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Y E A S T

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Herman J. Phaff, Editor
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Herman J. Phaff
Editor

NOTICE TO OUR READERS

The office of the Editor has been informed that invoice payments for the Yeast Newsletter by subscribers in foreign countries are subject to high service charges by their banks if payment is made directly to the Yeast Newsletter, Dept. of Food Science & Technology, University of California, Davis.

We have explored with the University of California the possibility of direct transfer of the subscription fee on the bank account of the University of California. Unfortunately, this is not possible because of the large size of the University on nine campuses in the State of California with its numerous accounts. It is suggested that subscribers may wish to purchase dollars and pay cash in order to save the high service charge or use a postal money order.

H.J. Phaff
Editor

I. Centraalbureau voor Schimmelcultures, Yeast Division, Julianalaan 67a, 2628 BC Delft, Netherlands. Communicated by M. Th. Smith.

Below follows:

1. A list of recently acquired yeasts

2. A list of papers which have been published or are in press

1. Recently acquired yeasts by the CBS

Bullera oryzae Nakase & Suzuki: CBS 7194 = IFO 10166 = JCM 5281, T, ex dead leaf of Oryza sativa L, Japan, T. Nakase;

Candida tsuchiyae Nakase & Suzuki: CBS 7195 = IFO 10167 = = JCM 1638, T, ex moss, Japan, T. Nakase;

Cryptococcus fuscescens Golubev: CBS 7189 = VKM Y-2600, T, ex saline takyr soil, U.S.S.R., V.I. Golubev;

Endomycopsella crataegensis (Kurtzman & Wickerham) v. Arx: CBS 7190 ex grapes with sour rot, Italy, mating type alpha, R. Marchetti;

Schizosaccharomyces octosporus Beijerinck: CBS 7191, ex feed of mason bee Osmia rufa, Denmark, J.P. Skou;

Spororbolomyces subbrunneus Nakase & Suzuki: CBS 7196 = IFO 10168 = JCM 5278, T, ex dead leaf of Oryza sativa, Japan, T. Nakase.

2. The following papers have been published or are in press.

a. Yamada, Y. and Smith, M.Th. 1985. The coenzyme Q system in strains of species in the genera Stephanoascus and Sporopachydermia (Saccharomycetaceae). Trans. mycol. Soc. Japan 26:247-251.

Summary

Thirteen strains of Stephanoascus and Sporopachydermia species and five strains of Candida steatolytica were examined for the Co-Q system. All the test strains had Q-9. The data are discussed from the taxonomic point of view.

b. Smith, M.Th. 1986. Zygoascus hellenicus gen. nov., sp. nov., the teleomorph of Candida hellenica (= C. inositophila = C. steatolytica). Antonie van Leeuwenhoek 52: (1) (in press).

Summary

The anamorphic yeast species Candida hellenica, C. inositophila and C. steatolytica were found to constitute haploid mating types of an undescribed, filamentous heterothallic Endomycete. The new genus Zygoascus is proposed for the teleomorph. Descriptions are given of the genus and the type species, Z. hellenicus.

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II. Laboratory of Applied Microbiology, The Institute of Enology and Viticulture, Yamanashi University, Kofu, 400 Japan. Communicated by Shoji Goto.

The following articles were recently published.

1. Masashi Yamazaki, Shoji Goto, and Kazuo Komagata. 1985. Taxonomical studies of the genus Tilletiopsis on physiological properties and electrophoretic comparison of enzymes. Trans. mycol. Soc. Japan 26:13-22.

Summary. A taxonomic study of the genus Tilletiopsis Derx was made by comparing the physiological properties and the electrophoretic patterns of ten enzymes. The enzymes studied were glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glutamate dehydrogenase (EC 1.4.1.4), malate dehydrogenase (EC 1.1.1.37), succinate dehydrogenase (EC 1.3.99.1), catechol oxidase (EC 1.10.3.1), tetrazolium oxidase, esterase (EC 3.1.1.1), glutamate-oxaloacetate-transaminase (EC 2.6.1.1), alkaline phosphatase (EC 3.1.3.1) and fumarase (EC 4.2.1.2). Two strains of T. washingtonensis Nyland showed different physiological properties and enzyme patterns. One strain of T. albescens Gokhale was characterized by its ability to assimilate inositol or ethanol as the sole carbon source and to grow in a vitamin-free medium. One strain of T. washingtonensis and the strain T. albescens produced identical enzyme patterns and showed the same physiological properties. The T. minor Nyland var. minor strains tested fell into two categories based on their physiological properties and enzyme patterns. A strain of T. minor Nyland var. flava Tubaki was characterized by its inability to utilize melezitose as the sole carbon source. A strain of T. fulvescens Gokhale and one group of T. minor var. minor emitted soluble brownish pigment into the medium, but they were clearly distinguishable from each other by their enzyme patterns. One strain of T. pallescens Gokhale differed from the other species in its ability to assimilate L-sorbose and its lack of growth at 30°C. Six isolates showed the same enzyme patterns as those of a strain of T. lilacina Tubaki. Each species of the genus Tilletiopsis was distinct in its enzyme patterns, therefore, electrophoretic comparisons of enzymes should prove a useful tool for the classification and identification of species of this genus.

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2. Masashi Yamazaki and Shoji Goto. 1985. An Electrophoretic Comparison of Enzymes in the Genera Lipomyces and Myxozyma. J. Gen. Appl. Microbiol., 31:313-321.

A taxonomic study of the genera Lipomyces Lodder et Kreger-van Rij and Myxozyma van der Walt, Weijman et von Arx was made by comparing the electrophoretic patterns of nine enzymes. The enzymes studied were glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glutamate dehydrogenase (EC 1.4.1.4), malate dehydrogenase (EC 1.1.1.37), lactate dehydrogenase (EC 1.1.1.27), fumarase (EC 4.2.1.2), tetrazolium oxidase, catechol oxidase (EC 1.10.3.1), esterase (EC 3.1.1.1), and glutamate-oxaloacetate-transaminase (EC 2.6.1.1).

Lipomyces lipofer Lodder et Kreger-van Rij and Lipomyces tetrasporus Nieuwdorp, Bos et Slooff had a uniform pattern within each species. On the other hand, Lipomyces kononenkoae Nieuwdorp, Bos et Slooff and Lipomyces

starkeyi Lodder et Kreger-van Rij each varied considerably in their enzyme patterns. Though about 50% similarity was observed between L. starkeyi and L. tetrasporus, five Lipomyces species, Lipomyces anomalus Babjeva et Gorin, L. lipofer, L. kononenkoae, L. starkeyi, and L. tetrasporus, differed from each other in their enzyme patterns. Myxozyma melibiosi (Shifrine et Phaff) van der Walt, Weijman et von Arx and Myxozyma mucilagina (Phaff, Starmer, Miranda et Miller) van der Walt, Weijman et von Arx each had a uniform pattern which differed from the five Lipomyces species. The enzyme patterns of six Myxozyma sp. strains were dissimilar to those of two Myxozyma species. One strain of Myxozyma sp. and two strains of L. starkeyi showed about 60% similarity. The enzyme patterns of other Myxozyma sp. strains were dissimilar to those of the five Lipomyces species.

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3. Keiko Tokuoka, Takasuke Ishitani, Shoji Goto, and Kazuo Komagata. 1985. Identification of Yeasts Isolated From High-Sugar Foods. J. Gen. Appl. Microbiol., 31:411-427.

Yeast strains were isolated from 265 samples of high-sugar foods and related materials, and 93 representative strains were identified. Fifty-four strains of ascosporogenous yeasts were identified as 12 species belonging to eight genera. Thirty-nine strains of asporogenous yeasts were identified as 26 species belonging to five genera. The major species identified were Zygosaccharomyces rouxii (23 strains), Hansenula anomala (eight strains), Debaryomyces hansenii (five strains), and Saccharomyces cerevisiae (four strains). More than 60% of the isolates were sugar-tolerant yeasts, and all strains of Zygosaccharomyces rouxii were osmophilic. Approximately 80% of the isolates fermented glucose. It is suggested that these yeasts cause pouch swelling and produce alcohol in gas-exchange packaged foods or vacuum-packaged foods.

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III. Department of Microbiology, University of the Orange Free State, Bloemfontein, South Africa. Communicated by J.C. du Preez, J.L.F. Kock and I.S. Pretorius.

Some of the research topics in this laboratory are:

1. YEAST TAXONOMY

The following are recent publications.

1.1 J.L.F. Kock, P.M. Lategan, P.J. Botes and B.C. Viljoen. 1985. Developing a rapid statistical identification process for different yeast species. J. Microbiol. Methods 4: 147-154.

Abstract. A yeast identification procedure was developed with the aid of statistical methods. In this process cellular fatty acids of ten yeast species grown on yeast nitrogen base medium were extracted from yeast cells by saponification and analyzed as methyl esters by gas-liquid chromatography. Each species produced a distinctive fatty acid 'fingerprint' characterized by certain fatty acid compositions. A statistical procedure was developed in order to transform the 'fingerprints' into a yeast profile which resulted in a

marked reduction in variation between profiles of the same yeast species. With this identification method, it was possible to identify the ten species within 4 h after they were obtained from cultures of yeasts grown for 48 h on glucose yeast nitrogen base medium as compared with 7-10 days for the more conventional methods.

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1.2 M. Cottrell, J.L.F. Kock, P.M. Lategan, P.J. Botes and T.J. Britz. 1985. The long-chain fatty acid compositions of species representing the genus Kluyveromyces. FEMS Microbiol. Lett. 30: 373-376.

Abstract. The cellular long-chain fatty acids of 32 strains representing 10 species of the genus Kluyveromyces were extracted by saponification and analyzed as methyl esters by gas chromatography. The Kluyveromyces strains were characterized by the presence of palmitate, palmitoleate, oleate, and linoleic acid as the major fatty acids. These strains were divided into 3 groups on the basis of fatty acid content. The first group was characterized by a high percentage of linolenic acid, the second group of a lower percentage and the third group by the absence of linolenic acid.

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Other papers on the application of gas chromatography of fatty acids to yeast taxonomy are listed below.

1.3 Kock, J.L.F. and J.P. van der Walt. 1986. Fatty acid composition of Schizosaccharomyces Lindner. Syst. Appl. Microbiol. (in press).

1.4 Cottrell, M., B.C. Viljoen, J.L.F. Kock and P.M. Lategan. 1986. The long-chain fatty acid compositions of species representing the genera Saccharomyces, Schwanniomyces and Lipomyces. J. Gen. Microbiol. (in press).

1.5 Cottrell, M., J.L.F. Kock, P.M. Lategan and T.J. Britz. 1986. Long-chain fatty acid composition as an aid in the classification of the genus Saccharomyces. Syst. Appl. Microbiol. (in press).

1.6 Kock, J.L.F. and P.M. Lategan. 1985. A rapid method to differentate between four species of the Endomycetaceae. J. Gen. Microbiol. 131:3393-3396.

1.7 Viljoen, B.C., J.L.F. Kock, P.M. Lategan, P.J. Botes and T.J. Britz. 1986. The systematics of the Saccharomycetaceae, Endomycetaceae, Metchnikowiaceae and Saccharomycodaceae: Long chain fatty acid composition as an aid in classification. Antonie van Leeuwenhoek (in press).

1.8 Kock, J.L.F., M. Cottrell and P.M. Lategan. 1986. A rapid method to differentiate between 5 species of the genus Saccharomyces. Appl. Microbiol. Biotechnol. (in press).

1.9 Kock, J.L.F. and P.M. Lategan. 1986. A rapid method to differentiate between 4 species of the genus Kluyveromyces. J. Microbiol. Methods (in press).

1.10 Viljoen, B.C., J.L.F. Kock and P.M. Lategan. 1986. Fatty acid composition as a guide to the classification of selected genera belonging to the Endomycetales. J. Gen. Microbiol. (in press).

1.11 Viljoen, B.C., J.L.F. Kock and P.M. Lategan. 1986. The influence of culture age on the cellular fatty acid composition of four selected yeasts. J. Gen. Microbiol. (in press).

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2. YEAST GENETICS

As part of a more extensive study into the bioconversion of starch to single cell protein/ethanol, yeast glucoamylase was genetically, physically and biochemically characterized. These experiments were carried out by I.S. Pretorius in the laboratory of Dr. Julius Marmur at the Albert Einstein College of Medicine in New York. The following three papers report on this work.

2.1 Pretorius, I.S., T. Chow, D. Modena and J. Marmur. 1986. Molecular cloning and characterization of the STA2 glucoamylase of Saccharomyces diastaticus. Mol. Gen. Genet. (in press).

Abstract. This report describes the molecular cloning by complementation of a DNA-fragment containing the STA2 gene, which encodes glucoamylase II of S. diastaticus. The restriction map of STA2 shows that it is very similar and thus closely related to STA1 and STA3. We also describe the subcloning of the STA2 gene, its fusion to β-galactosidase, its partial characterization and the integrative disruption of this gene.

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2.2 Pretorius, I.S., T. Chow and J. Marmur. 1986. Identification and physical characterization of yeast glucoamylase structural genes. Mol. Gen. Genet. (in press).

Abstract. Subcloned fragments of the STA2 gene provided probes and made it possible to correlate the physical structure of each of the three STA loci with its starch fermentation phenotype. The linkage relationship between a non-functional sequence and STA1 was also explored. The physical and genetic analysis of the STA multigene family form the basis of this report. Allelism between the STA and DEX loci, as well as the MAL5 locus, was investigated.

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2.3 Pretorius, I.S., D. Modena, M. Vanoni, S. Englard and J. Marmur. 1986. Transcriptional control of glucoamylase synthesis in vegetatively growing and sporulating Saccharomyces yeast. Mol. Cell. Biol. (in press).

Abstract. Genetic and physiological factors affecting glucoamylase were analyzed. Glucoamylase synthesis was continuous throughout the growth phases and any of the following modulated glucoamylase expression, primarily at the level of mRNA accumulation : the carbon and nitrogen sources, the presence of

functional mitochondria, the presence of the STA10 gene and the mating type configuration. In STA2/STA2 MATa/MATα diploids glucoamylase expression was repressed, but when sporulated glucoamylase mRNA and enzyme activity were restored in a time dependent manner. A 2.0 kb mRNA and its encoded sporulation-specific glucoamylase were coinduced and detectable in sta° sporulating diploids as well.

