B. Kohlhaw (1976), 3-Hydroxy-3-Methylglutaryl-CoA Reductase of Yeast: Another Enzyme which is Specifically and Reversibly Inactivated by CoA, Fed. Proc. 35, 1472.

4. J. W. Tracy and G. B. Kohlhaw (1975), Reversible, Coenzyme-A-Mediated Inactivation of Biosynthetic Condensing Enzymes in Yeast: A Possible Regulatory Mechanism, Proc. Natl. Acad. Sci. USA, 72, 1802-1806.

Abstract

α-Isopropylmalate synthase [EC 4.1.3.12], the enzyme catalyzing the first committed step in leucine biosynthesis, and homocitrate synthase [EC 4.1.3.21], the first enzyme in lysine biosynthesis in yeast, are rapidly inactivated in the presence of low concentrations of coenzyme A, a product of both reactions. Closely related compounds like 3'-dephospho-coenzyme A or oxidized coenzyme A are almost without effect, as are other sulfhydryl compounds. Citrate (si)-synthase [EC 4.1.3.7] appears to be completely resistant against inactivation by coenzyme A. Inactivated α-isopropylmalate and homocitrate synthases can be reactivated by dialysis, but not by adding excess substrate. Protection against coenzyme-A-mediated inactivation is provided by relatively high concentrations of the α-keto-acid substrate or the specific end product inhibitor of each of the two enzymes. The

coenzyme-A-mediated inactivation of α-isopropylmalate synthase has been more closely investigated. It requires the presence of divalent metal ions, with Zn^{++} being most effective. The inactivation does not require molecular oxygen. It occurs in the presence of low concentrations of substrates and is observed in toluene-treated cells. These results, together with evidence that α-isopropylmalate synthase and homocitrate synthase are located in the mitochondria, suggest a mechanism by which increasing intra-mitochondrial coenzyme A concentrations might serve as a signal of decreasing acetyl-coenzyme A levels, triggering a temporary inactivation of biosynthetic acetyl-coenzyme A-consuming reactions in order to channel the available acetyl-coenzyme A into the citrate cycle.

XXIII. Institute of Biochemistry, Section of Molecular Genetics, Bulgarian Academy of Sciences, Sofia, 1113, Bulgaria. Communicated by Pencho V. Venkov.

Below follow summaries of our recent studies concerning the further characterization of the fragile Sacch. cerevisiae mutants isolated in our laboratory several years ago.

1. Electron Microscopic Study of the Lysis of an Osmotic-Sensitive Yeast Mutant. Zoya Mateeva, Peter Petrov, Pencho Venkov, Asen Hadjiolov, J. Microscopie Biol. Cell. (1976) 26, 73.

The ultrastructural events accompanying the lysis of Sacch. cerevisiae VY 1160 cells in water were studied. Under the effect of osmotic shock, the cell wall is swollen to a different extent. Local ruptures in the cell wall are clearly visible forming holes through which the cellular content is flowing out. In the lysed cells the cell membrane is detached from the cell wall and shows defects in its continuity at the sites of cell wall ruptures. The results obtained show that the osmotic-sensitive yeast mutant is characterized by local alterations in the structure of the cell wall which are revealed under osmotic shock.

2. Effect of toyocamycin on the biosynthesis and maturation of ribosomal RNA in an adenosine-utilizing mutant of Sacch. cerevisiae. Pencho V. Venkov, Lubomira Stateva, Asen A. Hadjiolov, Biochim. Biophys. Acta (published at the end of 1976).

Toyocamycin is an analogue of adenosine. We made one of our fragile yeast strains auxotrophic for adenine and by new mutagenesis of the same strain we isolated adenosine-utilizing colonies. After starvation for adenosine the synthesis of RNA in these cells becomes dependent upon exogeneously added adenosine. If instead of adenosine, toyocamycin is added to such cells, the drug is incorporated into the chains of pre-ribosomal RNA replacing adenosine residues. The extent of this replacement depends on the concentration of added toyocamycin. Low doses of toyocamycin accumulate 27S and 20S pre-ribosomal RNA and these precursors of ribosomal RNA can process to 25S and 18S matured ribosomal RNA, but at a reduced rate. At higher concentrations, toyocamycin blocks completely the last steps of pre-ribosomal RNA processing, i.e., the conversions: 27S pre-rib RNA →25S mature RNA and 20S pre-rib RNA →18S mature RNA. The obtained results are consistent with the idea that the main sites of action of toyocamycin are the last steps in the maturation of ribosomal RNA, while the transcription and primary processing of 37S pre-rib RNA are only slightly affected by the drug.

XXIV. Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts, 02154. Communicated by H. O. Halverson.

Our laboratory has continued its interest in RNA regulation and the cell cycle of yeast.

Mr. C. Saunders and Dr. S. Sogin have obtained two forms of poly(A) polymerase from Saccharomyces cerevisiae, one nuclear (form I) and another predominantly cytoplasmic (form II). Both forms catalyze the addition of AMP residues to the 3' end of RNA's. Form II, however, has an absolute requirement for a 3' oligo(A) sequence on the primer. As suggested by Haff and Keller, form I is likely responsible for the primary polyadenylation of the transcript in the nucleus while form II may be involved in maintenance of poly(A) in the cytoplasm.

An altered form of the nuclear enzyme obtained after phosphocellulose chromatography initiates chains with normal velocity, but fails to elongate. This elongation activity is partially (~50%) restored by addition of the P-C flow-through material. There is a loss of form II polymerase as the cells enter stationary phase. The activity fails to reappear as cells sporulate. Co-extraction experiments argue against this loss being an artifact of extraction.

There is also a 2-fold decrease in the poly(A) content of stationary phase cells, as measured by poly(U) hybridization. This loss can be partially accounted for by an observed increase in the amount of small poly(A) (20-35) relative to the large poly(A) (90) in these cells.

Dr. Gail Roberts has joined our research group this fall. She is developing the methods for isolation of histone mRNA in yeast for use as a probe in cell cycle experiments.

XXV. Universiteit van Amsterdam, Laboratorium voor Microbiologie, Plantage Muidergracht 14, AMSTERDAM - NEDERLAND. Communicated by H. Aiking.

The Occurence of Polyphosphates in Candida utilis NCYC 321, Grown in Chemostat Cultures Under Conditions of Potassium- and Glucose-limitation.

H. AIKING, A. STERKENBURG and D. W. TEMPEST. FEMS - Microbiology Letters (in press).

Summary:

It was found that phosphate-limited C. utilis contained only 0.5% (w/w) of cell-bound phosphorus at a dilution rate (D) of 0.1 h^{-1}, which was completely accounted for by cellular nucleic acid content.

However, potassium-limited cells, grown at the same dilution rate, contained 4% (w/w) phosphorus, which could, of course, not be present as inorganic phosphate for reasons of osmotic pressure. By a fractionation procedure it was found that about 2% (w/w) was present as polyphosphates, thus representing half of the total phosphorus content of the cells.

Surprisingly, similarly grown glucose-limited cells contained about 3% (w/w) phosphorus, of which one third (1%, w/w) turned out to be present as polyphosphates.

It was concluded that polyphosphates were probably present as an energy sink for the turnover of that amount of ATP generated in excess of basic growth requirement. The fact that they also occurred in glucose-limited cells was assumed as further evidence for the idea that glucose-limitation is probably a carbon-limitation and not an energy-limitation.

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Influence of Specific Growth Limitation and Dilution Rate on the Phosphorylation Efficiency and Cytochrome Content of Mitochondria of Candida utilis NCYC 321.

