Y E A S T

A Newsletter for Persons Interested in Yeast

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Herman J. Phaff
Editor

I. National Collection of Yeast Cultures (N.C.Y.C.), Agricultural Research Council, Food Research Institute, Colney Lane, Norwich NR4 7UA, England. Communicated by Barbara Kirsop.

National Collection of Yeast Cultures

The NCYC has now been established at the Agricultural Research Council's Food Research Institute in Norwich, UK, for over a year. A new catalogue has been produced, which is available at a cost of £4.00.

On January 31, 1982 the NCYC was accepted as an International Depository Authority under the terms of the Budapest Treaty for the deposit of microorganisms that are the subject of patent procedures.

The main research effort of the NCYC is directed towards obtaining greater information on strains maintained in the collection and includes screening for such characteristics as thermotolerance and extrachromosomal DNA. In addition, criteria leading to maximum survival following storage in liquid nitrogen have been established as a preliminary to using the method as the back-up method of choice for maintaining collection strains. Freeze-drying remains the primary method of maintenance.

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

The strains listed have been added to the ATCC since October 30, 1981. Complete information for these strains may be obtained upon request from the Mycology Department of ATCC.

Candida cariosilignicola ATCC 46048	Dr. T. Kato Institute for Fermentation Japan
Candida susciphila ATCC 46049	"
Pichia ohmeri ATCC 46050-46053	M. Miranda University of California Davis, California
Saccharomyces cerevisiae ATCC 46054-46057, 46524-46526	Dr. P.K. Maitra Tata Institute of Fundamental Research India
Hansenula anomala ATCC 46058	Ding-ling Wei ATCC Rockville, Maryland
Hansenula polymorpha ATCC 46059	Dr. L. Eggeling Julich Germany

Saccharomycopsis lipolytica ATCC 46067-46070	Dr. D. Ogrydziak University of California Davis, California
Hansenula anomala ATCC 46131	Dr. J.J. Ellis NRRL Peoria, Illinois
Blastoschizomyces pseudotrichosporon ATCC 46132	Dr. I.F. Salkin Nelson A. Rockefeller Empire State Plaza New York, New York
Rhodosporidium paludigenum ATCC 46157-46158	Dr. J.W. Fell University of Miami Miami, Florida
Saccharomyces cerevisiae ATCC 46159-46161	Dr. B. Weiss-Brummer University of München Germany
Candida albicans ATCC 46167	Dr. R. Guinet Institute Pasteur Lyon France
Saccharomyces cerevisiae ATCC 46171	Dr. D.B. Sprinson Roosevelt Hospital New York, New York
Saccharomyces cerevisiae ATCC 46172	Dr. J. Jacques-Panthier INA-PG France
Saccharomyces cerevisiae ATCC 46174-46196	Dr. B. Dujon CNRS France
Saccharomyces cerevisiae ATCC 46227-46228, 46329	Dr. A.J.S. Klar Cold Spring Harbor Lab Cold Spring Harbor, New York
Saccharomyces bisporus ATCC 46246	Dr. J.J. Ellis NRRL Peoria, Illinois
Candida sp. ATCC 46247	"
Saccharomyces rouxii ATCC 46251	"
Saccharomycopsis fibuligera ATCC 46252-46253	"

Torulopsis etchellsii ATCC 46254	Dr. J.J. Ellis NRRL Peoria, Illinois
Saccharomyces rouxii ATCC 46261	Dr. K. Kuichi National Food Research Institute Japan
Torulopsis versatilis ATCC 46263	Dr. J.J. Ellis NRRL Peoria, Illinois
Pityrosporum orbiculare ATCC 46265-46268	Dr. P. Sohnle Medical College of Wisconsin Milwaukee, Wisconsin
Saccharomyces cerevisiae ATCC 46273-46276	Dr. Shoda Hara National Research Institute of Brewing Japan
Saccharomyces cerevisiae ATCC 46303-46307	Dr. K.A. Bostian Brandeis University Waltham, Massachusetts
Cryptococcus luteolus ATCC 46308	Czechoslovak Collection of Yeasts Czechoslovakia
Saccharomyces cerevisiae ATCC 46309-46313	Dr. M.C. Kielland-Brandt Carlsberg Research Laboratory Denmark
Saccharomyces cerevisiae ATCC 46328	Dr. R. Serrano University Autonoma Madrid Spain
Saccharomycopsis lipolytica ATCC 46330	Dr. H. Prauser IMET Culture Collection East Germany
Cryptococcus albidus ATCC 46394	Dr. E.J. Middelbeek University Mijmegen The Netherlands
Holtermannia corniformis ATCC 46395-46398	Dr. H.B. Maruyama Nippon Roche Research Center Japan
Cryptococcus asgardensis ATCC 46399	Dr. H.S. Vishniac Oklahoma State University Stillwater, Oklahoma
Cryptococcus baldrensis ATCC 46400	"

Cryptococcus hempflingii ATCC 46401	"
Cryptococcus vishniacii var. asocialis ATCC 46402	"
Cryptococcus vishniacii var. vladimirii ATCC 46403	"
Cryptococcus vishniacii var. wolfii ATCC 46404	"
Cryptococcus tyrolensis ATCC 46405	"
Cryptococcus wrightensis ATCC 46406	"
Hanseniaspora nodinigri ATCC 46412-46414	Dr. M.A. Lachance University of Western Ontario Canada
Saccharomyces cerevisiae ATCC 46427	Dr. H. Bussey McGill University Canada
Saccharomyces aceti ATCC 46433	Ding-ling Wei ATCC Rockville, Maryland
Saccharomyces cerevisiae ATCC 46439	Dr. W. Stocklein Weihenstephan West Germany
Saccharomycopsis malanga ATCC 46357	Dr. J.J. Ellis NRRL Peoria, Illinois
Saccharomyces cerevisiae ATCC 46440-46441	Dr. L.H. Johnston Division of Microbiology England
Trichosporon cutaneum ATCC 46446	Dr. M. Itoh Nagoya University Japan
Candida lipolytica ATCC 46482-46484	Dr. D.G. Ahearn Georgia State University Atlanta, Georgia
Trichosporon cutaneum ATCC 46489-46490	Dr. H.Y. Neujah Department of Biology Chemistry and Biotechnology Sweden

Candida tropicalis ATCC 46491	"
Saccharomyces rouxii ATCC 46494	Dr. J.J. Ellis NRRL Peoria, Illinois
Candida boidinii ATCC 46498	Dr. T. Urakami Mitsubishi Gas Chemical Company Japan
Saccharomyces cerevisiae ATCC 46523	Dr. W.J. Middelhoven Wageningen The Netherlands
Schizosaccharomyces pombe ATCC 46529-46531	Dr. M. Yamamoto University of Tokyo Japan
Saccharomyces cerevisiae ATCC 46534	Dr. P.K. Latta National Research Council Canada
Candida tropicalis ATCC 46535-46536	D. Yarrow CBS, Delft The Netherlands
Kluyveromyces fragilis ATCC 46537	"

III. Centraalbureau voor Schimmelcultures, Baarn (Netherlands). Communicated by J.A. von Arx.

Below follow two summaries of recently published work.

1. J.A. von Arx: The genera of fungi sporulating in pure culture. 3rd Ed., 424 pp (1981). J. Cramer, In den Springackern 2, 3300 Braunschweig, Germany (price DM 120,--).

The yeasts and some yeast-like fungi are treated on p. 66-105. The asci-forming yeasts are classified in the Endomycetales in the families Dipodascaceae (1 genus), Endomycetaceae (12 genera), Saccharomycetaceae (21 genera), Metschnikowiaceae (5 genera), Saccharomycodaceae (4 genera) and Schizosaccharomycetaceae (1 genus). The basidiomycetous yeasts are divided in the families Sporobolomycetaceae (7 genera) and Filobasidiaceae (10 genera). The Candidaceae comprise the anamorphous (imperfect) ascomycetous yeasts only (Candida, Kloeckera, Geotrichum, etc.). Dichotomous keys to all accepted genera are given. About 40 species are illustrated by line drawings.

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2. J.A. von Arx, J.P. van der Walt[1] and N.V.D.M. Liebenberg[1]. The Classification of Taphrina and other Fungi with Yeast-like Cultural States. ex Mycologia 74:285-296 (1982).

[1]Microbiology Research Group, Council for Scientific and Industrial Research, Pretoria, South Africa.

Abstract

TEM studies show that species of Taphrina and Protomyces have cell walls with a thick, electron-transparent inner layer, similar to those of the Endomycetales, e.g., of species of Saccharomyces or Hanseniaspora. The budding is holoblastic at the attenuated end or ends of the cell and is rather similar to that of the apiculate yeasts. Members of the genera Microstroma and Exobasidium have an electron opaque, often multilayered cell wall and show enteroblastic budding, similar to that of the "red yeasts" (Sporobolomycetales) and Ustilaginales. The Taphrinales are consequently classified in the Endomycetes (Ascomycotina) while Microstroma and Exobasidium are placed in the Basidiomycetes. Species of the genera Kabatiella and Aureobasidum have ascomycete-like cell walls and are therefore not related to Microstroma. The classes of the Ascomycotina and Basidiomycotina are discussed briefly.

IV. Institute of Biochemistry and Physiology of Microorganisms. USSR Academy of Sciences, Pushchino, Moscow region 142292, USSR. Communicated by V.M. Blagodatskaya.

Ascospores in Candida famata (Harrison) Meyer et Yarrow.

Asci and ascospore formation were observed on Gorodkova agar and McClary's acetate agar after 1 or 2 weeks at 18° - 20°C in seven strains of C. famata (Syn. Torulopsis candida (Saito) Lodder) but not including the type strain CBS 940. Heterogamous conjugation between mother cell and bud, precedes ascus formation, isogamous conjugation also occurs. The ascospores are spherical with a warty wall. Type strains of Torulopsis famata (Harrison) Lodder et Kreger van Rij, T. minor (Pollacci et Nannizi) Lodder, and Candida flareri (Ciferri et Redaelli) Langeron et Guerra formed spores in such a manner; hence they should be regarded as synonyms of Debaryomyces hansenii (Zopf.) Lodder et Kreger van Rij, but not Debaryomyces marama di Menna.

V. Inst. Biochem. and Physiol. of Microorganisms, USSR Acad. of Sci., Pushchino, Moscow region, 142292, USSR. Communicated by W.I. Golubev.

Recent publications:

Golubev, W.I., 1981. Species and varieties of yeasts and yeast-like organisms described in Russia (1884-1980). Mikologia i phytopathologia, 15, N 5, 375-379.

72 new species and 14 new varieties are listed.

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Golubev, W.I., 1981. New combinations in yeast fungi of the genus Cryptococcus. Mikologia i phytopathologia, 15, N 6, 467-468.

Seven new combinations are proposed: Cryptococcus amylolentus, Cr. curvatus, Cr. humicolus, Cr. magnus var. heveanensis, Cr. marinus, Cr. podzolicus and Candida melibiosophila nov. nom.

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Blagodatskaya, V.M., Trotsenko, R. Sh., 1981. The ability of Torulopsis spp. to assimilate D-glucuronic acid, D-glucono-δ-lactone, 5-and 2-keto-D-gluconate. Mikrobiologia, 50, N 4, 598-602.

Most of 42 species and varieties of the genus Torulopsis assimilate glucono-δ-lactone, with the exception of species that are capable of assimilating 5-ketogluconate. Only two species, T. ingeniosa and T. fragaria, of basidiomycetous affinity, assimilate glucuronic acid.

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Vorob'ev, A.V., Blagodatskaya, V.M., 1981. The effect of different culturing conditions on sexual agglutination and conjugation of cells in the yeast Metschnikowia pulcherrima. Mikrobiologia, 50, N 5, 857-863.

The optimum incubation temperature and pH for agglutination and conjugation in M. pulcherrima are 24°C and pH 5. The presence of glucose is necessary for conjugation but agglutination does not require an energy source. Only conjugation is stimulated by metal ions. Only pantothenic acid of the vitamins showed a small positive effect on both agglutination and conjugation. The boiled cells did not agglutinate.

VI. Department of Plant Science, University of Western Ontario, London, Ontario, Canada, N6A 5B7. Communicated by M.A. Lachance.

Marc-Andre Lachance. Sporopachydermia quercuum, a New Yeast Species Found in Exudates of Quercus rubra (submitted).

Abstract

A new species of Sporopachydermia Rodrigues de Miranda has been isolated from exudates of red oaks (Quercus rubra). Morphologically, it resembles S. cereana by producing curved cells. Physiologically, it is closer to S. lactativora. It differs from both species by its lower maximum temperature of growth. The epithet quercuum is proposed to indicate its origin.

VII. Institut für Biochemie und molekulare Biologie, Abt. Botanik. Technische Universität Berlin, H 80, Strasse des 17. Juni 135, 1000 Berlin. Communicated by G. Kraepelin.

Below follows the summary of a manuscript to be submitted soon for publication.

G. Kraepelin and U. Schulze. Sterigmatosporidium gen. n., a new heterothallic yeast related to Sterigmatomyces Fell.

Abstract

Sterigmatosporidium gen. n. isolated from drenched pitwood planks in an ore mine (12°C) is described as a new basidiomycetous yeast-like fungus with the single species S. polymorphum sp. n. for which a latin diagnosis and a preliminary life cycle is presented. The main character distinguishing the new genus from the imperfect Sterigmatomyces is the development of a dikaryotic mycelium with clamp connexions producing lateral chlamydospores and apical sexual spores as well as vegetative blastospores. The dikaryotic phase could be induced by crossing compatible haploid clones of the heterothallic (bipolar) fungus, which are similar to Sterigmatomyces but not identical with any known species.

VIII. Université de Lyon, Laboratoire de Biologie Végétale, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France. Communicated by M.C. Pignal.

Since the last issue of the Yeast Newsletter, one paper has been published and a second one is in press. Abstracts follow below.

1. R. Montrocher. Serological Relationships Among Sporogenous and Asporogenous Yeasts. Cellular & Molecular Biology 28(1), 1-3 (1982).

Abstract. Antigenic analyses of 13 species among the genera Candida, Torulopsis, Kluyveromyces and Saccharomyces were carried out by the immunoprecipitation method.

With the exception of C. intermedia, six species of the 'pseudotropicalis' group demonstrate very close serological affinities; in addition, antigenic structures of C. pseudotropicalis and C. macedoniensis are identical to those of their ascosporogenous forms K. fragilis and K. marxianus, respectively.

In the 'glabrata' group, composed of five thermophilic yeasts, T. glabrata appears clearly a distinct species. The close relationships between T. bovina and S. telluris are confirmed while C. slooffii is found more related to these two species than T. pintolopesii.