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3. PENTOSE FERMENTATION

As part of an ongoing cooperative programme on the utilization of sugar cane bagasse, recent work has focused on the fermentation of xylose to ethanol. The following are recent papers on this subject.

3.1 Du Preez, J.C. and B.A. Prior. 1985. A quantitative screening of some xylose-fermenting yeast isolates. Biotechnol. Lett. 7: 241-246.

3.2 Du Preez, J.C., J.L.F. Kock, A.M.T. Monteiro and B.A. Prior. 1985. The vitamin requirements of Candida shehatae for xylose fermentation. FEMS Microbiol. Lett. 28: 271-275.

3.3 Du Preez, J.C., M. Bosch and B.A. Prior. 1986. Fermentation of hexose and pentose sugars by Candida shehatae and Pichia stipitis. Appl. Microbiol. Biotechnol. 23: 228-233.

Abstract. Both Candida shehatae and Pichia stipitis produced ethanol from D-glucose, D-mannose, D-galactose and D-xylose. Only P. stipitis fermented D-cellobiose, producing 6.5 g.ℓ^{-1} ethanol from 20 g.ℓ^{-1} cellobiose within 48 h. Neither yeast produced ethanol from L-arabinose, L-rhamnose or xylitol. Diauxie was evident during the fermentation of a sugar mixture. Following the depletion of glucose, P. stipitis fermented galactose, mannose, xylose and cellobiose simultaneously with no noticeable preceding lag period. A similar fermentation pattern was observed with C. shehatae, except that it failed to utilize cellobiose even though it grew on cellobiose when supplied as the sole sugar. In general, P. stipitis exhibited a higher volumetric rate and yield of ethanol production. Unlike C. shehatae, P. stipitis had no absolute vitamin requirement for xylose fermentation, but biotin and thiamine enhanced the rate and yield of ethanol production significantly.

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3.4 Du Preez, J.C., M. Bosch and B.A. Prior. 1986. Xylose fermentation by Candida shehatae and Pichia stipitis: Effects of pH, temperature and substrate concentration. Enzyme Microb. Technol. (in press).

Abstract. The effects of temperature, pH and xylose concentration on the fermentation parameters of Candida shehatae and Pichia stipitis were evaluated. The optimum pH was in the region of pH 4 - 5.5, with an optimum fermentation temperature of 30°C. Maximum fermentation rates were reached at 50 g.ℓ^{-1} xylose. The ethanol yield of C. shehatae decreased considerably when cultivated above 30°C or when the xylose concentration was increased. Xylitol accumulated concomitantly. Xylitol production by P. stipitis was observed only during cultivation at 36°C. Whereas the ethanol yield of C. shehatae was

usually about 75% of the theoretical maximum, it was 85 - 90% with P. stipitis.

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

The following papers have been published or are in press since the listing in the Yeast Newsletter of June 1985 (Vol. 34, No. 1).

1. H.J. Phaff. 1985. Biology of yeasts other than Saccharomyces. In A.L. Demain and N.A. Solomon (eds) Biology of Industrial Microorganisms - Biotechnology Series. Chapter 18:537-562. The Benjamin/Cummins Publ. Co., Menlo Park, California (reprints not available).

2. H.J. Phaff. 1986. Ecology of yeasts with actual and potential value in biotechnology. Microb. Ecology 12:31-42 (special issue).

3. H.J. Phaff and W.T. Starmer. 1986. Yeasts associated with plants, insects, and soils. In A.H. Rose and J.S. Harrison (eds). The Yeasts, 2nd ed., Vol. 1, Academic Press, London (in press).

4. C.P. Kurtzman and H.J. Phaff. 1986. Molecular taxonomy of yeasts. In A.H. Rose and J.S. Harrison (eds). The Yeasts, 2nd ed., Vol. 1, Academic Press, London (in press).

5. Herman J. Phaff[1], Mary Miranda[1], William T. Starmer[2], Joanne Tredick[1] and J. Stuart F. Barker[3]. 1986. Clavispora opuntiae, a new heterothallic yeast occurring in necrotic tissue of Opuntia species. Int. J. Syst. Bacteriol. 36(3) July issue (in press).

Department of Food Science and Technology, University of California, Davis, California 95616[1], Department of Biology, Syracuse University, Syracuse, New York 13210[2] and Department of Animal Science, University of New England, Armidale, N.S.W. 2351, Australia[3]

Abstract. We describe Clavispora opuntiae, a new cactophilic species of yeast with a wide distribution in necrotic tissue of Opuntia species. The more than 200 strains recognized thus far come from eastern Australia, Hawaii, Venezuela, Spain, various islands in the Caribbean Sea, Mexico, and southwestern areas of the United States. The new species is heterothallic and occurs in nature exclusively in the haploid state. Upon conjugation of complementary mating types asci are produced with one or two clavate, warty ascospores (as observed by electron microscopy) that are very rapidly released from the asci. Testing for mating types has indicated that one mating type (arbitrarily designated h+) predominates in Australia, Haiti, Dominican Republic, and Montserrat, whereas in all other areas both mating types are found, sometimes in the same plant. The range of guanine-plus-cytosine content of the nuclear deoxyribonucleic acid of 11 strains is 43.0-44.1 mol% (average 43.5, standard deviation 0.4 mol%). The type strain of Cl. opuntiae is UCD-FST 77-279 (=ATCC 42172 = CBS 7068) and the complementary mating type is UCD-FST 78-540A (= ATCC 42173 = CBS 7069).

6. William T. Starmer[1,2], J.S.F. Barker[1], Herman J. Phaff[3], and James C. Fogleman[4]. 1986. The adaptations of Drosophila and yeasts: their interactions with the volatile, 2-propanol, in the cactus-microorganism-Drosophila model system. Austral. J. Biol. Sci. (in press).

[1]Department of Animal Science, University of New England, Armidale, N.S.W. 2351, Australia, [2]Department of Biology, Syracuse University, Syracuse, NY 13210, USA, [3]Department of Food Science & Technology, University of California, Davis, Davis, CA 95616, and [4]Department of Biological Sciences, University of Denver, Denver, CO 80208, USA.

Abstract. The interactions of yeasts growing in decaying cactus tissue with and without the alcohol 2-propanol, were studied with respect to the costs and benefits provided to three cactophilic Drosophila species (D. mojavensis, D. arizonensis and D. buzzatii). Two common cactus yeasts, Candida sonorensis and Cryptococcus cereanus, which can tolerate and metabolize 2-propanol, provide benefits to the three Drosophila species in the presence of the alcohol, as compared with another common cactus yeast, Pichia cactophila, which has less tolerance and cannot metabolize 2-propanol.

Because 2-propanol is commonly found in decaying cactus tissue and C. sonorensis and Cr. cereanus are also frequently recovered from the rotting tissue being utilized by the Drosophila, the interactions described here are viewed as possible adaptation in which the yeast provides benefits to one of its vectors by metabolism of 2-propanol in the habitat.

7. William T. Starmer[1], Philip F. Ganter[1], and Herman J. Phaff[2]. 1986. Quantum and continuous evolution of DNA base composition in the yeast genus Pichia. Evolution (accepted for publication).

Biology Department, Syracuse University, Syracuse, NY 13210[1] and Department of Food Science and Technology, University of California, Davis, Davis, CA 95616[2].

Abstract. This paper investigates the non-continuous nature and evolution of the base composition of the nuclear DNA (expressed as mol% guanine + cytosine) in species of the yeast genus Pichia sensu Kurtzman 1984b. The pattern of change in the G+C contents in species of this genus, which range from about 27 to 52 mol%, was evaluated. When specifically those species of Pichia were analyzed that have evolved in necroses of cactus species and associated Drosophila, a periodic change in the G+C contents of approximately 3.0-3.2 mol% was detected by a "bootstrapping" method, Fourier analysis, and a non-linear trigonometric model. Pichia species occurring in exudates of broad-leaved deciduous trees or associated Drosophila and substrates such as soil and water ("other") showed a periodicity of 2.5-2.6 mol%, whereas species associated with conifers and associated bark beetles showed no significant periodicity. Periodicity in the most recent association (cactus and resident Drosophila) as compared to the lack of periodicity in the oldest association (conifer-beetle) may indicate mixed evolutionary processes. Low mol% G+C values appear more frequently in the relatively recent cactus and Drosophila-associated yeast species. In addition, low mol% G+C species do not display the ancestral bud-meiosis mode of sexual reproduction which occurs frequently in medium to high mol% G+C yeasts. It was found that the mol% G+C content of the Drosophila- and cactus-associated Pichia species is positively correlated with the number of compounds fermented

or respired by these yeast species.

Possible reasons for the periodic changes in mol% G+C content accompanying speciation, include aneuploidy, allopolyploidy, the presence of nuclear plasmids, and regular differences in moderately repetitive portions of DNA. Since significant DNA complementarity is virtually limited to species within a relatively narrow G+C group, this suggests that there are at least two processes which alter the G+C content between species, one saltational and one continuous.

8. William T. Starmer[1], Marc-Andre Lachance[2], and Herman J. Phaff[3]. A comparison of yeast communities found in necrotic tissue of cladodes and fruits of Opuntia stricta on islands in the Caribbean Sea and where introduced into Australia. Submitted to Microbial Ecology.

[1]Department of Biology, Syracuse University, Syracuse, NY 13210,
[2]Department of Plant Science, University of Western Ontario, London, Ontario N6A 5B7
[3]Department of Food Science and Technology, University of California Davis, Davis, California 95616

Abstract. Yeast communities growing in the decaying tissues (cladodes and fruits) of Opuntia stricta (prickly pear cactus) and associated with yeast vectors (Drosophila species) were compared in two geographic regions (Caribbean and eastern Australia). The Australian yeast community provides an interesting comparison to the Caribbean community, because the host plant O. stricta was introduced to Australia over 100 years ago. Many of the yeasts found in the Australian system were introduced during a period of Biological Control (1926-1935) when they accompanied rotting prickly pear cladodes and insects shipped to Australia from the Americas. The yeast community composition (proportion of each species) is compared at several levels of organization, i) within and between regions, ii) across seasons and years, and iii) within and between tissue types. The yeast species composition of the cladode communities are similar from locality to locality, season to season and year to year, with the region to region similarity slightly less. The composition of the fruit-yeast communities are distinct from region to region and only show limited overlap with the cladoes within regions when collected simultaneously in the same locality. It is suggested that the cladode-microorganism-Drosophila system is relatively closed (little extrinsic influence) while the fruit-microorganism-Drosophila system is open (large extrinsic influence).

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9. Herman J. Phaff. 1986. My Life with Yeasts. Ann. Rev. Microbiol. 40:1-28 (prefatory chapter).

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10. Mendonca-Hagler, L.C., A.N. Hagler, H.J. Phaff, and J. Tredick. 1985. DNA relatedness among aquatic yeasts of the genus Metschnikowia and proposal of the species Metschnikowia australis comb. nov. Can. J. Microbiol. 31:905-909 (for abstract see Yeast Newsletter 34, No. 1, p. 11, 1985).

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V. Czechoslovak Academy of Sciences, Institute of Microbiology, 142 20 Praha 4 - KRC, Videňska 1083, Czechoslovakia. Communicated by Eva Streiblová.

Below follows a list of our publications and the summary of our recent paper that will appear in European Journal of Cell Biology (Vol 41, No 2).

1. Streiblová, E. (1984). The yeast cell wall: a marker system for cell cycle controls, p. 127-142; in: The Microbial Cell Cycle (P. Nurse and E. Streiblova, eds.), Boca Raton, CRC Press.

2. Streiblová, E., Hašek, J. (1985). How can cell wall pattern in yeast reflect spatial cell cycle controls, pp. 499-505; in: Environmental Regulation of Microbial Metabolism, Pushchino, USSR, June 1-7, 1983 (I.S. Kulajev, E.A. Dawes, D.W. Tempest, eds), London, Acad. Press.

3. Hašek, J., Svobodová, J., Streiblová, E. (1985). The microtubular pattern in cells and protoplasts of Saccharomyces uvarum. Europ. J. Cell Bioll. 38, suppl. 9,11.

4. Streiblová, E., Hašek, J. (1985). The cytoskeleton and control of the yeast cell cycle. Microbial. Sciences 2, 139-143.

5. Hašek, J., Svobodová, J., Streiblová, E. (1986). Immunofluorescence microscopy of the microtubular skeleton in growing and drug-treated yeast protoplasts. Europ. J. Cell Biol., 41, No. 2.

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Abstract. The microtubular system in growing protoplasts of Saccharomyces uvarum was visualized by immunofluorescence using the monoclonal antitubulin antibody TU 01. We confirmed the co-existence of regular spindle configurations and extensive cytoplasmic networks in growing protoplasts and also observed a distinct distortion of cytoplasmic microtubules in association with wall removal. After a short period for recovery of protoplasts in nutrient medium a restitution of cytoplasmic microtubules and their resumed contact with the protoplast surface was observed. Treatment of growing protoplasts with nocodazole resulted in the disappearance of spindle and cytoplasmic microtubules in the relevant fraction of the protoplast population. In carbendazime (MBC) - arrested protoplasts spindle microtubules were absent but cytoplasmic microtubules associated with spindle pole bodies were clearly visible. Microtubule reassembly on spindle pole bodies occurred within 30 min after washing out nocodazole as well as carbendazime. The approach using protoplasts suggests a simple way in which the differential effect of antimicrotubule agents can be experimentally tested and the microtubule organizing activity of yeast protoplasts visualized at the population level.

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VI. The University of Western Ontario, Department of Plant Sciences, Biological & Geological Building, London, Canada N6A 5B7. Communicated by Richard B. Gardiner and Alan W. Day.

Below follow abstracts of recent publications from our laboratory.

1. Gardiner, R.B., and Day, A.W. 1985. Fungal Fimbriae. IV. Composition and properties of fimbriae from Ustilago violacea. Experimental Mycology 9:334-350.

Abstract. The fimbriae of Ustilago violacea consist of long protein fibrils of 7-nm diameter. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the fimbriae of this species and of several other species of Ustilago and Rhodotrula demonstrated that they are composed of a protein of 74,000 Da which can spontaneously assemble into 7-nm fibrils. No carbohydrate moiety was detected. Fimbrial protein retained both its fibrillar structure and antigenicity when exposed to a variety of chemical treatments, and even when autoclaved. Concentrations of cations greater than 10^{-1} M (monovalent cations) or 5 x 10^{-2} M (divalent cations) resulted in a loss of fimbriae, while the effect of chelators suggested that calcium is important to the structural integrity of fimbriae. Ouchterlony tests using antisera prepared against the fimbriae of U. violacea and R. rubra indicated that while there are differences in the antigenic sites, the fimbrial protein of different basidiomycete species is highly conserved. Fimbrial protein did not react with several mammalian antisera directed against cytoskeletal proteins.