H. AIKING, A. STERKENBURG and D. W. TEMPEST. Archives of Microbiology (in press).

Abstract. With Candida utilis cells that had been removed directly from a 6 liter chemostat culture, in steady state, well-coupled mitochondria generally could be isolated. This required a modified snail-gut enzyme procedure that allowed the total processing time to be decreased to 3 h, or less. Examination of these mitochondria in an oxygraph showed the presence of 3 sites of energy conservation when the cells were grown at various dilution rates between 0.1 and 0.45 h^{-1} in environments that were, successively, glucose-, ammonia-, magnesium-, phosphate- and sulphate-limited. Potassium-limited cells also apparently possessed 3 sites of oxidative phosphorylation when growing at dilution rates greater than 0.2 h^{-1}, but only 2 sites when growing at lower dilution rates. Analysis of cytochrome spectra obtained with these intact mitochondria revealed large quantitative (but not qualitative) differences, depending on the environmental conditions under which the yeast had been cultured. In particular, comparison of the ratio of cytochrome b to cytochrome a showed a pattern of change with dilution rate in mitochondria from potassium-limited cells that was distinctly different from those evident in mitochondria from cells that had been limited in their growth by the availability of other nutrients.

XXVI. Miami University, Department of Microbiology, Oxford, Ohio 45056. Communicated by J. K. Bhattacharjee.

The following papers have been published in recent years from my laboratory:

1. Kurtz, M. and J. K. Bhattacharjee. 1975. Biosynthesis of lysine in Rhodotorula glutinis. Role of pipecolic acid. J. Gen. Microbiol. 86:103-110.

2. Coleman, J. S. and J. K. Bhattacharjee. 1975. Regulation of citrate synthase activity of Saccharomyces cerevisiae. Antonie van Leeuwenhoek, J. Microbiol. Serol. 41:249-256.

3. Burand, J. P., R. Drillien and J. K. Bhattacharjee. 1975. Citrate synthaseless glutamic acid auxotroph of Saccharomyces cerevisiae. Molec. Gen. Genet. 139:303-309.

4. Coleman, John S. and J. K. Bhattacharjee. 1976. Regulation of acetyl-CoA synthetase of Saccharomyces cerevisiae. Can. J. Microbiol. 22:762-764.

5. Bhattacharjee, J. K., T. Malavich and G. S. Gray. 1976. Glucose negative mutants of S. cerevisiae. Microb. Genet. Bull. 40:3-4.

6. Gray, Gary S. and J. K. Bhattacharjee. 1976. Biosynthesis of Lysine in S. cerevisiae: Regulation of Homocitrate Synthase in Analogue Resistant Mutants. J. Gen. Microbiol. 97:117-120.

7. Gray, Gary S. and J. K. Bhattacharjee. 1976. Biosynthesis of Lysine in S. cerevisiae: Properties and Spectrophotometric Determination of Homocitrate Synthase Activity. Can. J. Microbiol. 22:1664-1667.

8. Foy, James J. and J. K. Bhattacharjee. 1977. Gluconeogenesis in S. cerevisiae: determination of Fructose-1,6-bisphosphatase Activity in Cells Grown in the Presence of Glycolytic Carbon Sources. J. Bacteriol. 129:978-982.

9. Haidaris, Constantine G. and J. K. Bhattacharjee. 1977. High lysine excreting mutants of S. cerevisiae. J. Ferm. Technol. (in press).

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

Below follow titles and abstracts of three publications which have been accepted for publication.

THE EFFECT OF DIFFERENT CELL CYCLES IN YEAST ON EXPRESSION OF THE CYTOPLASMIC PETITE MUTATION

Byron F. Johnson, Allen P. James, Norman T. Gridgemen,
C. V. Lusena and Eng-Hong Lee

Chapter for a monograph: Cell Cycle Regulation
Academic Press (Jeter et al. eds)

ABSTRACT

In pedigrees, the expression of spontaneous petite mutations is asymmetric. Pedigrees also exhibit asymmetry of cell volumes, based on the unbalanced nature of growth of budding yeasts. The asymmetries are comparable, for the largest cell in a pedigree is the pedigree progenitor; this cell is also least apt to express the petite mutation. Furthermore, the older (and larger) the progenitor, the lower the frequency of petites among the progeny. A model for the different expressions of the petite mutation is proposed, based upon the different extents of replication and recombination of mitochondrial DNA in cells of different sizes.

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FUSION AND EROSION OF CELL WALLS DURING CONJUGATION IN THE FISSION YEAST (SCHIZOSACCHAROMYCES POMBE)

G. B. Calleja, Bong Y. Yoo and Byron F. Johnson
J. Cell Science (1977).

SUMMARY

Conjugation in Schizosaccharomyces pombe was studied by transmission electron microscopy. Mural and nuclear events were scored from induction, the initial event, to meiosis I, the start of sporulation. These morphogenic markers were separately identifiable as flocculation, copulation, conjugation-tube formation, cross-wall formation, cross-wall erosion, conjugation-tube expansion, cytoplasmic fusion, de-differentiation of site of union, nuclear migration and karyogamy. The following were identified as new structural elements: sex hairs, which presumably mediate hydrogen bonding between cells during flocculation; crimp at the site of union; dark patch, which presumably serves as a leak-proof seal at the time of cross-wall erosion; suture, an electron-dense seam formed by the union of a copulant pair; and small electron-dense particles close to the site of wall erosion. No special structures on the cell wall could be identified as indicative of specific sites for potential copulatory activity. The discontinuity of the 2 cell walls at the site of union became so de-differentiated after fusion and erosion that it was no longer possible to pinpoint the site of union.

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CONJUGATION-INDUCED LYSIS OF SCHIZOSACCHAROMYCES POMBE

G. B. Calleja, Bong Y. Yoo and Byron F. Johnson
In press: J. Bacteriol.

ABSTRACT

About 15% of conjugating cells of Schizosaccharomyces pombe were observed to lyse spontaneously during the conjugation process. Lysis occurred at the site of union.

XXVIII. Waksman Institute of Microbiology, P. O. Box 759, Piscataway, N.J. 08854. Communicated by J. O. Lampen.

Although much of the attention of my research group has been taken up with the problem of penicillinase formation by Bacillus licheniformis during the last couple of years, I have maintained a modest program on yeast, much of it dealing with the antibiotic tunicamycin which has been proven to be a useful and specific inhibitor of glycoprotein synthesis.

The following papers on yeast have appeared from my laboratory during the last year or two:

1. Tkacz, J. S., and Lampen, J. O. Tunicamycin inhibition of polyisoprenyl N-acetylglucosaminyl pyrophosphate formation in calf-liver microsomes. BBRC 65:248-257 (1975).

2. Kuo, S. -C., and Lampen, J. O. Tunicamycin inhibition of ^{3}H-glucosamine incorporation into yeast glycoproteins: Binding of tunicamycin and interaction with phospholipids. Arch. Biochem. Biophys. 172:574-581 (1975).

3. Lampen, J. O. Secretion of enzymes by microorganisms: The nature of the process. ASM Meeting, Orlando, Fla. Conf. on Genetics of Molecular Biology of Industrial Microorganisms. Microbiology - 1976, pp. 540-542.

4. Gallili, G., and Lampen, J. O. Large and small invertases and the yeast cell cycle: Pattern of synthesis and sensitivity to tunicamycin. BBA 475: 113-122 (1977).

5. Tkacz, J. S., S.-C. Kuo, and J. O. Lampen. Inhibition of glycoprotein synthesis in yeast by the antibiotic tunicamycin. Volume honoring Dr. Suomalainen's 60th birthday. In: Alcohol - Industry and Research, pp. 197-205 (1977), Helsinki, Finland.

Dr. Takemitsu Mizunaga from the Department of Agricultural Chemistry, University of Tokyo, Japan, joined our laboratory group in October 1976 on an International Public Health Service Fellowship. He has previously investigated the regulation of acid phosphatase biosynthesis by yeast and is currently studying the effects of tunicamycin on the biosynthesis of acid phosphatase and invertase.

Dr. Joseph S.-C. Kuo, who was with our laboratory for about five years, has joined the research group of Lederle Laboratories.

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

ON THE ISOLATION, ENZYMES AND LIPID COMPOSITION OF THE PLASMA MEMBRANE AND CELL WALL OF BAKER'S YEAST (SACCHAROMYCES CEREVISIAE)

YEAST 6-76

Timo Nurminen

Thesis, Helsinki University of Technology, 1976, 46 pp.

