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Y E A S T

A Newsletter for Persons Interested in Yeast

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NOTICE TO OUR READERS

After having served as Editor of the Yeast Newsletter since 1954 I felt that a change in editorship was desirable. Mr. M.A. Lachance, Department of Plant Sciences, University of Western Ontario, London, Ontario N6A5B7, Canada has graciously consented to take over the editorship from me in 1988. I have enjoyed the work involved with the publication of the Newsletter and want to express my appreciation for the contributions made by the Associate Editors to the success of the Newsletter.

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

H.J. Phaff
Editor

I. Centraalbureau Voor Schimmelcultures, Yeast Division, Juliana 67a, 2628 BC DELFT (Netherlands). Communicated by M. Th. Smith.

Below follows a list of new acquisitions at the CBS.

Recent acquisitions: Yeasts

Candida bombicola (Spencer et al.) Meyer & Yarrow: CBS 7267 = CSIR-Y1060, ex concentrated grape juice, South Africa, J.P. van der Walt.

Candida dulciaminis Tokuoka et al.: CBS 7288 = Tokuoka 155-CA = IAM 13114, T, ex sponge cake, Japan, K. Komagata.

Candida floricola Tokuoka et al.: CBS 7289 = Tokuoka 20-50A = IAM 13115, T, CBS 7290 = IAM 13116, ex flower of dandelion (Taraxacum platycarpum), Tokyo, Japan, K. Komagata.

Candida glucosophila Tokuoka et al.: CBS 7287 = Tokuoka 29-25A = IAM 13112, ex Taiwanese brown sugar, K. Komagata.

Candida lyxosophila van der Walt et al.: CBS 7268 = CSIR-Y1062, ex forest soil, Mount Sheba Forest Reserve, Transvaal, CBS 8194, ex surface woodland soil, South Africa, J.P. van der Walt.

Candida methanolophaga Kumamoto & Yamamoto: CBS 7297 = S-79 = IAM 13156, ex oil field, Akita Prefecture, Japan, K. Komagata.

Candida ooitensis Kumamoto & Yamamoto: CBS 7299 = S-214 = IAM 13158, ex slimy mud, Ooita Prefecture, Japan, K. Komagata.

Candida ovalis Kumamoto & Yamamoto: CBS 7298 = S-54 = IAM 13157, ex soil, Kanagawa Prefecture, Japan, K. Komagata.

Candida shehatae Buckley & van Uden: CBS 7261 = CSIR-Y1047, ex forest soil, CBS 7263 = CSIR-Y980, ex forest soil, J.P. van der Walt.

Candida sorbophila (Nakase) Meyer & Yarrow: CBS 7266 = CSIR-Y1050, ex uncultivated soil, Pretoria District, J.P. van der Walt.

Kluyveromyces marxianus (Hansen) van der Walt var. marxianus: CBS 6923 = CSIR-Y808.

Kluyveromyces thermotolerans (Filipov) Yarrow: CBS 6924 = CSIR-Y809, J.P. van der Walt.

Pichia methylovora Kumamoto & Yamamoto: CBS 7300 = S-97 = IAM 13159, T, ex rotten tree, Iwate Prefecture, Japan, K. Komagata.

Rhodosporidium toruloides Banno: CBS 7291 = IAM 13505, CBS 7292 = IAM 13512,CBS 7293 = IAM 13073, K. Komagata.

Rhodotorula glutinis (Fres.) Harrison: CBS 7294 = IAM 12966, K. Komagata.

Rhodotorula minuta (Saito) Harrison: CBS 7295 = IAM 12968, CBS 7296 = IAM 12975, K. Komagata.

Saccharomyces cerevisiae Meyen ex Hansen: CBS 7302 = A364A, a ade1 ade2 ural tyr1 his7 lys2 gal1 mkt1 (HOK) (NEX) K+R+, K1 killer, CBS 7303 = S7, alpha gal1 DET1 K-R-, CBS 7304 = L599-2D, alpha ade2-1 his4-580 met-8-1 Lo Mo K-R-, non-killer lacking double-stranded RNA, CBS 7305 = 1384, alpha his4 (HOK) K2+R2+, K2 killer, M.J. Leibowitz (Georgopoulos & Leibowitz 1987, Yeast 3:117-129).

Saccharomycopsis capsularis Schioenning: CBS 7262, ex soil, J.P. van der Walt.

Saitoella complicata Goto et al.: CBS 7301 = YK 112 = IAM 12963, T, ex soil, Laya, Bhutan, K. Komagata.

Schizosaccharomyces pombe Lindner var. pombe: CBS 7264 = NCYC 1354, h-, CBS 7265 = NCYC 1355, h+, NCYC.

Sporobolomyces griseoflavus Nakase & Suzuki: CBS 7284 = JCM 5653, T, ex dead leaves of Sasa, sp., Japan, T. Nakase.

Sporobolomyces naganoensis Nakase & Suzuki: CBS 7286 = JCM 5978, T, ex dead leaf of Sasa sp., Japan, T. Nakase.

Sporobolomyces sasicola Nakase & Suzuki: CBS 7285 = JCM 5979, T, ex dead leaves of Sasa sp., Japan, T. Nakase.

Sporobolomyces miscanthi Nakase & Suzuki: CBS 7282 = JCM 5773, T, ex dead leaves of Miscanthus sinensis, Japan, T. Nakase.

Sporidiobolus salmonicolor Fell & Statzell Tallman: CBS 7260 = VKM Y-2290, V.I. Golubev.

Sporobolomyces subroseus Nakase & Suzuki: CBS 7283 = JCM 5735, T, ex dead leaves of Miscanthus sinensis, Japan, T. Nakase.

Sporobolomyces weijmanii Nakase & Suzuki: CBS 7281 = JCM 5651, T. ex dead leaves of Miscanthus sinensis, Japan, T. Nakase.

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II. American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852-1776. USA. Communicated by S.C. Jong.

The strains listed below have been added to the ATCC since April 15, 1987. Complete information on these strains may be obtained upon request from the Mycology and Botany Department of ATCC.

New Yeast Strains

NAME	ATCC NO.	DEPOSITOR & STRAIN	Significance & Reference
Candida albicans	64124	C.F.H. Vickers, Darlington	Isolate from mouth swap (J. of Gen. Microbiology 132:2421-2431, 1986).
Candida albicans	64385	C. Nombela, 1001	Production of exo-1-3-β glucanase (J. Gen. Microbiol. 133:609-617, 1987).

Candida curvata	64321	A. Kockova-Kratochvilova, CCY29-18-3	Mushroom isolate (Ceska Mykologie 38:218-229, 1984.
Candida lipolytica	64042	M. Ohsugi, AKU 4582	Produces pimelic acid (Agric. Biol. Chem. 48 (7):1881-1882, 1984).
Candida lusitaniae	64125	W. G. Merz, CL-30	Human pathogen - resistant to nystatin & amphotericin B (J. Clin. Microbiol. 24:No, 4, 581-584, 1986).
Candida pintolopesii	64177	D.C. Savage, 108-1	Isolated from mouse's stomach (Appl. & Environ. Microbiol. 53:No, 2, 345, 1987)
Candida valida	64113	T. Higashihara, KU-1	Food for rotifer Brachionus pilcatilis (Bull. Japan Soc. Fish. 49:1001-1013, 1983).
Cryptococcus neoformans var gattii	64062	E. J. Bottone, Serotype B	First serotype B isolate in New York (J. of Clinical Microbiol. 23:No. 1, 186, 1986).
Hanseniaspora uvarum	64294-64297	F. Radler, 469, 470, 471, 478	Killer yeast (FEMS Microbiology Letters 29:269-272, 1985).
Hansenula polymorpha	64209	K. Ohta, CK-1	Produces anti-yeast substance (Agric. Biol. Chem. 50(4):827-832, 1986).
Kloeckera africana	64322	A. Kockova-Kratochvilova, CCY25-8-3	Mushroom isolate (Ceska Mykologie 38:218-229, 1984).
Kloeckera corticis	64328	A. Kockova-Kratochvilova, CCY25-3-3	Mushroom isolate (Ceska Mykologie 38:218-229, 1984).
Kloeckera vanudenii	64329	A. Kockova-Kratochvilova, CCY43-11-1	Mushroom isolate (Ceska Mykologie 38:218-229, 1984).
Lipomyces starkeyi	64135-64137	D. Yarrow, CBS 1809, CBS 2516, CBS 6047	Production of enzymes (Appl. Microbiol. Biotech. 22:352-358, 1985).
Metschnikowia pulcherrima	64323	A. Kockova-Kratochvilova, CCY69-2-8	Mushroom isolate (Ceska Mykologie 38:218-229,1984).
Pichia heedii	64324	A. Kockova-Kratochvilova, CCY39-53-1	Mushroom isolate (Ceska Mykologie 38:218-229, 1984).
Pichia humboldtii	64325	A. Kockova-Kratochvilova, CCY39-52-1	Mushroom isolate (Ceska Mykologie 38:218-229, 1984).
Pichia kluyveri	64298-64303	F. Radler, 475, 476, 484, 485, 487, 488	Killer yeast (FEMS Microbiology Letters, 29:269-272, 1985).
Pichia ohmeri	64326	A. Kockova-Kratochvilova, CCY39-30-4	Mushroom isolate (Ceska Mykologie 38:218-229, 1984).

Pichia strasburgensis	64327	A. Kockova-Kratochvilova, CCY39-34-3	Mushroom isolate (Ceska Mykologie 38:218-229, 1984).
Pityrosporum ovale	64061	P. Marichal, B 39387	Drug studies (J. Med. & Vet. Mycology 24:487-489, 1986).
Rhodotorula rubra	64041	M. Ohsugi, AKU 4817	Produces pimelic acid (Agric. Biol. Chem. 48 (7):1881-1882, 1984).
Saccharomyces bisporus	64332	A. Kockova-Kratochvilova, CCY21-23-2	Mushroom isolate (Ceska Mykologie 38:218-229, 1984).
Saccharomyces cerevisiae	62985 62994- 62996	R.K. Latta, NRC 5044, NRC 5045, NRC 5140 NRC 5692	Genetic engineering (J. of Bacteriology 166:No. 2, 484-490, 1986).
Saccharomyces cerevisiae	64030- 64035	F. Karst, erg 8-1, erg 9-1, erg 10A-1, erg 10B-1, erg 11-1, erg 12-1	Genetic study (Biochem. & Biophys. Res. Comm. 123:No. 2, 424-430, 1987).
Saccharomyces cerevisiae	64236	G.P. Kalle, B-2	Produces glycerol from molasses (Biotech. & Bioengr. XXIX:1173-1175, 1987).
Saccharomyces cerevisiae	64252	B. Stilinovic, TT-1	Baker's yeast (Acta Bot. Croat. 40:127-131, 1981).
Saccharomyces diastaticus	62987- 62993	R.K. Latta, NRC 5771, NRC 5709, NRC 5708, NRC 5707, NRC 5706, NRC 5705, NRC 5704	Genetic engineering (J. of Bacteriology 166:No. 2, 484-490, 1986).
Saccharomyces rosei	64331	A. Kockova-Kratochvilova, CCY21-51-1	Mushroom isolate (Ceska Mykologie 38:218-229, 1984).
Schizosaccharomyces pombe	62986	R. K. Latta, NRC 2447	Genetic engineering (J. of Bacteriology 166:No. 2, 484-490, 1986).
Sporobolomyces albo-rubescens	64330	A. Kockova-Kratochvilova, CCY19-13-1	Mushroom isolate (Ceska Mykologie 38:218-229, 1984).

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III. Japan Collection of Microorganisms, The Institute of Physical and Chemical Research Hirosawa, Wako-shi, Saitama 351, JAPAN. Communicated by T. Nakase.

1. Takashi Nakase and Motofumi Suzuki. 1987. J. Gen. Appl. Microbiol., 33, 177-196. Studies on Ballistospore-forming Yeasts From the Dead Leaves of Miscanthus Sinensis With Descriptions of the New Species Sporobolomyces miscanthi, Sporobolomyces subroseus, and Sporobolomyces weijmanii.

One hundred and one strains of ballistospore-forming yeasts isolated from 48 samples of dead leaves of a weed, Miscanthus sinensis, were identified as 16 species in the genera Bullera, Sporobolomyces and Tilletiopsis: Forty-six of 51 strains of Bullera were assigned to B. megalospora (18 strains), B. crocea (15 strains), B. alba (3 strains), B. piricola (3 strains), B. oryzae (3 strains), B. pseudoalba (3 strains) and

B. derxii (1 strain). Five strains resembled unidentified yeasts isolated from the dead leaves of Oryza sativa. Forty-one of 47 strains of Sporobolomyces were assigned to Sp. roseus (37 strains), Sp. salmonicolor (2 strains) and Sp. shibatanus (2 strains). Four strains represented 3 new species and were described as Sp. miscanthi (2 strains), Sp. subroseus (1 strain), and Sp. weijmanii (1 strain). These new species had Q-9 as the major ubiquinone. The remaining 2 strains of Sporobolomyces represent a single species, still unidentified. Three strains of Tilletiopsis were assigned to T. lilacina. The frequency of isolation of each species from Miscanthus sinensis is compared with that from Oryza sativa.

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2. Takashi Nakase and Motofumi Suzuki. 1987. Sporobolomyces griseoflavus and Sporobolomyces sasicola, Two New Species of Ballistosporous Yeasts Isolated From Dead Leaves of Sasa Sp. In Japan. J. Gen. Appl. Microbiol., 33, 167-175.

Two new species of ballistospore-forming yeasts, Sporobolomyces griseoflavus and Sporobolomyces sasicola, were isolated from dead leaves of Sasa sp. collected on a mountain in Japan. They form pale colored colonies and bilaterally symmetrical ballistospores, have Q-10 as the major ubiquinone and do not contain xylose in the cells. The taxonomic relationship of these new yeasts to Sporobolomyces singularis are discussed.

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3. Takashi Nakase and Motofumi Suzuki. 1978. Sporobolomyces naganoensis, a New Species of Ballistosporous Yeast Equipped With Ubiquinone-9, Isolated From a Dead Leaf of Sasa sp. in Japan. Trans. mycol. Soc. Japan 28:1-8.

Summary

A new species of ballistosporous yeast, Sporobolomyces naganoensis, sp. nov., was isolated from a dead leaf of Sasa sp. collected on a mountain in Japan. This yeast produced bilaterally symmetrical ballistospores, produced pale pink colonies, possessed Q-9 as the major homologue of ubiquinone, and did not contain xylose in the cells. These characteristics resemble those of Sporobolomyces miscanthi, Sporobolomyces subroseus, Sporobolomyces weijmanii, and Sporobolomyces intermedius. These five species differ from the typical species of the genus Sporobolomyces and are assumed to form a natural taxonomic group.