The serological characteristics of the 'robusta' group are quite different from those of the 'pseudotropicalis' and 'glabrata' groups.

The great affinities among species, the close similarities with some perfect genera and the low relatedness between the groups point out the heterogeneity and complexity of the genus Candida. These data, in correlation to those obtained from other molecular approaches confirm that immunoprecipitation is an appropriate criterion for systematics based on natural relationships.

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2. R. Montrocher. Serological Studies Among Sporogenous and Asporogenous Yeasts: Antigenic Structures of Some Candida and Related Taxa. Cellular & Molecular Biology, 1982 (in press).

Abstract. Serological identification tests performed using the immunoprecipitation technique were carried out with 22 species among the genera Candida and Pichia. Antigenic structures of the assumed pair of perfect/imperfect species P. kudriavzevii/C. krusei are identical and distinct from that of C. sorbosa. C. lambica, only remotely related to C. krusei, must be regarded as the anascosporogenous form of P. fermentans. No significant relatedness is observed between the two round roughened ascospore-forming yeasts P. terricola and P. kudriavzevii. The two serologically very similar species P. norvegensis and C. norvegensis must be considered as another perfect/imperfect pair with a tendency to P. kudriavzevii/C. krusei. The relatedness of both clusters in the norvegensis group is rather low. The serological affinities between sporogenous and asporogenous yeasts as well as the diversity of the antigenic structures point out the suggested heterogeneity of the genus Candida and the taxonomic significance of immunoprecipitation.

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

The following are abstracts of papers that have been accepted for publication:

1. A.I. Estrela, M. Lemos and I. Spencer-Martins. A note on the effect of growth temperature on the production of amylases by the yeast Lipomyces kononenkoae. Journal of Applied Bacteriology 52, 000-000, 1982.

The production of glucoamylase and α-amylase by the yeast Lipomyces kononenkoae IGC 4052 at growth temperatures between 15 and 36°C has been studied. Glucoamylase production was found to be independent of growth temperature. α-Amylase production had a temperature optimum in the range 25-28°C. The yield of growth on starch was not influenced by growth temperature.

* * *

2. I. Spencer-Martins and N. van Uden. The temperature profile of growth, death and yield of the starch-converting y##st Lipomyces kononenkoae. Zeitschrift für Allgemeine Mikrobiologie (accepted).

A strain of Lipomyces kononenkoae earlier proposed for industrial starch bioconversion was found to have a dissociative temperature profile. The Arrhenius plot of sustained exponential growth displayed a single branch between the optimum (32-33°C) and the maximum (about 35°C) temperature for growth while the extrapolated Arrhenius plots of growth and thermal death intersected at a biologically non-significant value. The yield of L. kononenkoae on glucose did not decrease at supraoptimal temperatures while the associative yeast Saccharomyces cerevisiae suffered yield

decreases above the optimum temperature for growth with increasing temperature.

* * *

3. A. Madeira-Lopes and N. van Uden. The temperature profile of Cryptococcus neoformans. Sabouraudia (accepted).

   A strain of Cryptococcus neoformans was found to have a dissociative temperature profile. The Arrhenius plot of sustained exponential growth displayed a single branch between the optimum (about 34°C) and the maximum (about 40°C) temperatures for growth while the extrapolated Arrhenius plots of growth and death intersected at a biologically non-significant rate value. The yield on glucose was temperature independent in the supraoptimal range.

* * *

4. M.C. Loureiro-Dias and J.D. Arrabaça. Flow microcalorimetry of a respiration deficient mutant of Saccharomyces cerevisiae. Zeitschrift für Allgemeine Mikrobiologie (accepted).

   In aerobic batch cultures in mineral medium with glucose of a respiration-deficient mutant of Saccharomyces cerevisiae, growth parameters were estimated and the heat evolved was measured by a flow microcalorimeter. A growth enthalpy of -163.6 joule per mol of glucose consumed was measured. Under anaerobic conditions, the value was -134.6 joule, closer to the expected for alcoholic fermentation alone. The difference was found to be due to cyanide-resistant respiration under aerobic conditions.

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5. J.D. Arrabaça and M.C. Loureiro-Dias. Cyanide-resistant respiration in a respiration-deficient mutant of Saccharomyces cerevisiae. Zeitschrift für Allgemeine Mikrobiologie (accepted).

   A respiration-deficient mutant of Saccharomyces cerevisiae grown aerobically with glucose as the sole source of energy and carbon, showed residual respiration resistant to cyanide. The differential sensitivity of cell fractions to inhibition by hydroxamic acids and carbon monoxide suggested that more than one type of organelle, probably mitochondria and microsomes, was involved in the consumption of oxygen.

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6. C. Leão and N. van Uden. Effects of ethanol and other alkanols on the kinetics and the activation parameters of thermal death in Saccharomyces cerevisiae. Biotechnology and Bioengineering 24, 000-000, 1982.

Ethanol, isopropanol, propanol, and butanol enhanced thermal death in Saccharomyces cerevisiae by increasing $\Delta S^{\ddagger}$, the entropy of activation of thermal death while $\Delta H^{\ddagger}$, the enthalpy of activation was not significantly affected. The relation between $\Delta S^{\ddagger}$ and alkanol concentration was linear with a different slope for each alkanol: $\Delta S_X^{\neq} = \Delta S_o^{\neq} + C_E^A \; X$, where X is the alkanol concentration and C_E^A the entropy coefficient for the aqueous phase defined as increase in entropy of activation per unit concentration of the alkanol. C_E^A was correlated with the lipid-buffer partition coefficients of the alkanols while C_E^M , the entropy coefficient for the lipid phase, was nearly identical for the four alkanols and averaged 37.6 entropy units per mol of alkanol per kilogram of membrane. As predicted by these results, the specific death rates (k_d) at constant temperature were an exponential function of the alkanol concentration and behaved in agreement with the following equation: $\ln k_d^X = \ln k_d^o + (C_E^A R)X$, where R is the gas constant. It was concluded that the alkanols enhanced thermal death through non-specific action on membrane structure.

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7. V. Loureiro and N. van Uden. Effects of ethanol on the maximum temperature for growth of Saccharomyces cerevisiae: A model. Biotechnology and Bioengineering 24, 000-000, 1982.

A model is presented, experimentally verified with an industrial wine yeast, that expresses the maximum temperature for growth in the presence of ethanol as a function of its concentration. The other parameters of the model are the enthalpy of activation of thermal death and the entropy coefficient with respect to death enhanced by ethanol.

X. Zymology Laboratory, School of Biological Sciences, University of Bath, Bath, BA2 7AY. Communicated by A.H. Rose.

Two main areas of yeast research are being conducted in the Zymology Laboratory. The first of these is the relationship between composition and

function in the plasma membrane of Saccharomyces cerevisiae. The second is the physiological basis for ethanol tolerance in strains of Sacchromyces cerevisiae. Currently visiting the laboratory are Dr. Paul A. Henschke from the University of Adelaide, Australia, Dr. Yasuyuki Uzuka from Yamanashi University, Japan, and Dr. Philippe Thibauld from the National Institute for Agricultural Research, Toulouse, France. The following are summaries of three papers which will appear later this year in the Journal of General Microbiology.

1. Production and Tolerance of Ethanol in Relation to Phospholipid Fatty-Acyl Composition in Saccharomyces cerevisiae NCYC 431

By Michael J. Beavan, Claudine Charpentier and Anthony H. Rose

Accumulation of ethanol in supernatants from anaerobic cultures of Saccharomyces cerevisiae NCYC 431 closely paralleled growth during the early exponential phase of batch growth, and continued after growth had ceased. During the 8-64 h period of the fermentation, the intracellular ethanol concentration was greater than the extracellular concentration. Ethanol was very rapidly extracted from organisms by washing with water. During growth up to 32 h, there was a progressive decrease in fatty-acyl unsaturation in phospholipids, and a corresponding proportional increase in saturation. Thereafter, the trend was very slightly reversed. Supplementing cultures with ethanol (0.5 or 1.0 M) after 8 h incubation retarded growth rate, while supplementation with 1.5 M-ethanol immediately stopped growth. In cultures supplemented with 0.5 or 1.0 M-ethanol, viability was not lowered, but supplementation with 1.5 M-ethanol caused a rapid decline in viability. Supplementation of cultures with ethanol at any of the three concentrations led to an increase in the proportion of mono-unsaturated fatty-acyl residues in cellular phospholipids, especially in C_{18} residues, which was accompanied by a decrease in the proportion of saturated residues.

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2. Effect of Plasma-Membrane Phospholipid Unsaturation on Solute Transport into Saccharomyces cerevisiae NCYC 366.

By Michael H.J. Keenan, Anthony H. Rose and Bernard W. Silverman

A comparison was made of kinetics of solute accumulation by Saccharomyces cerevisiae NCYC 366 grown anaerobically under conditions that lead to enrichment of the plasma membrane with ergosterol and either oleyl or linoleyl residues. Values for K_T and V_{max} were identical for accumulation of L-asparagine, L-glutamine, $H_2PO_4^-$, Ca^{2+} and SO_4^{2-} while, for accumulation of D-glucose, the V_{max} value differed slightly but not significantly. Values for K_T for accumulation of L-lysine, by both the low- and high-affinity systems, decreased when oleyl residues were replaced by linoleyl residues. Under these conditions, V_{max} values for the high-affinity systems decreased while that for the low-affinity system increased. An Arrhenius plot for accumulation of lysine by the high-affinity system revealed a discontinuity when membranes were enriched in linoleyl residues. However, no discontinuity was evident on plots of lysine accumulation when membranes were enriched in oleyl residues.

Similar plots for accumulation of L-asparagine, which was used as a control, showed that substitution of linoleyl for oleyl residues significantly raised the transition temperature, but had little effect on the activation energy at temperatures below the discontinuity. When palmitoleyl residues were incorporated into the yeast plasma membrane, the K_T value for L-lysine accumulation by the high-affinity system was hardly altered, although the V_{max} value was lowered, as compared with organisms with membranes enriched in oleyl residues. Replacement of oleyl by palmitoleyl residues lowered both the K_T and V_{max} values for accumulation of L-asparagine. A modified statistical method is described for calculating confidence limits for transition points on Arrhenius plots.

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3. Accumulation of L-Asparagine by Saccharomyces cerevisiae X-2180

By Malcolm E. Gregory, Michael H.J. Keenan and Anthony H. Rose

At concentrations up to 1mM, L-asparagine was accumulated by Saccharomyces cerevisiae X2180-A2 at 30°C against a concentration gradient. Values for K_T and V_{max} were, respectively, $3.5 \cdot 10^{-4}$ M and 33 nmol (mg dry wt)$^{-1}$ min^{-1}. At concentrations below 0.1 mM, a convex curve was obtained on a Woolf- Hofstee plot, possibly indicating the presence of two L-asparagine-binding sites. Autoradiograms of extracts of organisms that had accumulated labelled L-asparagine revealed only one spot with an R_F value identical with that of L-asparagine. Four mutant strains lacking the ability to synthesize the general amino-acid permease system grew and accumulated L-asparagine at similar rates to the parent. The rate of accumulation of L-asparagine from a 0.2 mM solution was greatest at pH 4.5, with the decrease in accumulation rate greater at values below than above 4.5. L-Glutamine, L-histidine, L-methionine, L-threonine and L-tryptophan caused appreciable inhibition of the rate of L-asparagine accumulation. With the exception of L-methionine, the inhibition caused by these amino acids was competitive. Several other amino acids, including D-asparagine and L-aspartic acid, caused little or no inhibition of L-asparagine accumulation.

XI. The University of Kansas, School of Medicine, Department of Biochemistry, 39th and Rainbow Blvd., Kansas City, Kansas 66103, U.S.A. Communicated by Wilfred N. Arnold.

The following are recent papers from our laboratory:

1. Arnold, W.N., and Johnson, B.P. Effects of polyenes, detergents, and other potential membrane perturbants on an osmotolerant yeast, Saccharomyces rouxii. Appl. Environ. Microbiol. 43:311-318 (1982).

2. Arnold, W.N. Glutaraldehyde-fixation of yeast cells: kinetics of a model system for enzyme cytochemistry. Current Microbiol. 6:305-308 (1981).

3. Arnold, W.N. will be on sabbatical leave June 15-December 15, 1982, at the University of Queensland, Brisbane, Australia 4067. He will collaborate with Dr. Burt Zerner on iron-activated acid phosphatases in yeast and animals.

XII. Purdue University, Department of Biochemistry, West Lafayette, Indiana 47907. Communicated by G.B. Kohlhaw.

The four items listed below describe various aspects of work going on in our laboratory.

1. Yun-Pung Hsu and Gunter B. Kohlhaw, Overproduction and Control of the LEU2 Gene product, β-Isopropylmalate Dehydrogenase, in Transformed Yeast Strains. J. Biol. Chem. 257:39-41 (1982).

Abstract Two transformed yeast strains, 21D/pYT14-LEU2 and AH22/CV9-2, were found to produce β-isopropylmalate dehydrogenase to such an extent that the enzyme constitutes 2 and 1%, respectively, of the total extractable protein. This is 30 and 15 times, respectively, above wild type level. β-Isopropylmalate dehydrogenase was purified from strain 21D/pYT14-LEU2 to a purity of about 95% in essentially three steps. Strain 21D/pYT14-LEU2 carries the LEU2 gene on a vector that also contains the yeast 2-μm plasmid and therefore replicates autonomously, whereas strain AH22/CV9-2 carries multiple copies of the LEU2 gene integrated at its normal chromosomal location. Despite the different genetic arrangements, regulation of LEU2 gene expression by leucine and leucine plus threonine was normal. Immunotitration showed that the decrease in specific activity caused by leucine and threonine corresponded to a decrease in immunoreactive material.

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2. Yun-Pung Hsu, Gunter B. Kohlhaw, and Peter Niederberger. Evidence that α-Isopropylmalate Synthase of Yeast is Under the "General" Control of Amino Acid Biosynthesis. J. Bacteriol. 150, in press (1982).

Abstract The specific activity and the immuno-reactive amount of α-isopropylmalate synthase were more than three times above wild type values in a mutant (cdr1) with constitutively derepressed levels of enzymes known to be under the general control of amino acid biosynthesis. The specific activity was also higher in lysine- and arginine-leaky strains when these were grown under limiting conditions, and in wild type cells grown in the presence of 5-methyltryptophan. A low specific activity was found in a mutant (ndr1) unable to derepress enzymes of the general control system. Neither isopropylmalate isomerase nor β-isopropylmalate dehydrogenase responded to general control signals.