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2. Svircev, A., Smith, R., Gardiner, R.B., Racki, I.M., and Day, A.W. 1985. Fungal fimbriae, V. Protein A-gold immunocytochemical labeling of the fimbriae of Ustilago violacea, Experimental Mycology 9:19-27.

Abstract. Protein A-gold immunocytochemical labeling of long protein appendages (fimbriae) on the surfaces of cells of Ustilago violacea is described. The specificity of the technique is confirmed by a series of serological and morphological controls. The technique allows specific identification and localization of fimbrial antigens in electron microscopic studies of a variety of fungi in both pathogenic and saprophytic situations.

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VII. Barnes Laboratory, Department of Biology, University of Chicago, Chicago, Illinois 60637. Communicated by E.D. Garber.

Garber, E.D. and A.W. Day (1985). Genetic mapping of a phytopathogenic Basidiomycete, Ustilago violacea. Bot Gaz. 146:449-459.

The heterothallic phytopathogen Ustilago violacea has haploid uninucleate sporidia that multiply by budding on complex or minimal medium to produce a yeastlike colony. The diploid teliospore from smutted anthers of diseased plants of Silene alba germinates on nutritive medium, undergoes meiosis and gives a linear tetrad of basidiospores on a short promycelium. The basidiospores form sporidia by budding and the sporidia multiply by budding so that the yeastlike teliospore colony has four sporidia clones. Each clone has the same genotype as the original basidiospore in the tetrad. We recently published a review of a number of meiotic and mitotic mapping strategies for this species (1) to assign mutations to specific linkage groups, (2) to determine centromere-linkage values, and (3) to map mutations in their respective linkage groups. These strategies should have application in the genetic study of yeast and yeastlike species.

Address reprint requests to E.D. Garber.

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VIII. Miles Laboratories, Cutter Biologicals Division, 4th and Parker Sts., P.O. Box 1986, Berkeley, CA 94701. Communicated by Richard F. Hector.

Below follows a communication from our laboratory.

Richard F. Hector and Phyllis C. Braun. 1986. The Synergistic Action of Nikkomycins X and Z with Papulacandin B on Whole Cells and Regenerating Protoplasts of Candida albicans. Antimicrob. Agents Chemotherapy 29(3):389-394.

Abstract

Combinations of nikkomycin X (NX) or nikkomycin Z (NZ), known inhibitors of chitin synthesis in fungi, together with papulacandin B (PB), an inhibitor of beta-glucan synthesis, were tested for synergistic activity against four isolates of Candida albicans both by broth micro dilution checkerboard technique and by a method to assess the regeneration of cell wall material in protoplasts. Construction of isobolograms from the data generated by the checkerboard determinations revealed a synergistic effect for the two classes of compounds against all strains. The combination of NX and PB was more effective than NZ and PB, perhaps a reflection of the lower Ki value of NX. While the presence of nikkomycins reduced chitin synthesis, as determined by staining with calcofluor white and assaying with a microfluorimeter, cells treated with PB demonstrated increased synthesis of chitin. Protoplast regeneration experiments using similar concentrations of the two classes of compounds resulted in comparable findings. The combination of NX and PB resulted in greater inhibition of chitin synthesis than equivalent combinations of NZ and PB. These data suggest that combinations of agents active against cell wall synthesis in fungi may prove more useful as chemotherapeutic agents than such compounds used singly.

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IX. Botanisches Institut der Universität Düsseldorf, Universitätsstrasse 1, D-4000 Düsseldorf, Federal Republic of Germany. Communicated by G. Michaelis.

Below follows the abstract of an article from my laboratory. The paper will be published in the EMBO Journal.

E. Pratje[1] and B. Guird[2]. 1986. One nuclear gene controls the removal of transient presequences from two yeast proteins: one encoded by the nuclear the other by the mitochondrial genome. EMBO J. (in press).

[1]Botanisches Institut der Universität Düsseldorf, D-4000 1, Federal Republic of Germany.
[2]Centre de Génétique Moleculaire du C.N.R.S., F-91190 Gif-sur-Yvette, France.

Abstract. The proteolytic processing of the mitochondrially encoded subunit II of cytochrome oxidase is prevented by the yeast mutation ts2858

(Pratje et al., 1983, EMBO J. 2, 1049-1054). We now report that the mutant is, in addition, temperature sensitive for the processing of cytochrome b_2, a protein encoded by nuclear DNA. Thus the same mutation affects the removal of presequences from a mitochondrially encoded inner membrane protein and from an imported soluble protein located in the intermembrane space. The mutation blocks the second processing step of cytochrome b_2. The cytochrome b_2 intermediate accumulates in the mutant at 36°C and assumes its enzyme activity. At 23°C the conversion to the mature protein is considerably slower than in wild type cells. The similarity of the cleavage sites asn-asp and asn-glu of the precursors for cytochrome oxidase subunit II and cytochrome b_2 respectively, suggests a sequence specific recognition by one protease or factor activating a protease. On the other hand maturation of cytochrome c peroxidase, another enzyme of the intermembrane space, is not affected by the pet ts2858 mutation.

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

Below follows the abstract of a paper now being written in our group entitled "Four GRC genes of yeast involved in the resting/growing-decision of the cell cycle" by Peter Briza, Marcella Eichler, and Michael Breitenbach.

Abstract. We have isolated a set of six temperature-sensitive yeast mutants which display pleiotropic phenotypes being deficient in ascospore germination, vegetative growth rate, entry into stationary phase, sporulation, or sexual conjugation. The mutants were originally isolated as germination-deficient using a selection scheme based on a "haploid meiosis"-strain harboring both the spo13 and ste9 mutations. They did not reach a defined terminal phenotype during the outgrowth of the ascospores, but rather seemed to undergo one to several cell cycles before they stopped growing.

At the restrictive temperature, vegetative cells of all six mutants grew more slowly than wild type. Two of the six mutants multiplied indefinitely at a constant rate, but the remaining four stopped growing after a limited number of cell cycles. After reaching the stationary phase, or on specialized starvation media, the mutants lost viability. Five of the six mutants were sporulation-deficient and one mutant was also sterile of the restrictive temperature. Survival under starvation conditions, sporulation, and mating all require arrest of the start point of the cell cycle. Therefore, this behaviour suggested a defect of the cells to arrest properly at start and/or to resume growth after a period of prolonged starvation. Consequently, the genes involved were designated GRC (for growth control).

All of the above mentioned phenotypes were temperature-sensitive and strictly co-segregated over several rounds of outcrossings. They were independent of the presence of the markers spo13 and ste9: Diploids homozygous for the respective grc mutations but wild type with respect to SPO13 and STE9 showed a phenotype very similar to the one of the original mutants. The six mutants were recessive and defined four genes as shown by two independent methods: a) complementation tests using the loss of viability-phenotype, and b) testing for recombination in all possible pairwise crosses of the six mutants. The locus GRC 1 was represented by three alleles. At least two of

them are different as judged by their phenotype. None of the four GRC genes was linked to its respective centromere.

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

Below follow five abstracts dealing with our currently active research projects.

1. A. Aguilera & F.K. Zimmermann. Characterization of the deletion mutation of the PGI1 gene coding for phosphoglucose isomerase in Saccharomyces cerevisiae.

The structural gene PGI1 coding for the phosphoglucose isomerase glycolytic enzyme of Saccharomyces cerevisiae was cloned from a genomic library constructed in the YEp13 vector containing the 2μ replication origin (Aguilera and Zimmermann, 1986, Mol. Gen. Genet 202:83-89). Southern blot experiments showed the existence of only one gene per haploid genome. After characterizing the coding region by S1 mapping more than 70% of the PGI1 coding region was removed from the chromosomal DNA and replaced by the LEU2 gene. The pgi1Δ mutants were still viable, indicating that this gene is not essential in yeast. But these mutants, whose known phenotype is the incapacity to grow on glucose media, are, unlike mutants with residual phosphoglucose isomerase activity, unable to grow on fructose as sole carbon source. The wild type growth rate could be restored by adding 0.1% glucose to this medium. On the other hand pgi1Δ/pgi1Δ diploid strains did not sporulate on the usual acetate medium. This defect could be alleviated by the addition of 0.05% glucose to the sporulation media. These results suggest that a) the phosphoglucose isomerase reaction is the only step catalyzing the interconversion of glucose-6-P and fructose-6-P; b) glucose-6-P is essential in yeasts, and c) the oxidation of the glucose-6-P through the glucose-6-P dehydrogenase reaction is not sufficient for supporting growth in yeasts (Aguilera, Mol. Gen. Genet., in press).

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2. J. Heinisch and F.K. Zimmermann. Physiological consequences of disrupting the genes coding for phosphofructokinase in yeast.

Yeast phosphofructokinase is an octameric enzyme composed of 4 α- and 4 β-subunits. The genes coding for these subunits are called PFK1 and PFK2, respectively. Mutants in one of the two genes lack detectable phosphofructokinase activity in vitro but are still able to ferment glucose. This has been attributed to a "leakyness" of the pfk2-mutation or the existence of a "particulate PKF" sharing one of the subunits with the cytoplasmic form. The genes PFK1 and PFK2 have been isolated. Disruption of the two genes led to the same phenotype as obtained with classical mutants. This suggests that neither of the two hypotheses cited above can account for glucose-fermentation by pfk-single mutants. Two models explain this feature: Either one of the two PFK-genes is sufficient for providing a catalytic function or there is a "bypass" to the reaction catalyzed by phosphofructokinase. The first model seems unlikely because there is no phosphofructokinase-activity detectable in vitro and catalytic function has

been assigned to the β-subunits. Evidence for the existence of a "bypass" has been published (Breitenbach-Schmitt et al., 1984a,b; Mol. Gen. Genet. 195, 530, 536).

Recent Publications: J. Heinisch and F.K. Zimmerman (1985): Is the Phosphofructokinase-reaction Obligatory for Glucose Fermentation by Saccharomyces cerevisiae? - Yeast, 1, 173-175; J. Heinisch (1986): Isolation and characterization of the two genes coding for phosphofructokinase in yeast - Mol. Gen. Genet., 202, 75-82.

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3. Stefan Hohmann. Invertase Genes of Saccharomyces cerevisiae.

Six different locations in the yeast genome can carry the structural information for invertase: SUC1 - SUC5 and SUC7. SUC1,2 and 7 have been cloned by Carlson et al. (1985). We isolated SUC2 again from a genomic library of Nasmyth using complementation of a suc° strain. By Southern analysis using cloned SUC2 the other SUC genes were located on Sph I restriction fragments. This allowed to clone SUC4 and SUC5 from minipools containing restriction fragments of the characteristic length. A DNA sequence adjacent to cloned SUC4 showing homology to corresponding regions of the other SUC genes was used to construct a plasmid which could be used to specifically "evict" other SUC genes. This plasmid was targeted for integration next to these genes and then cut out of chromosomal DNA with Xho I or Bgl II restriction endonuclease. A 5.6 kb fragment carrying structural information for invertase was obtained from a strain with SUC3 and a 17.3 kb fragment from a strain with SUC5.

The individual SUC genes were identified and characterized by genetic analysis of the donor strains, by Southern analysis of genomic DNA and by extensive restriction mapping.

We are going to clone SUC1 and a larger fragment of the SUC3 locus. The isolated invertase genes can be used for further investigations: regulation of invertase synthesis (carbon catabolite repression), glycosylation and excretion of invertase, excretion of other proteins using invertase leader sequences.

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4. T. Seehaus and F.K. Zimmermann. Isolation of a new class of pyruvate decarboxylase mutants in Saccharomyces cerevisiae.

Pyruvate decarboxylase mutants in Saccharomyces cerevisiae have been isolated previously (Lam and Marmur, 1977, J. Bacteriol. 130: 746-749; Lancashire et al., 1981, Mol. Gen. Genet. 181: 409-410; Schmitt and Zimmermann, 1982, J. Bacteriol. 151: 1146-1152).

Besides mutations in the genes pdc1 and pdc2 mutants in a new gene (PDC4) affecting pyruvate decarboxylase activity could be isolated. These recessive mutations are not linked to PDC1 and PDC2 as shown by tetrad analysis. They are unable to grow on a medium containing glucose and Antimycin A to block respiration. The residual pyruvate decarboxylase activity as measured in crude extracts is reduced to 4-15% wild type activity. Pyruvate excretion into the medium as well as the intracellular pyruvate concentration is

increased 6-12 fold over the wild type level. Concentrations of other glycolytic metabolites are normal. Pyruvate decarboxylase in these mutants is the same as in wild type in respect to substrate kinetics and in affinity for thiaminpyrophosphate.

Finally, a 5.4 kb sequence complementing the pdc4 defect was isolated from the gene pool of Nasmyth and Tatchell (1980, Cell 19: 753-764). The location of the coding region is under present investigation.

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5. F.K. Zimmermann, R.E. Taylor-Mayer* and U. Holzwarth. Chemically induced aneuploidy, mitotic recombination and reverse mutation in strains D61.M and D7 of Saccharomyces cerevisiae.

We have previously shown that aprotic polar solvents like ethyl acetate, methyl ethyl ketone, acetonitrile are strong inducers of aneuploidy in yeast when growing cells are inubated first at 30°C, then stored in an ice bath overnight and finally incubated at 30°C before plating (Mutation Research 149, 339-351, and 150, 203-210, 1985). We could also show (Mutation Res. 149, 333-338) that such agents interfered with the aggregation in vitro of porcine brain tubulin. This suggested that the primary targets for the induction of aneuploidy in yeast cells are the microtubules of the mitotic spindle which contain tubulin also in yeast. A more detailed analysis of the amplifying effects of cold storage on the induction of mitotic aneuploidy by aprotic polar solvents showed that the actual cold shock temperature optimum differs between chemicals in the range from ice bath temperature to 16°C. Cold shock is effective only in the very narrow range around 4°C with methyl pyrrolidinone, 6°C for γ-valerolactone whereas hardly any variation was observed for 4-acetylpyridine. The well established destabilization of microtubules at lower temperatures is considered to be a specific factor in the action of these chemicals which are hypothesized to act via an interference with the solvation coat covering the tubulin monomers, but collapses above a certain temperature and thus allows for aggregation. We have also tested a large number of pesticides and found that captafol is as potent an inducer of mitotic crossing-over, mitotic gene conversion and reverse mutation in strain D7, as EMS, however with a lowest effective dose of 0.05 ppm. Captafol did not induce aneuploidy in strain D61.M.