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4. Motofumi Suzuki, Takashi Nakase, Wanchern Daengsubha,[*] Montri Chaowsangket,[*] Puangpen Suyanandana,[*] and Kazuo Komagata[**]. 1987. Identification of Yeasts Isolated From Fermented Foods and Related Materials in Thailand. J. Gen. Appl. Microbiol., 33, 205-220.

[*]Thailand Institute of Scientific and Technological Research (TISTR), Bangkok 10900, Thailand.

**Institute of Applied Microbiology, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

Eighty yeast strains were isolated from 39 samples of 29 kinds of fermented food (fish, shrimp, meat, cereals, fruit, etc.) and other related materials in Thailand, and identified by conventional and chemotaxonomical methods. These strains were identified as Debaryomyces hansenii var. fabryi (1 strain), Pichia anomala (3 strains), Pichia farinosa (2 strains), Pichia membranaefaciens (1 strain), Pichia ohmeri (1 strain), Saccharomyces cerevisiae (9 strains), Saccharomycopis fibuligera (2 strains), Stephanoascus ciferrii (1 strain), Candida guilliermondii (4 strains), Candida kefyr (1 strain), Candida krusei (34 strains), Candida membranaefaciens (1 strain), Candida parapsilosis (1 strain), Candida sorbosa (8 strains), Candida sorboxylosa (2 strains), Candida tropicalis (5 strains), Candida valida (1 strain), Candida sp. (1 strain), and Geotrichum candidum (2 strains).

Candida krusei was the most frequently isolated species from the various kinds of fermented foods and other related materials. Candida sorbosa was isolated only from fermented fruit. The distribution of yeast species in these source materials is discussed.

* * *

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

Below follow abstracts of three recent publications from our Group.

1. J.P. van der Walt, J.A. von Arx, N.P. Ferreira, and P.D.G. Richards. 1987. Zygozyma gen. nov., a New Genus of the Lipomycetaceae, System. Appl. Microbiol. 9, 115-120.

[2]Het Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. [3]Histological Services, Biological Evaluation Division, National Institute for Food Research, Council for Scientific and Industrial Research, Pretoria, South Africa.

Summary

The yeast Zygozyma oligophaga gen. et sp. nov., was isolated from frass of an unidentified bark beetle in Northern Natal. It is related to Dipodascopsis and Lipomyces, and is distinguished from the former genus by the absence of septate hyphae and from the latter by allantoid, smooth ascospores. The family Lipomycetaceae is redefined and restricted to the three mentioned genera. Myxozyma includes anamorphs of Zygozyma. All species of the three genera form mucoid colonies with amyloid material and utilize imidazol as sole source of nitrogen.

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2. J.P. van der Walt, Y. Yamada[2], T. Nakase[3], and P.D.G. Richards[4]. 1987. Myxozyma geophila and Myxozyma lipomycoides spp. nov., Two New Anamorphic, Lipomycetaceous Yeasts from Southern Africa.

[2]Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Shizuoka University, Shizuoka, Japan.

[3]Japan Collection of Microorganisms, Institute of Physical and Chemical Research, (RIKEN), Wako, Saitama, Japan.
[4]Histological Services, Biological Evaluation Division, National Institute for Food Research, Council for Scientific and Industrial Research, Pretoria, South Africa.

Summary

Two undescribed species of the genus Myxozyma have been recovered from surface soils and arboricolous lichen, from South African habitats. The two new species, Myxozyma geophila and Myxozyma lipomycoides, are described and their possible relationship with the genera Lipomyces and Zygozyma is considered. A key for the genus Myxozyma is given.

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3. J.P. van der Walt, N.P. Ferreira and P.D.G. Richards[2]. 1986. New basidiomycetous yeasts from Southern Africa I: Sporobolomyces kluyveri-nielii sp. nov. Antonie van Leeuwenhoek 52:431-436.

[2]Division of Biological Evaluation, National Institute for Food Research, Pretoria, South Africa.

During a survey of yeasts associated with the phyllosphere of indigenous trees in the Transvaal, a single strain of an undescribed species of the genus Sporobolomyces Kluyver & van Niel (1925) was recovered. A description of the new species, Sporobolomyces kluyveri-nielii, is given.

Sp. kluyveri-nielii is distinguished from all accepted species of the genera Sporobolomyces and Sporidiobolus Nyland, physiologically by its inability to utilize any of the di- or trisaccharides used for differentiation, and morphologically by its unusually large, distinctive ballistospores.

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V. Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo, Japan 113. Communicated by K. Komagata.

The following two papers were published recently:

1. Makiko Hamamoto, Junta Sugiyama, and Kazuo Komagata. 1987. DNA-DNA Reassociation Studies of Strains in the Genera Rhodosporidium and Rhodotorula. J. Gen. Appl. Microbiol. 33:57-73.

The genetic relatedness of 60 strains in the basidiomycetous yeast genera Rhodosporidium and Rhodotorula was studied by DNA-DNA hybridization using the nitrocellulose filter technique. Degrees of the intraspecific reassociation among the DNAs from the strains of Rhodosporidium infirmominiatum, Rhodosporidium diobovatum, Rhodosporidium sphaerocarpum, and Rhodosporidium dacryoidum ranged from 64 to 99%. The mating-type strains of Rhodosporidium toruloides were divided into four homology clusters. The degrees of DNA-DNA reassociation between the mating-type strains and the self-sporulating strains in Rhodosporidium toruloides were very low (18%) indicating the absence of relatedness between them. The strains of Rhodotorula glutinis were divided into five homology clusters.

Among these strains, some, including the type strain, showed close relatedness to Rhodosporidium diobovatum, some to Rhodosporidium toruloides (self-sporulating), and some to Rhodotorula rubra, and Rhodotorula pilimanae. The strains of Rhodotorula minuta were divided into four homology clusters. A close relationship was found between some strains of Rhodotorula minuta including the type strain and a strain of Rhodotorula pallida. There was a very close relationship between the Rhodotorula sinensis strain and the strains of Rhodosporidium infirmominiatum.

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2. Keiki Tokuoka*, Takasuke Ishitani, Shoji Goto**, and Kazuo Komagata**. 1987. Four New Yeast Species Belonging to the Genus CANDIDA. J. Gen. Appl. Microbiol., 33:1-10.

*National Food Research Institute, 2-1-2, Kannondai, Yatabe, Ibaraki 305 Japan.

**The Institute of Enology and Viticulture, Yamanashi University, 1-13-1, Kitashin, Kofu 400, Japan.

Four new yeasts, Candida glucosophila from brown sugar, Candida dulciaminis from confectionery, Candida floricola from dandelion and azalea flowers, and Candida vaccinii from blueberry flowers are described on the basis of morphological, physiological, and biochemical characteristics. C. dulciaminis is considered to be a basidiomycetous yeast.

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VI. Laboratory of Applied Microbiology, Dept. of Agricultural Chemistry, Shizuoka University, Shizuoka 422, Japan. Communicated by Yuzo Yamada.

The following papers have been published recently:

1. Yuzo Yamada and Kana Aizawa. 1987. Electrophoretic comparison of enzymes in strains of species in the genera Myxozyma (Cryptococcaceae) and Zygozyma (Lipomycetaceae). Trans. mycol. Soc. Japan 28:163-170.

Summary

A taxonomic study, below the generic or at the specific level, was made of the electrophoretic patterns of seven enzymes in fourteen strains of Myxozyma and Zygozyma species. The seven enzymes were glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, hexokinase, phosphoglucomutase and fumarase. When a dendrogram based on calculated similarity values was drawn by the complete linkage method, five strains of M. geophila that had a uniform electrophoretic enzyme pattern (s=100%) were linked to the type strain with a similarity value of 86%. The calculated similarity value was 100% between strains of M. mucilagina (including the type strain) which were linked to the strain of Z. oligophaga and further to the type strain of M. melibiosi with respective similarity values of 28% and 14%. Another strain of M. mucilagina had electrophoretic enzyme patterns that differed from the above

two M. mucilagina strains (s=0%). The only Q_9-equipped strain of M. lipomycoides was unique, the similarity value being 0%. These data are discussed in terms of taxonomy.

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2. Yuzo Yamada and Isao Banno*. 1987. Hasegawaea Gen. Nov., An Ascosporogenous Yeast Genus for the Organisms Whose Asexual Reproduction is by Fission and Whose Ascospores Have Smooth Surfaces Without Papillae and Which Are Characterized by the Absence of Linoleic Acid in Cellular Fatty Acid Composition[1]. J. Gen. Appl. Microbiol., 33:295-298.

*Institute for Fermentation, Osaka, Jusohon-machi, Yodogawa-ku, Osaka 532, Japan.

Since the genus Schizosaccharomyces Lindner was established (2), four species have been accepted in the genus (3): Schizosaccharomyces pombe Lindner (1893) (type species), Schizosaccharomyces octosporus Beijerinck (1894), Schizosaccharomyces japonicus Yukawa et Maki (1931), and Schizosaccharomyces malidevorans Rankine et Fornachon (1964).

This paper proposes Hasegawaea gen. nov., a new ascosporogenous yeast genus for the organisms assigned to the third species which are characterized morphologically by the smooth ascospores without papillae and chemotaxonomically by either the absence of CO-Q or the presence of linoleic acid. The genus is named for Dr. T. Hasegawa, the ex-Director of Institute for Fermentation, Osaka, Osaka, Japan, in recognition of his contributions to yeast taxonomy.

Cells are globose, ovoid, ellipsoid to cylindrical, reproducing by fission. Mycelium is formed. Asci arise by conjugation of vegetative cells. Ascospores are smooth, globose, ellipsoid to reniform, 6-8 per ascus and without papillae. Fermentation. Coenzyme Q system is absent. Linoleic acid is present.

Type species: Hasegawaea japonica (Yukawa et Maki) Yamada et Banno comb. nov. (Basionym: Schizosaccharomyces japonicus Yukawa et Maki, Kyushu Teikoku Daigaku Nogakubu Gakugei Zasshi 4:223. 1931).

In the species, Hasegawaea japonica, the following two varieties are presently recognized.

Hasegawaea japonica (Yukawa et Maki) Yamada et Banno var. japonica.

Typus: CBS 354.

Hasegawaea japonica (Yukawa et Maki) Yamada et Banno var. versatilis Wickerham et Duprat, J. Bacteriol. 50:606. 1945 (nom. invalid. art. 36) ex Slooff, The Yeasts ed. 2. 740. 1970.

Typus: CBS 103.

The genus Hasegawaea is placed in the family Schizosaccharomycetaceae.

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

Below follows an abstract of a recent publication:

Shoji Goto, Hiroyoshi Iwasaki, Yutaka Okuma, Yoshio Ito, and Akira Endo. 1987. New Species Belonging to the Genera Pichia and Candida. J. Gen. Appli. Microbiol., 33, 275-286.

Abstract

Detailed taxonomic studies were applied to six strains utilizing ethanol and having acid and ethanol tolerance; they were isolated from soils and feces. It was concluded that one strain, IM-10, which has helmet-shaped ascospores, coenzyme Q-7, and 35.6 mol% GC content of DNA, is a new species, Pichia galeiformis. Five other anascosporogenous yeasts with coenzymes Q-7 were divided into three new species belonging to the genus Candida: one strain of C. hinoensis (DBB test -, 35.1 mol% GC), three of C. soli (DBB test -, 38.8 mol% GC), and one of C. solicola (DBB test -, 42.5 mol% GC).

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VIII. All-Union Collection of Microorganisms, Inst. Biochem. Physiol. of Microorganisms, USSR Academy of Sciences, Pushchino I42292, USSR. Communicated by W.I. Golubev.

The following are recent publications from our Institute.

1. Semenova S.A., Golubev W.I., 1986. Pedogamic conjugation in the yeast Nadsonia commutata. - Mikrobiologiya, 55, N 5, 765-767.

The conjugating bud of N. commutata is an independent mature cell which remains near the parent cell solely due to the local attachment through the edges of a bud scar. Both a mother cell and a bud form conjugation outgrowths which grow together to yield a conjugation tube. The contents of a bud pass into the mother cell, and the zygote is separated from the bud with a novel septum.

* * *

2. Golubev W.I., Bab'eva I.P., Vinovarova M.E., Tyurin V.S., 1987. New strains of rare species of the yeast genus Nadsonia. - Mikrobiologiya 56, N 3, 454-459.

Two new strains of N. fulvescens, so far represented by a single strain in the collections, and a new strain of N. commutata were found. The latter was isolated from soil of the Carpathian reservation (USSR) and this species had been found before only once, in soil of East Folkland Island (UK). The morphological and physiological properties of these strains were studied that made it possible to reveal the most stable characteristics to be used for species differentiation within the genus Nadsonia Sydow.

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3. Golubev, W.I., 1986. Sporobolomyces philippovii Krassilnikov 1933, an anamorph of Sporidibolus salmonicolor Fell et Tallman 1981. - Mykologiya i Phytophatologiya, 20, N 6, 449-451.

* * *

IX. University of East Anglia, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England. Communicated by James A. Barnett.

New Yeast Identification Program

Yeast Identification: PC Program - version 1 - December 1987.

By J.A. Barnett, R.W. Payne & D. Yarrow

The program is based on data (updated & revised) from their book, Yeasts: Characteristics and Identification (1983). It (1) identifies yeasts; (2) selects yeasts with chosen characteristics; (3) is for use in industry, medical mycology & research; (4) is available for IBM PC & compatibles.

This personal computer program simplifies the process of identifying yeasts, avoiding time-consuming searches through identification keys or descriptions of species.

After entering the results of tests and observations into the computer, lists can be obtained of the following.

1. All species with a matching set of characteristics.
2. Yeasts with characteristics that most nearly match the entered set, together with a statement of the characteristic(s) that differ.
3. Further tests necessary to complete the identification.

With the program you can also allow for mistakes in the test-results, display the description of a selected species and select any yeasts with particular characteristics.

Results can be either entered directly from the keyboard or read from a file.

This program contains the following improvements to that published in 1985 by Cambridge University Press.

1. You can create your own data file, and use it in addition to the default SPECIES.DAT file.
2. Data can be read from an input form. The form can be created from a transcript file, so that results that have been entered do not need to be re-typed when processing them on a later occasion.
3. You can display on the screen the description of any species.
4. The data file has been updated with recently described and revised taxa.