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3. Hampsey, D. Michael, Ph.D. Dissertation, Purdue University, May, 1982. Yeast α-Isopropylmalate Synthase: CoA Inactivation, Leucine Inhibition and Subcellular Location. Major Professor: Gunter Kohlhaw.

Abstract Yeast α-isopropylmalate (α-IPM) synthase (E.C. 4.1.3.12) is inactivated by micromolar concentrations of CoA in the presence of Zn^{2+}. It is reported here that rapid reactivation of inactivated enzyme occurred in the presence of millimolar concentrations of ATP or ADP, using permeabilized cells. For the reactivation of purified, CoA-zinc inactivated enzyme, a chelator was required in addition to ATP. Reactivation was also possible by processes that remove CoA from equilibrium; however, these processes were slow. Conditions are defined that result in protection against CoA-zinc inactivation. Since other nucleoside triphosphates are less effective than ATP it is concluded that the ATP effect is a specific adenylate effect.

Studies on the mechanism of CoA inactivation showed that inactivation does not involve covalent modification, but is more likely the result of the formation of an enzyme CoA zinc complex held together by noncovalent forces.

α-IPM synthase was previously shown to be mitochondrially-associated. Using isolated, intact mitochondria it is reported here that the enzyme was unaffected by externally-added proteases and inaccessible to its substrates. Subfractionation of the mitochondria showed that the enzyme remained with the mitoplasts. Thus, α-IPM synthase is not just mitochondrially-associated, but is located in the matrix.

Cell free synthesis of α-IPM synthase showed that this enzyme is not synthesized as a larger molecular weight precursor.

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4. The LEU2 gene and its flanking regions have been sequenced, and the sequences are presently being analyzed (A. Andreadis, Y.-P Hsu, G.B. Kohlhaw, M. Hermodson, W.C. Herlihy, and P.R. Schimmel, manuscript in preparation).

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

The following paper has recently been published from The Biological Institute of the Carlsberg Foundation.

1. Birte Kramhøft and H.A. Andersen. Regulation of RNA Synthesis in Fission Yeast. The effect of a Tetrahymena peptide factor on RNA synthesis in exponentially multiplying yeast cells. Cell Biology International Reports, Vol. 5 No. 11 (1981) 1019-1026.

Abstract

Exponentially multiplying cultures of the fission yeast Schizosaccharomyces pombe were treated with a peptide factor obtained from the protozoan Tetrahymena pyriformis. It was found that the rate of RNA synthesis was reduced by this factor, whereas cell multiplication and protein synthesis were unaffected. These results confirm previous results obtained with protoplasts of the same yeast.

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Furthermore, our laboratory has been represented at the 1st International Symposium on: Regulation of gene expression by low molecular weight peptides. Cosenza, Italy, 1982, with the following presentation:

2. Birte Kramhøft and H.A. Andersen. The Effect of Tetrahymena Peptide and other Low Molecular Weight Peptides on RNA Synthesis in the Fission Yeast Schizosaccharomyces pombe.

Abstract

The rate of RNA synthesis in exponentially multiplying yeast cells as well as in yeast protoplasts is markedly reduced after addition of a small peptide (MW 1,000) secreted by the ciliated protozoan Tetrahymena pyriformis. Protein synthesis is unaffected. The RNA synthesis is only inhibited when the yeast cells are growing on a rich nutrient medium (yeast extract plus glucose).

In minimal medium the RNA synthesis is unaffected by the Tetrahymena peptide, whereas addition of "unspecific" peptides of about the same size (obtained by trypsin digest of bovine serum albumin) stimulate the rate of RNA synthesis. The stimulatory peptides have no effect on cell multiplication or protein synthesis within two hours after addition.

These observations are analogous to results with Tetrahymena cultures.

Our results suggest that small peptides may play a general role in the regulation of the RNA synthesis in eukaryote cells.

XIV. Department of Microbiology, Faculty of Sciences, University of Salamanca, Spain. Communicated by Tomas G. Villa.

The following papers were recently published:

Angeles Sanchez, Julio R. Villanueva and Tomas G. Villa. Saccharomyces cerevisiae Secretes 2 Exo-β-Glucanases, FEBS Letters 138:209-212 (1982)

This work shows 2 different enzymic forms in Saccharomyces cerevisiae with exo-β-glucanase activity. Evidence for different M_r-, K_m-values and substrate specificity has been obtained.

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Francisco del Rey, Tomas G. VillaTomas Santos, Isabel Garcia-Acha and

*Cesar Nombela. Purification and partial characterization of a new, sporulation-specific, exo-β-glucanase from Saccharomyces cerevisiae (1982). Biochem. Biophys. Res. Comm. 105:1347-1353.

*Dept. of Microbiology, Faculty of Pharmacy, University of Madrid, Spain.

Abstract

A new exo-β-glucanase, sporulation-specific, was purified from sporulating S. cerevisiae (AP/1, a/α). Characterization of this new activity shows that the enzyme is a glycoprotein with substrate specificities, kinetic parameters and amino acid composition clearly different from those of its vegetative counterpart.

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

The following is an abstract of our recent work concerning the biosynthesis of vacuolar proteinases (this work is submitted for publication).

1. B. Mechler, M. Müller, H. Müller, F. Meussdoerffer, and D.H. Wolf. In Vivo Biosynthesis of Vacuolar Proteinases in Wild Type and Proteinase Mutants of Saccharomyces cerevisiae.

Immunochemical experiments revealed that proteinase A and proteinase B, two vacuolar enzymes in S. cerevisiae, are synthesized as larger precursors, whose molecular weight is approximately 52,000 and 42,000, respectively. These precursor molecules are processed to their mature forms of M_r=42,000 for proteinase A and M_r=33,000 for proteinase B. In vivo application of tunicamycin (an inhibitor of the synthesis of protein-asparagine-linked carbohydrate moieties) leads to a reduction within two steps of the molecular weights of the proteinase A precursor and authentic proteinase A. Molecular weight of the proteinase B precursor undergoes a partial reduction upon tunicamycin treatment, whereas the authentic proteinase B molecule remains unchanged. As mature proteinase B still contains 10% carbohydrate, linkage of this carbohydrate to the protein must be of different nature. Maturation of pro-proteinase A and pro-proteinase B is neither due to cleavage of asparagine linked carbohydrate nor is maturation dependent on the presence of this kind of protein bound carbohydrate moiety. Together with the findings for maturation of carboxypeptidase Y (1), another vacuolar enzyme, we consider reduction of M_r=10,000 in case of proteinase A and M_r=9,000 in case of proteinase B during maturation due to cleavage of a peptide chain. The molecular forms of proteinase A, proteinase B, and carboxypeptidase Y were studied in mutants (2,3,4) defective in genes which appear to be the structural genes of the respective enzymes. Among the mutants isolated strains were found in which only inactive enzyme forms of molecular weights similar to the precursor molecules could be detected immunochemically. These mutant forms might lead to elucidation of the pro-piece function of the enzymes during vacuole biogenesis.

(1) Hasilik, A. and Tanner, W. (1978) Eur. J. Biochem. 85, 599-608.
(2) Wolf, D.H. and Fink, G.R. (1975) J. Bacteriol. 123, 1150-1156.
(3) Wolf, D.H. and Ehmann, C. (1978) FEBS Lett. 92, 121-124.
(4) Mechler, B. and Wolf, D.H. (1981) Eur. J. Biochem. 121, 47-52.

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2. Dieter H. Wolf (1982). Proteinase action in vitro versus proteinase function in vivo: mutants shed light on intracellular proteolysis in yeast. Trends in Biochemical Sciences, January, 1982, Vol. 7, No. 1, pp. 35-37.

By using strains of Saccharomyces cerevisiae deficient in certain proteolytic enzymes it has been possible to uncover discrepancies between the in vitro action and the in vivo function of known proteolytic enzymes and to detect many new proteinases in yeast.

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

We are currently studying the interaction of cancerostatic drugs with DNA at the molecular level. Below follow the abstracts of four publications dealing with the interaction of cyclophosphamide with yeast DNA.

1. R. Fleer and M. Brendel. Toxicity, interstrand cross-links and DNA fragmentation induced by 'activated' cyclophosphamide in yeast. Chem.-Biol. Interactions, 37, 123-140 (1981).

Treatment of yeast cells with 4-hydroperoxy-cyclophosphamide (4-OOH-CP), the chemically activated form of cyclophosphamide, results in cell killing, induction of DNA interstrand cross-links and DNA fragmentation. Toxicity of 4-OOH-CP is greatly influenced by the cell's capacity of DNA dark-repair: genetic blocking of non-epistatic pathways of DNA repair results in an increase of sensitivity by several orders of magnitude. DNA primary lesions have been measured using a haploid, excision deficient, dTMP-uptaking mutant of S. cerevisiae. In this strain, a significant extent of DNA cross-linking can already be observed at a survival of 88%. At a concentration of 100 nmol/ml 4-OOH-CP, renaturability of DNA increases up to 12 h of drug exposure and drops to lower values upon further incubation. In contrast to the time course of renaturability, DNA double-strand breakage is seen at later stages of drug treatment and continuously increases as a function of incubation time. Whereas inactivation of cells and induction of strand breakage continue upon postincubation of cells, comparable effects are much less pronounced for DNA renaturability.

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2. B. Ogorek, R. Fleer, E. Mutschler and M. Brendel. Comparative study on the effects of cyclophosphamide on yeast in vitro and in the host-mediated assay: DNA damage and biological response. Chem.-Biol. Interactions, 37, 141-154 (1981).

Cyclophosphamide (CP), whether applied in its chemically activated form as 4-hydroperoxy-cyclophosphamide (4-OOH-CP) in vitro or in the host-mediated assay (HMA) using rats, exhibits toxic and mutagenic effects on excision deficient yeast cells. The expression of these effects is examined during a prolonged postincubation in buffer and compared with the ability of activated CP to induce interstrand cross-links and DNA fragmentation. At comparable doses, we observe a close similarity of biological and biochemical effects in either test system.

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3. R. Fleer and M. Brendel. Toxicity, interstrand cross-links and DNA fragmentation induced by 'activated' cyclophosphamide in yeast: Comparative studies on 4-hydroperoxy-cyclophosphamide, its monofunctional analogon, acrolein, phosphoramide mustard, and nor-nitrogen mustard. Chem.-Biol. Interactions, 39, 1-15 (1982).

Activated cyclophosphamide (CP) is known to achieve its cytotoxic and alkylating capacity upon spontaneous hydrolytic breakdown of the oxazaphosphorine ring structure. Treatment of yeast cells with the chemically activated form of CP (4-hydroperoxy-CP, 4-OOH-CP) and with several potentially toxic cleavage products revealed that cytotoxicity is closely linked to the formation of DNA interstrand cross-links and to DNA fragmentation. While this holds true for 4-OOH-CP and its bifunctional alkylating breakdown products, phosphoramide mustard (PM) and nor-nitrogen mustard (NNM), equimolar concentrations of acrolein and the monofunctional analogon of activated CP were inactive. NNM, the ultimate cleavage product within the successive degradation of the oxazaphosphorine structure was five times more toxic than 4-OOH-CO, whereas the cytotoxic action of PM was only slightly enhanced. The high cytotoxicity of NNM was matched by its ability to induce DNA interstrand cross-links: at concentrations and treatment times producing equal cell killing, 4-OOH-CP and NNM produced the same extent of cross-linking and DNA fragmentation. Biochemical potency of NNM is in contrast to data found with the NBP colorimetric assay which suggest that NNM loses its alkylating activity at neutral pH. 4-OOH-CP and PM are much more stable than predicted from half-life measurements performed via the NBP colorimetric assay; they retain a considerable fraction of their cytotoxic and cross-linking activity in spite of a 12-h preincubation at pH 7 and 36°C.

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4. R. Fleer, A. Ruhland and M. Brendel. The cytotoxic action of 'activated' and 'non-activated' cyclophosphamide in yeast: Comparison of induced DNA damage. Chem.-Biol. Interactions in the press (1982).

The cytotoxic and DNA-damaging effects of Cyclophosphamide (CP) and its "activated" derivative 4-OOH-CP were studied using a series of strains of S. cerevisiae which allow a phenotypical classification of genotoxic characteristics as well as direct physicochemical demonstration of key DNA lesions. The concurring results of biological and biochemical experiments indicate that (i) non-activated CP has a weak but detectable monofunctional alkylating potency, leading to DNA strand breaks and (ii) 4-OOH-CP has the ability to induce both DNA strand breaks and interstrand cross-links. The activity of CP is probably due to spontaneous decomposition in aqueous solution.

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Apart from general genetic analysis we have started to characterize the most promising of a large number of yeast mutants sensitive to the

bifunctionally alkylating agent nitrogen mustard. Below follow the abstracts of three papers published recently or in press.

5. W. Siede and M. Brendel. Isolation and characterization of Yeast mutants with thermoconditional sensitivity to the bifunctional alkylating agent nitrogen mustard. Current Genetics, 4, 145-149 (1981).

Selection of mutants of Saccharomyces cerevisiae sensitive to the DNA cross-linking agent nitrogen mustard (HN2) at two temperatures (23°C and 36°C) yielded two isolates with thermoconditionally enhanced (ts) sensitivity to the mutagen. Both were due to single recessive nuclear genes. Mutant allele snm1-2^{ts} showed mainly ts-sensitivity to HN2, whereas mutant allele snm2-1^{ts} conferred ts-sensitivity to HN2, half mustard (HN1) and UV. In temperature-shift experiments it was determined that the functions of SNM1 and SNM2 are needed for recovery within 6 to 7 h after mutagen exposure during incubation at 23°C on YEPD when HN2 and UV are applied. After HN1 treatment the SNM2 coded function is required for recovery for about 14 hrs. This possibly indicates a handling of UV- and HN2-induced lesions different from that of HN1-induced lesions.

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6. W. Siede and M. Brendel. Interactions among genes controlling sensitivity to radiation (RAD) and to alkylation by nitrogen mustard (SNM) in yeast. Current Genetics, in the press (1982).

Three haploid yeast mutants (snm) sensitive or thermoconditionally sensitive to the DNA cross-linking agent nitrogen mustard (HN2) were crossed with four rad strains representing mutations in the three pathways of DNA dark repair. The resulting haploid double and triple mutant strains were tested for their sensitivity to UV, HN2 and HN1. From the observed epistatic or synergistic interactions of the combinations of mutant alleles we could derive the relation of the SNM1 and SNM2 genes to the postulated repair pathways. Alleles snm1-1 and snm1-2^{ts} were found epistatic to genes of the rad3 group, whereas snm2-1^{ts} was epistatic to rad6. The snm1 and snm2 mutant alleles interacted synergistically. From these data it is concluded that the SNM1 gene product plays a cross-link specific role in excision repair while the SNM2 gene product may be involved in a system of error-prone repair.