*On sabbatical leave from the Department of Biology, Slippery Rock University, Slippery Rock, PA.

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XII. Alko Research Laboratories, P.O. Box 350, SF-00101, Helsinki 10, Finland. Communicated by Matti Korhola.

Below follows a list of our work published since December 1985.

1. Hannele Ruohola, Pirkko L. Liljeström, Tuula Torkkeli, Helena Kopu, Pirjo Lehtinen, Nisse Kalkkinen and Matti Korhola. 1986. Expression and Regulation of the Yeast MEL1 gene. FEMS Microbiology Letters 34: 179-185.

The Saccharomyces cerevisiae var. uvarum MEL1 gene, conferring production

of α-galactosidase, has been cloned to study its expression. Increasing amounts of α-galactosidase protein and activity closely followed the induction of functional MEL1 transcripts (1.65 kb). The initial appearance of the transcript occurred within 3-6 min and the corresponding α-galactosidase activity within 8-10 min of galactose addition. Therefore, regulation of α-galactosidase synthesis operates primarily at the level of MEL1 transcription. No transcription is detectable in glucose-grown cultures. α-Galactosidase activity is found in the periplasmic space and in the extracellular medium. Enzyme from either location exhibited a molecular size of 76 kDa, which was reduced to 53 kDa after deglycosylation with endoglycosidase H. The N-terminal sequence of purified α-galactosidase reveals cleavage of putative 18-amino-acid signal sequence for secretion.

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2. M. Korhola, A. Vainio and K. Edelmann. 1986. Selenium Yeast. Annals of Clinical Research 18: 65-68.

Baker's yeast is able to assimilate carbon, nitrogen, phosphrus and sulphur sources together with a great number of minerals and trace elements into a palatable, nutritious product. The metabolism of yeast is precisely controlled during the production growth phase and thus it is possible to determine the composition of the product by controlling the raw materials.

Because of existing deficiencies in the availability of certain trace elements, mainly selenium, in Finnish diets, we started testing the possibilities for enriching yeast with this essential trace element about five years ago. We have succeeded in developing a special yeast product with a selenium concentration of 500 mg/kg dry matter.

Selenium was expected, because of its structural similarity to sulphur, to replace sulphur in the biosynthetic reactions of the yeast cell.

We have recently studied the incorporation and distribution of selenium in yeast with radioactive selenium (^{75}Se). Analysis of the protein fraction of selenium yeast has shown that selenium is present in all the major soluble proteins. Selenomethionine was identified as the major selenium-containg compound in the protein fraction as well as in the whole cell.

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3. Roy S. Tubb and Pirkko L. Liljeström. A Colony-colour Method Which Differentiates α-Galactosidase-Positive Strains of Yeast. J. Institute of Brewing (in press).

5-Bromo-4-chloro-3-indolyl-α D-galactoside (X-α-gal) has been used as a chromogenic substrate for differentiating α-galactosidase-positive yeast strains on agar media. The method can be applied to monitoring culture purity in breweries which use both ale and lager strains of yeast.

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The following publication has appeared since the last communication. The abstract of the report has been given in Yeast Newsletter 34 (1985): 2, 55.

Pirkko Liljestrom, The nucleotide sequence of the yeast MEL1 gene. Nucleic Acids Research 13 (1985), 7257-7268.

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XIII. Vrije Universiteit, Biochemisch Laboratorium, P.O. Box 7161, 1007 MC Amsterdam, The Netherlands. Communicated by J. Klootwijk.

The following manuscripts from our group have been accepted for publication:

1. Tarek T.A.L. El-Baradi, Carine A.F.M. van der Sande, Willem H. Mager, Hendrik A. Raue, and Rudi J. Planta.
The cellular level of yeast ribosomal protein L25 is controlled principally by rapid degradation of excess protein. Current Genetics, in press.

Abstract. When the gene dosage for the primary rRNA-binding ribosomal protein L25 in yeast cells was raised about 50-fold, the level of mature L25 transcripts was found to increase almost proportionally. The plasmid-derived L25 transcripts were structurally indistinguishable from their genomic counterparts, freely entered polysomes in vivo and were fully translatable in a heterologous in vitro system. Nevertheless, pulse-labeling for periods varying from 3-20 min. did not reveal a significant elevation of the intracellular level of L25 protein. When pulse-times were decreased to 10-45 sec, however, we did detect a substantial overproduction of L25.

We conclude that, despite the strong RNA-binding capacity of the protein, accumulation of L25 is not controlled by an autogenous (pre-)mRNA-targeted mechanism similar to that operating in bacteria, but rather by extremely rapid degradation of excess protein produced.

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2. Lambertus P. Woudt, August B. Smit, Willem H. Mager, and Rudi J. Planta. Conserved sequence elements upstream of the gene encoding yeast ribosomal protein L25 are involved in transcription activation. The EMBO J., in press.

Abstract. Previous studies have revealed the occurrence of two closely linked conserved sequence elements, designated as HOMOL1 and RPG-box, in front of most yeast ribosomal protein (rp) genes examined.

To investigate whether these conserved nucleotide elements play a role in the regulation of rp gene expression, we performed deletion analysis of the DNA region upstream of the gene encoding ribosomal protein L25. To that end we constructed a hybrid gene consisting of the pertinent 5'-flanking sequence and the E. coli galK marker gene. The effects on the transcription of this fusion gene of Bal31-generated deletions were measured by Northern analysis of RNA isolated from the respective transformed yeast cells. The results demonstrate that removal of one box has a detrimental effect on the level of transcription, whereas after the deletion of both boxes hardly any transcription can be observed.

Subsequently, we inserted synthetic oligonucleotides in the upstream region of an L-25 gene from which the original boxes had been removed.

Expression of the inactivated hybrid gene turned out to be restored even by insertion of one RPG-element. Moreover, the RPG-box functions in both orientation, though not with equal efficiency.

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XIV. Instituto de Investigaciones Biomedicas del C.S.I.C., Facultad de Medicina U.A.M., Arzobispo Morcillo 4;28029, Madrid, Spain. Communicated by Carlos Gancedo.

The following work has been recently published:

Jose M. Siverio, Marta D. Valdes-Hevia and Carlos Gancedo. 1986. Toxicity of 3-O-methyl glucose in yeast is due to its phosphorylation by glucokinase. FEBS Letters 194:39-42.

Abstract. 3-O-Methyl glucose inhibited the growth of S. cerevisiae on non-fermentable carbon sources only in strains possessing glucokinase. Accumulation of a phosphorylated derivative of 3-O-methyl glucose occurred only in strains carrying glucokinase. Phosphorylation of the sugar in vitro was observed with partially purified preparations of glucokinase but not with equivalent preparations of isoenzymes PI or PII of hexokinase. The K_m value of glucokinase for 3-O-methyl glucose was 5 mM and the V_{max} was ~1/100 of that found for glucose. A mutant resistant to 3-O-methyl glucose was isolated from a strain possessing only glucokinase. This mutant had only about 10% of the glucokinase found in the parental strain.

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A review of Catabolite repression mutants will be published in FEMS Microbiology Reviews

Catabolite repression mutants of yeasts, Juana M. Gancedo and Carlos Gancedo.

Summary. The mechanism of catabolite repression in yeast is not well understood, although it has been established that cAMP does not play a role as it does in Escherichia coli. To identify the elements implicated in catabolite repression in yeast, a variety of mutants affected in this process have been isolated by different research groups. A systematic review of the results reported in the literature is presented. The conclusion that can be drawn is that the mechanism of catabolite repression is a complex one, with no single gene controlling all the genes subject to repression. The expression of a given gene or set of genes is controlled by several regulatory genes, but it is not yet known whether these genes act cooperatively or sequentially.

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A FEBS Advanced Course on "Biochemistry and Genetics of yeasts" will take place in Jerez de la Frontera (Spain) (Sherry area) from September 7 to 24, 1987. Detailed information will be available at the end of 1986 and may be obtained from C. Gancedo, Instituto de Investigactiones Biomedicas, Facultad de Medicina UAM, Arzobispo Morcillo 4, 28029 Madrid, Spain.

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XV. Centre National De La Recherche Scientifique, Laboratoire D'Enzymologie, 91190 Gif Sur Yvette (France). Communicated by J. Schwencke.

Below follows a summary of a short paper published recently in Histochemical Journal 17 (1985) 535-537 in collaboration with Dr. Vorisek (Prague).

"Electron cytochemical reaction for dipeptidyl aminopeptidase in the yeast Saccharomyces cerevisiae". J. Vorišek (1), J. Schwencke (2) and A. Kotyk (1).

1. Institute of Microbiology, Czechoslovak Academy of Sciences, Videnska 1083, 14220 Prague, Czechoslovakia.

2. Laboratoire d'Enzymologie, CNRS, 91190 Gif-sur-Yvette, France.

Abstract

Saccharomyces cerevisiae pep4-3 haploid cells which have low levels of proteinases A and B and carboxypeptidase Y (Hemmings et al. (1980) Arch. Biochem. Biophys. 202, 657-660) contain a membrane-bound X-prolyl dipeptidylaminopeptidase activity (Suarez-Rendueles et al. (1981) FEBS Letters 131, 296-299). Using the same strain we have designed an ultracytochemical procedure to analyse the intracellular location of this enzyme in more detail. Late exponentially growing cells, fixed with glutaraldehyde and permeabilized by deoxycholate were incubated with L-ala-L-Pro-4-methoxy-β-naphthyl-amide as substrate plus hexazotized pararosaniline. In vacuolated cells, the reaction product appears clearly in the tonoplast as previously found by biochemical methods (Bordallo et al. (1984) FEBS Letters 173, 199-203). The reaction product appeared also in a variety of membranous structures such as the nuclear envelope; polar layers of the endoplasmic reticulum membranes; lipoprotein bodies; the membranes and or the lumen of membrane vesicles (diameter 30-90 nm) and with high density in the core or the surface layer of small vesicles (diameter 25-40 nm). Some of these vesicles may correspond to chitosomes as found separately by biochemical methods (J. Schwencke et al. (1984) In: Microbial cell wall synthesis and autolysis (C. Nombela Editor) Elsevier Science Publishers B. V. Amsterdam (1984) pp. 121-130) after sub-cellular fractionation.

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XVI. University of Guelph, College of Biological Science, Department of Molecular Biology and Genetics, Guelph, Ontario, Canada N1G 2W1. Communicated by R.E. Subden.

The following is the abstract of a paper recently submitted to J. Bacteriology.

Osothsilp, C., and R.E. Subden. Malate transport in Schizosaccharomyces pombe.

Abstract

The transport of malate was studied in Schizosaccharomyces pombe wild type and mutant strains unable to utilize malic acid. Two groups of such mutants, viz. malic enzyme and malate transport deficient mutants, were

differentiated by ^{14}C-labeled-L-malate transport assay and starch gel electrophoresis followed by activity staining for malic enzyme and malate dehydrogenase. Transport of malate in S. pombe was constitutive and strongly inhibited by inhibitors of oxidative phosphorylation and of the formation of proton gradients. The transport was a saturable function of the malate concentration. The apparent Km and Vmax values for transport by the parent were 3.7 mM and 40 nmoles per min per mg protein, respectively, while those for malic enzyme mutant were 5.7 mM and 33 nmoles per min per mg protein. Malate transport was pH and temperature dependent. Rates of transport at various pH values suggest that the monoionic species is the molecular form preferentially transported. Specificity of the transport was studied with various substrates including mono- and dicarboxylic acids and the possibility of the existence of a common transport system for dicarboxylic acids is discussed.

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XVII. Albert-Ludwigs Universität, Biochemisches Institut, Hermann-Herder Str. 7, D-7800 Freiburg I. Br., Fed. Republic of Germany. Communicated by Claudio Purwin.

The following abstract summarizes our recent work about control of adenylate cyclase activity in the yeast Saccharomyces cerevisiae.

Claudio Purwin[1], Klaas Nicolay[2], W. Alexander Scheffers[3], and Helmut Holzer[1,4]. 1986. Mechanism of control of adenylate cyclase activity in yeast by fermentable sugars and carbonyl cyanide m-chlorophenylhydrazone. J. Biol. Chem. (in press).

[1]Biochemisches Institut der Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg, West Germany, [2]Institute of Molecular Biology, University of Utrecht, Padualaan 8, NL-3584 CH Utrecht, The Netherlands, [3]Department of Microbiology, Delft University of Technology, Julianalaan 67a, NL-2628 BC Delft, The Netherlands, [4]Gesellschaft fur Strahlen- und Umweltforschung Abteilung für Enzymchemie, Ingolstädter Landstr. 1, D-8042 Neuherberg bei München, West Germany.

Abstract. The phosphorylation of fructose-1,6-bisphosphatase is preceded by a transient increase in the intracellular level of cyclic AMP which activates a cyclic AMP-dependent protein kinase (Pohlig, G. and Holzer, H. (1985), J. Biol. Chem. 260, 13818-13823). Possible mechanisms by which sugars or ionophores might activate adenylate cyclase and thereby lead to an increase in cyclic AMP concentrations were studied. Studies with permeabilized yeast cells demonstrated that neither sugar intermediates nor carbonyl cyanide m-chlorophenylhydrazone (CCCP) were able to increase adenylate cyclase activity. In the light of striking differences between the effects of fermentable sugars and of CCCP on parameters characterizing the membrane potential, it seems not reasonable to assume that the activity of adenylate cyclase is under control of the membrane potential. Rapid quenching of 9-aminoacridine fluorescence after addition of fermentable sugars to starved yeast cells indicated an intracellular acidification. The ^{31}P-NMR technique showed a fast drop of the intracellular pH from 6.9 to 6.55 or to 6.4 immediately after addition of glucose or CCCP. The time course of the decrease of the cytosolic pH coincide with the transient increase of cyclic AMP concentration and the 50% inactivation of fructose-1,6-bisphosphatase

under the conditions of the NMR experiments. Kinetic studies of adenylate cyclase activity showed an approximately 2-fold increase of activity when the pH was decreased from 7.0 to 6.5, which was the result of a decrease in the apparent K_m for ATP with no change in V_{max}. These studies suggest that activation of adenylate cyclase by decrease in the cytosolic pH starts a chain of events leading to accumulation of cyclic AMP and phosphorylation of fructose-1,6-bisphosphatase.