Fragments of plasma membrane and cell walls have been isolated from the cell envelopes of Saccharomyces cerevisiae and characterized morphologically and biochemically.

Saccharase and various acid phosphohydrolases were mainly located outside the plasma membrane. The oligomycin-insensitive adenosine triphosphatase and the lipase, apparent in the cell envelopes, were located in the plasma membrane only, whereas a part of lysophospholipase was also present in the cell wall. An adenylate cyclase was shown to occur in the cell envelopes.

The main phosphoglycerides of the plasma membrane were phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol. The major envelope fatty acids in aerobic S. cerevisiae were palmitoleic and oleic acids. The principal very long-chain fatty acids were 2-hydroxy-n-hexacosanoic and cerotic acids. They largely originated from a glycosphingolipid containing inositol, phosphorus and mannose. The minor glycolipids included ceramides, sterol glycosides, sulpholipids, cerebrosides and acylglucoses. The cell-wall lipids largely consisted of neutral lipids, principally acylglycerols. The plasma membrane also contained acylglycerols

and most of the envelope sterols. The aerobic plasma membrane contained much more unsaturated fatty acids and sterol and much less squalene than the anaerobic membrane. The main sterol in the aerobic membrane was free ergosterol. Zymosterol and three other minor sterols were mostly esterified.

Zonal centrifugations of homogenates in sucrose and iso-osmotic Ficoll were studied for the larger scale isolation of plasma membranes. Light vesicles of plasma membrane were found at around the density 1.10 (g/cm^3). Heavy fragments of plasma membrane were found in Ficoll at the densities 1.12-1.15 and in sucrose at around the density 1.25. They contained much more carbohydrate, especially mannose, than the lighter vesicles of plasma membrane.

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CHARACTERIZATION OF AN ADENOSINE 3$^-$:5$^-$-CYCLIC MONOPHOSPHATE PHOSPHODIESTERASE FROM BAKER'S YEAST. ITS BINDING TO SUBCELLULAR PARTICLES, CATALYTIC PROPERTIES, AND GEL-FILTRATION BEHAVIOUR

John Londesborough
Biochem. J. 163 (1977) (in press).

The 3$^-$:5$^-$-cyclic AMP phosphodiesterase in the microsomal fraction of baker's yeast is highly specific for cyclic AMP, and not inhibited by cyclic GMP, cyclic IMP or cyclic UMP. Catalytic activity is abolished by 30 μM-EDTA. At 30°C and pH 8.1, the k_m is 0.17 μM, and theophyline is a simple competitive inhibitor with k_i 0.7 mM. The pH optimum is about 7.8 at 0.25 μM-cyclic AMP, so that over the physiological range of pH in yeast the activity changes in the opposite direction to that of adenylate cyclase [pH optimum about 6.2; Londesborough & Nurminen (1972) Acta Chem. Scand. 26, 3396-3398]. At pH 7.2, dissociation of the enzyme from dilute microsomal suspensions increased with ionic strength and was almost complete at 0.3 M-KCl. $MgCl_2$ caused more dissociation than did KCl or NaCl at the same ionic strength, but at low KCl concentrations binding required small amounts of free bivalent metal ions. In 0.1 M-KCl the binding decreased between pH 4.7 and 9.3. At pH 7.2 the binding was independent of temperature between 5 and 20°C. These observations suggest that the binding is electrostatic rather than hydrophobic. The proportion of bound activity increased with the concentration of the microsomal fraction, and at 22 mg of protein/ml and pH 7.2 was 70% at I 0.18, and 35% at I 0.26. Presumably a substantial amount of the enzyme is particle-bound in vivo. At 5°C in 10 mM-potassium phosphate, pH 7.2, the apparent molecular weight of KCl-solubilized enzyme decreased with enzyme concentration from about 200000 to 40000. In the presence of 0.5 M-KCl, a constant mol. wt. of about 55000 was observed over a 20-fold range of enzyme concentrations.

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DISTRIBUTION OF PLASMA-MEMBRANE FRAGMENTS DURING ZONAL CENTRIFUGATIONS OF HOMOGENATES FROM AEROBIC SACCHAROMYCES CEREVISIAE

T. Nurminen, L. Taskinen and H. Suomalainen
J. Gen. Microbiol. 98 (1977) 301-304

The distribution of several enzymes and other marker components after zonal centrifugations of whole homogenates from aerobic Saccharomyces

cerevisiae on sucrose and iso-osmotic Ficoll, and the composition and morphology of the fractions were investigated. After high-speed zonal centrifugation in sucrose or Ficoll, most of the protein, alkaline phosphatase, alkaline pyrophosphatase, adenosine monophosphatase, β-fructofuranosidase and an appreciable amount of acid phosphatase, NADPH-cytochrome c oxidoreductase, phospholipid and sterol were found at densities below 1.09. In Ficoll the RNA maximum was at ρ= 1.08 and the main peak of the mitochondrial marker, cytochrome c oxidase, was at ρ= 1.08-1.10 coincident with the main peak of oligomycin-sensitive Mg^{2+}-dependent adenosine triphosphatase (Mg-ATPase). The smooth membranes containing oligomycin-insensitive Mg-ATPase were found at around ρ= 1.12. In sucrose, the second protein peak at around ρ= 1.10 was rich in light membranes, including rough endoplasmic reticulum (RNA and NADPH-cytochrome c oxidoreductase maxima), vacuolar membranes (α-mannosidase maximum) and some partially oligomycin-insensitive Mg-ATPase. The main peaks of cytochrome c oxidase and oligomycin-sensitive Mg-ATPase indicated the presence of mitochondria at ρ= 1.16-1.18. A minor peak of phospholipid and sterol at around ρ= 1.26 contained smooth membranes rich in oligomycin-insensitive Mg-ATPase and carbohydrate, especially mannose. The membrane preparations containing Mg-ATPase were further purified on Urografin gradients. We concluded that the smooth membranes containing oligomycin-insensitive Mg-ATPase are plasma-membrane fragments, which can differ in density depending on their content of glycoprotein particles.

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PRODUCTION OF ESTERS BY DIFFERENT YEAST STRAINS IN SUGAR FERMENTATIONS

Lalli Nykänen and Irma Nykänen
J. Inst. Brew., London 83 (1977) 30-31

The production of the acetates of isoamyl alcohol and phenethyl alcohol and the ethyl esters of the C_6-C_{10} fatty acids was investigated in semiaerobic sugar fermentations by 56 strains of Saccharomyces cerevisiae and 3 strains of S. uvarum. The S. cerevisiae yeast generally produced more esters than the S. uvarum yeasts. Isoamyl acetate was the main component in the ester fractions examined and others in decreasing order, were ethyl caprylate, ethyl caproate, ethyl caprate and phenethyl acetate.

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DISTRIBUTION OF ESTERS PRODUCED DURING SUGAR FERMENTATION BETWEEN THE YEAST CELL AND THE MEDIUM

Lalli Nykänen, Irma Nykänen and Heikki Suomalainen
J. Inst. Brew., London 83 (1977) 32-34

The distribution of the esters formed during sugar fermentations between the yeast cells and the medium was investigated in fermentations by 5 strains of Saccharomyces cerevisiae and 3 strains of S. uvarum (carlsbergensis). The esters studied included the acetates of isoamyl alcohol and phenethyl alcohol and the ethyl esters of the C_6-C_{12} fatty acids. All of both acetates appeared in the medium. The proportion of the fatty acid ethyl esters transferred to the medium decreased with increasing chain length: all in the medium for ethyl caproate, 54-68% for ethyl caprylate, 8-17% for ethyl caprate, and all remaining in the yeast cell for ethyl laurate.

A higher proportion of the esters formed appeared to remain in the cells of the S. uvarum strains than in cells of S. cerevisiae.

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METABOLISM OF BAKER'S YEAST UNDER BIOTIN DEFICIENCY

Erkki Oura

Alcohol, Industry and Research, a volume dedicated to Professor Heikki Suomalainen on his 60th birthday January 26. 1977, Ed. by O. Forsander, K. Eriksson, E. Oura and Paula Jounela-Eriksson, Frenckellin Kirjapaino Oy, pp. 72-81 (Finnish), 235-236 (English synopsis).