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7. W. Siede and M. Brendel. Mutant gene snm2-1^{ts}, conferring thermoconditional mutagen sensitivity in Saccharomyces cerevisiae is allelic with RAD5. Current Genetics, in the press (1982).

Of two mutant genes (snm1-2^{ts} and snm2-1^{ts}) conferring thermoconditional mutagen sensitivity in Saccharomyces cerevisiae one (snm2-1^{ts}) is shown to be centromere-linked. At the restrictive temperature this allele reduces UV-induced back mutation frequency of the ochre allele his5-2 but has no influence on forward mutation at the CAN1 locus. Complementation tests and recombination analysis revealed snm2^{ts} to be allelic with rad5 (rev2).

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We are also studying the phenomenon of dTMP uptake in yeast. Below follow abstracts of four manuscripts recently submitted for publication.

8. W.W. Fäth, Q. Majid, E.M. Hartmann and M. Brendel. Nucleic acid metabolism in yeast. III. Identification of the dTMP-uptaking principle as a cytoplasmic membrane permease.

Summary. Evidence is presented for the existence of a Saccharomyces cerevisiae cytoplasmic membrane permease (TIP) importing exogenous dTMP at permissive conditions. The permease is specified by the following criteria: (1) Its activity depends on cellular energy metabolism, anaerobic route alone or aerobic route alone or anaerobic plus aerobic route. (2) The energy of activation of the TIP is 124 kJ. (3) There is a clear dependence of the TIP on the extracellular pH (maximal activity at pH 3.4; lowered activity at pH < 3.4 and at pH > 3.4). (4) The system's substrate constant is K_S = 20 µM dTMP, indicating rather a high substrate affinity. (5) The TIP can be adjusted to a multitude (>11) of discrete levels of activity, the minimal level, as realized in a seemingly wild-type yeast, being lower by a factor of at least 1000 as compared to the maximal level as verified in highly efficient utilizers of exogenous dTMP. (6) The yeast TIP is adjustable to discrete constitutive levels of activity as defined by discrete levels of v_{max}, i.e., it is not induced by any deficiency in the thymidylate biosynthesis pathway. (7) The TIP is distinguished by an extremely high specificity for the dTMP. Not even minimal alterations of this nucleotide are accepted by the permease. It is suggested that the eusubstrate of the TIP might be the dTMP of the physicochemical status prevailing at pH 3.4, i.e., the dTMP molecule with one negative charge on its 5'-coupled phosphate and the other physico-chemical features as resulting from a pH 3.4. The physiological significance of the TIP is discussed. It is concluded that dTMP import in S. cerevisiae is an "illicit" transport, and that TIP is a pseudonym for a yeast cytoplasmic membrane permease normally destined to perform functions different from importing exogenous dTMP.

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9. W.W. Fäth and M. Brendel. Nucleic acid metabolism in yeast. IV. A clue to the cell physiological function of the yeast cytoplsmic membrane permease importing exogenous dTMP.

Summary. A mutant of Saccharomyces cerevisiae, highly efficient utilizer of exogenous dTMP via a dTMP-specific cytoplasmic membrane permease, was characterized as a mutant with severely impaired activities in its cytoplasmic membrane P_i permeases (total deficiency in its low affinity P_i permease; significantly reduced activity in its high affinity cytoplasmic membrane P_i permease). The (tlr) mutant is constitutive for acid phosphatase/nucleotidase, especially for the acid phosphatase/nucleotidase species of yeast known to be relatively heat-sensitive and defined by a substrate constant K_S = .9 mM phosphomonoester, and extremely sensitive towards >> 10 µM dTMP and other (d)NMP. Exogenous dTMP at >> 10 µM inhibits import of P_i, competitively, in the (tlr) mutant. A

preliminary model of the yeast dTMP-uptaking principle (TIP) is presented, a model interpreting the TIP as a subfunction of the yeast cytoplasmic membrane low affinity P_i permease. The pehnomenon of dTMP-mediated cytostasis and cytotoxis, resp., as observed in some yeast (tlr) mutants is discussed. It is concluded that dTMP-mediated cytostasis can be explained by a blockage of P_i-import via the cytoplasmic membrane low affinity P_i permease; the cytotoxic effect of exogenous dTMP is interpreted as a consequence of blockage of the yeast cytoplasmic membrane low affinity P_i permease and of intracellular events subsequent to non-feedback-controlled dTMP import. - Yeast (tlr) mutants, sensitive or not sensitive towards exogenous dTMP, are understood as yeast (phoT) mutants.

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10. W.W. Fäth and M. Brendel. Nucleic acid metabolism in yeast. V. Excretion of thymidylate.

Summary. It is shown that highly efficient utilizers of exogenous dTMP of the yeast Saccharomyces cerevisiae are able to similarly efficiently excrete the nucleotide. Strains P_i-repressible in acid phosphatase/nucleotidase excrete dTMP at extracellular high P_i; strains constitutive for this enzymic activity excrete dThd. Excretion of thymidylate and dThd, resp., unlike import of exogenous dTMP, seems to be unaffected by the extracellular pH, by the extracellular presence of dTMP, and rather independent from the extracellular presence of a metabolizable carbohydrate such as D(+)-glucose. A model of the yeast dTMP-uptaking principle (TIP) is presented suggesting its also being responsible for export of endogenous thymidylate.

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11. W.W. Fäth and M. Brendel. Nucleic acid metabolism in yeast. VI. Utilization of exogenous dAMP.

Summary. It is shown that mutants of Saccharomyces cerevisiae able to highly efficiently utilize exogenous dTMP can also utilize exogenous dAMP. At extracellular conditions permissive for dTMP import label stemming from offered [8-^{3}H] dAMP preferentially is incorporated into alkali resistant high molecular weight material (putative DNA): only about 30% of high molecular weight cell-bound dAMP label were found to be sensitive towards mild alkali hydrolysis. This putative RNA label can be minimized to practically zero when $\geq$ mM Ade is employed in a dAMP labelling assay. Exogenous dAMP at an offer of >> 10 µM was found to be similarly cytostatic as >> µM dTMP and to similarly effectively inhibit import of exogenous P_i. We conclude from our results that there exists a yeast cytoplasmic membrane permease able to import dAMP. A model of this hypothetical permease system is presented.

XVII. University of Bielefeld, Faculty of Biology, P.O. Box 8640, 4800 Bielefeld 1, West Germany. Communicated by G. Michaelis.

Below follows the summary of a paper which in in press in Molec. Gen. Genetics.

Georg Thierbach[+] and Georg Michaelis[++]. Mitochondrial and Nuclear Myxothiazol Resistance in Saccharomyces cerevisiae.

[+]Gesellschaft für Biotechnologische Forschung, Abteilung Mikrobiologie, Mascheroder Weg 1, D-3300 Braunschweig, Federal Republic of Germany.

[++]Universität Bielefeld, Fakultät für Biologie, Postfach 8640, D-4800 Bielefeld 1, Federal Republic of Germany.

Summary

Mitochondrial and nuclear mutants resistant to myxothiazol were isolated and characterized. The mitochondrial mutants could be assigned to two loci myx1 and myx2 by allelism tests. The two loci map in the box region, the split gene coding for apocytochrome b. Locus myx1 maps in the first exon (box4/5) whereas myx2 maps in the last exon (box6). The nuclear mutants could be divided into three groups: two groups of recessive mutations and one of dominant muations. Respiration of isolated mitochondria from mitochondrial mutants is resistant to myxothiazol. These studies support the conclusion that myxothiazol is an inhibitor of the respiratory chain of yeast mitochondria. The site of action of myxothiazol is mitochondrial cytochrome b.

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

The following are abstracts of work from our laboratory.

1. A. Brunner, N. Carrasco, and A. Pena, Correlation between Resistance to Ethidium Bromide and Changes in Monovalent Cation Uptake in Yeast. Archives of Biochemistry and Biophysics, Vol. 216, No. 2, July, 1982.

A mutant of Kluyveromyces lactis resistant to ethidium bromide was studied and found to have an impairment to transport the dye. As described for other mutants of this kind, the fluorescence changes of the dye that are observed when the cells transport it, were not observed in the mutant strain. Simultaneous to this difficulty to take up the mutagen, the cells showed a diminished ability to take up monovalent cations, as compared to the wild-type strains. The defect of the mutant strain does not seem to reside in the capacity to pump out protons, which also indicates that it has no alterations of the general energy conversion systems. This view is also supported by the fact that the growth yields are similar in both the mutant and the wild-type strains. Both ethidium and K^+ fail to stimulate respiration of the mutant yeast when present in the medium, as compared to the wild-type strains. The mutant strain shows a normal cation content, which indicates that the impairment to take up monovalent cations, although much decreased, may still be enough to maintain a normal content of cations within the cells. According to the investigation carried out, the mutant cells seem to be normal, except for the fact that they are unable to transport both ethidium and K^+ from the medium. The data support the hypothesis that ethidium bromide and K^+ may be transported by the same system in yeast.

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2. S. Uribe, R. Alvarez and A. Peña. Effects of beta-pinene, an allelopathic monoterpene, on yeast cells and isolated mitochondria.

SUMMARY

Beta pinene is an active inhibitor of yeast metabolism and many effects have been found in isolated mitochondria that point to these organelles as one of the main sites of action of the molecule.

All effects found can be explained by the binding of beta pinene to the cell membranes.

Other hydrophobic small molecules tested do not show the effects of beta pinene or limonene on yeast cells or mitochondria.

In mitochondria, beta pinene at low concentrations behaves as an uncoupler as shown by the stimulation of ATPase activity and state 4 respiration with succinate as substrate. The basis for this effect seems to be an increase of the passive permeability of the inner mitochondrial membrane.

At higher concentrations (from 400 μM) beta pinene inhibits respiration showing a more pronounced effect with site I substrates.

At the highest concentrations tested (600 to 1200 μM) beta pinene seems to produce a partial resealing of the mitochondrial membrane.

XIX. Brooklyn College of The City University of New York, Brooklyn, New York 11210. Communicated by Nasim A. Khan.

The following paper has been accepted for publication in Molecular and General Genetics: "Suppression of Maltose-Negative Phenotype by a Specific Nuclear Gene (PMU1) in Petite Cells of the Yeast Saccharomyces cerevisiae" Nasim A. Khan.

SUMMARY

A small percentage of the primary petites isolated from strain 1403-7A-P1, constitutive for maltase synthesis, simultaneously lost the ability to utilize maltose and alpha-methylglucoside. Further studies showed that these primary petites were not stable with respect to maltose utilization. Approximately 30% of the secondary petites when isolated from the primary petites after vegetative growth were found to papillate on maltose plates. Tetrad analysis data revealed that a nuclear gene had reverted in these papillae, which is responsible for suppression of the maltose negative phenotype in primary petites. We have designated this nuclear gene as the PMU1 gene (petite maltose utilizer). The functional form of the PMU1 gene is required in addition to the MAL4 gene for both constitutive maltase synthesis and maltose utilization in cytoplasmic petite cells derived from strain 1403-7A-P1.

XX. Instituto de Quimica, Universidade Federal do Rio de Janeiro, Cidade Universitaria, Ilha do Fundão, Bloco A-Lab 547, Rio de Janeiro, Brasil. Communicated by Anita Panek.

Below follow abstracts of two recently prepared publications from our laboratory.

1. A. Chvojka[1], M. Barlas[1], H. Ruis[1], G.R.C.B. Padrão[2], A.D. Panek[2], and J.R. Mattoon[3].

1. Institut für Allgemeine Biochemie der Universität Wien, Ludwig Boltzmann-Forschungsstelle für Biochemie, A-1090 Wien, Wahringer Strasse 38, Austria.

2. Department of Biochemistry, Institute of Chemistry, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Bloco A, Rio de Janeiro, Brasil.

3. Department of Biology, University of Colorado, Colorado Springs, Colorado 80907, USA.

Following our investigations into the pleiotropic character of the glc1 Mutation (Mol. Gen. Genet. accepted Feb/82) catalase formation was studied. Mutations at the GLC1 locus in Saccharomyes crevisiae result in a major deficiency in synthesis of catalase T, but do not affect catalase A. Three independent glc1 mutations were shown to have the same pleiotropic phenotype: catalase T deficiency, defective glycogen synthesis and defective trehalose accumulation. These three deficiencies appear to be determined by a single, nuclear gene. The possibility that glc1 mutations alter a protein kinase is considered.

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2. A.B. Freitas-Valle, R.R. Menezes, A.D. Panek and J.R. Mattoon*.

* Department of Biology, University of Colorado, Colorado Springs, Colorado, USA.

The conversion of glucose to extracellular succinic acid under non-proliferating conditions was measured in normal and mutant yeast cells. Cells exhibiting different levels of mitochondrial development were prepared by culturing normal yeast in media containing glucose, maltose and ethanol, respectively. Increased capacity for succinate excretion was correlated with increases in the respiratory quotient and in mitochondrial cytochrome levels in the different yeast preparations. Diversion of the Krebs cycle intermediate α-ketoglutarate to glutamate, by addition of ammonium ions, caused marked inhibition of succinate excretion. Derepressed cells containing different levels of cytochromes were obtained by growing a heml mutant, lacking 5-aminolevulinate synthase, in maltose media containing different concentrations of 5-aminolevulinate. Again, a positive correlation of succinate excretion with cytochrome levels was found. The results of this study indicate that succinate excretion by resting yeast cells provides a convenient means for observing the in vivo activity of the Krebs cycle in yeast. The data also suggest that the

biosynthesis of cytochromes and Krebs cycle enzymes might be coordinately regulated by porphyrin supply.

XXI. Institut für Genetik und Mikrobiologie der Universität München, Maria Ward Strasse 1a, 8000 Munich 19, Federal Republic of Germany. Communicated by Klaus Wolf.

The following is an abstract of recent work from our Institute.

Gertrud Seitz - Mayr and Klaus Wolf. An Extrachromosomal Mutator Inducing Point Mutations and Deletions in the Mitochondrial Genome of Fission Yeast. Proc. Nat. Acad. Sci. U.S., accepted.