Enquiries: Dr. J.A. Barnett 36 Le Strange Close Norwich NR2 3PW, UK.

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X. The University of Western Ontario, Department of Plant Sciences, Biological & Geological Building, London, Canada N6A 5B7. Communicated by M.A. Lachance (see also under H.J. Phaff, University of California, Davis.

The following papers have been published or accepted recently.

1. Butler, M.J., and M.A. Lachance. 1987. The use of N,N,N'-N'-tetramethylphenylenediamine to detect peroxidase activity on polyacrylamide electrophoresis gels. Anal. Biochem. 162:443-445.

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2. Dr. Michael J. Butler has successfully defended his doctoral thesis entitled "Melanin production by the black yeast Phaeococcomyces." He has gone on to join Dr. G. Lazarowits, at the Agriculture Canada Research Station, London, Ontario, to pursue postdoctoral studies on fungal melanins.

Abstract

A black yeast isolated from oak bark was identified as a species of Phaeococcomyces. The yeast produced black pigment constitutively which was identified as a pentaketide melanin, since its production was inhibited by the systemic fungicide tricyclazole. The black yeast melanin was produced as granules with a diameter of ca. 30 nm. Three types of pigmentation mutant were produced; albino mutants, which did not produce melanin, diffusion mutants which were not melanized and excreted 2-hydroxy-juglone and flaviolin in culture media, and cross feeder mutants which were not melanized and excreted scytalone, a component of the main pentaketide pathway, into culture media. Oxidation products of the pentaketide pathway, and main pathway phenolic intermediates, produced by treatment with tricyclazole, or which were excreted by mutants, were identified by High Performance Liquid Chromatography and Thin Layer Chromatography. The melanin of the black yeast protected against enzymatic cell wall lysis. Albino mutants were susceptible to enzymatic cell wall lysis. The melanin of the black yeast did not protect against toxic phenolic compounds, ultraviolet light irradiation, or desiccation. An azure A dye binding assay was developed to quantify cell wall melanin. A defined low pH ascorbate medium was developed which completely inhibited melanin production by Phaeococcomyces sp., while allowing normal growth. Polyacrylamide gel electrophoresis was used to identify a number of copper-containing phenoloxidases in cell wall enzyme and cytoplasmic enzyme extracts of the black yeast, which acted as melanin polymerases. An enzyme (referred to as convertase) which acted against scytalone, was found in cytoplasmic extracts of all but cross feeder mutants. The enzyme had a molecular mass of ca. 65000, a K_m of 1.7 mM for scytalone, an isoelectric point of 7.9, a pH optimum of 7.5, and did not appear to be glycosylated. The convertase enzyme had activity against 1,3,8-Trihydroxynaphthalene, the presumed product of scytalone conversion. The latter activity was inhibited by tricyclazole.

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3. Forrest, S.I., C.F. Robinow, and M.A. Lachance. 1987. Nuclear behaviour accompanying ascus formation in Debaryomyces polymorphus. Can. J. Microbiol. 33. (accepted August 1987).

Nuclear behavior in growing and dividing and in ascospore forming cells of a strain of Debaryomyces polymorphus, a member of the "Torulaspora" group of yeasts, has been studied by light microscopy of fixed Giemsa stained preparations. Many of the images seen were compatible with the suggestion, advanced by certain earlier students, that meiosis in this type of yeast is preceded by a process of self-diploidization involving the nucleus of a bud that despite its small size is already separated from the parent cell by a cross wall. Diploidization, in this view, is achieved by the return of the bud nucleus to the parent cell via a channel in the cross wall. The bud nucleus next fuses with the nucleus of the parent cell. Self-diploidization in Deb. polymorphus is thus achieved in the guise of heterogamous conjugation. This in turn is followed by meiosis. A lesser number of cell associations suggestive of isogamous conjugation has been encountered also.

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4. Vaughan-Martini,* A., D.G. Sidenberg, and M.A. Lachance. 1987. Analysis of a hybrid between Kluyveromyuces marxianus and Kluyveromyces thermotolerans by physiological profile comparison, isoenzyme electrophoresis, DNA reassociation, and restriction mapping of ribosomal DNA. Can. J. Microbiol. 33: (accepted August 1987).

*Dipartimento di BIologia Vegetale, Universita degli Studi, 1-06100 Perugia, Italy.

A hybrid yeast strain (LAC/MAL), obtained by Johannsen and van der Walt (1978. Arch. Microbiol. 118:45-48) by growing together strains of Kluyveromyces marxianus (LAC/mal) and Kluyveromyces thermotolerans (lac/MAL), was studied. The nutritional profiles of the hybrid and of some segregants derived from the hybrid indicated that they were more similar to K. marxianus than to K. thermotolerans. This was confirmed by the electrophoretic analysis of isoenzymes, by DNA/DNA reassociation studies, and by restriction endonuclease mapping of ribosomal DNA cistrons. These analyses further indicated that the parent strain identified as K. thermotolerans was atypical of that species. The accumulated evidence ruled out the possibility that the hybrid strain was the result of Mendelian recombination but suggested that some form of transformation might have taken place.

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5. Starmer,* W.T., V. Aberdeen,* and M.A. Lachance. 1987. The yeast community associated with decaying Opuntia stricta (Haworth) in Florida, with regard to the moth Cactoblastis cactorum (Berg).

*Biology Department, Syracuse University, Syracuse, New York 13244-1270.

Abstract

The yeast communities of decaying cladodes and fruits of two large stands of Opuntia stricta in the area of Cape Canaveral, Florida, were investigated. The yeast community was comparable in many ways with those of O. stricta in the Caribbean and in Australia. Many important differences were observed as well, some of which may be attributable to the absence, in Florida, of Cactoblastis cactorum, a moth used extensively elsewhere for the biological eradication of prickly pear cactus as a weed.

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

The following papers have been published since the listing in the Yeast Newsletter of June 1987 (Vol. 36. No. 1).

1. Starmer, W.T.[1], M.A. Lachance[2], and H.J. Phaff. 1987. 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. Microb. Ecol. 14:179-192.

[1]Department of Biology, Syracuse University, Syracuse, NY 13210, USA; [2]Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7, Canada.

ABSTRACT

Yeast communities growing in the decaying tissues (cladodes and fruits) of Opuntia stricta (prickly pear cactus) and associated 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 also 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: (1) within and between regions, (2) across seasons and years, and (3) 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 some overlap with the cladodes within regions when collected simultaneously in the same locality. It is suggested that the cladode-microorganisms-Drosophila system is relatively closed (little extrinsic influence) whereas the fruit-microorganism-Drosophila system is open (large extrinsic influence).

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2. H.J. Phaff, W.T. Starmer[1], and Joanne Tredick-Kline, Pichia kluyveri sensu lato. A Proposal for Two New Varieties and a New Anamorph.

[1]Department of Biology, Syracuse University, Syrazcuse, N.Y. 13210, U.S.A. From the Symposium "The Expanding Realm of Yeast-like Fungi", Amersfoort, The Netherlands, 3-7 August 1987. Proceedings to be published in book form.

ABSTRACT

During our ecological studies of yeasts inhabiting necrotic tissue of various cactus species, we have recovered a number of heterothallic strains that are close relatives of P. kluyveri. This strongly-fermenting species is not cactus specific but is recovered occasionally from necrotic cactus tissue and periodically from Opuntia fruit. One group of related strains that is cactus specific originated in rotting stems of Opuntia spp. in Arizona and Texas. These strains are essentially non-fermentative or ferment very weakly. Their mating types are interfertile with those of P. kluyveri and their DNA relatedness to P. kluyveri is about 66%. These strains are described as P. kluyveri var. eremophila var. nov. An anamorph of that variety is described as Candida eremophila. A second group of slowly-fermenting strains came from rotting arms of the columnar cactus Cephalocereus royenii on the island of Montserrat. These strains were also interfertile with P. kluyveri and showed about 72% DNA relatedness with that species, but were different from the variety eremophila to which they showed about 69% DNA relatedness. This group is described as P. kluyveri var. cephalocereana var. nov. Single-spore viabilities of the parental varieties varied from about 50 to nearly 100%. Intervarietal hybrids generally showed reduced spore viability. Fermentation velocity in P. kluyveri appears to be controlled by more than a single gene. The evolutionary divergence of the different varieties may have been promoted by host plant chemistry, as well as allopatry.

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3. Fuson, G.B., Presley, H.L. and Phaff, H.J. 1987. Deoxyribonucleic Acid Base Sequence Relatedness Among Members of the Yeast Genus Kluyveromyces. Int. J. Syst. Bacteriol. 37:371-379. For abstract see Yeast Newsletter 36 (NO.1) p.7.

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4. Phaff, H.J., Starmer, W.T., Tredick-Kline, J., and Aberdeen, V., 1987. Pichia barkeri, a New Yeast Species Occurring in Necrotic Tissue of Opuntia Stricta. Int. J. Syst. Bacteriol. 37:386-390. For abstract see Yeast Newsletter 36 (NO.1) p.7.

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XII. Georgia State University, Laboratory for Microbial and Biochemical Sciences, Atlanta, Georgia 30303. Communicated by D.G. Ahearn.

Recent communications:

1. Guého, E., R.B. Simmons, W.R. Pruitt, S.A. Meyer, and D.G. Ahearn. 1987. Association of Malassezia pachydermatis with systemic infections of humans. J. Clin. Microbiol. 25:1789-1790.

Abstract

Thirty-two Malassezia spp. isolates from human clinical specimens represented M. furfur and M. pachydermatis. Both species reportedly were obtained from patients with similar febrile systematic syndromes, including infections of the lungs or other tissues.

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XIII. Department of Microbiology, University of the O.F.S., P.O. Box 339, 9300 Bloemfontein, South Africa. Communicated by J.C. du Preez and J.L.F. Kock.

The yeast taxonomy group aims to develop rapid chemotaxonomic methods for the identification and classification of yeasts. The procedures used include analysis of cellular long-chain fatty acids, chromosomal band separation, uptake of sugars in mixtures and the analysis of organic acids in culture fluids.

The following papers on yeast taxonomy have recently appeared or are in print:

1. Muller, H.B. and J.L.F. Kock. (1986). Waltiozyma gen. nov. (Saccharomycetaceae), a new genus of the Endomycetales. S. Afr. J. Sci. 82, 491-492.

Abstract

The species described as Pichia mucosa differs from the type of the genus Pichia by the formation of Saturnoid ascospores, the CoQ_6-system and the formation of non-amyloid estracellular polysaccharides. Differences in the fatty acid composition of the type strain of P. mucosa emphasize these phenotypic differences. Since the combination of the definitive characters of P. mucosa excludes it from all recognized genera of the Endomycetales, it is proposed that this species can be assigned to the new genus Waltiozyma.

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2. Coetzee, J.C., J.L.F. Kock and G.H.J. Pretorius. (1987). The value of orthogonal-field-alternation gel electrophoresis as a rapid identification process for some species representing the genus Saccharomyces. J. Microbiol. Meth. (In Press).

Abstract

The chromosomal DNA band patterns for nine strains representing four species of the genus Saccharomyces were determined by an optimized orthogonal-field-alternation gel electrophoresis (OFAGE) method. These yeasts were grouped according to their similarities and then compared.

Consequently the Saccharomyces strains were clustered into four groups on the basis of their chromosomal DNA band patterns. The band patterns of each of the four species proved to be unique.

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3. Smit, E.J., J.P.J. van der Westhuizen, J.L.F. Kock, P.M. Lategan and F. Oberwinkler. (1987). A yeast identification method: The influence of culture age on the cellular long-chain fatty acid composition of three selected Basidiomycetous yeasts. Syst. Appl. Microbiol. 10:38-41.

Abstract

Cellular fatty acids were extracted from the cells of Agaricostilbum palmicolum, Filobasidiella neoformans and Tremella aurantia. The changes in relative fatty acid composition, at different growth stages, was measured by gas-liquid chromatography. A high degree of variation in relative amounts of long-chain fatty acids was observed during the exponential and early stationary phases of growth, while reproducible results and a stable fatty acid composition was obtained with cells harvested in the late stationary phase. The significance of the changes in fatty acid profiles during growth and the possible application thereof for identification purposes is evaluated.

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4. Viljoen, B.C., J.L.F. Kock, H.B. Muller and P.M. Lategan. (1987). Long-chain fatty acid compositions of some asporogenous yeasts and their respective ascosporogenous states. J. Gen. Microbiol. 133:1019-1022.

Abstract

Long-chain fatty acid compositions were used to measure the relatedness between seven different species within the genus Candida and among these species and their counterparts within the perfect genera. Close relationships were found between the fatty acids of seven perfect yeasts and their imperfect states: Candida shehatae and Pichia stipitis; Candida kefyr and Kluyveromyces marxianus; Candida lipolytica and Yarrowia lipolytica; Candida pelliculosa and Hansenula anomala; Candida pseudotropicalis and Kluyveromyces fragilis; Candida utilis and Hansenula jadinii; Candida parapsilosis and Lodderomyces elongisporus. According to these results, the long-chain fatty acid technique is considered to be a useful tool for the study of relationships between the perfect and imperfect states of the genus Candida.

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5. Van der Westhuizen, J.P.J., J.L.F. Kock, E.J. Smit and P.M. Lategan. (1987). The value of long-chain fatty acid composition in the identification of species representing the Basidiomycetous genus Rhodosporidium Banno. Syst. Appl. Microbiol. 10:31-34.

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Cellular long-chain fatty acids were extracted from cells of the basidiomycete Rhodosporidium toruloides at different stages of growth. Gas-liquid chromatography was used to measure changes in the relative fatty acid composition. Large variations in the relative amounts of fatty acids were observed during the exponential and early stationary growth phases, while greater reproducibility and stable fatty acid compositions were obtained with cells harvested in the late stationary phase. The cellular long-chain fatty acids of eight species of the genus Rhodosporidium, harvested in late stationary phase, were also determined by gas-liquid chromatography. The Rhodosporidium species were characterized by the presence of myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), magaric acid (C17:0), iso-magaric acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). These species were divided into two groups on the basis of fatty acid content. The first group was characterized by a higher mean percentage, and the second group by a lower mean percentage linoleic acid (C18:2).

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6. Viljoen, B.C., J.L.F. Kock and T.J. Britz. (1987). The significance of long-chain fatty acid composition and other phenotypic characteristics in determining relationships among some Candida, Kluyveromyces and Saccharomyces species. Syst. Appl. Microbiol. (In Press).