The effects of biotin deficiency on yeast metabolism are caused primarily by the decreased activity of pyruvate carboxylase. Owing to the limited functioning of this biotin-containing enzyme, the rate of formation of oxaloacetate is greatly decreased. When, in addition, oxaloacetate is drawn from the TCA cycle for synthetic purposes, the functioning of the TCA cycle as a catabolic mechanism is more or less limited. As a result, the metabolism at low biotin concentrations is strongly fermentative even in vigorously aerated continuous cultivations, and oxidative metabolism, i.e., the TCA cycle, still functions to only a limited extent even when the biotin concentration is 5 µg/1000 ml. The low rate of oxaloacetate formation means that the anabolic reactions necessary for the growth of yeast proceed slowly under biotin deficiency.

The formation of acetyl-CoA from pyruvate seems not to be affected by biotin deficiency. This can be concluded from the indifference to biotin deficiency of some reactions in which acetyl-CoA participates: (a) the formation of ethyl acetate is greater in biotin deficient yeast than in yeast grown with optimal biotin concentrations and (b) the formation of lipids and fatty acids in biotin deficient yeasts is not noticeably affected. According to these results one can assume that the biotin enzyme acetyl-CoA-carboxylase is not so sensitive to biotin deficiency as pyruvate carboxylase.

More of the limited amount of oxaloacetate formed during biotin-restricted growth seems to be used in the condensation reaction with acetyl-CoA to form citrate, and then via α-ketoglutarate to form glutamate, than is used in aspartate biosynthesis. This is shown by (a) there being much more glutamate than aspartate in the cellular amino acid pool, and (b) only traces of soluble arginine being observed in biotin deficient yeast, whereas the amount of citrulline in these cells is some 15 times higher than in cells grown with biotin. The high citrulline pool indicated that the formation of glutamate remains essentially unaffected, whereas the almost negligible arginine formation implies a strongly limited formation of aspartate.

The limited amount of aspartate in biotin deficient yeast cells also affects the biosynthesis of purine nucleotides, and the biosynthetic intermediates, diazoticable amines and hypoxanthine that would normally react with aspartate are instead excreted into the medium.

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GLYCEROL AND SUCCINIC ACID AS THE MAIN SIDE REACTION PRODUCTS DURING YEAST FERMENTATIONS

Erkki Oura
Process Biochem. 1977:April (in press)

During all yeast fermentations some 4-5% of the substrate is consumed in the formation of glycerol and succinate. To reduce the formation of glycerol and succinate and to increase the yield of ethanol by a corresponding amount one must clarify the mechanisms of their formation and any dependence of their formation of each other or on the reactions in yeast.

The following hypothesis has been formulated:
- Fermentation leads to an elevated energy charge in the cell, which will activate the pyruvate carboxylase.
- This leads to the formation of succinate and an excess of reduced respiratory nucleotides.
- This excess $NADH_2$ is oxidized in the formation of glycerol and in this way the redox state in the cell is balanced.
- Glycerol will also be formed in connection with the production of some other side products, the main part, however, is connected with the formation of succinate.

The following publications have appeared since the last communications. The abstracts of reports have been given in Yeast Newsletter 25 (1976):1, 10-11; 25(1976):2, 22.

Heikki Suomalainen and Timo Nurminen, Some aspects of the structure and function of the yeast plasma membrane. J. Inst. Brew., London 82 (1976), 218-225.

Kaija Varimo and John Londesborough, Solubilization and other studies on adenylate cyclase of baker's yeast. Biochem. J. 159 (1976), 363-370.

XXX. Service de Genetique - Institut National Agronomique, 16, Rue Claude Bernard, 75231, Paris Cedex 05, FRANCE. Communicated by H. Heslot.

PROTOPLAST FUSION AND RECOMBINATION IN THE YEAST *CANDIDA TROPICALIS*

M. Heslot, P. Fournier, and A. Provost
Archives of Microbiology (submitted for publication)

Candida tropicalis is an asexual yeast whose degree of ploidy is unknown. Auxotrophic mutants can be induced, but crossing between strains and recombination of markers cannot normally be obtained.

In an attempt to overcome these limitations, we chose two doubly auxotrophic strains (cyt$^-$ ade$^-$ and met$^-$ his$^-$) and prepared protoplasts out of them, using - with slight modifications - a method already described by our laboratory (Housset, Nagy and Schwenke, 1975 J. Gen. Microbiol. 90, 260-264), to obtain *S. pombe* protoplasts.

Treatment of cells involved dithiothreitol and snail enzyme, the conversion rate into protoplasts reaching 99%. These protoplasts were induced to fuse under the action of polyethyleneglycol, and regeneration of cell walls was achieved in a liquid hypertonic medium, containing mannitol.

The fusion products were selected by complementation on solid minimal medium. Nuclear staining revealed that these prototrophs were dikaryotic, but further cultivation either on complete medium or on minimal medium gave rise to segregation of the two parental nuclei.

In some cases however segregants were obtained carrying a single marker, either met^-, his^-, cyt^- or ade^-. Moreover stable prototrophs were selected after subcloning. These prototrophs consisted of uninucleate cells and could be shown to be heterozygous for all four markers: somatic segregation occured at a frequency of 10^{-4}, giving rise to a variety of recombinants such as $met^- ade^-$, $his^- cyt^-$, etc...

We conclude that protoplast fusion seems to be a valuable tool to induce genetic recombination in asexual yeasts.

XXXI. Departamento de Microbiologia, Facultades de Ciencias y Farmacia e Instituto de Microbiologia Bioquímica, C.S.I.C. Universidad de Salamanca, Salamanca, SPAIN. Communicated by Rafael Sentandreu.

The following are recent papers from this department:

1) VILLA, T. G., NOTARIO, V., BENITEZ, T. and VILLANUEVA, J. R. On the effect of glucono-δ-lactone on the yeast Pichia polymorpha Arch. microbiol. 109, 157-161 (1976).

2) VILLA, T. G., NOTARIO, V. and VILLANUEVA, J. R. Method of purifying β-(1-3)-glucanase from Candida utilis Appl. Environ. Microbiol. 32, 185-187 (1976).

3) VILLA, T. G., NOTARIO, V., BENITEZ, T. and VILLANUEVA, J. R. Purification of an exo-1,3-β-glucanase from Candida utilis Can. J. Biochem. 54, 927-934 (1976).

4) BENITEZ, T., VILLA, T. G., NOTARIO, V. and GARCIA ACHA, I. Studies of walls of Trichoderma viride using fluorescent brighteners. Trans. Br. mycol. Soc. 67, 485-489 (1976).

5) NOTARIO, V., VILLA, T. G. and VILLANUEVA, J. R. Purification of an exo-β-D-glucanase from cell-free extracts of Candida utilis. Biochem. J. 159, 555-562 (1976).

6) LARRIBA, G., ELORZA, M. V., VILLANUEVA, J. R. and SENTANDREU, R. Participation of dolichol phospho-mannose in the glycosylation of yeast wall manno-proteins at the polysomal level. Febs Letters 71, 316-320 (1976).

7) ELORZA, M. V., LOSTAU, C. M., VILLANUEVA, J. R. and SENTANDREU, R. Cell wall synthesis regulation in Saccharomyces cerevisiae: Effect of RNA and protein inhibition. Biochim. Biophys. Acta, 454, 263-272 (1976).

8) SENTANDREU, R. and VILLANUEVA, J. R. Enzymic mechanisms involved in the synthesis of yeast cell wall. In: Reflections on biochemistry. Ed. by A. Kornberg et al. Pergamon Press Oxford 1976, pp, 187-195.

9) ELORZA, M. V., VILLANUEVA, J. R. and SENTANDREU, R. The mechanism of catabolite inhibition of invertase by glucose in Saccharomyces cerevisiae. Biochim. Biophys. Acta, 475, 103-112 (1977).