Abstract

We report the isolation and characterization of a mutator mutant in the fission yeast Schizosaccharomyces pombe. This mutator is of extrachromosomal, very likely mitochondrial inheritance and acts exclusively on mitochondrial DNA. It greatly enhances the frequency of spontaneous mitochondrial drug resistance mutants compared to the wild type, but it is not obligatory for their occurrence. In contrast, mitochondrial respiratory deficient mutants can only be isolated from mutator strains. It could be shown that this mutator induces point mutations as well as deletions in the mitochondrial genome which lead to respiratory deficiency. This mutator might prove to have a novel function encoded by the mitochondrial DNA.

XXII. Institute of Genetics, University of Parma, Borgo Carissimi 10, 43100 Parma, Italy. Communicated by Pier Paolo Puglisi.

Below follow abstracts of papers recently published:

1. Ultrastructural analysis of the life cycle of an Apomictic (apo) Strain of Saccharomyces cerevisiae. I. Meiosis and Ascospore development. N. Marmiroli, C. Ferrari, F. Tedeschi, P.P. Puglisi and C. Bruschi. Biol. cell (1981) 41, 79-84.

The apomictic strain of Saccharomyces cerevisiae 4117-H2 (apo) produces in acetate sporulation medium asci with two diploid spores. The ultrastructural analysis of the sporulation process had evidenced that the apomict depended on a defect in apo meiosis and not on a mitosis associated sporulation. In sporulation medium the apo nucleus underwent all the early modifications of Meiosis I nucleus as in APO strains: axial cores, synaptonemal complexes and polycomplexes appeared at zygotene-pachytene; Meiosis I spindle pole body which was single till diplotene, duplicated in diplotene and, in diakinesis, separated into two spindle pole bodies that appeared elongated as in prophase of Meiosis I of APO strains. At this stage, spindle pole bodies and nucleus took on the characteristics of Meiosis II and the nucleus did not elongate but it divided before further division of the two spindle pole bodies; in this way the prespore wall formation enveloped nucleoplasmic material that had not been subject to reductional division. After apo meiosis, the two nuclei formed were properly encased by spore walls and we had not observed formation of additional spore wall components within the ascogenous cell, indicating

that prespore walls biosynthesis was controlled either by the number of nuclei formed or by the number of spindle pole bodies present after prophase II. The ultrastructural modifications that took place in other cellular components: mitochondria, vacuoles, cytoplasm, during sporulation of apo, resembled those observed in APO strains. During apo sporulation not only single cells but also multibudded cells could undergo meiosis and spore formation. Daughter cells usually were late in sporulation and produced only single-spored asci though the two-spored specific phenotype of apo was genetically determined.

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2. Ultrastructural analysis of the life cycle of an Apomictic (Apo) Strain of Saccharomyces cerevisiae. II. Mitotic reproduction. N. Marmiroli, F. Tedeschi, C. Ferrari, P.P. Puglisi and C. Bruschi. Biol. Cell (1981) 41, 85-90.

As described in the previous paper, SPIII sporulation medium supported both mitosis and meiosis; the switch from one type of cell division to the other occurred after several mitotic reproductive cycles. Therefore using the SPIII sporulation medium a continuous analysis of the morphological and ultrastructural modifications that take place during mitosis and meiosis and during the course of the switch from the former to the latter process can be made.

In this paper we report the results of morphological and ultrastructural observations on the reproduction of apo as it occurred until the sixth hour after the transfer in SPIII medium; these observations were also representative of the apo reproduction in YPD medium. The results obtained indicated that no structures resembling axial cores, synaptonemal complexes and polycomplexes were established or became detectable till the sixth hour in SPIII medium; moreover, duplication and modification of the nucleus, the spindle and the spindle pole body were different from those featured for apo after the sixth hour in SPIII medium. This fact confirms that after six hours in SPIII medium apo switched from mitosis to prophase of Meiosis I as it occurred, in the same condition, for APO cells. Up to the sixth hour apo divided mitotically although with some exceptions, as featured for budding yeasts in the Hartwell model. Two additional independent pathways for cell division, bypassing cytokinesis and cell separation, were proposed on the basis of the data reported; in addition, the existence of a true G1 period in the apo cell division cycle seemed to be doubtful; preparation for a new cycle can occur at any time during cell division. The relationship between apo meiotic defects and apo mitosis is discussed.

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3. IMP1/impl: a gene involved in the nucleo-mitochondrial control of galactose fermentation in Saccharomyces cerevisiae. F. Tassi, C. Donnini, C. Bruschi, P.P. Puglisi and N. Marmiroli. IN: "Developments in Biophysical Research" (1980) Plenum Publishing Corp. N.Y.

In some strains of Saccharomyces cerevisiae, the induction of enzymes of the Leloir pathway, galactose fermentation and growth on galactose

depend on mitochondrial function; mitochondrial dependence is elicited through the recessive allele impl of the nuclear gene IMP1. The genetic element IMP1 is not allelic to any of the known GAL genes; IMP1 strains can grow on and ferment galactose in respiratory-deficient (RD) condtion or in the presence of the mitochondrial inhibitors ethidium bromide and erythromycin; whereas, impl strains can grow on and ferment galactose only in respiratory-sufficient (RS) condition. The impl elicited mitochondrial dependence apparently involves regulation of the synthesis of the galactose catabolizing enzymes and synthesis of the galactose specific permease. IMP1 is not the only genetic determinant that elicits an interaction of the mitochondrion and the expression of the GAL system; the GAL3 gene, whose role in galactose utilization is demonstrated by the long-term adaptation phenotype of gal3 RS mutants, gives rise to a noninducible phenotype in RD condition or in the presence of mitochondrial inhibitors.

XXIII. Institut für Mikrobiologie - Genetik -, Technische Hochschule, Schnittspahnstr. 10, D-6100 Darmstadt, Fed. Rep. Germany. Communcated by F.K. Zimmerman.

Genetic Analysis of Yeast Phosphofructokinase - thesis project of Dr. I. Breitenbach-Schmitt. Mutation in two genes, PFK1 and PFK2, can eliminate totally phosphofructokinase activity as measured in vitro without suppressing glycolysis. Only double mutants with simultaneous defects in both genes also eliminated all glycolytic activity. A comparison between wild type and single pfk1 or pfk2 mutants showed that after a shift from an ethanol medium to a glucose medium, only wild type cells would start to ferment immediately, the pfk mutants started to produce fermentative CO_2 only after a lag period of more than 90 min. Comparing specific radioactivity in CO_2 formed from uniformly and 3,4-position-labelled glucose showed that a preferential or almost exclusive formation of fermentation CO_2 from glucose carbon atoms 3 and 4 occurred only during the first 2 - 3 hours, after that other carbon atoms contributed considerably. In pfk mutants with a single defect, CO_2 was formed from all carbon atoms at equal rates suggesting a complete randomization of the glucose atoms during glycolysis without phosphofructokinase activity. Starting from pfk1 or pfk2 single mutants which still fermented glucose, mutants were isolated that were completely blocked in glycolysis. There were mutants with additional pfk defects, other glycolytic blocks and finally some in a pathway called phosphofructokinase bypass. Such byp mutants all by themselves did not reveal any drastic effects, only in combination with a single pfk mutant did they block glycolysis. A new phosphorylated sugar was accumulated in pfk and byp single mutants and at very high level in pfk byp double mutants. This turned out to be sedoheptulose-7-phosphate. A byp single mutant would accumulate high levels of fructose-1,6-bisphosphate, the product of the phosphofructokinase reaction. All this was interpreted to mean that there are two pathways between fructose-6-phosphate and the triose phosphates: The classical phosphofructokinase aldolase reactions and now the newly discovered bypass reaction with sedohepuptulose-7-phosphate as an intermediate. The second reaction takes about 2 h to become established after a shift from gluconeogenic carbon sources to glucose medium.

Genetic Analysis of Yeast Pyruvate Decarboxylase - current thesis project of H.D. Schmitt. Pyruvate decarboxylase mutants have been isolated and

shown to affect a structural gene PDC1 (mutants with altered enzyme properties) and a presumptive regulatory gene PDC2 (reduced levels of normal enzyme). Yeast pyruvate decarboxylase is not a constitutive enzyme, it is formed at high levels only in the presence of fermentable sugars, even though (aside from a single pdc1-8 mutant) all mutants showed considerable residual activity after several hours of incubation in glucose medium. After growth in the absence of glucose, enzyme levels were very low. Mutant strains carrying either pdc1 or pdc2 defective mutant alleles were transformed with plasmid YRp7 into which were ligated DNA fragments from a partial Sau 3A yeast DNA digest. After selection for glucose utilization, 4 transformants were obtained for the pdc1 and 2 for the pdc2 defective recipient mutant strains. All 4 pdc1 transformants had the same 5.5 kb insert. This hybrydized to a ply(A) RNA of 2 kb. This PDC1 mRNA was high in glucose grown cells, low after growth on ethanol, low in pdc2 mutants but normal in pdc1 (structural) gene mutants. This was interpreted to mean that PDC2 is in fact a regulatory gene. When PDC2 DNA was used as a probe for mRNA, no hybridization could be detected either to pol(A) or total RNA. The length of the PDC2 insert was 2.5 kb. Stable transformants were obtained for both fragments. Tetrad analysis revealed that the two fragments were inserted at or very close to the location of their original genes. This was taken as a final evidence supporting the claim that the two fragments were indeed representing PDC1 and PDC2, respectively. As shown previously, mutants with a defect in the phosphoglucose isomerase gene PGI never reached the full glycolytic pyruvate decarboxylase activities when grown on fructose. However, this reduced enzyme activity was not reflected in reduced mRNA levels hinting at a possible regulation at a post-transcriptional stage. Consequently, pyruvate decarboxylase synthesis is under the transcriptional control of gene PDC2 and a possible post-transcriptional control mediated in some unknown way by gene PGI, the structural gene of phosphoglucose isomerase.

Genetic Analysis of Yeast Maltase Formation - current thesis project of C. Stüwer. A pool derived from yeast strain R234 with gene MAL1 (partial Sau 3A total yeast DNA digest) was used to transform yeast strains without any MAL genes. Maltase activities in strain R234 after growth on maltose media reached about 1 unit. Transformants between 0.58 to 0.8 units were selected for further investigation. Three types of fragments were found. Five fragments had a common 2.8 kb insert while two more were entirely different from those five and also between themselves. After re-isolation from transformed yeast, fragments were amplified in E. coli and used to transform other yeast strains without MAL genes. Among those re-transformed plasmids, the one with the longest inert was used to probe BamHI and SalI yeast DNA digests. No matter what the genotypes of the strains were, with MAL1, without MAL1 but without any other MAL gene, or with SUC1, almost allelic to MAL1, or with either MAL2, MAL3 and MAL4, in all cases, the recombinant plasmid hybridized to the TRP1 fragment which is part of YRp7 and an additional band. This additional band was the same in all cases. Consequently, at present it is quite obscure what the function of the isolated genome fragments are. However, they bestow on cells without a resident MAL gene the ability to form maltase. Stable transformants have been obtained and crossed to a MAL1 strain, tetrad analysis has still to be performed.

Mutagenicity Testing: A Search for Inducers of Mitotic Chromosomal Malsegregation. A diploid strain D61.M has been constructed with the following relevant markers.

$$\frac{cyh2}{+}\ \frac{+}{trp5}\ \frac{leu1}{+}\ \text{centromere}\ \frac{ade6}{+}\ \frac{ade2}{ade2}\ \frac{ilv1\text{-}92}{ilv1\text{-}92}$$

Mitotic chromosome loss results in the appearance of colonies on a medium with cycloheximide (recessive resistance), white colony color (expression of recessive ade6) and a requirement for leucine (recessive leu1). Mitotic recombination (crossing-over or gene conversion) or point mutation CYH2 to cyh2 also create the formation of resistant colonies which are preponderantly red thus monitoring induction of additional genetic events. Double mitotic recombinants between centromere and cyh2 and centromere and ade6 will also yield white resistant colonies. However, due to the short distance between leu1 and the centromere, most of those whites will not express leu1. The treatment protocol was: culture in a synthetic medium with minimal supplements starting with about 500 000 cells/ml with mutagen solution added is grown for about 16 -18 h to a final titer in the control of 2×10^7 cells/ml. This protocol provides a highly sensitive test. The lowest active dose for mitomycin C was below 0.55 ppm; EMS with less than 0.2 µl/ml, 4-nitroquinoline oxide less than 62.5 ng/ml. However, the effects were only on the induction of mitotic recombination. Evidence for a definite induction of mitotic chromosome loss was only obtained for p-fluorophenylalanine and MMS, the other mutagens gave only an erratic and occasional positive response. Mitotic recombination served as a positive internal control demonstrating that there was induction of genetic events even though not in respect to mitotic chromosomal malsegregation. Such chemicals were: diepoxybutane, hydrazine sulfate (maybe a weak effect on chromosome loss), hycanthone, dibromoethylene, chloroform (very tricky), acridinium chloride, acridine orange, acriflavine (very potent inducer of mitotic recombination). Totally negative were: ethionine, canavanine, 5-fluorouracil, diethylstilbestrol, sodium dodecyl sulfate, sarcosyl, econazol nitrate (a membrane active antimycotic drug) and heavy metal salts: $CoNo_3$, $Hg\ Cl_2$, LiCl, K_2CrO_7, $MnCl_2$, $NiSO_4$, lead acetate). At present, it is not clear whether the treatment protocol, very sensitive for the induction of mitotic recombination, is adequate for the detection of mitotic chromosome loss since no agent has been identified that would efficiently induce this effect. As matters stand now, mitotic chromosome loss could be a very rare effect induced by even very potent mutagens.

XXIV. Department of Genetics, Microbiology and Biophysics, Faculty of Science, Charles University, Prague, Czechoslovakia. Communicated by Olga Bendová.

Below follow abstracts of two manuscripts.

1. Application of killer toxin in selection of hybrid strains in yeasts. Kupcova, L., Janderova, B., Bendova, O., Vondrejs, V.

Abstract

It was demonstrated by induced intraspecific and interspecific protoplast fusion of killer and killer sensitive strains of Saccharomyces sp., that killer toxin is a very useful tool for selection of hybrid

strains. The toxin production and the killer resistance can serve as the transient genetic markers for selection because hybrid strains can be easily cured by heat (in the same way as other killer strains of Saccharomyces cerevisiae).

The selection by means of killer toxin is highly recommended particularly for construction of new industrial strains because the killer toxin sensitivity is a common natural marker of parental industrial strains.

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2. A rapid assay of killer toxin interaction with sensitive yeast cells. Gaskova, D., Plasek, J., Vondrejs, V.