Abstract

Thirty one species representing the genera Candida, Kluyveromyces and Saccharomyces were grouped according to their cellular long-chain fatty acid compositions, carbon source utilization patterns and degree of cell differentiation. The coordinate use of these different phenotypic characteristics provided a convenient method to establish possible teleomorph and anamorph relations as well as similarities among yeasts. Yeasts with known teleomorph-anamorph relations were generally closely grouped while unrelated yeasts were positioned some distances from each other. An interesting correlation was also found between the different phenotypic characteristics of the species studied. The presence of linolenic acid correlated with the formation of pseudomycelium and/or true hyphae as well as the utilization of a large number of carbon sources while the absence of linolenic acid correlated with the formation of rudimentary pseudomycelium and/or single cells and the utilization of only a few carbon sources.

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7. Smit, E.J., J.P.J. van der Westhuizen, J.L.F. Kock and P.M. Lategan (1987). The long-chain fatty acid composition of the two varieties of Filobasidiella neoformans. Syst. Appl. Microbiol. 10:28-30.

Other recent publications from this department include the following:

8. Ligthelm, M.E., B.A. Prior and J.C. du Preez. The oxygen requirements of yeasts for the fermentation of D-xylose and D-glucose to ethanol. Appl. Microbiol. Biotechnol. (accepted).

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The effect of oxygen availability on D-xylose and D-glucose metabolism by Pichia stipitis, Candida shehatae and Pachysolen tannophilus was investigated. Oxygen was not required for fermentation of D-xylose or D-glucose, but stimulated the ethanol production rate from both sugars. Under oxygen-limited conditions, the highest ethanol yield coefficient ($Y_{e/s}$) of 0.47 was obtained on D-xylose with P. stipitis, while under similar conditions C. shehatae fermented D-xylose most rapidly with a specific productivity ($q_p max$) of 0.32 h^{-1}. Both of these yeasts fermented D-xylose better and produced less xylitol than P. tannophilus. Synthesis of polyols such as xylitol, arabitol, glycerol and ribitol reduced the ethanol yield in some instances and was related to the yeast strain, carbon source and oxygen availability. In general, these yeasts fermented D-glucose more rapidly than D-xylose. By contrast Saccharomyces cerevisiae fermented D-glucose at least three-fold faster under similar conditions.

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9. Horn, C.H., A. de Kock and J.C. du Preez. A comparative study of the amylolytic ability of Lipomyces and Schwanniomyces yeast species. Syst. Appl. Microbiol. 467 (in press).

Abstract

The starch degrading ability of the yeast genera Lipomyces and Schwanniomyces were evaluated on solid and in liquid media. Strains of Lipomyces kononenkoae, L. starkeyi and Schwanniomyces occidentalis utilized 100% of the starch supplied, with biomass yield coefficients of up to 0.52. There was little correlation between the clearing zone diameters on starch agar plates and the extracellular amylase activities. A derepressed mutant strain of L. kononenkoae had the highest α-amylase activity (more than three-fold higher than the other yeasts) and glucoamylase activity (more than five-fold higher), while L. starkeyi exhibited the highest debranching activity. The L. kononenkoae mutant was the only yeast insensitive to glucose repression of its amylases.

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10. Horn, C.H., J.C. du Preez and P.M. Lategan. Protein enrichment of banana plant wastes by yeast cultivation. Biomass (in print).

Abstract

Banana plant wastes, which are generated in copious amounts during normal agricultural practice, contain between 35 to 60% starch on a moisture free basis. A dextrin-containing liquid extract, obtained after liquefaction of the plant starch with a commercial thermostable α-amylase, was used as substrate for the cultivation of Candida utilis. Saccharification of the dextrins, to enable growth of C. utilis, was effected with either a commercial glucoamylase or by the co-cultivation of Lipomyces kononenkoae with C. utilis. The saccharification efficiency obtained with this amylolytic yeast proved comparable with that of the commercial glucoamylase, and a dry biomass concentration of 14.86 g/l with a crude protein content of 47.4% was reached in the hydrolysate.

Supplementation of the hydrolysate with banana peels resulted in a dry biomass concentration of 37.7 g/l (31.1% crude protein) and increased the yield to 0.23 g crude protein/g glucose equivalents.

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11. Kilian, S.G. and N. van Uden. 1987. Transport of xylose and glucose in the xylose-fermenting yeast Pichia stipitis. Appl. Microbiol. Biotechnol. (in press).

Abstract

A low-affinity and a high-affinity xylose proton symport operated simultaneously in both starved and non-starved cells of Pichia stipitis. Glucose competed with xylose for transport by the low-affinity system and inhibited xylose transport by the high-affinity system non-competitively. The low affinity system was subject to substrate inhibition when glucose but not when xylose was the substrate. The differences between the characteristics of monosaccharide transport by Pichia stipitis and its imperfect state, Candida shehatae, are discussed.

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XIV. Instituto de Quimica Universidade Federal do Rio de Janeiro, Cidade Universitaria, Ilha do fundão, Caixa Postal 1573, Rio de Janeiro, Brazil. Communicated by Anita D. Panek.

The following papers are in press:

1. Ana C. Panek, J. François and Anita Panek. Current Genetics, 0-0, 1987.

   New Insights Into A Mutant of Saccharomyces cerevisiae Having Impaired Sugar Uptake and Metabolism.

A regulatory mutant of Saccharomyces carlsbergensis unable to inactivate fructose-1, 6-bisphosphatase was shown to have a normal response to the glucose signal as measured by trehalase and 6-phosphofructose-2-kinase activities. Yet, fructose-2, 6-bisphosphate was found to be 4 to 5-fold lower when compared to a wild type strain. A rapid and drastic depletion in ATP was confirmed. A partial revertant for growth on glucose which retained its inability to grow on fructose, did not show normal levels of fructose-2, 6- bisphosphate, however, ATP levels were restored. Trehalose-6-phosphate synthase activity was found in its phosphorylated less active form. A high degree of phosphorylation, at the level of enzymatic activity, and of sugar phosphorylating systems might be responsible for the impairment of control between hexose transport and metabolism, as well as for the absence of trehalose accumulation.

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2. C. Coutinho, E. Bernardes, Durvalina Félix and Anita Panek. Journal of Biotechnology, 00, 1987.

   Trehalose as Cryoprotectant For Preservation of Yeast Strains.

Preservation of genetic banks of yeast strains as well as of any kind of eukaryotic cells during dehydration and subsequent rehydration depends upon the maintenance of the integrity of the cell membrane. Trehalose has been successfully used as a non-toxic cryoprotectant for plant cells (Bhandal et al., 1985), as well as for lobster sarcoplasmic vesicles (Rudolph and Crowe, 1985). The hypothesis underlying these observations is that the disaccharide avoids fusion of membranes by replacing water molecules in the bilayer (Crowe et al., 1984). The viability of yeast strains submitted to different drying techiques is reported in this paper. Mutant strains with defects in the regulation of the trehalose-6-phosphate synthase complex were compared. Yeast strains dried in layers at 37°C for 6 h. did not loose their viability, however, they died thereafter at 5°C, unless trehalose was used for resuspending the cells before drying. It should be noted that no trehalose accumulation was seen during drying at 37°C under our experimental conditions. In experiments in which cells were frozen at -120°C, addition of 10% trehalose to the suspending buffer had a significant protective effect. On the other hand, a mutant strain with an extremely high trehalose-6-phosphate synthase activity showed an intrinsic capacity for survival which did not depend upon addition of exogenous trehalose. This raises the question of the location of the internal trehalose pool and whether it could replace the externally added cryoprotectant.

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XV. Albert-Ludwigs-Universität, Biochemisches Institut, D-7800 Freiburg 1. BR., Hermann-Herder-Str.7, F.R.G. Communicated by Klaus Mittenbühler.

The following is a summary of a recent research project.

Klaus Mittenbühler[1] and Helmut Holzer[1),2)]. Purification and Characterization of Acid Trehalase from the Yeast suc-2 Mutant to be submitted by J. Biol. Chem..

1) Gesellschaft für Strahlen- und Umweltforschung, Projekt Inhalation, Ingostadter Landstr. 1, D-8042 Neuherberg, West Germany.

2) Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg, West Germany.

Summary

Acid trehalase (maximal activity at pH 4.5) was purified from the yeast suc-2 deletion mutant. After hydrophobic interaction chromatography, the enzyme could be purified to a single band or peak by a further step of either poly-acrylamide gel electrophoresis, gel filtration or isoelectric focusing. An apparent molecular mass of 218,000 Da was calculated from gel filtration. Polyacrylamide gel electrophoresis of the purified enzyme in the presence of sodium dodecyl sulfate suggested a molecular mass of 216,000 Da. Endoglycosidase H digestion of the purified enzyme resulted after SDS gel electrophoresis in one distinct band at 41,000 Da representing the mannose-free protein moiety of acid trehalase. Amino acid analysis indicated 354 residues/mol of enzyme including 9 cysteine moieties. The isoelectric point of the enzyme was estimated by gel electrofocusing to be approximately 4.7. The catalytic activity showed a maximum at pH 4.5. The activity of the enzyme was not inhibited by 10 mM each of $HgCl_2$, EDTA,

iodoacetic acid, phenanthroliniumchloride or phenylmethylsufonylfluoride. There was no activation by divalent metal ions. The acid trehalase exhibited an apparent K_m for trehalose of 4.7 + 0.1 mM and a V_{max} of 7.14 µmol of trehalose $min^{-1} \times ml^{-1}$ at 37°C and pH 4.5. The carbohydrate content of the enzyme was 86%. Studies with purified vacuoles demonstrated the acid trehalase to be a vacuolar enzyme. The rabbit antiserum raised against acid trehalase exhibited strong cross reaction with purified invertase and other glycoproteins in crude extract. These cross reactions were removed by affinity chromatography using invertase coupled to CNBr-activated Sepharose 4B. Immunoinhibition of purified acid trehalase was observed with the purified antiserum.

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XVI. Delft University of Technology, Department of Microbiology & Enzymology, Julianalaan 67, NL-2628 BC Delft, The Netherlands. Communicated by W. Alexander Scheffers.

The following paper has appeared:

1. W.A. Scheffers & J.P. van Dijken. 1987. Redox control in yeast. European Brewery Convention Symposium on Brewers' Yeast (Monograph-XII), Verlag Hans Carl (Brauwelt Verlag), Nurnberg, 1987, pp. 23-43.

ABSTRACT

The coenzyme couples NAD^+/NADH and $NADP^+$/NADPH play a central role in the redox reactions of carbohydrate metabolism in yeasts. Many of the enzymes involved in dissimilation and assimilation of sugars in yeasts are specific for either the NAD(H) or the NADP(H) system. This has consequences for product formation and growth of the yeasts.

As examples of redox control in yeasts: formation of acetic acid and glycerol, competition for reducing equivalents between fermentation and respiration, adaptation to anaerobiosis, and fermentation of xylose are discussed.

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The following paper is in press:

1. A. Noshiro, C. Purwin, M. Laux, K. Nicolay, W.A. Scheffers & H. Holzer. Mechanism of the stimulation of endogenous fermentation in yeast by carbonyl cyanide m-chlorophenylhydrazone. Journal of Biological Chemistry 262: in press.

ABSTRACT

Addition of the uncoupler and protonophnore carbonyl cyanide m-chlorophenylhydrazone (CCCP) to starved yeast cells starts endogenous alcoholic fermentation lasting about 20 min. Hexose 6-phosphates, fructose 2, 6-bisphosphate, and pyruvate accumulate in less than 2 min. after addition of CCCP from almost zero concentration to concentrations which correspond to 1/5 - 1/10 of the steady-state concentrations during fermentation of glucose. CCCP immediately causes a decrease of the intracellular cytosolic pH from 6.9 to 6.4. This change activates adenylate cyclase (Purwin, C., Nicolay, K., Scheffers, W.A., and Holzer, H. (1986) J.

Biol. Chem. 261, 8744-8749) and leads to the previously observed transient increase of cyclic AMP. It is shown here that the following enzymes known from in vitro experiments to be activated by cyclic AMP-dependent phosphorylastin are activated in the CCP-treated starved yeast cells in vivo: glycogen phophorylase trehalase (pH7), 6-phosphofructo-2-kinase. The activation of 6-phosphofructo-2-kinase leads to an accumulation of fructose 2,6-bisphosphate, which is known from in vitro experiments to activate 6-phosphofructo-1-kinase and to inhibit fructose-1,6-bisphosphatase. All effects observed in the intact yeast cells fit with the idea that the CCCP-initiated activation of adenylate cyclase leads to a sequence of events which by protein phosphorylation and allosteric effects initiates endogenous alcoholic fermentation.

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XVII. Research Laboratories of ALKO Ltd, P.O. Box 350. SF-00101 Helsinki, Finland. Communicated by Roy S. Tubb.

Below follow several recent publications:

1. Roy S. Tubb and Pirkko L. Liljeström. 1986. A Colony-Colour Method Which Differentiates α-Galactosidase-Positive Strains of Yeast. J. Inst. Brew., 92:588-590.

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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2. Roy S. Tubb. 1987. Cambridge Prize Lecture - Gene Technology for Industrial Yeasts. J. Inst. Brew., 93:91-96.

Yeast genetics is now available as a practical tool for the development of brewing industry practices. The contribution of Brewing Research Foundation work (1978-84) to recent advances is illustrated by the construction of brewing strains with superattenuating (amylolytic) or anti-contaminant properties. Approaches based on hybridisation (by rare mating) or recombinant DNA technology have been evaluated. Techniques developed for (i) gene transfer to brewing strains, (ii) ensuring stable inheritance of novel characteristics and (iii) exploiting the secretory ability of yeast strains, can be widely applied not only with brewing, distilling, baking or wine yeasts, but also in the use of yeasts to produce novel biotechnical products. "Spin-off" from these studies includes valuable methods of differentiating or enumerating wild yeasts in brewery quality control.

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3. Roy S. Tubb, Pirkko L. Liljeström, Tuula Torkkeli and Matti Korhola. MEL Gene Polymorphisms in Strains of Saccharomyces. 13th International Conference on Yeast Genetics and Molecular Biology, Banff, Alberta, Canada 1986. Yeast 2 (Spec. Iss.) 1986 S396.

The ability of strains to ferment melibiose (secrete α-galactosidase) has played an important role in the development of the taxonomy of the genus Saccharomyces. It has also led to much confusion in the naming of strains. Although the early studies of Winge and Roberts indicated that a MEL gene family might exist, only one gene (MEL1) has been the subject of further study. Therefore, we have examined molecular hybridization of a MEL1 probe to endonuclease fragments of DNA from more than 20 Mel^+ strains of Saccharomyces and closely related genera.