10) SENTANDREU, R., ELORZA, M. V. and VILLANUEVA, J. R. Molecular aspects of wall formation in Saccharomyces cerevisiae. Alcohol, industry and research. Ed. by O. Forsander, K. Eriksson, E. Oura and Paula Jounela-Eriksson. Helsinki 1977, pp. 190-196, 248.

XXXII. Zymology Laboratory, School of Biological Sciences, University of Bath, Bath, Avon, England. Communicated by A. H. Rose.

Three main areas of research on yeast are under investigation in this laboratory.

I. Relationship between composition and function in the plasma membrane of Saccharomyces cerevisiae. YEAST 6-76

Three ways have been developed for effecting specific alterations in the lipid composition in strain NCYC 366 of this yeast. The first of these exploits the anaerobic requirement for a sterol and an unsaturated fatty acid. The second technique involves supplying the medium with choline or ethanolamine which induces increased synthesis of phosphatidylcholine and phosphatidylethanolamine, respectively. The third method uses anaerobic growth of Saccharomyces cerevisiae in a medium supplemented with a sterol and a mixture of phospholipids. The following is a shortened abstract of a paper delivered before the Society for General Microbiology in March 1977 describing this method.

SUMMARY

Saccharomyces cerevisiae, grown under strictly anaerobic conditions, must be provided with those precursors or cellular constituents whose synthesis requires molecular oxygen. The anaerobically-induced requirement was discovered by Andreasen and Stier who demonstrated growth in cultures supplemented with ergosterol and oleic acid. Anaerobically-grown S. cerevisiae enriched in different sterols and fatty-acyl residues has been used in this laboratory to study the role of these lipids in yeast plasma-membrane function. We have now established that the fatty-acid requirement for anaerobic growth of S. cerevisiae can be replaced by a mixture of phospholipids from this yeast.

S. cerevisiae NCYC 366 was grown aerobically or anaerobically, lipids extracted from freeze-dried cells and separated into classes as reported by Hossack and Rose. Maximum growth rate was obtained in anaerobic cultures supplemented with ergosterol (5 mg) and at least 20 mg of a mixture of phospholipids from aerobically-grown cells. Growth rate decreased in cultures supplemented with lower concentrations of phospholipids. Cells grown anaerobically in phospholipid-supplemented cultures did not contain detectable traces of phospholipase activity, using phosphatidylcholine as substrate: phospholipase activity could be detected however in aerobically-grown S. cerevisiae

NCYC 366. Phospholipids from aerobically-grown cells were separated by thin-layer chromatography. Cells did not grow in media supplemented with a mixture of phospholipids from which phosphatidylcholine had been removed. Egg yolk lecithin could not replace the phosphatidylcholine from yeast cells.

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To date, the functions of the plasma membrane which have been studied, as affected by the lipid composition, are firstly its capacity to stretch [Hossack and Rose (1976) J. Bact. 127, 67-75; Hossack, Sharpe and Rose (1977) J. Bact. 129, 1144-1147]. Another function which has been studied is the capacity of the yeast to tolerate toxic concentrations of ethanol. Reproduced below is an abstract of a paper presented before the Society for General Microbiology in March 1977, describing this work:

SUMMARY

Although strains of S. cerevisiae remain viable in the presence of concentrations of ethanol that are lethal for most other microorganisms, nothing is known of the molecular basis of this tolerance. It is generally held, however, that the composition of the plasma membrane must be an important factor in the ethanol tolerance of this yeast. Using S. cerevisiae with plasma-membrane lipids enriched in specific sterols and fatty-acyl residues, we have established that the presence of certain sterols and unsaturated fatty-acyl residues substantially increases its ethanol tolerance.

To obtain cells of S. cerevisiae enriched in specific sterols and unsaturated fatty acids, strain NCYC 366 was grown anaerobically in media supplemented with oleic acid or linoleic acid and one of the sterols cholesterol, ergosterol, campesterol or stigmasterol. The free-sterol content in cells grown under these conditions was about 70% enriched by the exogenously provided sterol, while 55-60% of the cellular fatty-acyl residues were chemically identical with the fatty acid included in the medium. Plasma membranes were isolated from sphaeroplasts of S. cerevisiae and shown to be comparably enriched in the exogenously-provided sterol and fatty-acyl residues. Cells enriched in different sterols and fatty-acyl residues were suspended in 67 mM-KH_2PO_4 (pH 4.5) containing M-ethanol, and the drop in viability on incubation at 30°C followed by methylene blue staining and by plate counts.

Tolerance was always greater when the cells were enriched with linoleic-acid compared with oleic-acid residues. Cells with membranes enriched in oleic-acid residues were usually susceptible to ethanol irrespective of the sterol supplement. Cells with membranes enriched in linoleic-acid residues and a sterol with a saturated side chain (cholesterol or campesterol) were consistently less able to tolerate ethanol than those enriched in linoleic-acid residues and a sterol with an unsaturated side chain (ergosterol or stigmasterol).

It is suggested that the tight packing of sterols and phospholipid molecules in the yeast plasma membrane which is promoted by the presence of sterols with an unsaturated side chain may make the

membrane a more effective barrier to ethanol molecules, whereas the presence of highly unsaturated fatty-acyl residues in the interior of the membrane could increase the ethanol solubility in the membrane.

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Another plasma membrane function which is being studied is the action of detergents including sodium dodecyl sulphate, cetyltrimethyl ammonium bromide and Triton. Cells with plasma membrane enriched in phosphatidylethanolamine are much more susceptible to sodium dodecyl sulphate than those with plasma membranes enriched with phosphatidylcholine.

II. Envelope growth in Saccharomyces cerevisiae

Several workers have suggested that intracellular vesicles are involved in envelope growth in S. cerevisiae. A technique has been devised for separating two size classes of these vesicles from S. cerevisiae NCYC 366, and a study has been made of the lipid composition and enzyme activity of these vesicles. Reproduced below is an abstract of a paper submitted for publication.

SUMMARY

A mixture of small (0.43 μm diameter) and large (0.62 μm diameter) low-density vesicles from sphaeroplasts of S. cerevisiae was fractionated by rate centrifugation in a gradient of 0-8% (w/v) Ficoll to yield fractions rich (90-95%) in small or large vesicles. The large, but not small, vesicles swelled when diluted into mannitol solutions containing less than 0.4 M mannitol. The pH-electrophoretic mobility curve of the large vesicles showed that they are probably enclosed in a phospholipid-protein membrane. The dyes neutral red and toluidine blue, accumulated into large vesicles by intact cells and sphaeroplasts, were largely lost from large vesicles when these were separated from stained sphaeroplasts. Sudan Black III stained small and large vesicles, both classes of vesicle retaining the stain on separation. Fractions rich in large vesicles contained proportionately more phospholipid, and less free sterols, diacylglycerols and free fatty acids compared with those enriched in small vesicles. The two classes of vesicle contained about the same proportions of esterified sterols and triacylglycerols. The free fatty acids in both small and large vesicles were free from unsaturated fatty-acyl residues; diacylglycerols and triacylglycerols contained appreciable proportions of unsaturated fatty-acyl residues. Small vesicles were richer in lipase activity, while the large vesicles contained greater β-glucanase and α-mannosidase activities. Phospholipase activity could not be detected in any of the fractions.

Further analyses have concentrated on the composition of neutral lipids in S. cerevisiae and Kluyveromyces fragilis with a view to illuminating the role of triacylglycerols and sterol esters in envelope growth. The abstract of a paper describing this work, and which will be published in Archives of Microbiology later in 1977, is reproduced below:

SUMMARY

S. cerevisiae, grown aerobically or anaerobically under conditions which induce a requirement for a sterol and an unsaturated fatty acid, synthesized approximately the same amounts of neutral lipid and intracellular low-density vesicles, although the neutral lipids in aerobically-grown cells contained more esterified sterol and less triacylglycerol than those in anaerobically-grown cells. K. fragilis synthesized much less neutral lipid and a small quantity of low-density vesicles than S. cerevisiae whether grown at 30°C (generation time 1.1 h) or 20°C (generation time 2.1 h). Both yeasts, synthesized highly saturated triacylglycerols, relatively unsaturated phospholipids, and esterified sterols with an intermediate degree of unsaturation irrespective of the conditions under which they were grown. Free sterols in the yeasts were rich in ergosterol and 22(24)-dehydroergosterol, while the esterified sterol fractions were richer in zymosterol.