Abstract

The mechanism of action of the killer toxin from Saccharomyces cerevisiae T158 C was investigated with a lipophilic fluorescence probe N-phenyl-1-naphtyl-amine (NPN). After toxin is added to sensitive cells the probe fluorescence increases. The largest increase in intensity is at 430 nm. It depends particularly on toxin concentration, ageing of the toxin preparation and the cell titer. It was proved that the observed effect is killer specific and most likely caused by the interaction of toxin with the cytoplasmic membrane. When the active toxin is added to a cell suspension pretreated with the heat-inactivated toxin the stimulation effect is reduced.

XXV. Biology Departments, Thames Polytechnic and Goldsmiths' College of London University, London, England. Communicated by J.F.T. Spencer.

1. J.F.T. Spencer and Dorothy M. Spencer. The Use of Mitochondrial Mutants in Hybridization of Industrial Yeasts. III. Resortation of Mitochondrial Function in Petites of Industrial Yeast Strains by Fusion with Respiratory-Competent Protoplasts of Other Yeast Species. Submitted to Molecular and General Genetics.

Summary

Protoplasts of petites of strains 625-C^r of Saccharomyces diastaticus and NCYC 1085 of Saccharomyces cerevisiae, originally obtained from the National Collection of Yeast Cultures, England, were fused with protoplasts of Candida pseudotropicalis, Saccharomyces rosei, Saccharomyces montanus, Pichia membranaefaciens, Hansenula anomala, Hansenula capsulata, and Schizosaccharomyces pombe. The respiratory-competent products of the fusions were selected on the basis of using at least one of the carbon sources utilized by the petite parent and not by the other. The products of the fusion of C. pseudotropicalis x 1085(p^-) consisted of two cell types; an oval cell which utilized both lactose and maltose and fermented lactose vigorously, and a cylindrical form which fermented maltose slowly. The S. rosei x 1085(p^-) hybrids had acquired the ability to metabolize and ferment galactose, and to ferment maltose, from the petite parent. The P. membranaefaciens x 625(p^-) hybrids acquired the ability to

metabolize galactose, sucrose and maltose, but fermented only glucose, weakly, like the P. membranaefaciens parent strain. The H. capsulata x 625(p^-) hybrids, unlike the hybrids with P. membranaefaciens of S. rosei, resembled the petite parent morphologically and also had the fermentative capacities of this strain (galactose, maltose, sucrose and starch), and the ability to ferment starch was considerably enhanced. The S. montanus x 625(p^-) hybrids acquired the ability to utilize starch. Schizosaccharomyces pombe x 625(p^-) hybrids resembled S. pombe morphologically, but had the ability to metabolize galactose and starch. Some of the asci produced by these hybrids contained abnormal numbers of ascospores. H. anomala x 625(p^-) hybrids fermented starch, though weakly.

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2. J.F.T. Spencer, Dorothy M. Spencer and Renee Miller. Loss of Ability to Utilize Sucrose by the Petite Mutant of a Distiller's Yeast.

We have found a strain of Saccharomyces cerevisiae which appears to be unusual in its failure to metabolize sucrose when converted to the petite form. We have made mutants both by acriflavin and ethidium bromide treatment, and in both cases the petite mutant does not metabolize this sugar. We have sporulated this strain, which is a homothallic diploid or near-diploid and produces, normally, two viable spores/ascus, and tested the segregants, and these, too, fail to utilize sucrose when converted to petites. We have also crossed this strain with three different mating diploids (two $\alpha\alpha$ and one aa mating types), and have observed that from one $\alpha\alpha$ strain, the segregants from the hybrid were all suc^- in the petite form, from the other, the suc^- character segregated 2:2, and in the hybrid with the aa diploid, the segregants were all suc^+ in the petites.

We have tested the cell-free extract from the suc^- petite, and found that it contained an active invertase. We have not determined whether the enzyme present was the glycosylated or non-glycosylated form. There was no invertase activity in the pellet of cell walls and unbroken cells. Thus, the petite mutation apparently interferes with transport of the enzyme out of the cell, or, possibly, with glycosylation of the enzyme protein. This situation appears to be the opposite to that observed by Wilkie and Evans where the petite mutation prevents transport of galactose or maltose into the yeast cell.

Our strains differed from the mutants SUC2° isolated by Carlson et al. (1981), which did not synthesize invertase at all, and from the SNF mutants they described, which showed a pleiotrophic effect and did not utilize maltose, galactose or sucrose, but which formed the non-glycosylated form of invertase. Strains which lack the ability to metabolize sucrose when converted to the petite form appear to be relatively rare, only two such strains being known to us at present. Recently we have found a killer strain which possessed this character, however. Conversion to the petite form, though it abolished the ability to utilize sucrose, did not affect production of the killer toxin.

Marian Carlson, Barbara C. Osmond and David Botstein (1981). Mutants of yeast defective is sucrose utilization. Genetics, 98, 25-40.

XXVI. Technical Research Centre of Finland (VTT), Biotechnical Laboratory, Tietotie 2, 02150 Espoo 15, Finland. Communicated by Veijo Mäkinen.

The following papers have been submitted (or accepted) for publication:

1. Maija-Liisa Suihko and Veijo Mäkinen. Candida krusei in Baker's Yeast Production. European Journal of Applied Microbiology and Biotechnology 13: 113-116 (1981).

Summary

Candida krusei is a harmful contaminant in baker's yeast manufacture, because it grows much faster than Saccharomyces cerevisiae under production conditions. This investigation showed that C. krusei utilizes the ethanol produced by baker's yeast as sole carbon source when molasses is used as a substrate. When the alcohol concentration in the effluent air is used as a parameter for controlling the aeration of the culture, conditions become favourable for the dominance of wild yeast because some of the ethanol produced by the baker's yeast is consumed immediately by C. krusei and aeration is then automatically reduced, leading to increased growth of the wild yeast.

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2. J. Ahvenainen and V. Mäkinen. The Effect of Pitching Yeast Aeration on Fermentation and Beer Flavour. European Brewery Convention, Proceedings of the 18th Congress, Copenhagen 1981, 285-291.

Summary

Traditional, anaerobic pitching yeast was replaced by aerobically grown yeast. Lager-type beer of normal and high gravity worts was brewed in laboratory and pilot scale. Aerated yeast is rich in sterols and unsaturated fatty acids. Wort aeration is not needed and so the oxydation of flavour compounds in wort is avoided. The fermentation time was shortened by about 25 percent compared with the anaerobic pitching yeast. Concentrations of acetate esters in the final beer were reduced, whereas fusel alcohol concentrations were slightly increased. Vicinal diketone concentrations were strongly reduced after the primary fermentation. The yeast yield after the primary fermentation was increased by about 10 percent if the pitching rate was unchanged. No off-flavours were detected in beers produced by aerated pitching yeast. This work is part of the research concerning yeast lipids and beer flavour being carried out in this laboratory. In practical brewing situations pitching yeast aeration is useful, for example, when wort aeration is not advisable and/or when acetate ester concentrations cause flavour problems.

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3. J. Ahvenainen. Lipid Composition of Aerobically and Anaerobically Propagated Brewer's Bottom Yeast. Journal of the Institute of Brewing, accepted to be published 1982.

Summary

Aerobically grown pitching yeast was very rich in unsaturated fatty acids and sterol esters compared to traditional, anaerobic yeast. The principal fatty acids in aerobic yeast cells were unsaturated palmitoleic and oleic acids, whereas in anaerobic cells saturated palmitic acid predominated. The difference in fatty acid distribution between aerobic and anaerobic cells was most marked in the sterol esters. The fatty acids of phospholipids were more stable, although remarkable differences were observed. The sterols of aerobic cells were almost entirely in esterified form and zymosterol was the principal sterol. During the first hours of fermentation a rapid synthesis of palmitoleic acid was observed when anaerobic yeast was used for pitching and the wort was aerated. The synthesis of oleic acid required more oxygen and time than is available under normal brewing conditions. When aerobic pitching yeast was used no more unsaturated fatty acids were synthesized and the lipid stores of pitching yeast were distributed among the daughter cells. The decrease in acetate ester production by aerobic pitching yeast is concluded to be due to a decrease in acetyl CoA synthesis, which may be caused by the high proportion of unsaturated fatty acids in membrane lipids.

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4. A. Haikara and S. Boije-Backman. The Use of Optical Brighteners in the Detection of Bacterial and Yeast Contaminants in Beer. Brauwissenschaft 35, nr. 5 (1982).

Summary

A microcolony method for detecting both bacteria and yeast contaminants in beer is described. The beer sample is filtered through a membrane filter and the filter is incubated on agar containing 500 mg/l of the optical brightener Tinopal CBS-X (a derivative of distryryl-β-phenyl). After a short incubation time the filters are examined under a fluorescence microscope. Low levels of living bacteria and yeast cells can be detected as brightly fluorescing microcolonies.

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

Below follows a list of our work published since the December issue of the Yeast Newsletter (1981).

1. I. Molnar, E. Oura and H. Suomalainen. Study of Volatile Substances Produced During the Autolysis of Champagne Yeast. Acta Alimentaria 10: 27-36 (1981).

Intact and distintegrated Champagne Hautvillers yeast were added to tank-fermented as well as model sparkling wine and stored at different temperatures. Then, the changes in the composition of certain aroma substances were examined in the sparkling wine by gas chromatography. The concentrations of components of relatively high boiling point, viz., of

ethyl caproate, ethyl myristate, ethyl palmitate, ethyl palmitoleate, ethyl stearate, ethyl oleate, ethyl linoleate, cis-farnesol and trans-farnesol increased considerably. Raising of the temperature of seasoning enhanced the increase in the volatile components under study in the champagne. If sparkling wine had been aged in the presence of the same amount (expressed in yeast dry matter) of disintegrated yeast, the volatile substances would have reached 2 to 5 times as high concentrations in the sparkling wine as in the presence of intact yeast.

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2. Elke Parkkinen and Heikki Suomalainen. Esterases of Baker's Yeast. II. Substrate Specificities Towards Esters Formed During Sugar Fermentations. Journal of the Institute of Brewing 88: 34-38 (1982).

Ethyl esters of fatty acids (C_2 - C_{12}), isoamyl acetate and 2-phenethyl acetate were studied as substrates for yeast esterases and compared with the synthetic substrates, p-nitrophenyl esters and β-naphthyl esters. Intact yeast, the 55% and 55-75% ammonium sulphate precipitate of centrifuged yeast homogenate, and partly purified esterases were used for the determination of the hydrolytic activity towards the esters.

The results showed that the yeast esterases prefer to hydrolyse the ethyl esters with acyl chain length of C_5 to C_{12}. The acetate esters, ethyl acetate, isoamyl acetate and 2-phenethyl acetate are only very slowly hydrolysed or remain unaffected. The substrate specificity of different esterases varies and can be used for their characterization.

Investigating pH optimum curves using intact yeast and a crude esterase preparation and different substrates confirmed the earlier result that there are esterases on both sides of the plasma membrane. The specificity of intracellular and periplasmically located esterases, however, is different.

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3. Elke Parkkinen and Heikki Suomalainen. Esterases of Baker's Yeast. III. The Ester/Acid Ratio in Model Solutions. Journal of the Institute of Brewing 88: 98-101 (1982).

Ester/acid equilibria were studied for reaction mixtures containing ethyl alcohol, ethyl caprylate or caprylic acid, and baker's yeast or an esterase purified from baker's yeast in buffer. The equilibrium concentration of ethyl caprylate after an incubation of yeast or a yeast esterase preparation with caprylic acid was the same as in the case where yeast or esterase preparation was incubated with the ethyl caprylate. The equilibrium attained depends not only on the concentration of the ester and the alcohol but also on the pH, the final ester concentration remaining higher at lower pH. It could be shown that yeast esterase is responsible for the hydrolysis or synthesis of the ester. At equilibrium the molar ratio of ethyl caprylate/caprylic acid is about the same as that found in fermentation solutions under the same conditions.

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4. Heikki Suomalainen. Yeast - Alcohol Production and Bread Making. Advances in Biotechnology (vol. 4), Current Development in Yeast Research, ed. by Graham G. Stewart and Inge Russell, Proc. Int. Yeast Symp., 5th, London, Ontario, Canada 1980, Pergamon Press, pp. 3-8.

Aspects of fuel alcohol production, aroma of alcoholic beverages and leavening of bread, in all of which yeast plays a decisive role, are described.

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5. Erkki Oura and Reijo Tanner. Cellular Material and Water Pools in Baker's Yeast During Storage. Advances in Biotechnology (vol. 4), Current Developments in Yeast Research, ed. by Graham G. Stewart and Inge Russell, Proc. Int. Yeast Symp., 5th, London, Ontario, Canada 1980, Pergamon Press, pp. 47-50.

The dry matter content of baker's yeast during storage at room temperature for 7 days varied slightly owing to evaporation of water and simultaneous utilization of yeast organic material. The two processes acted in opposite directions and so tended to balance each other. Dry material decreased by 8% during this period, and the water loss was 7.4%. The water loss was exclusively extracellular, the amount of which decreased further because a part of it moved from outside the cells to the interior.

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6. K. Edelmann, P. Stelwagen and E. Oura. The Influence of Temperature and Availability of Oxygen on the Carbohydrates of Stored Baker's Yeast. Advances in Biotechnology (vol. 4), Current Developments in Yeast Research, ed. by Graham G. Stewart and Inge Russell, Proc. Int. Yeast Symp., 5th, London, Ontario, Canada 1980, Pergamon Press, pp. 51-56.

Compressed baker's yeast stored either in 50 g pieces or in 1 kg blocks at temperatures of 4, 10, 20, and 30°C was studied in respect of the consumption of reserve carbohydrates and the fermentative activity. It was found that the rate of consumption of the reserve carbohydrates, i.e. trehalose and acetic acid-soluble glycogen was significantly less in pieces than in blocks. This difference was reasoned to have been caused by the availability of atmospheric oxygen and thus on the type of metabolism (oxydative or fermentative) the yeast has to use to obtain energy for the maintenance functions.

The initial content of trehalose was 3.1 g/100 g of fresh yeast (28.1 g d.m.). This content dropped to half in 50 g pieces in 6 days at 30°C, 20 days at 20°C, 27 days at 10°C, and 47 days at 4°C. The half-life values in 1 kg blocks were 5, 17, 22, and 45 days, respectively. The same trend was found with the acetic acid-soluble glycogen.

The fermentative activity of the samples dropped in 1 kg blocks faster than in 50 g pieces; particularly so in a sugar-rich test dough.

Availability of air was found to have a positive effect on the maintenance of the fermentative activity.

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7. Heikki Suomalainen. Yeast in Alcohol Production and Bread Making. Paper presented by invitation of the Hungarian Academy of Sciences in Budapest, 1980. Acta Alimentaria 11 (1982): 3 (in press).