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XVIII. Technische Hogeschool Delft, Laboratorium voor Microbiologie, Julianalaan 67, NL-2628 BC Delft, The Netherlands. Communicated by W. Alexander Scheffers.

The following two papers, abstracts of which have already appeared in Yeast Newsletter Vol. XXXIV, Number II, now have been published:

1. P.M. Bruinenberg, J.P. van Dijken and W.A. Scheffers. A radiorespirometric study on the contribution of the hexose monophosphate pathway to glucose metabolism in Candida utilis CBS 621 grown in glucose-limited chemostat cultures. J. Gen. Microbiol. 132: 221-229.

2. C. Verduyn, J. Frank, J.P. van Dijken and W.A. Scheffers. 1985. Multiple forms of xylose reductase in Pachysolen tanophilus CBS 4044. FEMS Microbiol. Lett. 30: 313-317.

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The following papers have been accepted for publication.

3. J.P. van Dijken and W.A. Scheffers. 1986. Redox balances in the metabolism of sugars by yeasts. FEMS Microbiol. Reviews: in press.

Abstract. The central role of the redox couples NAD+/NADH and NADP+/NADPH in the metabolism of sugars by yeasts is discussed in relation to energy metabolism and product formation. Besides their physical compartmentation in cytosol and mitochondria, the two coenzyme systems are separated by chemical compartmentation as a consequence of the absence of transhydrogenase activity. This has considerable consequencs for the redox balances of both coenzyme systems and hence for sugar metabolism in yeasts.

As examples, the competition between respiration and fermentation of glucose, the Crabtree effect, the Custers effect, adaptation to anaerobiosis, the activities of the hexose monophosphate pathway, and the fermentation of xylose in yeasts are discussed.

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4. P.M. Bruinenberg, G.W. Waslander, J.P. van Dijken and W.A. Scheffers. 1986. A comparative radiorespirometric study of glucose metabolism in yeasts. Yeast 2: in press.

Abstract. A comparative radiorespirometric study of glucose metabolism in glucose-limited chemostat cultures of Saccharomyces cerevisiae, Candida

utilis and Rhodosporidium toruloides was performed in an attempt to estimate the contribution of the hexose monophosphate (HMP) pathway to glucose metabolism. Radioactively labelled glucose was administered directly to the cultures in a constant substrate feed, without disturbance of the steady state. The $^{14}CO_2$ yields from [1-^{14}C]- and [6-^{14}C]-glucose demonstrated that the HMP pathway activities for the three yeasts were very similar. Furthermore, a quantitative analysis of results indicated that the HMP pathway activities were close to the theoretical minimum needed to cover the NADPH requirement for biomass formation.

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5. J.P. van Dijken, E. van den Bosch, J.J. Hermans, L. Rodrigues de Miranda and W.A. Scheffers. 1986. Alcoholic fermentation by "non-fermentative" yeasts. Yeast 2: in press.

All type strains of "non-fermentative" yeasts, available in the culture collection of the Centraalbureau voor Schimmelcultures, were reinvestigated for their capacity to ferment glucose in the classical Durham tube test. Although visible gas production was absent, nearly all strains produced significant amounts of ethanol under the test conditions. Under conditions of oxygen-limited growth, even strong alcoholic fermentation may occur in a number of yeasts hitherto considered as non-fermentative. Thus, shake-flask cultures of Hansenula nonfermentans and Candida silvae fermented more than half of the available sugar to ethanol. It was concluded that the taxonomic test for fermentation capacity, which relies on detection of gas formation in Durham tubes, is not reliable for a physiological classification of yeasts as fermentative and non-fermentative species.

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6. C. Purwin, K. Nicolay, W.A. Scheffers and H. Holzer. 1986. Mechanism of control of adenylate cyclase activity in yeast by fermentable sugars and carbonyl cyanide m-chlorophenylhydrazone. J. Biol. Chem.: in press.

The abstract of this article is given in the previous section communicated by Claudio Purwin.

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

The following papers were published recently:

1. C. Cabeça-Silva, A. Madeira-Lopes and N. van Uden. 1985. The temperature profiles of growth, thermal death and ethanol tolerance of the cellobiose-fermenting yeast Candida wickerhamii. Journal of Basic Microbiology 2:221-224.

The temperature profiles of growth and thermal death of the cellobiose-fermenting yeast Candida wickerhamii was associative with the initial maximum, optimum and minimum temperatures for growth around 38°C, 35°C, 31°C and 3°C, respectively. Ethanol enhanced thermal death by increasing the entropy of activation (entropy coefficient 7.2 entropy units mol^{-1} l^{-1}), shifted the

supraoptimal part of the profile to lower temperatures without disrupting it and increased the minimum temperature for growth. The temperature profile of ethanol tolerance with respect to growth displayed a narrow temperature plateau (18-22°C) of maximum tolerance (limit 7.4%, v/v, ethanol) while the toxic effects of ethanol increased steeply on either side of the plateau.

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2. J. Jiménez and N. van Uden. 1985. Use of extracellular acidification for the rapid testing of ethanol tolerance in yeasts. Biotechnology and Bioengineering 27:1596-1598.

The exponential enhancement by ethanol of proton influx in Saccharomyces cerevisiae, observed earlier in our laboratory (Leao and van Uden, Biochim. Biophys. Acta 774, 43, 1984) was made use of to develop a rapid method for testing ethanol tolerance. Exponential enhancement constants calculated from the final pH values of acidification curves in the presence of ethanol obtained with a collection of strains isolated in Spain from wines and musts, correlated with K_i, the concentration of ethanol that reduced the specific growth rate by 50%.

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3. C. Leão and N. van Uden. 1985. Effects of ethanol and other alkanols on the temperature relations of glucose transport and fermentation in Saccharomyces cerevisiae. Applied Microbiology and Biotechnology 22: 359-363.

Ethanol, isopropanol, propanol and butanol exponentially inhibited the maximum velocity of the glucose transport system of Saccharomyces cerevisiae, determined by use of the non-metabolizable analogue D-xylose. While the exponential inhibition constants increased with the lipid solubility of the alkanols, they were independent of temperature in the range 21-35°C: the Arrhenius plots (modified according to the theory of absolute reaction rates) of the initial maximum rates of xylose transport were linear and parallel in both the absence and presence of alkanols. Thus, the alkanols did not affect the enthalpy of activation of the glucose transport system ($\Delta H^{\neq}$ was 12 189 cal mol^{-1}) but decreased the entropy of activation. The following entropy coefficients (decrease in activation entropy per unit concentration of alkanol) were obtained: ethanol, -0.84; isopropanol, -1.21; propanol, -1.41 and butanol, -3.18 entropy units per mole per liter. The temperature relations of glucose fermentation with and without ethanol by resting cells over the temperature range studied (15-35°C) were nearly identical with those of the glucose transport system, suggesting that the latter mediates the rate-limiting step of the former and that this relationship is maintained in the presence of ethanol.

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4. C. Leão and N. van Uden. 1986. Transport of lactate and other short-chain monocarboxylates in the yeast Candida utilis. Applied Microbiology and Biotechnology 23:389-393.

Lactic acid grown cells of the yeast Candida utilis transported lactate by an accumulative electroneutral proton-lactate symport with a proton-lactate stoicheiometry of 1:1. The accumulation ratio at pH 5.5 was about twenty.

The symport accepted the following monocarboxylates (K_s values at 25°C, pH 5.5 in brackets): D-lactate (0.06 mM), L-lactate (0.06 mM), pyruvate (0.03 mM), propionate (0.05 mM) and acetate (0.1 mM). The system was inducible and was subject to glucose repression. The affinity of the symport for lactate was not affected by pH over the range 3-6, while the maximum transport velocity was strongly pH dependent, its optimum pH being around pH 5. Undissociated lactic acid entered the cells by simple diffusion. The permeability for the undissociated acid increased exponentially with pH, the diffusion constant increasing 35-fold when the pH was increased from 3 to 5.5.

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5. C. Lucas and N. van Uden. 1985. The temperature profiles of growth, thermal death and ethanol tolerance of the xylose-fermenting yeast Candida shehatae. Journal of Basic Microbiology 25:547-550.

The temperature profile of growth and thermal death of the xylose-fermenting yeast Candida shehatae was dissociative. The Arrhenius plot of growth lacked a descending supraoptimal branch and the specific growth rate at the maximum temperature for growth (around 31°C) was not significantly different from its values at the other temperatures studied (down to 20°C). Ethanol enhanced thermal death by increasing its entropy of activation (entropy coefficient 16.1 entropy units mol^{-1} l^{-1}). The temperature profile of ethanol tolerance with respect to growth displayed a temperature plateau (10-17.5°C) of maximum ethanol tolerance (limit 6% v/v of ethanol) while the toxic effects of ethanol increased on either side of the plateau depressing the maximum temperature for growth from 31 to 17.5°C and increasing the minimum temperature for growth from 2.5 to 10°C.

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6. C. Lucas and N. van Uden. 1986. Transport of hemicellulose monomers in the xylose-fermenting yeast Candida shehatae. Applied Microbiology and Biotechnology (accepted).

Cells of Candida shehatae repressed by growth in glucose- or D-xylose-medium produced a facilitated diffusion system that transported glucose (K_s ± 2 mM, V_{max} ± 2.3 mmoles g^{-1} h^{-1}), D-xylose (K_s ± 125 mM, V_{max} ± 22.5 mmoles g^{-1} h^{-1}) and D-mannose but neither D-galactose nor L-arabinose.

Cells derepressed by starvation formed several sugar-proton symports. One proton symport accumulated 3-O-methylglucose about 400-fold and transported glucose (K_s ± 0.12 mM, V_{max} ± 3.2 mmoles g^{-1} h^{-1}) and D-mannose, a second proton symport transported D-xylose (K_s ± 1.0 mM, V_{max} 1.4 mmoles g^{-1} h^{-1}) and D-galactose, while L-arabinose apparently used a third proton symport. The stoicheiometry was one proton for each molecule of glucose or D-xylose transported. Substrates of one sugar proton symport inhibited non-competitively the transport of substrates of the other symports.

Starvation, while inducing the sugar-proton symports, silenced the facilitated diffusion system with respect to glucose transport but not with respect to the transport of D-xylose, facilitated diffusion functioning simultaneously with the D-xylose-proton symport.

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7. A. Madeira-Lopes. 1985. The influence of temperature on the relations between thermal death, growth and yield in Candida utilis. Journal of Basic Microbiology 25:39-42.

In the yeast Candida utilis an associative temperature profile was found with respect to thermal death and growth. The cardinal temperatures were the following: optimum temperature for growth, 36°C, minimum temperature of thermal death, 37.9°C; final maximum temperature for growth, 40.1°C; initial maximum temperature for growth, 40.7°C. The growth yield on glucose only decreased near the final maximum temperature for growth.

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8. A. Madeira-Lopes. 1986. Thermal death potentiation by amphotericin B in Cryptococcus neoformans and its dependence on pre-incubation temperature. Sabouraudia 24:35-40.

Thermal death of Cryptococcus neoformans in the presence of amphotericin B was strongly dependent upon the temperature of pre-incubation. The entropy coefficient, that is, the increase in entropy of activation of thermal death per square unit concentration of the drug in the medium, was 35 times higher after pre-incubation at 25°C than at 39°C. This means that C. neoformans cells grown at lower temperatures were much more sensitive to the temperature-dependent fungicidal effect of amphotericin B.

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9. A. Madeira-Lopes, M. Teresa Plácido and C. Cabeça-Silva. 1986. Comparative study of the temperature profiles of growth and death of the pathogenic yeast Cryptococcus neoformans and the non-pathogenic Cr. albidus. Journal of Basic Microbiology 26:43-47.

The temperature profiles of two species of Cryptococcus were compared. The pathogenic Cr. neoformans had a maximum temperature for growth of 39.8°C and the non-pathogenic Cr. albidus, of 30.2°C. The specific growth rates measured in the former were of an order of magnitude higher than in the latter, whereas the Arrhenius plots of the specific thermal death rates did not show a significant difference.

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10. J.M. Peinado and M.C. Loureiro-Dias. 1986. Reversible loss of affinity induced by glucose in the maltose-H^+ symport of Saccharomyces cerevisiae. Biochimica et Biophysica Acta (accepted).

Glucose represses and inactivates maltose transport in Sacch. cerevisiae. Inactivation has been described as an irreversible process involving proteolysis. We have studied the inactivation of the maltose-H^+ symport in this yeast and observed that the mechanism of inactivation depended on the physiological conditions. In resting cells there was a decrease of transport capacity. The rate of decrease was enhanced non-specifically by the presence of a sugar, glucose being more effective than maltose. In growing cells, glucose induced a decrease in affinity of the H^+-symport which could be recovered by starvation even in the presence of cycloheximide; there was no loss in capacity or, if present, could be fully explained by the dilution due to repression during growth on glucose. We submit that in growing cells

inactivation consists in a reversible modification of the permease not involving proteolysis.

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11. I. Sá-Correia and N. van Uden. 1986. Ethanol-induced death of Saccharomyces cerevisiae at low and intermediate growth temperatures. Biotechnology and Bioengineering 28:301-303.

The Arrhenius plots of the specific death rates of Saccharomyces cerevisiae in the presence of ethanol were composite and expressed the sum of ethanol-enhanced thermal death ($\Delta H^{\neq}$ around 90 kcal/mol) and ethanol-induced death ($\Delta H^{\neq}$ between 14 and 24 kcal/mol depending on the ethanol concentration). The former mode of death dominated at high physiological temperatures, the latter at low temperatures while both modes of death had measurable weight at intermediate temperatures.

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12. I. Spencer-Martins and N. van Uden. 1985. Catabolite interconversion of glucose transport systems in the yeast Candida wickerhamii. Biochimica et Biophysica Acta 812:168-172.