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Currently techniques are being developed for studying fusion of vesicles from plasma membrane preparations in order to examine transfer of lipid material from vesicles to the growing plasma membrane.

III. Flocculation in Saccharomyces cerevisiae

A paper by P. M. Jayatissa and A. H. Rose describing research from this laboratory on yeast flocculation has been published recently (J. Gen. Microbiol. (1976) 96, 165).

From the preliminary work reported in this paper, it was deduced that production of extra carboxyl groups in the yeast wall when cultures enter the stationary phase of growth is seminal for flocculation, as a prelude to the formation of calcium linkages between these groups in adjacent cells. These and recently obtained results suggest that phosphodiester linkages in wall phosphomannan act by repelling adjacent cells. Currently this research is being extended in collaboration with Dr. G. G. Stewart of Labatt Breweries of Canada Limited, London, Ontario.

XXXIII. Laboratoire d' Enzymologie, CNRS, 91190 Gif-sur-Yvette (France), and Fondation Curie, Institut du Radium-Biologie, 91405 Orsay (France). Communicated by J. Schwencke.

Yeast Protoplasts from Stationary and Starved Cells: Preparation, Ultrastructure and Vacuolar Development

J. Schwencke, N. Magña-Schwencke and J. Laporte
Annales De Microbiologie, 128A, 3-18 (1977)

SUMMARY

The conversion of stationary and starved yeast cells into protoplasts is described. The method is rapid, simple and can be applied to a variety of stationary yeast cells. Preincubation of yeast cells in the presence of Pronase was essential for effective conversion into protoplasts. Baker's yeast and seven defined yeast strains, including one "petite", were studied. All of them were efficiently transformed into protoplasts in 60 to 90 min,

depending on the strain, culture conditions and the age of the culture. Protoplasts may be obtained even from late-stationary cells which contain spores.

Saccharomyces cerevisiae cells subjected to complete starvation conditions in water, could also be completely transformed into protoplasts, even after 48 h of starvation.

Electron microscope examination of stationary protoplasts from three different yeast strains showed no evidence of a remaining cell-wall. S. cerevisiae stationary cells show a very developed vacuolar system, a number of "lipid granules" and a few altered mitochondria. Endomycopsis fibuligera and Candida tropicalis stationary protoplasts show a similar fine structure, but "lipid granules" were completely absent.

XXXIV. Chelsea College, Chemistry Department, University of London, Manresa Road, London, SW3 6LX, England. Communicated by A. E. Beezer.

Current research in progress by our group:

(1) Flow microcalorimetric identification of, and metabolism in yeasts.

(2) Storage, recovery and characteristics of yeasts held at liquid nitrogen temperatures.

(3) Metabolism and antibiotic interactions with C. albicans.

(4) Antifungal antibiotics--assay of, and interaction studies with.

Papers published this year include:

1. Beezer, A. E., R. D. Newell and H. J. V. Tyrrell. Application of flow microcalorimetry to analytical problems: The preparation, storage and assay of frozen inocula of Saccharomyces cerevisiae. J. Appl. Bacteriol., 1976, 41, 197-207.

2. Beezer, A. E., R. D. Newell and H. J. V. Tyrrell. Bioassay of nystatin bulk material by flow microcalorimetry. Analyt. Chem., 1977, 49, 34-37.

3. Beezer, A. E. Microcalorimetric studies of microorganisms in applications of calorimetry in life sciences, Ed. I. Lamprecht and B. Schaarschmidt, de Gruyter, Berlin, 1977.

Thesis. Ph.D. Newell, R. D. Bioassay of nystatin, London Univ. 1975.

We are nine in the group and have six more papers submitted for publication.

XXXV. Instituto de Biología, Universidad de México, Apartado Postal 70-600, México 20 D. F., México. Communicated by A. Peña.

The following paper has been submitted for publication in Biochem. Biophys. Res. Comm.

A Novel Method for the Rapid Preparation of Coupled Yeast Mitochondria

A. Peña, M. Z. Piña, E. Escamilla and E. Piña

SUMMARY

A new technique is described for the preparation of yeast mitochondria. The main difference with other methods is the disruption of the cells, which is achieved by means of a Ribi cell fractionator under special conditions. Afterwards, mitochondria are separated by a simple differential centrifugation pattern. The method takes approximately two hours for the preparation of mitochondria.

The study of the mitochondria obtained revealed a good degree of coupling, although some differences were observed, depending on the substrate employed. The method is rapid, and this, as well as its reproducibility in obtaining coupled mitochondria, are the main advantages over other methods reported.

XXXVI. Ecole Nationale Superieure Agronomique de Montpellier. Laboratoire de Recherches de la Chaire de Génétique I.N.R.A., MONTPELLIER, France. Communicated by P. Galzy.

The following articles have appeared recently or will soon be published.

J. KOLODINSKY, C. BIZEAU, P. GALZY. Study of the antigenic properties of the "smooth colony" mutants. - Can. J. Microbiol. (in press). A fraction of peptido-mannans extracted from the cell wall of Saccharomyces cerevisiae by the action of Helix pomatia juice exhibits antigenic differences between wild "rough" strains and "smooth colony" mutants.

G. MOULIN, J. F. ARTHAUD, R. RATOMAHENINA, P. GALZY. Remarques sur les métabolismes respiratoire et fermentaire de Candida kéfyr. - Mycopathologia, Vol. 59, 1, pp. 25-27, 1976. Study of the metabolic parameters of Candida kéfyr: glucose effect, Pasteur effect. A strong fermentation under aerobic conditions is observed.

G. MOULIN, P. GALZY. Une possibilité d' utilisation du lactosérum: la production de levure. - Ind. Alim. Agr. n° 11, Nov. 1976. A study of the production of yeast on whey after separation of protein by ultrafiltration or precipitation at the iso-electric point, is presented. The process and the selected strains are mentionned. Some economics remarks are made.

G. MOULIN, P. VARCHON, P. GALZY. Une nouvelle utilisation possible du lacto-sérum: la préparation de boisson á base de galactose. - Ind. Alim. Agr. n° 1, Janv. 1977. We present in this article a new process for the treatment of solutions containing lactose (whey or milk), able to produce a mass of single cell proteins and a solution of galactose, this by microbial culture of different strains.

XXXVII. Illinois Institute of Technology, Department of Biology, 3101 S. Dearborn, Chicago, IL, 60616. Communicated by Timothy J. Zamb and Robert Roth.

A MODIFIED DIPHENYLAMINE ASSAY FOR QUANTIFYING DNA CONTENT

Samples, each containing from 1.5 to 5 x 10^8 cells are recovered and transferred to 15.0 ml Corex centrifuge tubes. All of the following reactions and procedures may be carried out in these tubes, precluding unnecessary sample transfer. The samples are harvested by centrifugation and then frozen.

Subsequent to the collection of all samples, DNA content is determined utilizing the following semimicromodification of the colorimetric assay previously described by Croes (1967):

1) The frozen pellets are thawed and resuspended in 8.0 ml of PcA-EtOH (0.2M Perchloric acid in 50.0% ethanol). Vortex to homogenize clumped cells, and then incubate at room temp. for 40 min.

2) Centrifuge samples at 3500-4500 rpm for 10-12 min., decant supernatant and resuspend pellets in 4.0 ml of PcA-EtOH wash solution. Centrifuge immediately and discard supernatant.

3) Add a small amount of acid washed glass beads (120 μm in diam.) to each tube. Vigorously vortex the pellets to homogeneity.