Fuel Alcohol. The energy crisis has stimulated an unprecedented amount of research into how oil products can be replaced by renewable natural resources. Much interest has been directed towards the enzymatic hydrolysis and fermentation to ethanol of cellulosic materials. However, really extensive industrial applications are unlikely to materialise in the near future. The wood conversion industries in Northern Europe are already major consumers of wood, and experts in Finland believe that the exploitation of wood has almost reached its limit.

Beverages. Much of the work studying the properties of alcoholic beverages has been concerned with the factors responsible for the formation of the aroma, and many investigations have confirmed that a great variety of metabolic products is formed in yeast cells during fermentation. With regard to the esters, our results show that a part of the compounds formed by the yeast remains in the cell and part permeates through the cell membrane to the medium. Consequently, the ester composition may be different in the distillate if the distillation of the medium is carried out in the presence of the yeast or after its removal.

Baking. The latest estimate for the annual production of baker's yeast exceeds 1,400,000 tons (fresh weight) and is growing only slowly if at all in the industrialized countries. The developing countries, e.g., in Africa and the Islamic regions, have started to show interest in their own yeast production. In Finland consumption of baker's yeast has reached a steady state level at nearly 2 kg per person a year, with a third being used in home baking. The large consumption of rye bread is a Finnish characteristic.

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8. Erkki Oura and Heikki Suomalainen. Biotin-Active Compounds, Their Existence in Nature and the Biotin Requirements of Yeasts. Journal of The Institute of Brewing (1982) (in press).

After a short sketch of the discovery of biotin and the progress of early biotin research, we described the natural occurrence of biotin with particular consideration to the raw materials used in the fermentation industry and its products. Of the many known biotin derivatives, we have covered those appearing in nature and those which can be converted to biotin-active (or inactive) compounds by simple procedures. Ways to bypass the need for biotin in microbes is discussed. The importance of biotin in yeast production, the biotin requirements of yeast, and the effect of culture conditions on these requirements are reported. The close relationship between the participation of biotinylenzymes and the biotin requirements is noted.

Our "Biotin and the Metabolism of Baker's Yeast" has appeared earlier in this Journal as a review article supplemented with our own observations and experimental results. The present article is a continuation, and we intend to complete the series with an article concerning the determination of biotin and with a short description of the biosynthesis of biotin in yeast.

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9. John Londesborough and Lea Jonkkari. Low Km Cyclic AMP Phosphodiesterase of Yeast may be Bound to Ribosomes Associated with the Nucleus. Molecular and Cellular Biochemistry (in press).

Close to 65% of the low Km cyclic AMP phosphodiesterase was particle-bound in both spheroplast lysates and mechanical disintegrates of yeast. In disintegrates, the enzyme sedimented with the ribosomal fraction, from which it was solubilised by treatment with RNase, but in lysates it sedimented with the nuclear fraction (protein/DNA = 16). These results suggest the enzyme may be bound by ribosomes attached to the outer membrane of the nucleus.

XXVIII. Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Canada S7N 0W0. Communicated by W.M. Ingledew.

The following papers have been published or are in press. A number of these resulted from the Ph.D. thesis of J.J. Wilson entitled "Starch Utilization and Protoplast Fusion in the Yeast Schwanniomyces alluvius". University of Saskatchewan, 1981.

J.D. Burton, W.M. Ingledew. Yeast Blotters: Survival of Yeast and Bacteria. J. Inst. Brewing. 88: , 1982 (in press).

Abstract

The survival of yeast on yeast blotters with and without contaminating bacteria was monitored to determine whether or not microscopic analysis of yeast after shipment on blotters might be influenced by growth of such bacteria. To examine blotter yeast, they were resuspended and roughly balanced to known opacity by colorimeter - then precisely by hemacytometer. Suspensions were then analysed for survival by plating. It was determined that bacteria and yeast both die quickly on blotters but demonstrate varying levels of survival. It was verified that viable counts from blotters are not practical for enumeration purposes although qualitative knowledge can be gained from viable cell assessment.

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J.J. Wilson, G.G. Khachatourians, W.M. Ingledew. Protoplast Fusion in the Yeast Schwanniomyces alluvius. Molecular Gen. Genetics: , 1982 (in press).

Abstract

This first application of the technique of protoplast fusion to Schwanniomyces suggests that it is possible to overcome the genetic isolation of this genus imposed by its inability to undergo conventional intraspecific mating. The stability, increased ploidy, and cell volumes of such fusion hybrids over the parental strains indicate the possibility of construction of polyploid strains suitable for use in industry.

Nuclear fusion (karyogamy) appears to occur in intraspecific hybrids as evidenced by isolation of recombinants after mitotic segregation of parental auxotrophic genetic markers.

Intergeneric hybrids formed from Schw. alluvius and Saccharomyces spp. were unstable and spontaneously segregated into original auxotrophic parent cultures. Genetic diversity between these genera may be too great to allow stable co-existance of the two genomes within a single nucleus. Nuclear fusion in such cases could not be confirmed.

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J.J. Wilson and W.M. Ingledew. Isolation and Characterization of the Amylolytic Enzymes of Schwanniomyces alluvius. Appl. Environ. Microbiol. : , 1982 (in press).

Abstract

The extracellular amylolytic enzymes of Schwanniomyces alluvius were studied in order to study future optimization of this yeast for the production of industrial ethanol from starch. Both α-amylase and glucoamylase were isolated and purified. The α-amylase had an optimum pH of 6.3 and was stable from pH 4.5 to 7.5. The optimum temperature was 40°C but it was quickly inactivated at temperatures above 40°C. The K_m for soluble starch was 0.364 mg/ml. The molecular weight was calculated to be 61,900 ± 700. The α-amylase was capable of releasing glucose from starch, but not from pullulan.

The glucoamylase had an optimum pH of 5.0 and was stable from pH 4.0 to > 8.0. The temperature optimum was 50°C and although less heat sensitive than α-amylase, it was quickly inactivated at 60°C. K_m values were 12.67 mg/ml for soluble starch and 0.72 mM for maltose. The molecular weight was calculated to be 155,000 ± 3,000. The glucoamylase released only glucose from both soluble starch and pullulan.

Schw. alluvius is one of the very few yeasts to possess both α-amylase and glucoamylase as well as some fermentative capacity to produce ethanol.

* * *

J.J. Wilson, G.G. Khachatourians, W.M. Ingledew. Schwanniomyces: SCP and Ethanol from Starch. Biotech. Letters : , 1982 (in press).

Abstract

Application of Schwanniomyces yeasts to single cell protein or alcohol production is feasible based on cell yields of 60% aerobically, and ethanol yields of 86% of theoretical in associative fermentation.

* * *

Review articles of significance to industrial quality control have also been published.

G.P. Casey, W.M. Ingledew. The Use and Understanding of Media Used in Brewery Bacteriology I. Brewers Digest 56 (2): 26-32, 1981. II. Brewers Digest 56 (3): 38-45, 1981. III. Brewers Digest 56 (4): 24-32, 1981.

W.M. Ingledew, G.P. Casey. The Use and Understanding of Media Used in Brewing Mycology. I. Brewers Digest 57 (3): 18-22, 1982. II. Brewers Digest 57 (4): 22-26, 1982.

XXIX. Research Institute for Viticulture and Enology, 833 11 Bratislava, Matuskova 25, Czechoslovakia. Communicated by E. Minarik.

This is a summary of a paper accepted for publication in Wein-Wissenschaft (GFR) in 1982:

E. Minarik: The yeast flora of concentrated grape juice and its influence on wine stability.

The osmophilic yeasts Saccharomyces bailii and Torulopsis stellata are the predominant species in the yeast flora of concentrated grape juice. S. bailii var. bailii prevails in musts concentrated by sucrose, T. stellata in musts concentrated by heat. S. bailii could be found only sporadically on cellar equipment; yeasts of the genus Torulopsis, Candida and Hansenula, on the other hand, very frequently. Contaminations caused by S. bailii in bottled wines may thus originate very likely from secondary habitats in the wineries as well as from concentrated grape juice used in wine production.

XXX. The Institute of Enology, University of Bordeaux II and Inra, 351 cours de la Libération 33405 Talence, France. Communicated by S. Lafon-Lafourcade.

The following paper is in press in the "Comptes rendus de l'Académie des Sciences, section Microbiologie"

F. Larue, S. Lafon-Lafourcade and P. Ribereau-Gayon, 1982. Inhibition of Saccharomyces cerevisiae metabolism in grape must.

Summary

During the alcoholic fermentation of grape must, the intracellular hexose content of Saccharomyces cerevisiae decreases to nothing; there is less change in alcohol content. Addition of steroids into the must increases intracellular concentration of ethanol and also activates the fermentation of must. Thus it seems that, yeast cells are inhibited more

by the difficulty of penetration of sugars than an excessive accumulation of ethanol.

XXXI. Institut für Mikrobiologie und Weinforschung, Johannes Gutenberg-Universität Mainz, Postfach 3980, 6500 Mainz, West Germany. Communicated by F. Radler.

Below follow summaries of two publications on yeasts. Both papers are "in press".

1. J.T. Kuczynski and F. Radler, The Anaerobic Metabolism of Malate by Saccharomyces bailii and the Partial Purification and Characterization of Malic Enzyme, Arch. Microbiol. (1982).

Abstract

The main pathway of the anaerobic metabolism of L-malate in Saccharomyces bailii is catalyzed by a L-malic enzyme.

The enzyme was purified more than 300-fold. During the purification procedure fumarase and pyruvate decarboxylase were removed completely, and malate dehydrogenase and oxalacetate decarboxylase were removed to a very large extent.

Manganese ions are not required for the reaction of malic enzyme of Saccharomyces bailii, but the activity of the enzyme is increased by manganese.

The reaction of L-malic enzyme proceeds with the coenzymes NAD and (to a lesser extent) NADP.

The K_m-values of the malic enzyme of Saccharomyces bailii were 10 mM for L-malate and 0.1 mM for NAD.

A model based on the activity and substrate affinity of malic enzyme, the intracellular concentration of malate and phosphate, and its action on fumarase, is proposed to explain the complete anaerobic degradation of malate in Saccharomyces bailii as compared with the partial decomposition of malate in Saccharomyces cerevisiae.

* * *

2. P. Pfeiffer and F. Radler, Purification and Characterization of Extracellular and Intracellular Killer Toxin of Saccharomyces cerevisiae Strain 28, J. Gen. Microbiol. in press.

Abstract

The extracellular killer toxin of Saccharomyces cerevisiae strain 28 was concentrated by ultrafiltration of culture supernatants and purified by ion-exchange chromatography. Polyacrylamide gradient gel electrophoresis in sodium dodecyl sulphate indicated that the toxin is a glycoprotein with a molecular weight of about 16 000. Amino acid analysis revealed that the killer toxin contains 111 amino acid residues, equivalent to a molecular

weight of 14 045. The ratio of protein to carbohydrate in the molecule is therefore about 9 to 1. The isoelectric point of the killer toxin was pH 4.4 to 4.5. The toxin was unaffected by heating at 40°C for 1 h and its maximum activity against sensitive yeast cells was observed at pH 5.0. Cell-free extracts prepared from well washed cells of S. cerevisiae strain 28 were toxic for sensitive yeasts. The toxin present in these extracts (intracellular toxin) was partially purified by ultrafiltration and ion-exchange chromatography. The isoelectric points of the extracellular and intracellular killer toxin were similar.

XXXII. Division of Biology, Defence Materials and Stores Research and Development Establishment, Post Box No. 320, Kanpur - 208 013, India. Communicated by M.C. Bardiya.

The following is a recent report from our laboratory.

1. G.P. Tandon, H.C. Pant and M.C. Bardiya. Studies on Osmotrophic Yeast Strains.

A large number of osmotrophic yeast strains have been isolated from honey, raisins, candied fruits, Mahua flower, fruit jams, etc., by direct plating on Yeast Extract Peptone Sucrose (30%) medium. These strains have been tested for their sugar tolerance. Four strains isolated from spoiled fruit jam could ferment glucose and sucrose in concentration as high as 85 and 90% (w/v), respectively. Strain 3, 5, 9 could grow and initiate fermentation in YEP sucrose (90%) broth within 48 hours whereas strain 10 took 96 hours. The fermentation of YEP glucose (85%) was very slow and it started after 9 days of incubation. Growth rates of these four strains were compared in 2 and 30% sucrose broth. Strain 5, 9 and 10 grew faster in 30% sucrose broth compared to 2%, whereas strain 3 had almost similar growth rates in both of the media. The former three strains were found to grow on glycerol medium, therefore, these were respiratory sufficient whereas the latter one could not grow on glycerol medium. These strains were ovoid to rod shaped and cell size varied between 2.5 and 3.0 µm in length.

* * *

2. C.P. Tandon, H.C. Pant and M.C. Bardiya. Screening of Osmophilic Yeast Strains.

The screening program for selecting osmophilic yeast strains from the large number of yeast isolates is being continued. Four more strains of osmophilic yeasts have been studied. The two strains isolated from date-palm (DP1 and DP2) were polymorphic and produced pseudomycelia. Two other isolates from Mahua flowers were ellipsoidal (MH2) and rod-shaped with polar budding (MH3). The date-palm strains were top fermenting and Mahua strains were bottom yeasts. With regard to their osmotolerance, MH2 and MH3 have been found to ferment 90% sucrose and 85% glucose within 24 and 48 h of incubation, respectively. The other two strains DP1 and DP2 were slow in fermenting concentrated sugar solution. They could ferment 90% sucrose and 85% glucose only after the 5th and 8th day of incubation, respectively. These strains were found to utilize fructose preferentially compared to glucose (fructophilic). However, all these strains have shown

comparatively lower range of tolerance to sodium chloride (17.5% only). Only DP1 was found to sporulate on acetate medium. All the strains are respiratory sufficient.

XXXIII. Department of Food Science, Faculty of Agriculture, Al-Fateh University, Tripoli, Libya. Communicated by Amin S. El Nawawy.

1. Amin S. El Nawawy* and Said O. Gnan. Isolation and Propagation of New Methanol-Utilizing Bacteria and Yeasts. Submitted to Biotechnol. & Bioengineering.

*Permanent address after July 1, 1982: 8 Nakla ALMOTEI Street, Heliopolis, Cairo, Egypt.

Summary

A research program began in Libya (SPLAJ) aiming to isolate some microorganisms (Bacteria, Yeasts) from local sources, which can utilize methanol as carbon source for SCP production. Twenty bacterial cultures and 24 yeast cultures were isolated from natural sources. The isolates were screened according to their efficiency in utilizing methanol, salt tolerance, growth at temperatures higher than 30°C.