Candida wickerhamii IGC 3244 growing in glucose medium transported glucose by facilitated diffusion (at 25°C and pH 5, the K_s value was 1.7 mM and the V_{max} value was 1.6 mmol/h per g dry wt.), while cells grown under derepressed conditions produced a glucose proton symport (at 25°C and pH 5, the K_s value was 0.18 mM and the V_{max} value was 1.8-1.9 mmol/h per g dry wt.). In each case, the Lineweaver-Burk plot of initial uptake rates was linear, indicating the presence of a single system. In buffer with 2% glucose, the symport suffered catabolite inactivation while the facilitated diffusion system emerged concomitantly in such a way that the combined V_{max} remained nearly constant. During the conversion process, the Lineweaver-Burk envisions the proton symport as composed of the facilitated diffusion system in association with (an)other transport protein(s), the latter being sensitive to carbon catabolite repression and inactivation.

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13. I. Spencer-Martins and N. van Uden. 1985. Inactivation of active glucose transport in Candida wickerhamii is triggered by exocellular glucose. FEMS Microbiology Letters 28:277-279.

Under conditions of derepression the yeast Candida wickerhamii formed a high-affinity glucose proton symport. Glucose and glucose analogues induced inactivation of the glucose proton symport and its interconversion into a low-affinity facilitated diffusion system. The specific inactivation rate increased with the concentration of the inactivating sugar and did not obey satuation kinetics. This dependence was still pronounced at sugar concentrations far above saturation of the glucose transport systems. This suggested that the inactivation and interconversion mechanism was triggered by interaction of the inactivating sugar with receptor sites located on the cell surface.

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14. Review paper:

N. van Uden. 1985. Ethanol toxicity and ethanol tolerance in yeasts. Annual Reports on Fermentation Processes 8:11-58.

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XX. Department of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, Australia 2351. Communicated by K. Watson.

The following are summaries of two papers from this laboratory which have been submitted for publication.

1. High-Efficiency Carbohydrate Fermentation to Ethanol at Temperatures above 40°C by Kluyveromyces marxianus var. marxianus Isolated from Sugar Mills

P.J. Anderson, K. McNeil, and K. Watson. Appl. Environ. Microbiol. June, 1986.

A number of yeast strains, isolated from sugar cane mills and identified as strains of Kluyveromyces marxianus var. marxianus, were examined for their ability to ferment glucose and cane syrup to ethanol at high temperatures. Several strains were capable of rapid fermentation at temperatures up to 47°C. At 43°C, >6% (wt/vol) ethanol was produced after 12 to 14 h of fermentation, concurrent with retention of high cell viability (>80%). Although the type strain (CBS 712) of K. marxianus var. marxianus produced up to 6% (wt/vol) ethanol at 43°C, cell viability was low, 30 to 50%, and the fermentation time was 24 to 30 h. On the basis of currently available strains, we suggest that it may be possible by genetic engineering to construct yeasts capable of fermenting carbohydrates at temperatures close to 50°C to produce 10 to 15% (wt/vol) ethanol in 12 to 18 h with retention of cell viability.

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2. Intrinsic and Transient Thermotolerance and Ethanol Tolerance in Saccharomyces cerevisiae.

R. Cavicchioli and K. Watson. Submitted for publication.

Yeast cells, when subjected to a primary heat shock, defined as a temperature upshift from 23°C to 37°C for 30-60 min, acquired tolerance to heat stress (52°C/5 min) and ethanol stress (10-15% w/v). A protection against thermal injury of the plasma membrane transport system, as measured by uptake of ^{14}C-leucine and ^{14}C-glucose, by a primary heat shock implicated a functional role for membranes in the heat shock response.

Primary heat shocked cells incubated at 23°C for up to 6 h, progressively lost thermotolerance but retained high levels of the major heat shock proteins as observed on polyacrylamide gels. On the other hand, a temperature upshift back up to 37°C for 30 min fully restored thermotolerance. The major high molecular weight heat shock proteins (hsp) identified were of approximate molecular weight 100,000 (hsp 100), 80,000 (hsp 80) and 70,000 (hsp 70). 37°C grown cells were intrinsically more thermotolerant than 23°C grown cells. The

proportion of survivors following heat treatment increased as cells, growing at 23°C or 37°C, progressed from exponential growth to stationary phase. The effect was particularly marked for 23°C grown heat stressed cells, where viability increased from essentially nil at early log phase to 25% at stationary phase. Cycloheximide (1-100 $\mu g\ ml^{-1}$) pretreatment of 23°C grown cells (a) lead to an enhancement of thermotolerance and, to a lesser extent, ethanol tolerance and (b) did not result in a marked loss of tolerance induced by a primary heat shock. Overall, these results provide evidence that there is no direct causal relationship between heat shock induced ethanol and thermotolerance and presence of heat shock proteins.

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XXI. Technischen Universität Berlin (West), Fachbereich Lebensmitteltechnologie und Biotechnologie. Communicated by Siegfried Windisch.

Below follows the abstract of the doctoral dissertation by Peter M. Weidner, entitled "Beitrage zur Kenntnis der Osmotoleranz and Osmophilie bei Hefen", 1985, 131 pp.

Abstract

Summary: Thirty strains of yeast (osmotolerant, osmophilic and normal), belong to Candida apicola, C. haemulonii, C. halophilia, C. magnoliae, Citeromyces matritensis, Hanseniaspora osmophila, Hansenula anomala, Kluyveromyces marxianus, K. thermotolerans, Pichia sorbitophila, P. guilliermondii, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Torulaspora delbrueckii, Zygosaccharomyces bailii, Z. bisporus, Z. microellipsodes and Z. rouxii were examined. In media with a reduced water activity (a_w) the time for the onset of fermentation was measured. The reduction of a_w was achieved with fructose, glucose, glycerol, NaCl, polyethyleneglycol 200 and 400, xylitol, sorbitol and sucrose; to the non-fermentable solutions, 2% fructose was added. The measured times were characteristic of each strain and for the above-mentioned groups of yeasts. Glycerol allowed some of the osmotolerant and normal strains to ferment at a distinctly lower level of a_w; e.g. the type strain of S. cerevisiae was able to ferment at 0.70 a_w in glycerol, whereas its limit in fructose was at 0.85 a_w. The osmophilic and osmotolerant strains needed less time for fermentation both in media with a higher concentration of fructose and in those where the a_w was lowered with a non-utilizable substance than the normal strains. The osmophilic and most of the osmotolerant strains were able to ferment in higher concentrations of NaCl than the normal strains; they showed no need for a higher concentration of carbohydrates to overcome the low a_w due to NaCl. The differentiation of yeasts into the categories of osmotolerant (according to WINDISCH) and osmophilic yeasts (according to LODDER) has shown itself to be useful. The growth rates of 11 strains were measured, the osmophilic strains growing fastest at or below 0.975 a_w (fructose). For some strains, belonging to C. halophila, Z. bisporus and Z. rouxii, an increase in the growth rate was apparent if the water activity of a basal medium with 2% glucose (0.998 a_w) was lowered by the addition of the nonfermentable polyethyleneglycol 200 to 0.975 a_w. Eleven strains were assayed for the production of polyols. They all synthesized some (glycerol, sorbitol, xylitol, arabinitol), especially glycerol. Glycerol could almost always be detected in the broth after fermentation, while the higher polyols were retained within the cells. All higher polyols were synthesized by osmophilic yeasts.

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XXII. The University of New South Wales, P.O. Box 1, Kensington, N.S.W. Australia 2033. Communicated by G.H. Fleet.

Below follow abstracts and titles of recent publications from my laboratory.

1. Gillian M. Heard and Graham H. Fleet. 1985. Growth of Natural Yeast Flora during the Fermentation of Inoculated Wines. Applied and Environmental Microbiology 50:727-728.

The growth of yeasts that occur naturally in grape juice was quantitatively examined during the fermentation of four wines that had been inoculated with Saccharomyces cerevisiae. Although S. cerevisiae dominated the wine fermentations, there was significant growth of the natural species Kloeckera apiculata, Candida stellata, Candida colliculosa, Candida pulcherrima, and Hansenula anomala.

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2. G.M. Heard and G.H. Fleet. 1986. Occurrence and growth of yeast species during the fermentation of some Australian wines. Food Technology in Australia 38:22-25.

The growth of yeast species that occur naturally in grape juice was quantitatively examined during the fermentation of six wines that had been inoculated with Saccharomyces cerevisiae and in six wines undergoing natural fermentation by indigenous yeasts. Saccharomyces cerevisiae was the dominant yeast in most grape musts and dominated the fermentation of all wines. Significant growth of the yeast species Kloeckera apiculata, Candida stellata, C. colliculosa and C. pulcherrima was noted during fermentations conducted by inoculated and indigenous yeasts.

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3. Harry Puspito and Graham H. Fleet. 1985. Microbiology of sayur asin fermentation. Appl. Microbiol. Biotechnol. 22:442-445.

Summary. Sayur asin is a fermented mustard cabbage product of Indonesia. The cabbage is fermented naturally in the presence of brined water taken from boiled rice. Fermentation was characterized by a sequential growth of the lactic acid bacteria, Leuconostoc mesenteroides, Lactobacillus confusus, Lactobacillus curvatus, Pediococcus pentosaceus, and Lactobacillus plantarum. Starch degrading species of Bacillus, Staphylococcus and Corynebacterium exhibited limited growth during the first day of fermentation. The yeasts, Candida sake and Candida guilliermondii contributed to the fermentation. Lactic acid, acetic acid, succinic acid, ethanol and glycerol were products of fermentation. Glucose, generated by the degradation of rice starch and maltose, was metabolized by the species that grew.

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4. G.H. Fleet. 1984. The occurrence and function of endogenous wall-degrading enzymes in yeast. In C. Nombela (ed) Microbial Cell Wall Synthesis and Autolysis. pp. 227-238. Elsevier Science Publishers, Amsterdam.

Introduction -- Glucanases -- Mannanases
Chitinases -- Proteinases -- Future Research

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5. G.H. Fleet. 1985. Composition and Structure of Yeast Cell Walls. In M.R. McGinnis (ed) Current Topics in Medical Mycology. pp. 24-56. Springer-Verlag, New York.

Introduction - Wall Function - Preparation of Walls and Wall Components - Chemistry, Architecture, and Function of Wall Components - Sacch. cerevisiae (glucans, mannan, chitin, lipid) - Organization of Wall Components (Candida albicans and other Candida spp, Cryptococcus neoformans and other Cryptococcus spp.) - Other Yeast Species - Biosynthesis and Degradation of Yeast Walls - Future Research.

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XXIII. Research Institute for Viticulture and Enology, Matuškova 25, 833 11 Bratislava, Czechoslovakia. Communicated by E. Minárik.

The following are summaries of papers recently published or accepted for publication in 1986.

1. E. Minárik. Activation of alcoholic fermentation of grape must difficult to ferment by yeast wall addition (in German). Mitteilungen Klosterneuburg (Austria). Accepted for publication.

Yeast cell wall preparations (Fould-Springe, S.A., Maison-Alfort, France) substantially speed up the fermentation start as well as the whole course of fermentation of grape must. Compared with control musts without stimulator, substantially higher alcohol contents and a more complete fermentation may be achieved even under unfavorable fermentation conditions. Marked influence of yeast cell walls is evident in musts with high sugar concentration even in the presence of inhibitors such as caprylic and capric acids.

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2. F. Malík, A. Navara and E. Minárik, 1985. 5-Hydroxymethylfurfural contents in grape wines (in German). Mitteilungen Klosterneuburg (Austria), 35:45-47.

The 5-HMF-contents of 33 wine samples originating from two Slovak wine regions were examined. The highest 5-HMF concentration (4.22 $mg.l^{-1}$) could be determined in White Burgundy 1982. Harmfulness for human organism (2 $mg.kg^{-1}$ weight) could not be detected in the examined samples. The 5-HMF concentrations found in wine do not point out any negative influence on yeast activity either.

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3. A. Navara, F. Malík, and E. Minárik, 1986. 5-Hydroxymethylfurfural (5-HMF) in wines originating from the East Slovak and Czechoslovak Tokay wine region (in German). Mitteilungen Klosterneuburg 36:28-33.

A set of varietal and special wines of different vintages originating from the East Slovak and Tokay wine region of Czechoslovakia was examined regarding the content of 5-HMF. The 5-HMF-values did not attain by far concentrations inhibiting yeast growth or being harmful for human health. 5-HMF-values in wines were slightly higher in qualitative more favourable vintages compared with average or less favourable ones. In varietal wines maximum 5-HMF-values attained were 5.20 $mg.l^{-1}$, in ordinary and Samorodny Tokay 3.32 $mg.l^{-1}$, and in 3-vat-Tokay Aszu 37.50 $mg.l^{-1}$. The 5-HMF-content in Tokay Aszu and Samorodny is influenced rather by ecological factors than by long-term wine aging and storing.

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4. E. Minarik and Z. Silharova. Possibilities of alcoholic fermentation intensification of grape must by yeast cell walls (in Slovak). Vinohrad (Bratislava) 24: 1986 (in press).

The addition of 250 $mg.l^{-1}$ of a yeast cell wall preparation ANTIGARSTOP (Erbsloh, Geisenheim, GFR) to grape must prior to fermentation caused remarkable acceleration of the fermentation course even under unfavourable fermentation conditions (presence of fungicide residues, high sugar concentration of the must etc.). Basic requirements for technological use are discussed.

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XXIV. Instituto de Biotecnologia - Universidade de Caxias do Sul - Caixa Postal, 1352 - 95.070 - Caxias do Sul - RS - Brasil. Communicated by Juan L. Carrau.

Second Generation of Fusion Products Among Strains of Enological Interest

Pasqual, M.S.; Serafini, L.A.; Dillon, A.J.P.; Carrau, J.L.; Yazbek-Silva, L.C.A. and Zugno, M.T.

Abstract

Saccharomyces cerevisiae, O. Mendoza and MB 6 TC, have been successfully fused in our laboratories forming a genetically stable fusion product. The yeast MB 6 TC was obtained from the fusion of Benda I (Schizosaccharomyces pombe) and Montrachet (Saccharomyces cerevisiae). The recovery of the fusion products is directly performed on selective regenerative growth medium that uses only natural markers. These growth media are selective for fusion products and are used also to make these fusion products genetically stable. Segregation to the morphology of parentals occurs when the strains are allowed to grow in media without selective pressure.

Supported by Fapergs and CNPq - PIG V.

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XXV. Johannes Gutenberg-Universität Mainz, Institut für Mikrobiologie und Weinforschung Universität, Postfach 3980, D-6500 Mainz, Federal Republic of Germany. Communicated by F. Radler.

Below follow summaries of some recent publications from this institute.