The hybridization pattern expected for MEL1 is found not only in ATCC 9080 (=NCYC 74 - CECT 1317; i.e. the source of the gene cloned at Alko) and its derivitives, but also in CECT 1323 (Winge and Roberts strain 303-49) and 1453-3A (Berkeley strain; descended from Lindegren's C1A?) In all these cases a single bybridizing band is obtained on digestion with BamH1 (> 20 kb) or with a BamH1-Sal1 double digest (2.8 kb).

Strong hybridization to MEL1 is also seen with strains of S. cerevisiae var. oleaceus or oleaginosus. The MEL gene in these strains is carried on a 12 kb BamH1 fragment which contains no Sal1 site. However, the RNA transcript of this gene and the product of its translation in vitro are of sizes similar to those for MEL1.

Unique patterns of DNA hybridization are shown by an American whiskey strain and a strain of S. cerevisiae var. norbensis. Remarkably, DNA from brewing lager strains does not hybridize to MEL1 at high stringency, as is also the case for S. kluyveri (1 strain), Schwanniomyces alluvius (1 strain) and Zygosaccharomyces cidri (2 strains).

We conclude that a MEL gene family exists among strains now classified as Saccharomyces cerevisiae and that brewing lager strains form a unique group in which the the Mel^+ phenotype is conferred by a gene which is not closely related to MEL1.

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4. Tubb, R.S. and Hammond J.R.M. (1987). Genetics of brewing and distilling yeast. In Brewing Microbiology. Edited by F.G. Priest and I. Campbell. Elsevier Applied Science, London, pp. 47-82.

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5. Tubb, R.S. (1987). Yeast Genetics and Alcoholic Beverages. In Proceedings of 6th Australian Wine Industry Technical Conference. Adelaide, July 1986. Edited by T.H. Lee. Australian Wine Research Institute publication, in press.

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6. Knowles, J.K.C. and Tubb, R.S. (1987). Recombinant DNA: gene transfer and expression techniques with industrial yeast. In EBC Monograph XII: Brewers' Yeast. Brauwelt-Verlag, Nürnberg. pp. 169-185.

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XVIII. Technical Research Center of Finland, Biotechnical Laboratory, Titotie 2, SF-02150 Espoo, Finland. Communicated by Maija-Liisa Suihko.

The communicator for the Yeast Newsletter of the Technical Research Centre of Finland (VTT) Dr. Veijo Makinen retired at the end of January 1987 after his 25 years in yeast research. At the request of Dr. Tor-Magnus Enari the new communicator will be Dr. Maija-Liisa Suihko.

During the last year the following publications have been prepared about the construction, expression and properties of cellulolytic yeasts in VTT, Biotechnical Laboratory, Tietotie 2, SF-02150 Espoo, Finland:

Penttilä, M.E., André L., Saloheimo, M., Lehtovaara, P. & Knowles, J.K.C., Expression of two Trichoderma reesei endoglucanases in the yeast Saccharomyces cerevisiae. Yeast 3 (1987), 175-185.

Penttilä, M.E., Suihko, M.-L., Lehtinen, U. Nikkola, M. & Knowles, J.K.C., Construction of brewer's yeasts secreting fungal endo-β-glucanase. Curr. Genet. 12 (1987), in press.

Penttilä, M.E., André, L., Lehtovaara, P. Bailey, M.J., Teeri, T.T. & Knowles, J.K.C., Efficient secretion of two fungal cellobiohydrolases in Saccharomyces cerevisiae. Gene, in press.

Enari, T.-M., Knowles, J., Lehtinen, U., Nikkola, M., Penttilä, M., Suihko, M.-L., Home, S. & Vilpola, A., Glucanolytic brewer's yeast. Proc. 21st Congress of EBC, Madrid 1987, 529-536.

These original publications are summarized in:

Penttilä, M., Construction and characterization of cellulolytic yeasts. VTT Publications 39, Espoo 1987. 53 p. + app. 63 p.

Knowles, J., Penttilä, M., Teeri, T., André, L., Lehtovaara, P. & Salovuori, I., The development of cellulolytic yeasts and their possible applications, In: Biological Research on Industrial Yeasts, (eds. G.G. Stewart, I. Russell, R.D. Klein & R.R. Hiebsch), CRC Press, Boca Raton, Florida, 1987, Vol. 1, 189-199.

Penttilä, M., André, L., Lehtovaara, P., Suihko, M.-L., Niku-Paavola, M.-L. & Knowles, J., Construction of yeast strains secreting fungal cellulases. In: Industrial Yeast Genetics, Proceedings of the Alko Symposium on Industrial Yeast Genetics, 9-10 June 1987, Helsinki, Finland. (Eds. M. Korhola & H. Nevalainen), Foundation for Biotechnical and Industrial Fermentation Research, Vol. 5, in press.

This subject has also been reviewed in :

Knowles, J.K.C. & Tubb, R.S., Recombinant DNA: Gene transfer and expression techniques with industrial yeast strains. EBC-Symposium on Brewer's yeast, 24-25 November 1986, Vuoranta (Helsinki), Finland. EBC Monograph-XII, 169-184.

Suihko, M.-L., Improvement of brewer's yeasts with recombinant-DNA-technique. Mallas ja Olut 1987: 5, 139-148. (In Finnish with Swedish and English summary).

Knowles, J.K.C., Brewer's yeast, views on the EBC Symposium. Proc. 21st Congress of EBC, Madrid 1987, 123-132.

The following publications deal with the immobilization of yeasts:

Linko, M., Mäkinen, V. & Pajunen, E., Fermentation with immobilized yeast cells: methods, facts and prospects. Lecture presented in the course on brewing, Louvain, 22-26 September 1986. In: J. De Clerck, Chair II, Microbiology and the Brewing Industry from Barley to Beer, Louvain 1986, 12 pages.

Mäkinen, V. & Leikas, A., Production of beer with immobilized yeast. Mallas ja Olut 1987: 1, 4-16. (In Finnish with Swedish and English summary).

Pajunen, E., Mäkinen, V. & Gisler, R., Secondary fermentation with immobilized yeast. Proc. 21st Congress of EBC, Madrid 1987, 441-448.

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XIX. Institut für Mikrobiologie, J.W. Goethe-Universität, 6000 Frankfurt am Main, Fed. Rep. of Germany. Communicated by Martin Brendel.

Below follow abstracts of three publications, two of them in press and one submitted.

1. E. Bender and M. Brendel (1988). Effects of excess thymidylate on thymidylate low-requiring strains of Saccharomyces cerevisiae: high mutagenicity and absence of DNA strand breaks. Mutation Res., in press.

dTMP exposure concentrations of 0.1 mmolar or higher are genotoxic in exponentially growing cells of thymidylate low-requiring mutants of Saccharomyces cerevisiae. Mutagenicity of excess dTMP is highest in an exposure concentration tenfold of the one needed for external supplementation of endogenously blocked thymidylate synthesis. dTMP concentrations still higher act predominantly cytotoxical. The canavanine forward mutation system shows excess dTMP to be a mutagen as potent as irradiation by ultraviolet light. Mutagenicity of excess dTMP, however, differs from that of directly DNA-attacking mutagens in that it is highest in the absence of significant toxicity. Alkaline sucrose gradient centrifugation shows that excess dTMP does not induce significant numbers of DNA single- or double-strand breaks while conditions of thymidylate deprivation leads to DNA strand breaks and thymineless death.

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2. M. Mack, P. Gömpel-Klein, E. Haase, B.J. Hietkamp, A. Ruhland, and M. Brendel (1988). Genetic characterization of hyperresistance to formaldehyde and 4-nitroquinoline-N-oxide in the yeast Saccharomyces cerevisiae. Molec. Gen. Genet., in press.

The hyperresistance to 4-nitroquinoline-N-oxide and formaldehyde of yeast strains transformed with the multi-copy plasmids pAR172 and pAR184, respectively, is due to the two genes, SNQ and SFA, which are present on these plasmids. Restriction analysis revealed the maximal size of SFA as 2.7 kb and of SNQ of 2.2 kb, respectively, including transcription control elements. Presence of the smallest 2.7 kb subclone carrying SFA increased hyperresistance to formaldehyde fivefold over that of the original pAR184

isolate. No such increase in hyperresistance to 4-NQO was seen with the smaller subclones of the pAR172 isolate. Disruption of the gene SFA led to a threefold increase of sensitivity to formaldehyde as compared to wildtype. Expression of gene SNQ introduced on a multi-copy vector into haploid yeast mutants rad2, rad3, and snm1 did not complement either mutation that block excision repair.

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3. P. Sander and M. Brendel (submitted). Hyperresistance to formaldehyde of Saccharomyces cerevisiae seems not correlated with formation and removal of DNA-protein cross-links. Curr. Genetics, submitted.

Formation and removal of formaldehyde-mediated DNA-protein cross-linking was measured by CsCl density gradient analysis in yeast strains of differing resistance to formaldehyde. Wild-type cells and transformants made hyperresistant to formaldehyde by a multi-copy vector containing the yeast SFA gene were specifically labelled in their DNA and incubated in the presence of formaldehyde. Treatment with formaldehyde lead to formation of equal amounts of DNA-protein cross-links, and upon liquid-holding for 24 h nearly all DNA-protein cross-links were removed regardless of formaldehyde resistance status of the strains.

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

1. C. Jager-Magiera: Accumulation of fructose-2, 6-bisphosphate in various glycolysis mutants of Saccharomyces cerevisiae.

Fructose-2, 6-bisphosphate is considered to be a major regulator of glycolysis in yeast (Leder et al., Biochem. Biophys. Res. Comm. 103, 1281-1287, 1981). It was determined according to van Schaftingen et al. (Eur. J. Biochem. 129, 191-195, 1982) in the different mutants growing on a synthetic mineral medium supplemented with different carbon sources. The following results were obtained:

Strain	Fructose-2, 6-Bisphosphate in pmole/mg dry weight	
	on glucose medium	on ethanol-glycerol
no defect in glycolysis	9	2
pfk1::LEU2 of Heinisch	300	4
pfk2::LEU2 of Heinisch	76	2
byp1 of Breitenbach et al.	5	5
byp2 of Breitenbach et al.	302	2
byp3 of Breitenbach et al.	6	3
pfk2 byp1	100	8

pfk2::LEU2 byp1	290	10
pfk2::LEU2 byp2	194	8
pfk2-94 byp3	202	10
pgi1-Δ of Aguilera	16*	4**

Source of mutants: A. Aguilera (Molec. Gen. Genet. 204, 310-316, 1986; Breitenbach et al. (Molec. Gen. Genet. 195, 530-535 and 536-540, 1984); Heinisch (Curr. Genet. 11, 227-234, 1986). *The pgi1-Δ mutant was grown on 2% fructose plus 0.1% glucose or ** incubated for 3 h in a medium with 2% glucose. The results indicate that a defect in one of the structural genes coding for a subunit of phosphofructokinase (PFK1 or PFK2) or in the gene BYP2 required for glykolysis without phosphofructokinase activity (Breitenbach et al.: Molec. Gen. Genet. 204, 536-540, 84) leads to an accumulation of exceedingly large amounts of fructose-2, 6-bisphosphate. The same defects also lead to an accumulation of sedoheptulose-7-phosphate, a metabolite not detectable in wild type, but also found in the PGI-deletion mutant of Aguilera grown on fructose with a trace of glucose.

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2. Structural analysis of the 5'-regions of yeast SUC, genes revealed analogous palindromes in SUC MAL and GAL. Stefan Hohmann and Daniel Gozalbo. Molec gen Genet., in press.

In the yeast Saccharomyces cerevisiae six unlinked structural genes for invertase, the SUC genes, are known. We have sequenced about 800 bp of the 5' non-coding region and the first 220 bp of the coding region of the genes SUC1, SUC3, SUC4 and SUC5 and compared them with the previously sequenced genes SUC2 and SUC7 (Sarokin and and Carlson 1985a). All are highly homologous within the coding region but in the non-coding region SUC1 shows some differences and SUC2 is more highly diverged. Two different kinds of TATA-boxes were identified: the more strongly expressed genes SUC1, 2 and 4 have the sequence TATAAA and the more weakly expressed genes SUC3, 5 and 7 have TACAAA. Though the SUC1 sequence is in general more homologous to the other SUC genes the region between -140 and +100 of SUC1 is nearly identical to SUC2. This could be due to a gene conversion between SUC1 and the silent suc2° allele which occurs in the strains carrying SUC1. Within the upstream regions of all the SUC genes three regions with palindromic sequences analogous to stem and loop structures were identified. Comparable structures could be detected in similar positions in the upstream sequences of the divergently transcribed yeast gene pairs MAL6S-MAL6T and GAL1-GAL10. Implications for the importance of these structures in the regulation and initiation of transcription are discussed.

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3. The Thiamine pyrophosphate Pyruvate Enzyme Family: Sequence Homologies Reveal Common Origins. Communicated by Jeremy B.A. Green.

Pyruvate decarboxylase (PDC) catalyses the first committed step of ethanol synthesis by S. cerevisiae. Protein sequences of PDC derived from cloned genes of yeast (S. cerevisiae) and Zymomonas mobilis were compared

with each other and with protein and nucleotide sequence databases. Extensive sequence similarities were found between these two enzymes and with two others: cytochrome-linked pyruvate oxidase (EC1.2.3.3) from E. coli and acetolactate synthase (product of the ilvI and ILV2 genes of E. coli and S. cerevisiae respectively). All these enzymes catalyse decarboxylation of pyruvate and use thiamine pyrophosphate (TPP) as a cofactor. None of the sequences was homologous to the E1 component of pyruvate dehydrogenase from E. coli which also decarboxylates pyruvate with the help of TPP. Alignment of the homologous sequences revealed two especially well-conserved regions. The general overall homology suggests common ancestry for these enzymes.

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4. F.K. Zimmermann: Induction of chromosomal malsegregation in yeast strain D61.M.

Induction of mitotic chromosomal malsegregation in strain D61.M is detected by the simulataneous expression of three recessive markers flanking the centromere of chromosome VII: resistance marker cyh2, color marker ade6 on the other side of the centromere and centromere marker leu1. Colonies of this phenotype are usually selected on a cycloheximide medium and found at frequencies varying between somewhat less than 1×10^{-6} to about 3×10^{-6}. This range of frequencies is unexpectedly low and and could be an artifact caused by selecting on the cycloheximide medium. We have analysed the very rare white colonies which arise on a non-selective medium in almost 300 experiments performed with cultures each started with about 200 - 300 cells so that whatever white segregants were observed in different experiments were of independent origin. The control platings on non-selective medium yielded on the average about 800 colonies.