The most promising bacterial isolate was identified as belonging to the genus Pseudomonas, while that of yeast belongs to the genus Rhodotorula. Each strain was propagated in a fermentor under controlled pH, temperature, aeration, stirring in a batch culture. Pseudomonas could convert 40% of methanol in the medium (0.5%) to bacterial cells containing 65% true protein and 8.5% total nucleic acids, while Rhodotorala could convert 38.8% of methanol (0.6%) to yeast cells containing 43% true protein and 5.4% total nucleic acids.

2. Amin S. El Nawawy and Said O. Gnan. New Yeast Isolates from Natural Sources in Libya. XIIIth International Congress of Microbiology, Boston, 1982.

Ten strains of yeast were isolated from some natural sources available in Libya, i.e., soil, date syrup wastes, and citrus peel wastes. The ten isolates were identified according to the scheme mentioned in 'The Yeast' 1970. Four isolates belong to the genus Candida, three to Hansenula, one to Rhodotorula, one to Cryptococcus one to Saccharomyces. However, some of their physiological characteristics have some variations from the already known and described species and variants. Such differences are discussed.

One of the Hansenula isolates and Rhodotorula isolate proved to be efficient in utilizing methanol as sole carbon source. Other eight isolates range in their ability to utilize specific carbon sources, i.e., Xylose, Lactose, Sucrose and Starch; indicating the possibility of each strain for utilizing specific raw materials, i.e., methanol, dates syrup wastes, starchy wastes and wood shavings hydrolyzate. Nucleic acid contents of the isolates vary in the range of 2.5-6.5 percent.

International and National Meetings on Yeast

1. VIIIth International Specialised Symposium on Yeasts. ISSY VIII will be held January 24-28, 1983 in Bombay, India, with major emphasis on Yeast Technology.

   The National Organising Committee of the VIIIth ISSY and the Association of Microbiologists of India (AMI) wish to extend to all the scientists and technologists in the area of yeasts and yeast-like microorganisms and in allied specialisations, a cordial and warm welcome to India and request their participation in the VIIIth ISSY that has "Yeast Technology", as the major theme.

   The venue of the Symposium will be "Hotel Oberoi Towers" Bombay, India.

   The dates are Monday, January 24, 1983 through Friday, January 28, 1983 with January 26 as a free day.

   The VIIIth ISSY, jointly sponsored by the AMI, ICY and IUMS is supported by scientific research bodies, industries, research foundations, scientific associations and professional organisations, in India. The Symposium is hosted by the National Organising Committee of the VIIIth ISSY and the Association of Microbiologists of India.

   The official language of the Symposium will be English. No simultaneous translation facilities will be available.

   The official travel agents to the VIIIth ISSY are M/s, Trade Wings Ltd. 30 K. Dubash Marg, Bombay-400 023, (Tel. 244334 Telex: 11-2494 Cable: Travel, Bombay).

   To obtain more information on the scientific programme, please write to:

   Dr. T.V. Subbaiah
   Convener, VIIIth ISSY
   C/o, Research Division
   Alembic Chemical Works Co. Ltd.
   Baroda-390 003 (Gujarat)
   India

2. IXth International Specialized Symposium on Yeasts

   In accordance with the resolution of the International Commission for Yeasts at the meeting on September 24, 1981 in Valencia, Spain, the IXth International Specialized Symposium on Yeasts, Yeasts in the Human Environment, will be held on April 18-22, 1983 at Smolenice Castle near Bratislava, Czechoslovakia.

   The scientific program will comprise invited plenary lectures, short oral reports, posters and discussions on the following subjects:

1. Ecology of natural habitats
2. Ecology of technological habitats
3. Human and animal origin of yeasts
4. Influence of chemization on yeasts
5. Taxonomy and identification

All participants will be accomodated at Smolenice Castle, The House of Scientific Workers of the Slovak Academy of Sciences. Fullboard will be offered to all participants at the Castle.

Those who wish to attend this Symposium are requested to write for the Preliminary Application Form not later than May 31, 1982. The 2nd Circular will be forwarded only to those who returned the completed form to: Dr. A. Kockova-Kratochvilova, Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta, 84238 Bratislava, Czechoslovakia.

3. Sixth International Protoplast Symposium

August 12-16, 1983, Basel, Switzerland.

As a result of the discussions at the Fifth International Protoplast Symposium in Szeged, 1979, the next Symposium will take place in Basel, Switzerland under the joint organization of A. Hinnen, P.J. King, I. Potrykus of the Friedrich Miescher-Institut, Basel, and C.T. Harms and R. Hütter of the Departments of Crop Science and Microbiology, Swiss Federal Institute of Technology (ETH), Zurich.

The Symposium will provide a forum for discussion of basic and applied aspects of protoplast biology in bacteria, fungi and plants.

Applications for the First Circular should be addressed to: Dr. I. Potrykus, Friedrich Miescher-Institut, P.O. Box 273, CH-4002 Basel, Switzerland.

4. The Eleventh International Conference on Yeast Genetics and Molecular Biology will be held in Montpellier (France) from the 13th to the 17th of September 1982 (Monday morning to Friday evening).

Scientific program

The program is not yet completely established, it will cover a wide range of topics. A non-exhaustive list is given below: Chromosome structure, replication, recombination and conversion, transposable elements, mutagenesis and repair mechanisms, regulation of the gene expression, genetic engineering and transformation, cell cycle mating and sporulation, nitrogen and carbon metabolisms, protein synthesis, mitochondria, killers, evolution, applied aspects.

Invited speakers will deliver talks at the morning sessions, poster sessions will be held in the afternoon and concurrent workshops will be proposed in the evening. A final plenary session will allow all participants to listen to reports on the headlines of the workshops.

Address for application forms: 11th Yeast Conference. Centre de Génétique Moleculaire du C.N.R.S., 91190 Gif-sur-Wette, France.

5. The Eighteenth Harden Conference on 'Cell Cycles', Wye College, Ashford, Kent, England. September 5 - September 10, 1982.

Cell Cycles under the Chairmanship of J.M. Mitchison, Department of Zoology, University of Edinburgh.

Principal Topics: Cell Cycle Models for the Control of DNA Synthesis and Division Macromolecular Synthesis During the Cell Cycle.

Speakers and Chairmen will include: R.F. Brooks (London); W. Sachsenmaier (Innsbruck); B.L.A. Carter (Dublin); R.R. Schmidt (Gainsville, FL); W.D. Donachie (Edinburgh); S. Shall (Sussex); P.A. Fantes (Edinburgh); J. Smith (London); H.O. Halvorson (Waltham, MA); J.J. Tyson (Blacksburg, VA); L. Hereford (Waltham, MA); R. van Wijk (Utrecht); P.C.L. John (Belfast); A.E. Wheals (Bath); C.G. Johnston (Halifax, NS); D.H. Williamson (London); P. Nurse (Sussex); M.M Yeoman (Edinburgh); R.K. Poole (London); P. Young (Kingston, Canada)

Harden Lecturer: Professor D.M. Prescott (University of Colorado, Boulder, CO).

POSTER COMMUNICATIONS ARE INVITED

Conference Fee £80, cost of full board at Wye College £85, inclusive of VAT. Further information and application forms are available from the Meetings Officer, The Biochemical Society, 7 Warwick Court, London WC1R 5DP.

Applications to attend must be received by the Meetings Officer of the Biochemical Society before June 7, 1982.

Applicants will be notified of the results of their applications by the first week of July.

Six sponsored places will be available at each Conference by competition for applicants normally under 27 years. Preference will be given to senior graduate students and restricted to Members of the Biochemical Society and the sponsoring societies, that is: British Biophysical Society, British Pharmacological Society, British Society of Cell Biology, British Society for Immunology, Genetical Society, Physiological Society, Society for General Microbiology. Applications for these grants should be sent to the Biochemical Society from the supervisor and be accompanied by an independent reference.

6. XIVth Annual Conference of the Yeast Commission of the Czechoslovak Society of Microbiologists, held in Smolenice 17th to 19th February 1982. Communicated by Dr. Anna Kockova-Kratochvilova.

A. Main lectures:

Kocková-Kratochvílová, A.: Recent problems in taxonomy and identification of yeasts.
Kotyk, A., Horák, J.: Transport and its regulation in yeasts.
Káš, J.: Utilization of yeasts and their enzymes in analyses.
Ludvík, J.: Ultrastructure of immobilized yeasts.

B. Reports:

Svoboda, A.: Cytological aspects of protoplast fusion.
Kopecká, M.: Autoorganization of microfibrils of β-1, 3-glucans in vitro.
Vořišek, J.: Ultracytological staining of polyphosphates in S. cerevisiae.
Vondrejs, V.: Perspectives of the utilization of protoplast fusion methods in the improvement of industrial yeasts.
Beran, K., Holan, J.: The influence of Calcoflor White MRR New on biology of yeasts.
Šandula, J.: Chemotaxonomy in the genus Saccharomyces on the basis of the structure and immunological activity of surface polysaccharides.
Svorcová, L.: The occurrence of yeasts in bath traffic.
Kováčová, V., Vlčková, V.: Problems in the identification of yeast mutant allelism with the help of the complement tests.
Vlčková, V., Kováčová, V.: The following of spontaneous and UV-induced mutagenis in pet9 locus of the standard and UV-sensitive strain rad 18 of S. cerevisiae.
Janderová, B.: Selective processes utilizable in the improvement of new industrial yeasts.
Breierová, E.: The influence of SO_2 on yeast growth.
Minárik, E., Navara, A.: The influence of hyphal fungi of the type of Botrytis cinerea on the fermentation of grape juice.
Navara, A., Minárik, E.: The inhibition effect of temperature on the activity of wine yeasts.
Vernerová, J., Kurzová, B., Bendová, O.: The autolyzing ability of brewing yeasts.
Čepička, J., Čížková, H.: The influence of raw materials on changes in metabolic quotients of bottom brewing yeasts.
Zemek, J.: The influence of the structure of carrier on characters of immobilized biosystems.
Augustin, J.: β-mannanases in yeasts and yeast-like organisms.
Opekarová, M., Siegler, K.: Proton movement through the membrane in S. cerevisiae.
Vraná, D.: The aging of yeasts and its physiological expression.
Kadlečíková, B.: The transformation of saccharides by yeasts and yeast-like organisms.
Páca, J.: The influence of yeast concentration on changes in catabolic activity during starvation.
Peciarová, A., Biely, P.: The peculiarities of active site of cell wall β-glucosidase in Cryptococcus albidus.
Biely, P., Vršanská, M., Krátký, Z.: The structure of xylose oligosaccharides synthetized in transglycolysis reaction.

Baláž, Š., Sturdík, E., Škárka, B.: The modeling of interphase transport as the efficiency determining character of some antifungal compounds.

C. Posters

Kocková-Kratochvílová, A.: Life cycles in yeasts.
Svoboda, A.: Morphological mutants of Trigonopsis variabilis.
Nečas, O., Svoboda, A., Sládečková, A., Pivničková, E.: Polyethylenglycol as a cryoprotectans for yeasts.
Havelková, M., Malá, L., Křipalová, J.: Nuclei in regenerating yeast protoplasts expressed by mitramycin.
Hašek, J., Streiblová, E.: Tubulin in yeasts.
Bendová, O.: The interspecies fusion of protoplasts of S. cerevisiae with industrial strain S. uvarum.
Tomšíková, A.: Contribution to the serological identification of yeasts.
Vojteková, G.: Contribution to the ecology of yeasts in Little Carpatian vineyards.
Breierová, E.: The optimization of growth conditions of C. utilis, C. tropicalis, K. fragilis on lignocellulosic wastes.
Rybářová, J.: The evaluation of the waste free technology of fodder yeast from the viewpoint of final product.
Jurčová, Z.: The preservation of yeast and yeast-like microorganisms on gels of biopolymers.
Kováč, L., Poliachova, V., Bohmerova, E.: The specific effect of ionophoric antibiotics on mitochondria in intact yeast cells.
Ruml, T., Šilhánková, L.: Transport in yeasts.
Šmogrovičová, D., Augustin, J., Hal'ama, D.: The kind of uptake of C-1 to C-3 compounds by yeasts and yeast-like organisms.
Zámocký, J.: The affinity of proteolytic enzymes and yeast peptidases to modified proteins.
Horák, J., Říhová, O., Kotyk, A.: Transport of L-proline in S. cerevisiae.

D. Films

Gabriel, M.: Microkinematographic study of karyokinese and cytodierese in regenerating and reverting Schizosaccharomyces versatilis.

Brief News Items

After more than 40 years of active service, I shall retire at the end of January and leave my duties at Alko. I wish to express my sincere thanks to all my colleagues around the world, who through the years have given me so much support and an abundance of personal friendship and consideration.

At Alko Mr. Matti Kaukinen will succeed me as the Industrial Director and Deputy General Manager, and Dr. Kalervo Eriksson as

the Director of Production and Research. However, you can still reach me through Alko, if necessary.

Navigare necesse est ...

I thank you for your collaboration and send my kindest regards.

January 1982

Heikki Suomalainen

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SF-00101 Helsinki 10
Finland

2. I have been invited by the editors of Annual Review of Microbiology to write a review article on "DNA Plasmid in Yeast" for the 1983 issue (Volume 37). In the article, I will concentrate on 2 μm and newly isolated yeast plasmids (intracellular localization; function; biochemical and genetic studies; etc.) and on recombinant DNA and transformation of yeast (vectors; expression of cloned yeast and non-yeast genes; promoter and leader sequences; terminater; isolation of ars, centromeres, promoters; practical applications; etc.). Yeast is not confined to Saccharomyces cerevisiae.

I would like to make the article as up-to-date and full of information as possible. So I am writing to ask the readers of the Yeast Newsletter for valuable help. I would appreciate it very much if they could kindly send me any reprints or preprints of works on yeast plasmids, recombinant DNA/transformation and related fields.

I will start writing on August 1, 1982 and must submit the manuscript by the end of November, 1982. I would like to obtain information, if possible, by the end of July to include in the review.

Thank you in advance for any help.

Norio Gunge
Mitsubishi-Kasei Institute
of Life Sciences
11, Minamiooya, Machida-Shi
Tokyo, Japan

3. We have recently become involved in determining how genetically manipulatable is the food yeast, Candida utilis. There is very little published on mutating or mutants available of Candida. We have spent the past few months using classic Saccharomyces mutant

screening procedures and have very few mutants. If anyone is working in this area and may have helpful ideas, I would appreciate hearing from them.

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