1. F. Radler, K. Dietrich and I. Schönig. 1985. Mikrobiologische Prüfung von Trockenhefepraparaten für die Weinbereitung. Deutsche Lebensm. Rundsch. 81:73-77.

Summary

Simple methods were compiled that appeared suitable for the evaluation of the microbiological quality of active dry yeast for wine-making. The total cell count and the percentage of viable cells were determined by microscopy. The colony count yielded lower figures but was well correlated with the results of the microscopical enumeration of viable cells. Lysin agar and cycloheximde containing agar medium were used to count "non-Saccharomyces-yeasts" and lactic acid bacteria, respectively. The fermentation activity of the dried yeast preparation was measured manometrically or by the glucose induced pH shift. Besides the average values for the total cell count and colony count the standard deviation is given for up to 40 separate determinations. The preparations of active dry yeast contained per g 1.1 to 3.5×10^{10} yeast cells (colony count), up to 29×10^6 "non-Saccharomyces-yeasts" and up to 22×10^6 bacteria.

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2. F. Radler, P. Pfeiffer and M. Dennert. 1985. Killer toxins in new isolates of the yeasts Hanseniaspora uvarum and Pichia kluyveri. FEMS Microbiology Letters 29:269-272.

Summary

From various habitats (plant material, fruits, soil), yeasts belonging to the species of Pichia kluyveri and Hanseniaspora uvarum were isolated that showed killer activity. According to the activity spectrum against other yeasts these strains belonged to 11 different groups that were distinguishable from the killer strains K_1-K_{10}. The isoelectric points of the killer proteins were in the range of pH 3.5-3.9, the activity optimum was observed at pH 4.2-4.6. Above pH 5 and above a temperature of 25-35°C the killer proteins were inactivated.

Since the first description of killer toxin of yeast by Makower and Bevan, the genetic and biochemical aspects of this phenomenon have been primarily investigated. In Saccharomyces the production of the active toxin, an extracellular protein, depends on the presence of virus-like-particles, consisting of two species of protein-encapsidated ds-RNA. Killer strains have been found in yeast culture collections and have been isolated from natural habitats. So far, killer strains have been reported among strains of the genera Saccharomyces, Candida, Debaryomyces, Kluyveromyces, Hansenula, Pichia, Torulopsis and Cryptococcus. This paper describes an unexpected seasonal variation when it was attempted to isolate killer yeast strains from natural habitats. Among the strains isolated and identified, the species Hanseniaspora uvarum and Pichia kluyveri were found, of which the former is so far unknown to produce killer toxin. The killer activity was compared by

cross-reaction with known killer strains and the temperature and pH stability as well as the isoelectric point of the toxins were determined.

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XXVI. Bioresources Research Facility, University of Arizona, 250 E. Valencia Road, Tucson, AZ 85706. Communicated by Hunsa Punnapayak.

Below is the summary of a recently published paper:

Punnapayak, H. and G.H. Emert. 1986. Use of Pachysolen tannophilus in simultaneous saccharification and fermentation (SSF) of lignocellulosics. Biotechnol. Lett., 8:63-66.

Summary

A comparison was made between the effectiveness of Pachysolen tannophilus and Candida brassicae as fermenting organisms in the simultaneous saccharification and fermentation (SSF) process. The substrates were alkali-pretreated rice straw, corn cobs and simulated hydrolysates containing D-glucose:D-xylose in ratios comparable to rice straw or corn cobs. Under these pretreatment and SSF conditions, Pa. tannophilus gave higher ethanol yields than C. brassicae in all cases, producing at least 0.23 g/g from corn cobs, 0.17 g/g from rice straw, 0.43 g/g from simulated corn cob hydrolysate and 0.47 g/g from simulated rice straw hydrolysate. The relatively high ethanol yields of Pa. tannophilus came from the utilization of both glucose and xylose, while C. brassicae utilized only glucose.

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XXVII. Dept. of Microbiology CBS&H, G.B. Pant University of Agriculture & Technology, Pantnagar 263145, Dist. Nainital, India. Communicated by R.S. Rana.

Below follow abstracts of a Masters and a Doctoral thesis from our institution.

1. Utilization of mixed fruit juice of damaged guava and banana for alcohol production. Shruti Bhatt and R.S. Rana. Thesis, Master of Sciences (Microbiology), G.B. Pant Univ. of Agr. & Technol., Pantnagar, 117, pp. 1985.

Abstract

The pulp of damaged banana containing 69% moisture and 0.91% ash and guava pulp having 79% moisture and 1.10% ash were used for juice extraction. The juices of banana having 10.6% sugar and that of guava 0.9% were mixed and used for fermentation. Seven yeast cultures were isolated and the one giving higher ethanol yield was identified as Candida krusei. The ethanol yields and fermentation efficiency of this yeast were compared with Saccharomyces cerevisiae CDRI and NCIM 3095. The optimum conditions for fermentation were established at pH 4.7 and 0.75 g/l of phosphate for C. krusei. Nitrogen supplementation did not influence ethanol yield. C. krusei was found to be a better ethanol producer than S. cerevisiae CDRI and NCIM 3095 and compared well with the S. cerevisiae CDRI NTG strain for ethanol yield and biomass production.

* * *

2. Ethanol Production from Sugarbeet by Saccharomyces cerevisiae and Zymomonas mobilis strains. L.R. Nain & R.S. Rana, Ph.D. thesis, Department of Microbiology, College of Postgraduate Studies. G.B. Pant University of Agriculture and Technology, Pantnagar; India, 263145, 208 pp. 1985.

Abstract

Sugarbeet (Beta vulgaris L) is a high yielding and short duration crop with high sugar content, resistant to frost, mechanical abuse and high pH. It offers an excellent choice over traditional agricultural crops to serve as a raw material for ethanol production. Keeping in view the above facts, studies were conducted to evaluate the potential of sugarbeet for ethanol production. Sugarbeet and sugarbeet juice were analysed for proximate composition. Twenty three strains belonging to Saccharomyces species and four strains of Z. mobilis were screened for ethanol production on the basis of ethanol yield (0.416 g/g) and other fermentation kinetic parameters. S. cerevisiae CDRI NTG was selected for optimization of the fermentation conditions for ethanol production. The ethanol tolerance of this yeast strain was 10-15% (v/v). The percent inoculum, incubation temperature of 30°C, 0.0025% nitrogen supplementation as ammonium sulfate or urea and 0.003% phosphate supplementation as orthophosphoric acid were found to be optimum for ethanol production. After optimization of fermentation conditions ethanol yield was increased to 0.505 g/g with fermentation efficiency of 93.12% as compared to 0.479 g/g and fermentation efficiency of 89.41% in control during batch fermentation.

In single stage continuous fermentation of unsupplemented sugarbeet juice at a dilution rate of 0.02 h^{-1} the ethanol yield was observed to be 0.508 g/g with a fermentation efficiency of 94.42% as compared to ethanol yield of 0.479 g/g recorded during batch fermentation. The continuous fermentation is, therefore, considered better as compared to batch fermentation. The cost of ethanol production was calculated to be Rs. 2.50/l and economics was found to be favourable to use sugarbeet as a supplementary raw material for ethanol production.

* * *

XXVIII. University Claude Bernard, 43 Bd du 11 Nov. 1918, Section Levures, Bât. 405, Univ. Lyon I, 69622 Villeurbanne Cedex, France. Communicated by M.C. Pignal.

Below follows news from our laboratory.

Agnes Michel has just defended her thesis for her Ph.D. in microbiology entitled "Production of proteins from yeasts grown on crude lactoserum".

To purify the sweet crude lactoserum (not deproteinized and not pasteurized) and to make it economically valuable for the production of proteins from yeasts directly utilizable in animal and human nutrition is envisaged.

A yeast strain of the genus Candida has been cultivated in batch and continuous process (fermentors of 2 and 6 liters).

The total purification of the effluent is obtained with a dilution rate of 0.20 h^{-1}. The maximum protein productivity is reached at D = 0.35 h^{-1}.

The composition of the biomass has been analyzed: proteins, amino acids, lipids, fatty acids and B-vitamins.

* * *

Geneviève Billon Grand is still working for her thesis. The "biosystematic" aspect consists in researching the different intracellular carbohydrate hydrolases, nitrite and nitrate reductases, vitamin requirements, GC content and qualitative determination of coenzymes Q. This study was carried out with species of different genera of sporogenous yeasts: Pichia, Hansenula, Debaryomyces, Lipomyces, Schizosaccharomyces, Dekkera and Hanseniaspora, as well as their imperfect forms. The "physiology" part is essentially based on a study of the influence of the culture conditions of the yeast on the coenzyme Q. These studies will be broadened to include yeast-like fungi as well as some bacteria having respiratory ubiquinones.

* * *

Recent publications in preparation.

G. Billon Grand, 1985. Coenzyme Q of some species of the genus Pichia; qualitative and quantitative determination.

R. Montrocher and M.L. Claisse. Spectrophotometric analyses of some Candida species and related yeasts: significance in taxonomy. In press in Cellular and Molecular Biology.

J.B. Fiol, Z. Hmama, R. Montrocher and G. Billon Grand. Pichia species with spherical spores: biochemical and immunological study. In preparation. The presence of intracellular carbohydrate hydrolases, of nitrate and nitrite reductases, vitamin requirements, quantitative determination of respiratory ubiquinones, absorption spectra of cytochromes, the G+C contents and serological comparisons confirm the heterogeneity of this group. Three categories are shown to exist, and one of them includes strains belonging to the new genus Issatchenkia. Their relationships with neighboring genera are discussed.

* * *

XXIX. Brief News Items

1. The Commonwealth Mycological Institute is at present compiling a set of descriptions of certain yeast species potentially pathogenic to humans. These will be published later this year as part of the series CMI Descriptions of Pathogenic Fungi and Bacteria (obtainable from CMI, Ferry Lane, Kew, Surrey) and will include the following species:

Candida albicans C. tropicalis

C. guilliermondii	C. viswanathii
C. kefyr	C. zeylanoides
C. krusei	Torulopsis glabrata (C. glabrata)
C. parapsilosis	T. candida (C. famata)

Yours faithfully

Ms. Carole Davis
(Mycologist)
Commonwealth Mycological Institute
(incorporating UK National Collection
of Fungus Cultures)
Ferry Lane : Kew : Surrey TW9 3AF : UK

2. Change of address:

Dr. K. Watson
Dept. Biochemistry/Microbiology/Nutrition,
University of New England
Armidale, N.S.W. 2351
Australia

(Former address: Dept. Biochemistry, University of North Queensland, Townsville, Australia).

3. Professor Smith Shadomy, Medical College of Virginia, Virginia Commonwealth University, was the recipient of the 1986 Meridian Award for Excellence in clinical Mycology, awarded by the Medical Mycological Society of the Americas.

4. Y.D. Hang, C.Y Lee, and E.E. Woodams. 1986. Solid-state fermentation of grape pomace for ethanol production. Biotechnol. Letters 8:53-56. Grape pomace was used as a substrate and the yield of ethanol amounted to greater than 80% of the theoretical, based on the fermentable sugar consumed.

5. Mercedes R. Edwards and Katherine E. Fritz. 1985. Detection of an antigenic cell wall layer in Histoplasma capsulatum - An immunoelectron microscopic study. Arch. Microbiol. 142:242-247.

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XXX. Meetings

1. Minutes of ICY meeting held on 19 March 1986 in Lisbon, Portugal, during the XI ISSY meeting organized by our colleague, Dr. Van Uden.

It was confirmed at this meeting that a specialized symposium will be held in Louvain, Belgium, in September 1989. The topic will be "Yeast Technology. Relationship to Alcoholic fermentation".

It was also confirmed that the symposium on "Taxonomy of yeast" will be held in Smolenice, Czechoslovakia, in September 1990.

The Commission discussed the amendment of article 7 of the constitution, proposed by several of our colleagues (see proposed amendment in the letter dated 3 March 1986 that was sent to all members of the Commission by P. Galzy and J.-M. Bastide). Dr. Rose proposed to reject the amendment; his proposition was accepted by the majority of the members present. The Commission then decided that a member of the Commission who leaves his/her country of domicile will be considered as having resigned from the Commission. It follows that a member of the Commission is a member for life except if he/she leaves his/her country of domicile or if he/she has submitted a letter of resignation. Each country's delegation to the ICY shall consist of up to 3 residents.

The Commission recalled that the ICY constitution makes no provision for the designation of new members of the Commission. The Commission ruled that the ICY is sovereign and co-opts its members. However, the ICY agreed that a country may propose a new member to its delegation.

Dr. Forage (Ireland) submitted a letter of resignation and proposed that he be replaced by Dr. Cantwell. The ICY ratified this proposal.

Dr. H. Koch (GDR) has left his country of domicile; he will be replaced by Dr. H. Weber who was nominated by the Commission upon proposal of the delegation of the German Democratic Republic.

The "Symposium on Taxonomy of Yeast-Like Fungi" which will be held in Amersfoort, Netherlands, was announced at the Lisbon meeting. This symposium will be organized by Dr. de Hoog on 3-7 August 1987.

Professor P. Galzy
Chairman of ICY

Professor J.-M. Bastide
Secretary of ICY

* * *

Members Present at the 19 March 1986 Meeting of ICY in Lisbon, Portugal.

H. Verachtert (Belgium); A. Panek (Brasil); P. Venkov (Bulgaria); G. Stewart (Canada); A. Kotyk (Czechoslovakia); A. Stenderup (Denmark); M. Karkola (Finland); P. Galzy (France); D. Birnbaum (GDR); A. Rose (England); A. Martini (Italy); N. van Uden (Portugal); J.C. Du Preez (South Africa); J.M. Gancedo (Spain); H. Neujahr (Sweden); O. Käppeli (Switzerland); W.A. Scheffers (Netherlands); M. Beker (USSR); S. Barbaric (Yugoslavia).

The following members sent their apologies for absence.

Klaushoffer (Austria); I. Russel (Canada); B. Johnson (Canada); E. Minarik (Czechoslovakia); J.F.T. Spencer (England); T.M. Enari (Finland); J.-M. Bastide represented by R. Ratomahenina (France); Novak, Deak and Ferenczy were represented by T. Török (Hungary); M.G. Shepherd (New Zealand); H. Oberman (Poland); J.P. van der Walt (South Africa); F. Sherman (USA); N. Elinov (USSR); V. Johanides (Yugoslavia).