A total of 223 215 unselected colonies were obtained of which 62 - 2.78×10^{-4} - expressed only ade6, the color marker; 3 expressed ade6 and leu1 but not cyh2 - 1.35×10^{-5} and finally a single colony expressing ade6 and leu1 and cyh2, the type expected to result from chromosomal malsegregation which is a frequency of 4.48×10^{-6}. This is almost within the range of frequencies obtained with selection for cycloheximide resistance and shows that selection for the products of mitotic chromosomal malsegregation does not very strongly bias the results. It is quite remarkable that segregants expressing ade6 on one side and leu1 but not cyh2 - both on the other side of the centromere - occurred relatively frequently. On the other hand, no segregants were obtained which expressed ade6 and cyh2 but not centromere marker leu1. This suggests that the segregants expressing ade6 and leu1 but not cyh2 are the result of events of gene conversion covering very long spans of the chromosome and not events of double crossing-over.

Usually, mitotic malsegregation is studied in strains where the markers flanking the centromeres are both very close and a simultaneous expression of such markers is taken as evidence for chromosomal malsegregation and frequencies observed are usually much higher than those observed in strain D61.M for chromosome VII. This discrepancy could be due to a relatively frequent occurrence of mitotic gene conversion which involves long stretches and could even extend beyond centromeres.

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XXI. Institut für Genetik und Mikrobiologie, Maria-Ward-Strasse 1a, 8000 München 19, FRG. Communicated by Klaus Wolf.

The following papers from our laboratory have been published in 1987:

1. Merlos-Lange, A.-M., Kanbay, F., Zimmer, M., Wolf, K. 1987. DNA-splicing of Mitochondrial Group I and II Introns in Schizosaccharomyces pombe. Molec. gen. Genet. 206, 273.

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2. Wolf, K. and Del Giudice, L. 1987. Horizontal Gene Transfer Between Mitochondrial Genomes. Endocyt. C. Res. 4,103.

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3. Zimmer, M., Welser, F., Oraler, G., Wolf, K. 1987. Distribution of Mitochondrial Introns in the Species Schizosaccharomyces pombe and the Origin of the Group II Intron in the Gene Encoding Apocytochrome b. Curr. Genet. 12:329-336.

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The following papers will appear this or at the beginning of next year:

1. Wolf, K., Del Giudice, L. The Variable Mitochondrial Genome of Ascomycetes. Organization, Mutational Alterations, and Expression. Advances in Genetics, in press.

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2. Wolf, K. Mitochondrial Genes of the Fission Yeast Schizosaccharomyces pombe. IN: Gene Structure in Eukaryotic Microbes. SGM Special Publication 22. Editor: J.R. Kinghorn. IRL Press (Oxford), 1987, in press.

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3. Wolf, K., Ahne, A., Del Giudice, L., Oraler, G., Kanbay, F., Merlos-Lange, A.M., Welser, F., Zimmer, M. Introns as Key Elements in the Evolution of Mitochondrial Genomes in Lower Eukaryotes. IN: Cytochrome Systems: Molecular Biology and Bioenergetics, Papa et al., eds. Plenum Publishing Corporation, New York - London - Washington-Bosten, in press.

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4. Munz, P., Kohli, J., Wolf, K., Leupold, U. Genetics Overview. IN: The Molecular Biology of the Fission Yeast. A. Nasim et al., eds., Academic Press, in press.

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5. Lückemann, G., Merlos-Lange, A.M., Del Giudice, L., Wolf, K. Genetic and Physical Analysis of Transmission, Segregation and Recombination of Mitochondrial Genomes in the Fission Yeast Schizosaccharomyces pombe. Life Science Advances, in press.

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XXII. Ecole Nationale Supérieure Agronomique de Montpellier, Chaire de Génétique, et de Microbiologie, 34060 Montpellier, France. Communicated by Pierre Galzy.

The following articles have been recently published by my co-workers.

1. Leclerc, M., Arnaud, A., Ratomahenina R. and Galzy, P. 1987. Yeast -glucosidases. Biotechnology and Genetic Engineering Reviews (GB), 5:269-295.

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2. Leclerc, M., Chemardin, P., Arnaud, A., Ratomahenina, R., Galzy, P., Gerbaud, C., Raynal, A. and Guerineau, M. 1987. Comparison of the properties of the purified β-glucosidase from a transformed strain of Saccharomyces cerevisiae TYK F2 with that of the donor strain Y 610. Biotechnol. Appl. Biochem. (USA), 9 (in press).

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3. Boze, Hélène, Moulin, G., and P. Galzy. 1987. A study of galactose and lactose transport in a strain of Kluyveromyces lactis van der Walt. Folia Microbiol., 32:107-111.

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4. Boze, Hélène, Moulin, G. and Galzy, P. 1987. Influence of culture conditions on the cell yield and amylases biosynthesis in continuous culture by Schwanniomyces castellii. Arch. Microbiol., 148:162-166.

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5. Boze, Hélène, Moulin, G. and Galzy, P. 1987. A comparison of growth yields obtained from Schwanniomyces castellii and an alcohol dehydrogenase mutant. Biotechnol. Letters, 9:461-466.

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XXIII. THE UPJOHN COMPANY - Molecular Biology Research. Kalamazoo, Michigan 49001, USA. Communicated by Ronald D. Klein.

The following updates our work on the yeast Schwanniomyces occidentalis. The major goal of our laboratory is to elucidate aspects of the molecular biology of this industrially important yeast. We have cloned the gene encoding orotidine 5' phosphate decarboxylase (ODC) by complementation in S. cerevisiae using the ADHI based expression vector pYcDE8. The ODC gene has been localized to a 1.1 kb fragment by standard deletion analysis. Expression of the ODC gene product is independent of the ADHI promoter and suggests that Schwann. occidentalis promoters are recognized and expressed in S. cerevisiae making the standard routine of complementation an efficient approach for cloning genes from this

organism. Details of this work have been accepted for publication.

We have also completed work on the development of a fast protein liquid chromatography (FPLC) system for the purification of the secreted amylolytic enzymes from Schwann. occidentalis namely α-amylase and glucoamylase in a rapid two step process: size exclusion (Superose 12) and anion exchange (Mono Q). Detailed biochemical characterizations of the purified proteins are in disagreement with previously published results of other laboratories. We have demonstrated that glucoamylase and α-amylase isolated from supernatants of cells grown on a variety of substrates contain only 12% N-linked carbohydrate. In addition, our results confirm earlier reports of Wilson and Ingledew regarding the properties of glucoamylase and α amylase in that the observed activities can be attributed to a single protein species, and that the native molecular weights (for Schwann. alluvius) they report are identical to those observed by us.

Recent publications of our lab include:

R.D. Klein and Lori L. Roof, Cloning of the Orotidine 5'-phosphate decarboxylase (ODC) gene of Schwanniomyces occidentalis by complementation of the ura 3 mutation in S. cerevisiae. Current Genetics. Accepted for publication.

M.R. Deibel, R.R. Hiebsch and R.D. Klein, Secreted Amylolytic Enzymes from Schwanniomyces occidentalis: Purification by Fast Protein Liquid Chromatography FPLC). Preparative Biochemistry. Accepted for publication.

R.D. Klein, P.G. Zaworski and R.R. Hiebsch, Transformation and cloning systems in Non-Saccharomyces yeasts, to appear in Yeast Strain Selection (C.P. Panchel, ed.) in the series Bioprocess Technology, W.C. McGregor (ed.), Marcel Dekker Inc., New York.

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XXIV. National Autonomous University of Mexico, Research Center of Genetic Engineering and Biotechnology and Department of Food Science, Faculty of Chemistry. A.P. 70-479 Mexico D.F. 04510. Mexico. Communicated by Mariano Garcia-Garibay.

The following are works done in our Institution during 1987.

1. Garcia-Garibay M., Arriaga E., Fernandez M., Casad L.T., Galindo E., Gonzalez A. and de la Vega H. Taxonomical and Functional Characterization of Yeast Strains for Rum Production.

This work was done for Bacardi y Cia (Mexico) for the taxonomical identification of their yeast strains, the functional characterization (i.e. evaluation of their capabilities for the elaboration of rum), and the evaluation of several techniques of preservation in order to find the best one for long-term maintenance. It was found that all the strains belong to the species Saccharomyces cerevisiae, according to the criteria of The Yeast 3rd Ed. (Kreger-van Rij, 1984) and DNA hybridization. All the yeast showed very good performance for the production of rum; in any case they were better than the type strains used for comparison such as ATCC 26603.

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2. Garcia-Garibay M., Gomez-Ruiz L. and Barzana E. Studies on the Simultaneous Production of Single-Cell Protein and Polygalactouronase from Kluyveromyces fragilis. Biotechnology Letters 9(6), 411-416 (1987).

Kluyveromyces fragilis produces polygalactouronase on a lactose medium. Although the enzyme is normally repressed at high aeration levels, significant amounts of PG can be produced under such conditions when pectin is added as inducer. The productivity and yield of cell mass were not significantly affected by the presence of inducer, suggesting potential applications to current single cell protein processes from whey.

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3. Garcia-Garibay M., Gomez-Ruiz L. and Barzana E. Simultaneous Production of Single Cell Protein and Pectinase from Whey. Proceedings of the 1987 Food Processing Waste Conference. Georgia Tech Research Institute. Atlanta GA. Sept. 1-2, 1987.

A simple modification to current processes for the production of single cell protein from whey allows the simultaneous production of endo-polygalacturonase as a secondary product improving the attractiveness of those processes.

Endo-polygalacturonase is completely repressed under aerobic conditions necessary to produce high amounts of biomass. In this work it is demonstrated that pectin acts as an inducer of the enzyme under those conditions. Using pasteurized whey, it was possible to produce up to 13.68 g of yeast and 800 units of enzyme per liter. Cell yield and final lactose load were unaffected by the presence of pectin.

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4. Gomez-Ruiz L., Garcia-Garibay M. and Barzana E. Utilization of Endo-Polygalacturonase from Kluyveromyces fragilis for the Clarification of Apple Juice. Submitted for publication.

5. Garcia-Garibay M., Torres J., Lopez-Munguia-Canales A. and Casas L.T. Influence of Oxygen Transfer Rate on Beta-Galactosidase Production from Kluyveromyces marxianus. Biotechnology Letters 9(6), 417-420 (1987).

Beta-galactosidase specific production from K. marxianus was shown to be related to kLa at low rates. A power type curve fitted very well when specific activity was plotted vs kLa. Significant difference in total activity yields among the kLa treatments were not found, as every increase in specific activity was compensated by a decrease in biomass concentration.

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XXV. Molecular Biology Group, Central Laboratories of Key Technology, Kirin Brewery Co., Ltd. 3 Miyahara-cho, Takasaki, Gunma 370-12, Japan. Communicated by Jun-ichi Tanaka.

The following papers concerning expression of ALDC gene in brewer's yeast have been recently published or completed for publication:

1. Hidetaka Sone, Toshio Fujii, Keiji Kondo and Jun-ichi Tanaka. 1987. Molecular Cloning of the Gene Encoding α-acetolactate Decarboxylase from Enterobacter aerogenes. Journal of Biotechnology, 5:87-91.

Abstract

Enterobacter aerogenes genomic library has been constructed using cosmid pJB8 in Escherichia coli. The gene encoding α-acetolactate decarboxylase (ALDC) has been isolated from this library by direct measurement of enzyme activity. The expression of the ALDC gene in E. coli appears to originate from its own promoter. Subsequent subcloning revealed that the ALDC gene locates within 1.7 kb BamHI-PstI fragment.

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2. H. Sone, K. Kondo, T. Fujii, F. Shimuzu, J. Tanaka and T. Inoue. 1987. Fermentation properties of brewer's yeast having α-acetolactate decarboxylase gene. Proceedings of the European Brewery Convention Congress. Madrid, 1987. 545-552.

Abstract

Cloning of α-acetolactate decarboxylase (ALDC) gene of Enterobacter aerogenes and its expression in brewers' yeast have been carried out to repress the diacetyl formation.

The fermentation test with the brewers' yeast having ALDC activity revealed the following results. (1) Total diacetyl content of young beer with the yeast was considerably lower than that with the control yeast. (2) No significant difference in fermentation properties (attenuation degree, amino acids composition and volatile compounds) was observed between the yeast and the control yeast. (3) The YEp type plasmid containing ALDC gene was stably retained in the brewers' yeast.

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3. Hidetaka Sone,* Toshio Fujii, Keiji Kondo, Fumio Shimizu, Jun-ichi Tanaka, and Takashi Inoue. 1988. Nucleotide Sequence and Expression of the Enterobacter aerogenes α-Acetolactate Decarboxylase Gene in Brewer's Yeast. Applied and Environmental Microbiology, Jan. Issue (in press).

Abstract

The nucleotide sequence of a 1.4-kilobase DNA fragment containing the α-acetolactate decarboxylase gene of Enterobacter aerogenes was determined. The sequence contains an entire protein-coding region of 780 nucleotides which encodes α-acetolactate decarboxylase having 260 amino acids. The DNA sequence coding for α-acetolactate decarboxylase was placed under the control of the alcohol dehydrogenase I promoter of the yeast Saccharomyces cerevisiae in a plasmid capable of autonomous replication in both S. cerevisiae and Escherichia coli. Brewer's yeast cells transformed by this plasmid showed α-acetolactate decarboxylase activity and were used in laboratory-scale fermentation experiments. These experiments revealed that the diacetyl concentration in wort fermented by the plasmid-containing yeast strain was significantly lower than that in wort fermented by the

parental strain. These results indicated that the α-acetolactate decarboxylase activity produced by brewer's yeast cells degraded α-acetolactate and that this degradation caused a decrease in diacetyl production.

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

The following papers are in press or have been published since the last yeast newsletter.

1. Ingledew, W.M., C.A. Magnus and F.W. Sosulski. 1987. Influence of Oxygen on Proline Utilization During the Wine Fermentation. Amer. J. Enol. Vitic. 38(3):246-248.