4) Add 4.0 ml of an Ether-EtOH solution (1 part anhydrous ether to 3 parts absolute ethanol; volume to volume). Vortex and then incubate at 60°C for 5 min. At the midpoint of this incubation the samples are mildly vortexed.

5) Centrifuge samples and carefully remove supernatant by aspiration. Then repeat step 4.

6) Subsequent to the second lipid extraction, as much supernatant as possible is aspirated from the pellets. The samples are then oven dried at 55.0°C (which takes 4-8 hrs; but up to 24 hrs. of drying will not alter samples).

7) DNA standards: Using a solution of calf thymus DNA, at a concentration of 100 μg/ml of saline citrate (1.5 mM sodium citrate in 15.0 mM sodium chloride), prepare a duplicate set of tubes containing 0.0, 3.0, 5.0, 10.0 and 15.0 μg of DNA (i.e., 0.0 to 0.15 ml of DNA stock solution). Take these standard samples to dryness at 55°C as in step 6 (may take up to 24 hrs. for the larger volumes).

8) After drying, remove all tubes from 55° and allow to cool to room temp. To all tubes (DNA standards, and cell pellets) add exactly 1.0 ml of 1.0 M PcA, and vortex to homogeneity.

9) Incubate tubes for 30 min. in a 70°C water bath, vortexing each tube periodically.

10) After the 70° incubation, again allow the tubes to cool, and then to each add exactly 1.0 ml of DPA reagent (4.0% Diphenylamine dissolved in glacial acetic acid; to which is added 80 µg of acetaldehyde/ ml of reagent, immediately prior to the application of the reagent to the samples). Vortex each sample and cover with Parafilm.

11) Incubate at 30°C for 17-19 hrs. to allow color development.

12) After development, centrifuge the cell samples and transfer supernatants to appropriately labeled tubes and discard pellets. The standard samples need not be centrifuged or transferred.

13) Read cell samples and standards spectrophotometrically at 595 and 650 nm to three place accuracy. The difference of the two readings is proportional to the DNA concentration.

Further notes:

All centrifugations are performed in 15 ml Corex tubes (Corning Glass) with a Sorvall Angle Centrifuge at 3500-4500 rpm for 10-12 min.

On resuspension of pellets throughout the procedure (steps 1, 2, 3 and 8) care should be taken to break cell clumps and to obtain homogeneous suspensions.

Elevated drying temperatures (step 6) may induce charring of cell pellets which will alter OD readings.

After completion of step 11, the samples should appear bright blue in color. Any hint of a greenish cast is suggestive of water contamination during incubation (step 11) or use of contaminated ether, either of which produces inaccurate results.

Reagents:

Perchloric acid 70% (Fisher-Reagent A.C.S.) Ethanol (Rossville-Gold Shield) Ether (Fisher-Anhydrous) Note, after opening, the ether should not be used for longer than one month. Calf thymus DNA (Sigma) Sodium Citrate FW. 294.10 (Fisher-Certified A.C.S.) Diphenylamine (Fisher-Certified A.C.S. - special indicator grade) Glacial acetic acid (Fisher-Reagent A.C.S.) Acetaldehyde (Eastman)

Stock saline citrate solution is prepared volumetrically (15 mM sodium citrate in 150 mM sodium chloride) and adjusted to pH 7.0 with dilute HCl. Upon preparation of DNA standard solutions (100 µg/ml, equal to an OD of 2.00 at 260 nm) the stock saline citrate is diluted 1:10 with distilled water. These DNA stock solutions may be stored indefinitely at 0°C.

Stock acetaldehyde solutions are prepared to a concentration of 1.6% (by weight) and maintained at room temp.

The DPA reagent is unstable and must be prepared just prior to use. This reagent is activated by addition of the acetaldehyde stock at a rate of 0.1 ml/20 ml of DPA reagent.

References:

Croes, A. F., Planta, 76, 209-226 (1976).
Kuenzi, M. T., and R. Roth, Exptl. Cell Resh., 85, 377-382 (1974).
Roth, R., and H. O. Halvorson, J. Bact., 98, 831-832 (1969).

XXXVIII. The University of Kansas Medical Center, Department of Biochemistry, Rainbow Boulevard at 39th, Kansas City, Kansas 66103. Communicated by Wilfred Arnold.

Philip Bestic and I noticed that our standard curves for light extinction (Klett) versus yeast concentration (dry weight per unit volume) are rectangular hyperbolas. Such plots become linear on double-reciprocal coordinates and a set of constants can then be derived either graphically or computationally. Accordingly,

$$\text{Concentration} = \frac{1}{\frac{p}{\text{Klett}} - q}$$

$$\text{where } p = \frac{1}{\text{slope}} \text{ and } q = \frac{\text{intercept}}{\text{slope}}$$

Conversion of subsequent Klett values (cf absorbances) into concentrations is then easily handled on contemporary desk calculators. The ultimate in convenience is to have a tabulated computer printout of Klett values and concentrations (over the useable range). Our experience has been with Saccharomyces cerevisiae, S. rouxii and Candida albicans, in conjunction with a Klett Summerson photoelectric colorimeter or a Zeiss PM6 spectrophotometer. Private communications indicate the usefulness of this linear transformation for some other species. We have published a short note on the method in Applied and Environmental Microbiology 32:640-641 (1976).

Readers who use a turbidimetric method to routinely monitor growth may wish to explore the applicability of this transformation to their standard curves. Should an interested laboratory not have access to a computer we would be happy to generate a table for them based on their constants.

XXXIX. NATIONAL AND INTERNATIONAL MEETINGS

1. The Second International Mycological Congress will be held at the University of South Florida, Tampa, U.S.A. from 27 August-3 September 1977. Information on this Congress may be obtained by writing to Professor M. S. Fuller, Department of Botany, University of Georgia, Athens, Georgia 30602, U.S.A. On 3 September, Dr. C. P. Kurtzman will be chairing a symposium on yeast taxonomy with the following participants: N. J. W. Kreger-van Rij (ultrastructure), J. A. Barnett (nutritional characteristics), T. Tsuchiya (serological techniques), J. N. Baptist (electrophoresis of cellular enzymes), and H. J. Phaff (DNA taxonomy).

2. The Fifth International Specialized Symposium on Yeasts dealing with various aspects of taxonomy and ecology will take place September 12-15, 1977 in Keszthely, Hungary. For further information, please write to Dr. J. Zsolt, Fifth International Symposium on Yeasts, Office for Conference Organization, Motesz, H-1361, Budapest POB 32, Hungary.

3. 6th I.S.S.Y. - 6th International Specialized Symposium on Yeast

Metabolism and Regulation of Cellular Processes

Prof. P. Galzy, Chairman

Executive Committee:

A. Arnaud
C. Bizeau
R. Galzy
J. Guiraud
G. Moulin
F. Vezinhet

Correspondence:

Secretariat du Sixth International Specialized Symposium on Yeast:
Chaire de Génétique et Microbiologie
Ecole Nationale Supérieure Agronomique
Place Viala
34060 MONTPELLIER Cedex, France

First Circular

In accordance with the resolution of the fourth International Symposium on Yeasts in Vienna in 1974, the 6th International Specialized Symposium on Yeast (metabolism and regulation of cellular processes) will be held at Montpellier, France, 2nd to 8th July, 1978. The old city of Montpellier (Mons puellarum) is a nice town standing at eight kilometers of the Mediterranean seaside and fifty kilometers of the Cevennes mountains. July is usually hot and very sunny.

Membership

Membership will be open to all persons interested in the scientific programme. In addition, accompanying members are also welcome.

Scientific programme

Suggested topics are as follows:*

1) Nutrition and growth.
2) Oxidative and fermentative metabolism.
3) Subcellular structure biochemistry.
4) Biological cycle.
5) Biosynthesis and regulation.
6) Enzymology and mechanism of enzyme action.
7) Technics for genetical and biochemical studies of yeast.
8) Histochemistry and cytology.

Language

The language of the symposium will be English.

Social programme

Social programme will be organized both for active and accompanying members.

Accommodation

Possibilities will be indicated in a further circular.