Statute of the International Commission for Yeasts

Article 1. History

In the year 1966 the Council for Yeast Research, composed of prominent specialists in the field of yeasts was established in Bratislava. In 1971 the Council was transferred into the International Association of Microbiological Societies (IAMS) and in 1982 into the International Union of Microbiological Societies (IUMS).

Article 2. Name

The name of the organization shall be the International Commission for Yeasts (ICY).

Article 3. Structure

The International Commission for Yeasts shall be an organization within the IUMS. For this reason the ICY is subject to the statute of IUMS and shall determine only specialized by-laws.

Article 4. Affiliation

Any regional yeast commission organized within their national microbiological societies irrespective of size or location may seek affiliation with ICY.

Article 5. Headquarters of ICY

Headquarters of ICY shall be the offices of the Chairman and Secretary of ICY.

Article 6. Selection of the Chairman and Secretary

The Chairman and Secretary of ICY for the term of four years shall be the Chairman and Secretary of the Organizing Committee of the last General Symposium. The Vice-Chairman is automatically the retiring Chairman of the previous General Symposium.

Article 7. Membership

ICY shall consist of up to three members from each country.

Article 8. Objectives

The general objectives of ICY shall be: To establish an effective liaison between persons and organizations concerned with yeast investigations, and between them and the practical users of results of investigations, including yeast culture collections.

ICY will use the "Yeast Newsletter" as a means of communication.

ICY shall sponsor conferences and symposia on topics and problems of common interest. Every five years a General Symposium and if possible each year in the interum a Specialized Symposium shall be held. Members of ICY shall be informed about regional conferences of yeasts.

Article 9. Activities

The activities of the ICY shall be conducted through the Executive Board (EB), composed of the Chairman, Vice-Chairman and Secretary.

Article 10. Duties of the ICY and its officers

The Chairman shall preside at meetings of the ICY. The Vice-Chairman shall, in the absence of the Chairman, perform the duties and exercise the powers of the Chairman. The Secretary shall record the minutes of meetings of the ICY. He shall be responsible generally for the maintenance of effective liaison between the ICY and affiliated organizations. He shall prepare reports of EB for presentation to the ICY and send invitations for its meetings.

Article 11. Meetings

Meetings of the Commission (ICY) shall be held at every general and specialized symposium. The members of the Commission will officially be invited by the Headquarters to every meeting.

Article 12. Rights, privileges and obligations of membership

All members shall enjoy full participation in the affairs of the ICY except where otherwise stated in statutes.

No member shall use his connection with the ICY to further interests of his or any other organization except as is provided for in the statutes. The ICY shall not be responsible for the utterance or acts of its individual members.

Article 13. Amendments to the statutes

Any proposals for amendments to the statutes may be made in writing to the Secretary of the ICY at least six months prior to a meeting of the ICY.

Updated List of Members of ICY

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* * *

2. XIIth International Specialized Symposium on Yeast. "Genetics of Non-Conventional Yeasts", September 13-19, 1987, Weimar, German Democratic Republic. Chairmen: H. Weber, Jena and F. Böttcher, Greifswald.

First Announcement

The International Commission for Yeast decided at its meeting in Bombay (1983) that the XIIth International Specialized Symposium on Yeasts will be held on September 13-19, 1987 in Weimar, GDR.

The Scientific Programme will focus on the genetics of non-conventional yeasts. Results of genetic research on Saccharomyces cerevisiae and Schizosaccharomyces pombe have been discussed during several recent yeast meetings. It is, therefore, the aim of this symposium, to bring together scientists working on the genetics of yeasts other than these two species and to discuss latest results and trends of genetic studies on these yeasts with emphasis on industrially important features. The scientific program will comprise plenary lectures and original communications presented orally or as posters.

Proposed topics:

1. Life cycles, sexuality, sporulation, parasexuality
2. Genome structure, recombination, extrachromosomal elements
3. Gene transfer and cloning
4. Regulation of gene expression
5. Applications in biotechnology

The Language of the Symposium will be English.

Accompanying guests are also welcome. Social Program will be organized both for active and accompanying guests.

For further information, please write to:

Prof. Dr. H. Weber
Central Institute of Microbiology
and Experimental Therapy of the
Academy of Sciences of the GDR
GDR-6900 Jena
Beutenbergstr. 11, East Germany

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3. XVIIth Annual Conference on Yeasts, held in Smolenice castle 12-14 February 1986. Commmunicated by Dr. A. Kocková-Kratochvilová, Institute of Chemistry, Slovak Acad. Sci., Bratislava CSSR.

Section: Cytoskeleton, coordinated by O. Necas and E. Streiblova.

O. Nečas: Principles of the functional organization of the cell skeleton.
E. Streiblová: The importance of the study of the skeleton on yeast models.
J. Hašek: The polymerization and depolymerization in the cell cycle of yeasts and the influence of inhibitors.
J. Svobodová, J. Hašek, E. Streiblová: Saccharomyces uvarum protoplasts and their application in studies of the topology of its microtubular system.
A. Svobodá, E. Ouředníček: The regeneration of yeast-like protoplasts in alginate gels.
V. Farkaš: The biosynthesis of chitin in the mutant cdc 24-1 of the cell cycle of Saccharomyces cerevisiae.
M. Hrmová, V. Farkaš: The metabolic characteristics of protoplasts lacking nuclei.
M. Gabriel, M. Kopecká: Relation of incomplete cell wall formation to the septation in flattened protoplasts of Schizosaccharomyces japonicus var. versatilis.
M. Kopecká: Electron-microscopy of purified glucan and mannan components of Saccharomyces cerevisiae cell walls.
M. Kopecká, M. Gabriel, E. Streiblová: The effect of Kongo red on the biogenesis of microfibrils and cell walls of yeasts and protoplasts of Saccharomyces cerevisiae.
M. Kopecká, D.R. Kreger: The molecular metabolism leading to origin of β-1-3-D-glucan microfibrils of protoplasts.

Section: Taxonomy, ecology and immunology, coordinated by A. Kockova-Kratochvilova, V. Stollarova and A. Tomsikova.

A. Kocková-Kratochvilová: The actual importance of yeast culture collections.
E. Sláviková, A. Grabińska-Lonievska: Yeasts and yeast-like organisms in the biocenose of a denitrification unit.
E. Breierová, R. Delgado: The survival of Candida species in liquid nitrogen.
E. Minarik: New knowledge of yeasts and yeast-like organisms of primary and secondary habitats in wine production.
V. Stollarová: The influence of synthetic retardants on the yeast community during the application on selected fruit trees.
R. Kovačovská, L. Švorcová: The identification of yeasts from galls of patients with hepatitis.

Z. Jesenská: Yeasts from the viewpoint of hygiene.
A. Tomšiková: The adherent ability of Candida albicans to the epithelium of mucous membrane and its relationship to the pathogenicity.
A. Kocková-Kratochvilová, E. Sláviková, R. Kovačovská: Taxonomy and classification of teleomorphic and anamorphic genera of yeasts.
V. Čurpakovicova: The microflora of musts and vines of the Tokaj region.
E. Paulovičová, J. Šandula: The determination of Ig-isotopes specific against Candida albicans and mannan antigen test Elisa.
E. Paulovičová, J. Šandula: The characterization of cross reactivity of antigens of pathogenic strains of the genera Candida and Torulopsis by heterogeneous EIA.
G. Kogan, L. Masler: Comparative physico-chemical and structural studies of mannans pathogenic for yeasts.
V. Pavliak, J. Šandula: Comparative immunochemical studies of mannans of the pathogenic yeasts C. albicans and C. parapsilosis.

Section: Biotechnology, coordinators O. Bendova, D. Vrana and F. Malik.

D. Vraná: Cell cycle, physiological state and biotechnology.
O. Bendová, B. Janderová: Yeast strains and cultivating media in classical technologies.
J. Votruba: Introduction into the mathematical physiology of yeasts.
M. Sobotka: The survey of measuring methods of monitoring of physiological state of yeasts for purposes of the construction of mathematical models.
I. Havlik: Systems "fermentor-computer" for the simulation of the behaviour in big fermentation equipment under laboratory conditions for the purposes of enlargement.
J. Hrnček, F. Malik: The propagation and autoselecting effect of yeasts.
V. Báleš, I. Langfelder: A mathematical model of a bioreactor with immobilized yeasts.
I. Havlik, V. Vošahlik: Practical exhibition of mathematical modeling of the behaviour of a yeast population by the application of a microcomputer.
S. Michalčáková, F. Malik, E. Sturdik, E. Minarik: Biochemical and physiological characteristics of wine yeasts.

Section: Varia, coordinated by F. Malik.

M. Rychtera: The comparing of immobilized yeasts with yeast cells free living in the ethanol production in distilleries.
A. Navara: The influence of yeast cells on the nitrate and nitrite content in young wines.
P. Kotal: The application of yeasts in experimental medicine.
M. Lišková, I. Labudová: The transformation of yeasts by the fusion of protoplasts and DNA.
I. Pavliček, V. Vondrejs: The analysis of hybrids of Schizosaccharomyces pombe by the application of benomyl.
D. Tomešková, V. Vondrejs: Fusion of living and dead protoplasts of Schizosaccharomyces pombe.
B. Večerek, B. Janderová: Hybrids and cybrids received from the fusion of protoplasts.
V. Vlčková, V. Kováčová: The use of Saccharomyces cerevisiae for the testing of potential and genetical hazard of newly synthetized chemical compounds.
J. Šperl: Biotechnological production of porphyrins by immobilized yeasts.
P. Griač: Electrofusion of Saccharomyces cerevisiae protoplasts.
Vojtková-Lepšilová, A., E. Machová, Z. Kossaczká: Characterization of the biomass of yeasts cultivated on waste xylose syrup.

N. Obermayerová, Y. Gbelská: The improvement of baker's yeast.
P. Sulo: The transfer of mitochondria into protoplasts by fusion.
B. Běhalová, K. Beran, L. Doležalova, J. Zajíček, Č. Novotny: The influence of nitrogen concentration in the medium on the synthesis of sterols in S. cerevisiae.
J. Šajbidor, Heriban V., J. Augustin: The biotransformation of higher n-alkanes into fatty acids and acids of the Krebs cycle in Candida lipolytica.
P. Biely, H. Lee, H. Schneider: The first-hand fermentation of xylan into ethanol.
M. Vrbová, L. Šilhánková, M. Rychtera: The influence of some herbicides on the technological activity of Saccharomyces cerevisiae.

* * *

4. "The expanding realm of yeast-like fungi" An international symposium on the perspectives of taxonomy, ecology and phylogeny of yeasts and yeast-like fungi. Amersfoort, The Netherlands, 3-7 August 1987.

Information can be obtained from the organizing committee:

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Aims and Scope

There is still a wide gap between the methods employed for the study of yeasts and of moulds, while it is increasingly realized that different parts of the life-cycle of one and the same organism may be found on both sides of this "borderline". The confrontation of these dissimilar areas of research opens new horizons, both on fundamental research and on industrial application.

In order to arrive at a balanced programme, there will be invited plenary lectures with three main topics: (1) a taxonomic review of yeast-like fungi and related organisms, (2) frontiers of development of a selection of analytical methods, and (3) ecology and the species problem.

Free poster contributions on the above or related topics are also accepted.

The conference is also scheduled as a post-congress activity of the XIVth International Botanical Congress to be held in West Berlin from 24 July to August 1, 1987.

Preliminary Scientific Programme

A taxonomic survey
Convenors: M. Th. Smith, F. Oberwinkler, G.S. de Hoog

1. Survey of the established yeasts (J.P. van der Walt)
2. Agaricales (H. Prillinger)
3. Filobasidiales (K.J. Kwon-Chung)
4. Tremellales, Auriculariales (R.J. Bandoni)
5. Dacrymycetales, Exobasidiales, Septobasidiales, Tulasnellales (F. Oberwinkler)
6. Sporidiobolales (J.W. Fell)
7. Uredinales (R. Bauer)
8. Ustilaginales (G. Deml & T. Boekhout)
9. Taphrinales (C.L. Kramer)
10. Endomycetales, Ophiostomatales (J.A. von Arx)
11. Onygenales (D. Malloch)
12. Dothideales (M.R. McGinnis & G.S. de Hoog)
13. synthesis and phylogeny: geography (K.A. Pirozynski)
14. synthesis and phylogeny: ultrastructure (R.T. Moore)

Taxonomic methods
Convenors: A.C.M. Weijman, P.A. Blanz

15. relevant structures TEM
16. mitosis (I.B. Heath)
17. meiosis (D.J. McLaughlin)
18. relevant and irrelevant DNA (J. de Ley)
19. ribosomal RNA (P.A. Blanz)
20. mitochondrial DNA (G.D. Clark-Walker)
21. co-enzyme Q systems (Y. Yamada & H. Kuraishi)
22. cellular carbohydrates (A.C.M. Weijman)
23. physiology (A.H. Scheffers)
24. capsular fimbriae (A.W. Day)
25. extracellular mannans (K.-H. Rademacher)
26. serology (L. Kaufman)
27. isozymes at species level
28. isozymes below species level (J.J. Burdon)

Ecology and the species problem
Convenor: J.P. van der Walt

29. isolation and maintenance (T. Nakase)
30. psychrophilic species (H.S. Vishniac)
31. xero- and osmophilic species (W.T. Starmer)
32. insect-symbionts (L.R. Batra)
33. mating experiments (G.J. Wong)
34. protoplast fusion (M. Sipiczki)
35. DNA reassociation (C.P. Kurtzman)
36. genetics of Basidiomycetes (K. Wells)
37. genetics of Ascomycetes (G.J. Naumov)

Closing remarks (B. Kendrick)

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5. 7-13 September, 1986. Microbe 86 - XIV International Congress of Microbiology, Manchester, UK.
Scientific programme consists of 62 symposia (33 bacteriology, 10 mycology, 8 virology, 11 interdivisional); 17 round tables; 3 virology workshops; 80 poster sessions. There will also be an extensive programme of IUMS COMCOF meetings.
Contact: For registration form, abstract form for posters and final circular: A Yates, Trading Services, UMIST, PO Box 88, Manchester M60 1QD, UK.

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6. The 4th European Congress on Biotechnology will be held in the Internationaal Congrescentrum RAI in Amsterdam from June 14-19, 1987.

For all information on the congress please contact:
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Europaplein 12
1078 GZ Amsterdam
The Netherlands
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