ABSTRACT

In order to overcome sluggish or stuck fermentations, it may be necessary to utilize oxygen and yeast-utilizable nitrogen sources found in some yeast foods. Government regulations in the US preclude the use of large amounts of yeast foods - even those derived from yeast or proteins often employed as food ingredients. The present work describes the conditions under which proline, an amino acid naturally present in high concentrations in musts, can be utilized fully as a source of nitrogen. Oxygen is required for proline degradation; it is also a requirement to ensure sufficient yeast growth and to avoid sluggish fermentations.

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2. Thomas, K.C., G.G. Khachatourians, and W.M. Ingledew. 1987. Production and Properties of Beauveria bassiana conidia cultivated in submerged culture. Can. J. Microbiol. 33:12-20.

Abstract

Under submerged growth in a defined medium (TKI broth), the entomopathogenic fungus, Beauveria bassiana, produced conidia; it produced only blastospores in complex media. Production of such "submerged" conidia depended on the nature of the carbon source and the presence of nitrate as a nitrogen source. Maximum yield of conidia (5×10^8 mL) was obtained when glucose was the carbon source and when the glucose to nitrate ratio was 5:1. Other carbon sources gave rise to both conidia and blastospores. Reducing the phosphate concentration resulted in the production of conidia which resembled "aerial" conidia in morphology and germinatin rates. The surfaces of "submerged" conidia were relatively smooth, but had a tendency to acquire the rough, warty, brittle surface characteristics of aerial conidia. Blastospores produced in defined media gave rise to conidia through microcycle conidiation without going through the vegetative phase of growth. In more complex media, blastospores did not undergo microcycle conidiation.

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3. Ingledew, W.M., C.A. Magnus, and J.R. Patterson. Yeast Foods and Ethyl Carbamate Formation in Wine. Amer. J. Enol. Vitic. 38(4): (in press).

ABSTRACT

Fermentation experiments have inidcated that ethyl carbamate was not formed during fermentation, even in the presence of urea, ammonium phosphate, or amino-acid-containing yeast foods at 12 times allowable levels. Heating of end fermentation broths, however, led to ethyl carbamate formation, but only from fermentation supernatants where urea was used.

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4. Connie E. Smith, Gregory P. Casey, and W.M. Ingledew. The Use and Understanding of Media Used in Brewing Microbiology. Update 1987. Brewers Digest 62: (in press).

An extensive review of media used by the brewing industry was published in 1981 and 1982 by Casey and Ingledew. Since that time a significant number of new papers have been published, and this report will summarize new developments in media used in brewing microbiology.

* * *

XXVII. Research Laboratories, ALKO LTD, POB 350, SF-00101 Helsinki, Finland. Communicated by Matti Korhola.

Below follows a list of our work published since June 1987.

1. M. Korhola. 1987. Yeast in the Alcohol Industry. IN: European Brewery Convention: Proceedings of the 21st Congress, Madrid 1987. Oxford: IRL Press Limited, p. 105-121.

For the purpose of this presentation the alcohol industry is defined as comprising fermented, distilled alcoholic products, i.e., spirits beverages and fermented industrial alcohol.

The lecture covers:

1. Characteristics of distillers' yeast, i.e., mainly Saccharomyces cerevisiae, but to some extent also Schizosaccharomyces pombe and Kluyveromyces fragilis (lactis), and from the point of view of the whisky industry Saccharomyces uvarum, and S. carlsbergensis as well.

2. Technological aspects of yeast propagation, substrate fermentation, and effluent treatment.

3. Problems with process contaminants and yeast genetic instability.

4. Future developments in currently non-utilizable raw materials (e.g. pentoses) and in anticontaminant yeasts.

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2. S. Knuth. 1986. Patent Protection of Yeasts. IN: European Brewery Convention, Monograph-XII. Nurnberg: Verlag Hans Carl (Brauwelt-Verlag), 1987, p. 209-220. (E.B.C. - Symposium on Brewers' Yeast, Vuoranta (Helsinki), Finland.

Patent protection of yeasts can be obtained for example for new yeast strains, for methods where these strains are used, or for novel uses of known yeast strains. Where genetically engineered yeasts are concerned the patent protection may include that for the new genetic material (new genes, new vectors etc.) transferred into a yeast strain. Protection of a new product (compared to the production method) is most desirable, because it is the strongest form of patent protection. By product protection the product per se is protected irrespective of the method by which it is produced or purpose of its use. Product protection is the most difficult to get and this is especially true for micro-organisms. One of the main problems in patenting invention concerning micro-organisms is repeatability of the invention. If the micro-organism in question is not available to the public and cannot be described literally in such a manner that the invention can be used by a person skilled in the art, the micro-organism must be deposited. In different countries the deposit rules are still diverging and the patent applicant easily gets lost in the jungle of rules.

The protection of an invention must be carefully constructed. Before applying for a patent one should seek answers, in particular, to the following questions: What is the invention? What is the purpose of the patent? Is it possible to police the patent rights?

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3. J.K.C. Knowles and R.S. Tubb. 1987. Recombinant DNA: Gene Transfer and Expression Techniques With Industrial Yeast Strains. IN: European Brewery Convention, Monograph-XII. Nurnberg: Verlag Hans Carl (Brauwelt-Verlag), 1987, p. 169-185. (E.B.C. - Symposium on Brewers' Yeast, Vuoranta (Helsinki), Finland 1986.

A number of recent technological developments have facilitated the application of recombinant DNA technology to industrial yeast strains. Developments include: "dominant" selectable markers for yeast transformation; a range of different vectors for stabilizing introduced genetic material; expression systems which allow the production of the gene product at an appropriate moment in the industrial process; and methods which ensure that the gene product is localized in the correct cellular compartment.

* * *

4. Matti Korhola. 1987. Genetic Improvement of Baker's Yeast Strains. Kemia-Kemi 14 3:197-202.

The baker's yeast manufacturer and the baker have many important practical requirements for the proper performance of baker's yeast strains in their processes. These requirements include the ability to utilize fully the supplied raw materials for cell material build-up, yield in industrial production, good storage stability, freezing and drying stability, good leavening ability, tolerance to osmotic pressure, ethanol and low pH, production of appropriate flavour compounds and genetic stability.

The most difficult problem the geneticist faces in industrial strain improvement besides technical difficulties is the poor knowledge about the biochemical and genetic basis of all the above requirements. Very few of them are explicitly defined in a way that permits the direct application of recombinant DNA techniques.

However, we have constructed by genetic engineering new and stable strains of baker's yeast that contain the MEL1 gene and can utilize melibiose. MEL1 baker's yeast gives a slightly increased yield of cell mass from beet molasses and consequently reduces the effluent load of the yeast plant. The public acceptance of MEL1 baker's yeast strains should be guaranteed, since the gene was cloned from a brewer's yeast into baker's yeast; both considered useful organisms for centuries.

The article also discusses briefly possibilities for using baker's yeast in producing useful by-products, mainly foreign proteins.

* * *

XXVIII. University of Guelph, Department of Environmental Biology, Guelph, Ontario, Canada N1G 2W1. Communicated by J.D. Cunningham.

The following reports summarize current departmental studies on yeasts as related to the fermentation industries.

1. Studies on Xylose-Metabolizing Yeasts for Ethanol, P. Bicho, J.D. Cunningham.

The utilization of pentose sugars by selected yeasts in the bioconversion of plant biomass (cellulosics, hemicellulosics) to ethanol has been investigated. D-glucose and D-xylose substrate utilizations were determined by selected Pichia stipitis and Pachysolen tannophilus strains on the basis of predilection of sugars, alcohol tolerance level, and inhibition or induction of xylose reductase and xylitol dehydrogenase enzyme activities.

D-glucose or D-Mannose at 4% levels inhibited both enzymatic activities of these yeasts with approximately 60 to 100% suppression. Cellobiose, L-Arabinose and D-Galactose did not appear to inhibit induction of either of these enzymes in these yeast species.

Further investigations in development of acclimatized yeast strains capable of fermenting mixed sugars as they exist in lignocellulosic materials have been planned in order to achieve more efficient bioconversions and increase ethanol recovery as an alcohol fuel from cellulosics.

* * *

2. Effect of Fungicide Residues on the Yeast Fermentation of Artificial and Natural Grape Must Substrates

Three wine yeasts have been obtained for preliminary studies in order to determine the possible effects of fungicidal residues on selected fermentations. Fungicides such as Captan, Bayleton, Benomyl, Bravo, Ferbam and Rovral and possible combinations thereof have been considered for the

preliminary screenings and determination of inhibitory/suppressive effects on strains of Saccharomyces cerevisiae. Fungicide concentrations representative of levels from 0 to 100 mg active ingredient/litre are being investigated in order to: (a) simulate possible field conditions and carry-over of a residual fungicide in the grape must and early yeast fermentation; (b) determine the effect, if any, on the wine yeasts and rate or efficiency of yeast fermentation; and (c) determine fungicide longevity, disappearance, degradation, or uptake by yeast metabolism during the fermentation period.

Unexplained "stuck" fermentations or delayed fermentations experienced in industry are also deemed warranted in this investigation with the hopes of developing close liaison with industry during the vintage season.

* * *

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

The following is the summary of a paper recently published.

1. E. Minarik: Stimulation of alcoholic fermentation of grape must (in Slovak). Vinohrad 25, 1987 (10).

The ability of yeast ghosts (yeast cell wall preparations) to adsorb toxic substances produced by yeasts (short chain fatty acids and their esters) in fermenting grape must may be compared with the "survival factor" in the sense of Lafon-Lafourcade et al. They show a positive effect on the survival of living yeast cells of a given yeast culture. Microcrystalline cellulose showed a similar influence as yeast ghosts when added to grape must prior to fermentation. Yeast ghosts have a favorable effect also if added to incompletely fermented young wine with residual sugar in order to complete the fermentation. Practical aspects for wine making are discussed.

* * *

This is a summary of a paper recently accepted for publication in 1987 in Przemysl Fermentacyjny i Owoczowo-Warzywny (Warsaw).

2. E. Minárik and O. Jungová: Recent experience with the application of yeast ghosts in grape must fermentation under unfavorable conditions (in Polish).

Yeast ghosts are able to intensify and accelerate alcoholic fermentation of grape must under unfavorable fermentation conditions, e.g. high sugar concentration of the must, residues of inhibitors (pesticides) etc. Higher alcohol levels and less unfermented residual sugar in the wine may be achieved. This is due to the adsorption of short chain fatty acids and their esters by yeast ghosts enabling "cleaning" the surface of toxic fatty acid fractions and esters of cell walls of living yeasts. The function of yeast ghosts may thus be described as "survival factor" prolonging the viability of yeasts.

* * *

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

New Fusion Products for Demalification During Wine Making.

Serafini, L.A., Dillon, A.J.P., Carrau, J.L., Pasqual, M.S.

Abstract

Two new series of genetically stable fusion products have been obtained in a second generation of fusions among enological yeasts. The yeasts were Saccharomyces cerevisiae (Montrachet) with MB 6TC; and Saccharomyces cerevisiae (KI) with MB 6TC (Genetically stable fusion product obtained between Benda I Schizosaccharomyces pombe and Montrachet, Saccharomyces cerevisiae, Carrau and col. 1981, Carrau 1985). From the first fusion resulted the MB II series and from the second the MEK series.

Recovery and establishment of the fusion products was done in selective medium using only natural markers.

Many of the fusion products, being tested have shown important enological characteristcs, such as: good L-malic acid degradatin capacity, good fermentation capacity, and absence of Schizosacharomyces taste in wines produced. Strains are tested at a laboratory and semi-industrial scale.

Until this moment, enologically usefull fusion products have not expressed the killer character.

Supported by FAPERGS and CNPq. - PIG V.

* * *

XXXI. BASS PLC, RESEARCH LABORATORY, 137 HIGH STREET, BURTON-ON-TRENT, STAFFORDSHIRE DE14 1JZ, ENGLAND, COMMUNICATED BY DAVID QUAIN.

The following are abstracts of recent publications from our laboratory.

1. D.E. Quain and M.L. Duffield, 1985. A Metabolic Function for Higher Alcohol Production by Yeast. Proc. European Brewery Convention, Helsinki, 307-314.

Abstract

Unbalanced production of higher alcohols during fermentation perturbs beer flavour. Although the pathways for higher alcohol production in yeast are identified, there is scant knowledge of the metabolic significance of such processes. It is proposed that these synthetic systems exert a fine control over the cellular redox balance and possibly aid control of pH, by removing keto acid precursor substances. This proposal is discussed in relation to observations of the effect of oxygen on the quantities of individual higher alcohols in beer and attention is drawn to the inverse relationship between levels of higher alcohols and of glycerol.

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2. D.E. Quain, W.G. Box and E.F. Walton, 1985. An Inexpensive and Simple Small-Scale Laboratory Fermenter. Lab. Practice February, 84-85.

Abstract

A stirred small-scale fermenter is described which is both inexpensive and simple to assemble. Anaerobic or aerobic conditions are easily established and maintained. The fermentation system is particularly applicable to investigations not conveniently performed in large-scale fermenters such as extensive feasibility studies or routine screening of existing or new strains of micro-organisms.

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3. D.E. Quain, 1986. Differentiation of Brewing Yeast. J. Inst. Brew. 92, 435-438.

Abstract

Current methods for the differentiation of brewing yeasts are described and their performance critically discussed. Although these methods are widely used they are non-specific and require confirmation by other tests. Emergent approaches such as pyrolysis gas chromatrography or the use of gene cloning techniques are considered and their application reviewed. It is concluded that development of such methods could offer a unique "fingerprint" for each brewing yeast based on a single test.

* * *

4. D.E. Quain and C.A. Boulton, 1987. Growth and Metabolism of Mannitol by Strains of Saccharomyces cerevisiae J. Gen. Microbiol. 133, 1675-1684.

Of 40 polyploid strains of Saccharomyces cerevisiae screened for growth on D-mannitol (5%w/v) half grew well (5-20 mg dry biomass ml^{-1}). Certain of these strains were unable to grow on low concentrations of mannitol (1-2% w/v) and others, initially unable to grow on mannitol, exhibited long-term adaptation to growth. A NAD^+dependent D-mannitol dehydrogenase (EC1.1.1.67) was detected in mannitol-grown yeast. Growth was dependent on mitochondrial function and was obligately aerobic. Measurement of products of metabolism and respiratory activity indicated that growth on mannitol allows catabolite derepression.

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5. D.E. Quain and C.A. Boulton, 1987. The Propagation of Yeast on Oxidative Carbon Sources - Advantages and Implications. Proc. European Brewery Convention, Madrid, in press.