Preliminary registration

The enclosed preliminary application form is requested to be completed and returned before February 28th, 1977. Further announcement will be sent to those who return this form.

*Suggestions for further topics, free sections or panel discussions on special themes will be accepted.

4. 9th Annual Conference on Yeasts

Smolenice, 1st - 4th March 1977
CSSR
with the participation of the GDR microbiologists delegation

Communicated by A. Kocková-Kratochvílová

Program:

Section 1: Selection of productive cultures

A. Kockova-Kratochvílová: The importance of regional collections of cultures.
J. Zemek, Ľ. Kuniak, J. Augustin, A. Kocková-Kratochvílová: A new method for determination of glycan-glycanohydrolases and glycosidases of yeasts and yeast-like microorganisms.
A. Kocková-Kratochvílová, E. Slávíková, J. Zemek, J. Augustin and Ľ. Kuniak: The cellulolytic activity within the genus Trichosporon.
J. Augustin, J. Zemek, A. Kocková-Kratochvílová and Ľ. Kuniak: Alpha-amylases produced by yeasts and yeast-like microorganisms.
P. Biely, Z. Krátky, Š. Bauer and A. Kocková-Kratochvílová: Selection of yeast strains capable of growing on wood xylan.
M. Černáková and A. Kocková-Kratochvílová: Preliminary information on Pullularia pullulans strains utilizing some lignin components.
J. Zámocký, J. Zemek, J. Augustin and Š. Kucár: Proteases and esterases of yeasts and yeast-like microorganisms.
J. Zemek, V. Bílik, J. Augustin, Ľ. Zákutná: Effect of molybdenum on growth of Saccharomyces cerevisiae.

Section 2: Immunology and pathogenicity

A. Tomšíková: Immunological aspects with vaginal candidiasis.
M. Valaskova, L. Masler, J. Šandula: Immunological studies of peptido-mannans from Cryptococcus species.

Section 3: Cytology

O. Necas: Ultrastructure of plasma membrane in yeast protoplasts.
M. Kopecká, J. Horák and H. Maršíková: Biogenesis of the fibrillar wall component in the cell cycle of Saccharomyces cerevisiae.
A. Svoboda: Polyethylene glycol induced fusion of yeast protoplasts.
Y. Koch and K. H. Rademacher: Morphological and biochemical investigations on the cell wall of yeasts.
Z. Holan, A. Gemperle, J. Baldrian and V. Pokorný: A study of the glucan-chitin complex of Saccharomyces cerevisiae by electron-diffraction and X-ray diffraction.
E. Streiblova: The importance of fluorescent techniques for yeast study.

Section 4: Biochemistry

D. Vraná: DNA synthesis during the cell cycle of Candida utilis daughter cells in dependence on the growth rate of the population.

J. Lieblova and K. Beran: Synthesis of RNA during maturation of daughter cells of S. cerevisiae.

R. Borris: Synthesis of multiple glucamylases by Endomycopsis bispora.

H. Reinbothe, J. Miersch and H. Lapp: Ribitol metabolism by Candida guilliermondii.

D. Birnbaum, F. Bottcher, I. A. Samsonová, R. Bode and A. Hofmann: Some aspects of tryptophan degradation in yeasts.

R. Bode: Regulation of biosynthesis of aromatic amino acids in Hansenula henricii CCY 38-10-2.

Section 5: Technology

E. Minárik and A. Navara: Occurrence of Saccharomycodes ludwigii Hansen in sulphited young wines with low alcohol content.

V. Svejcar: Utilization of the de-acidification effect of Schizosaccharomyces in biological acid decomposition.

O. Bendová: Sensibility of brewer's yeasts to killer strains.

H. Krauel and H. Weide: Production of dicarboxylic acids by Candida guilliermondii strain H17 from n-alkanes.

D. Franke-Rinkers: Enzymatic investigation on citric acid accumulating yeasts.

M. Rychtera and V. Gregr: The growth of Candida utilis on the ethanol and acetic acid at different pH values of the cultivation media.

J. Paca and V. Gregr: Effect of ethanol concentration in the feed on growth of Candida utilis in a multistage tower fermentor.

B. Behalova and K. Beran: Autolysis of disintegrated yeast cells.

R. Barták, D. Haláma and D. Nevludová: Health hazards connected with utilization of animal waste hydrolyzates.

Section 6: Minisymposium "Genetic effects on biochemical processes in yeasts"

B. Škárka: Yeasts as a model for genetic and biochemical studies.

F. Böttcher and I. A. Samsonova: Genetical studies on Rhodosporidium yeasts.

V. Farkas: Use of mutants in the study of cell wall biosynthesis in yeast.

L. Šilhanková: Genetic impairment of glycolysis in S. cerevisiae.

J. Súbik: Genetic control of mitochondrial functions in S. cerevisiae.

A. Kotyk: Genetic aspects of membrane transport in yeasts.

M. Povázaj and B. Škárka: The genetic and biochemical aspects of yeasts and yeast-like microorganisms and their application in practice.

Lindner R., Weber H.: Genetic instabilities in yeast.

H. Weber and G. Barth: Studies on conjugation and sporulation of Saccharomycopsis lipolytica.

G. Straube and W. Fritsche: Investigations of the riboflavin overproduction in purine auxotrophic mutants of Candida guilliermondii.
L. Silhankova: The effect of the combination of ultrasonic waves and UV radiation on the genetic material of S. cerevisiae.
E. Ujcova and Z. Fencl: Fluor-acetate-sensitive mutants of yeasts producing citric acid.
E. Gunther, J. Kopp, U. Zuck and R. Brandt: Mutants of Candida guilliermondii with changes in amino acid content.

5. As most of you are now aware, the Fifth Global Impacts of Applied Microbiology Conference, which was to have been held in Kuala Lumpur in March 1976, has had to be transferred, for political reasons, to Bangkok, and now everything is settled for November 22-27, 1977 at Mahidol University under the direction of Professor Pornchai. Associated with the Conference is a special Symposium on Fermented Foods organized by Professor Steinkraus, in which I am involved. All the preparatory work of GIAM V was handed over to Professor Pornchai earlier this year and this left me free to pick up a longstanding offer from my host at the Technical University, to be at the Institute of Biochemical Technology, in Graz as a centre at which to spend my study leave. Graz is at the geographical centre of Europe and, apart from the congenial atmosphere of the institution, residence here has proved invaluable for catching up with a number of research developments.

It was not known until late 1975 that GIAM V would not be held in Kuala Lumpur. The winding-up and transfer continued well into 1976 and the transfer created problems in connection with the publication of the pre-Conference book. However, even this is now making progress and the book entitled "Global Impacts of Applied Microbiology: State of Art 1976" is already being prepared in galley proof by the University of Malaya Press and will be out well in advance of the Conference. There are 37 contributions from eminent microbiologists from all over the world and the details will be sent on request.

W. R. Stanton
School of Biological Sciences
University of Malaysia
Kuala Lumpur, Malaysia

XL. BRIEF NEWS ITEMS

1. The Editor announces with deep regret that Mrs. Gertrude Lindegren died on March 21, 1977 after an illness of several months; but up until almost the end, she continued her work in the laboratory. Her complete dedication to a lifetime career in yeast genetics will be remembered by her friends and colleagues for many years to come.

2. The following publications from our laboratory have recently appeared or are in press:

N. J. W. Kreger-van Rij and M. Veenhuis. 1976. Ultrastructure of the ascospores of some species of the Torulaspora group. Antonie van Leeuwenhoek 42:445-455.

N. J. W. Kreger-van Rij. 1977. Electron microscopy of sporulation in Schwanniomyces alluvius. Antonie van Leewenhoek 43(1) (in press).

3. Postdoctoral position available

Effective immediately a postdoctoral associateship, sponsored by NIH, will be available in the laboratory of Professor Wilfred Arnold, Dept. of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas 66103. Tel. 913-588-7056. The stipend is up to $13,000 per year. The project is a continuing study on the biochemistry and ultrastructure of the yeast cell envelope. Interested persons are invited to apply to Prof. Arnold at the above address.