Abstract

Newly propagated yeast often performs poorly during initial fermentation, a consequence of pitching insufficient yeast of poor physiological status. Propagation on a non-fermentative carbon source such as mannitol results in yeast of superior quality to that produced by existing systems. Although growth on such substrates is dependent on

oxygen, yeast at the end of the process is rich in sterols. As these lipids determine yeast growth during fermentation, the pitching rate into aerated wort may be reduced. Further, such yeast can be stored for many months without any loss in viability. Aspects of this work are subject to patent applications.

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6. C.A. Boulton and D.E. Quain, 1987. Yeast, Oxygen and the Control of Brewery Fermentations. Proc. European Brewery Convention, Madrid, in press.

Abstract

Greater consistency and efficiency of batch brewery fermentation can be achieved by control regimes based on measurement of a predictive parameter at the very earliest stages of fermentation. This measurement is used to adjust conditions such that the fermentation proceeds to a predetermined optimum course. Rates of oxygen consumption by defined mixtures of yeast and wort, measured in-line during vessel filling, can be used to select appropriate pitching rates and levels of wort oxygen. The husbandry of the pitching yeasts and its physiological status is crucial to the success of this control loop.

* * *

XXXII. A.Ü. Ziraat Fakültesi, 06110 Diskapi, Ankara, Turkey. Communicated by M. Hilmi Pamir.

A Study on Increasing the Biological Value of Waste Breads Through Bioconversion.

The evaluation of waste breads in Turkey, whose people consume a great deal of bread and other flour products, is already being investigated. The goal of this study is to produce biomass protein and biochemical (ethanol) from the waste breads. As an amylolytic yeast, Saccharomycopsis fibuligera CBS 2523, as a protein yeast, Candida tropicalis ME 50, and a alcohol yeast, Saccharomyces cerevisiae RXII were chosen for this purpose. These yeasts were tested individually or in certain associations under the following assay conditions. The sustrate containing 10% finely ground bread medium was inoculated with these yeasts at the rate of 1% either alone or in association and incubated at 30°C. In this assay both the continuous (150 rpm) and limited shaking methods (2 times a day) were used. The mixing rate (1:1) of associated yeasts were adjusted at 640 nm. None of the yeasts tested alone produced more biomass protein than the associated yeasts.

As to the mixed cultures of S. fibuligera + C. tropicalis and also S. fibuligera + Sacch. cerevisiae, the amount of biomass protein increased 39% and 47%, respectively. Meanwhile, the ethanol production increased 89% in the case of the associations of S. fibuligera with Sacch. cerevisiae when the limited shaking method was used, whereas the continuous shaking method appeared to be less effective (84%) for the production of ethanol as expected.

According to the evaluation of these results it has been accepted that the biological value of waste breads will be improved to a great extent for animal nutrition through bioconversion using the associated yeasts without

any pretreatment. Also this method can be used directly for ethanol production from starch without any enzymatic or chemical pretreatment for hydrolysis in the near future (to be published).

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XXXIII. Kuwait Institute for Scientific Research, P.O. Box 24885, Safat 13109, Kuwait. A.S. ElNawawy, E. ElRayes and R.Al-Daher. Enzymatic Conversion of Cellulose in Cardboard for Yeast Production*. *Paper given at the "International Conference on Wood and Cellulosics, Industrial Utilization, Biotechnology, Structure and Properties (Cellucon '86)" Held at Wrexham, North Wales, U.K., 14-18 July, 1986. Communicated by A.S. ElNawawy.

Trichoderma reesei (QM 9414) was propagated in a Fermenter (7 l capacity) on Mendles and Webers medium at 28°C containing 1% cardboard (CB) as sole carbon source. After 8 days, the cellulases in the medium were precipitated with ammonium sulfate (80% saturation), centrifuged in a refrigerated centrifuge at 12,000 g/20 minutes. The precipitates were dissolved in 0.01 M sodium citrate buffer (317.5 ml), desalted by dialyzing in collodium sacks against the same buffer. The resulting cellulase activity was 1150 U/ml, giving total activity of 365125U/317.5 ml. This amount was used to convert 105 g cellulose present in 160 g cardboard within 48 hours at 50°C. The enzymes were able to produce 72 g reducing sugar, i.e., 68.6% of the cellulose in CB. The sugar solution was adjusted by evaporation to give 2 % reducing sugar, supplemented with ammonium sulfate 0.6% and 0.2 % K_2HPO_4 + 0.1% yeast extract.

A strain of Candida utilis was propogated for 48 hours at 30°C. The yeast yield was 56% of the reducing sugar and having 44% protein. In other words, the material balance of 160 g cardboard is 36 g yeast = 15.9 g protein. When dried yeast is mixed with CB residue (88 g), the protein present of the mixture will be 12.8%.

* * *

XXXIV. Yeast Research in Nigeria. Submitted by R.N. Okagbue, Department of Microbiology, University of Nigeria, Nsukka, Nigeria.

In NIgeria, yeasts have been studied indirectly for nearly three decades, as part of the general microbiology of food and beverage fermentations. More specific studies emphasizing yeast ecology and activity in food production and spoilage, and in human and animal health and disease, are relatively rare and a few research articles and other papers (published, submitted or accepted for publication) written by Nigerian workers are presented below:

1. Okafor, N. 1972, Palm wine yeasts from parts of Nigeria. Journal of the Science of Food and Agric 23:1399-1407.

2. Njoku-Obi, A.N.U., Okafor, J.I. and Gugnani, H.C. 1976. Yeast-like fungi recovered from normal human skin in Nsukka (Nigeria) Antonic van Leeuwenhoek 42:101-105.

3. Ibe, L.S.N. and Ike, C.U. 1982. The effect of Candida tropicalis and Pichia membranaefaciens on the germination of seeds of Capsicum annuum. Nigerian J. of Microbial 2:190-195.

4. Nwachukwu, S.U. and Akpata, T.V.I. 1987. Utilisation of carbohydrate and protein by Candida famata during spoilage of snail meat (Archachatina marginata Swainson) J. Food and Agric. 1:27-30.

5. Okagbue, R.N. A note on the leavening activity of yeasts isolated from Nigerian palm wine. J. Appl. Bacteriol. (In Press).

6. Okagbue, R.N. Locust bean pulp agar: a medium for isolation and enumeration of yeasts. Nigerian J. of Microbiol. (Accepted).

7. Okagbue, R.N. 1986. Active dried bakers yeast: perspectives in its handling and use in Nigeria. Nig. Journal of Home Economics 2:42-46.

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XXXV. TEACHING COURSE IN TAXONOMY

Intensive International Course on Molecular Methods in Yeast Taxonomy.

1. Teaching Staff:

C.P. Kurtzman
N.R.R.L.
Microbial Culture Collection
U.S. Department of Agriculture
Peoria, Illinois U.S.A.

Paul Blanz
University of Bayreuth
Bayreuth, F.R.G.

M.A. Lachance
University of Western Ontario
London, Ontario, Canada

T. Nakase
Japan Collection of
of Microorganisms,
Riken,
Wako-shi, Saitana,
JAPAN

Date: 8th - 26th August, 1988

Location: The Gulbenkian Institute of Science which is located in Oeiras, Portugal, about 20 Km west of Lisbon at walking distance from the Atlantic Ocean.

Participants: The number will be limited to twenty. The course is primarily of interest to research workers in yeast taxonomy and to staff members of microbial culture collections who wish to learn and make use of molecular methods in advanced taxonomy.

Financial aspects: The course is financed by the Calouste Gulbenkian Foundation. No fees are charged to the participants. Lunch may be taken at the Gulbenkian Institute of Science (Monday through Friday) at a subsidized price.

Housing: Successfull applicants should make their own hotel reservations through a travel agency or otherwise. Many hotels are located at a convenient distance from the institute (Estoril,

Cascais, Carcavelos or Lisbon). A number of single rooms with shower in a student hostel near the institute is available free of charge. Applicants interested in such a room should indicate this on their application-for-admission form.

Synopsis: During the three weeks'full-time course lecturing and laboratory work will focus on the following topics:

- Methods for determining mol% guanine plus cytosine in nuclear DNA
- Interstrain DNA hybridization by spectrophotometry
- Interstrain DNA hybridization with labelled DNA
- 5 S ribosomal RNA sequencing
- Large subunit ribosomal RNA partial sequencing
- Restriction mapping of highly repeated nuclear DNA
- Co-enzyme Q determination.

Deadline for application: 1st May, 1988
Information and application-for-admission forms:

Prof. N. van Uden
Department of Teaching
Gulbenkian Institute of Science
Apartado 14
2781 OEIRAS Codex
Portugal

* * *

XXXVI. MEETINGS

International Commission of Yeasts (ICY). The following minutes were prepared by Professor Bastide (Secretary of ICY) and Professor P. Galzy (Chairman of ICY).

As planned, the Symposium was held in Weimar (GDR), on 12-18 september 1987: 150 participants representing 18 countries attended the symposium; 92 communications (lectures and posters) were presented.

We would like to thank Professor Weber and his colleagues for their remarkable organization.

The importance of the papers presented shows that the topic chosen and proposed by Professor Weber and accepted by the Commission was judicious.

The main topics touched upon at the symposium were:

- genetic ingineering techniques for all yeast species other than saccharomyces.
- sexual cycle and parasexuality
- gene expression regulation
- the genome structure and extrachromosomal elements
- applications in biotechnology

The main species studied at the symposium were: Yarrowia lipolytica, Kluyveromyces lactis, Hansenula polymorpha, Candida maltosa, Pichia pastoris.

It seems that genetic engineering techniques can now be applied to numerous species.

This meeting was held at on important an important moment. The study of these new species corresponds again to a "pioneering step".

It appears that there are important differences in the metabolism and the behavior between some of these yeasts and Saccharomyces. It is possible to imagine that new genetic systems or new mechanisms of sexuality will appear in the course of the development of new studies.

The I.C.Y. meeting was held on 17 september 1987 at the Dornburg castle; the following members were present at this meeting:

Prof. Bendova O., Russel I., Dr. Stateva, Prof. Shalowski, Prof. Weber and Prof. Galzy P. It should be recalled Czechoslovakia is officially represented by Dr. Kockova-Kratochvilova, but that Dr. Bendova has been substituting for her in these meetings for several years, with the agreement of the Commission and the Czechoslovakian Academy of Sciences.

Upon the request of the GDR, Dr. W. Nordheim, who has changed his field of research, will be replaced by Dr. Birnbaum.

The I.C.Y. commission and the organizers of the Symposium sincerely thank Dr. Stewart, treasurer of the IUMS for the financial help accorded to the symposium.

Dr. Sheperd proposed to organize a specialized symposium in 1991 in New Zealand on the following subject: "Cell surface of Saccharomyces cerevisiae and Candida albicans". The commission agreed with this project but suggested to extend the title: "Cell surface of yeasts: Saccharomyces cerevisiae, Candida albicans, etc. ...".

Provided that this is confirmed at the I.C.Y. meeting in PERUGIA (Italy), the next symposia will be held in:

- 1989: LOUVAIN - BELGIUM on "Fermented beverages".
- 1990: CZECHOSLOVAKIA on "Evolution and Taxonomy of yeast".
- 1991: NEW-ZEALAND on "Cell surface of yeast".

An informal discussion then followed. It was recalled that many members of the Commission wished that a general symposium organized by Dr. Panek be held in Brazil.

Professor Shalowski expressed his desire to study the possibility of organizing a specialized symposium in USSR on "Regulation of metabolism".

* * *

The 20th Annual Meeting of the Yeast Genetics and Molecular Biology Society of Japan was held from August 20th through 22nd, 1987 at the Hiroshima Garden Palace in Hiroshima. Following ninety-three topics were presented and discussed in fourteen sessions: Session

I, Secretion (topics 1-7); II, Repair (8-11); III, Life cycle (12-21); IV, OFAGE (22-25); V, Application (26-29); VI, RAS.cAMP (30-37); VII, Cell cycle (38-41); VIII, Biochemistry (42-52); IX, Cell Structure, (53-58); X, Plasmid (59-61); XI, Evolution and Database (62, 63); XII, Chromosome (64-69); XIII, Organella (70-77); XIV, Gene Regulation (78-93). The abstracts of these presentations will be published in Japanese as "Yeast Genetics and Molecular Biology News, Japan" at the end of 1987. Communicated by Osami Niwa, Department of Biophysics, Kyoto University, Kyoto 606, Japan.

1. A. Sakai, Y. Shimizu and F. Hishinuma (Mitsubishi-Kasei Institute of Life Sciences). Characterization of oversecretion mutant rgr1 in Saccharomyces cerevisiae.

2. M. Tokunaga, A. Kawamura, N. Wada and F. Hishinuma (Lab. of Mol. Genet. Mitsubishi-Kasei Insti. of Life Sciences). Expression and secretion of the killer toxin encoded on yeast linear plasmids. 4. The expression system of 28K killer subunit.

3. Kimihisa Ichikawa, Katsunori Suzuki*, Tadaatsu Nakahara and Yoshifumi Jigami* (Natl. Chem. Lab. Ind.*, Inst. Appl. Biochem., Univ. Tsukuba). Isolation and characterization of Human Lysozme super-secreting mutants in Saccharomyces cerevisiae.

4. T. Nagasu, H. Kato (EISAI Co., Ltd.). Selectable cloning of the promoter and secretion signal of S. cerevisiae.

5. T. Mizunaga, Y. Katakura, H. Sato, S. Nagayoshi, H. Tachikawa, Y. Maruyama (Dept. Agric. Chem., Univ. of Tokyo). Effects of Amino Acids Substitutions at the Signal Peptide Cleavage Site on the Secretion of the Repressible Acid Phosphatase of Saccharomyces cerevisiae.

6. A. Nakano (Dept. Chem., Natl. Inst. Health Japan). Interaction between secretory gene SEC12 and its suppressor gene SAR1 in S. cerevisiae.

7. S. Miyake and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Cloning of the yhol gene in fission yeast: a homolog to S. cerevisiae YP11 and SEC4.

8. N. Usami, *K. Kobayashi* and S. Ishizaka (Inst. Biol. Sci., Univ. Tsukuba, *Photon Factory, Natl. Lab. High Energy Physics). Radiation sensitivity of Saccharomyces cerevisiae labeled with BrdUMP.

9. K. Kobayashi[1)], N. Usami[2)], K. Hieda[3)], H. Maezawa[4)], Y. Furusawa[4)] and T. Ito[5)] ([1)] Photon Factory, Natl. Lab. High Energy Physics, [2)] Inst. Biol. Sci., Univ. Tsukuba, [3)] Dept. Biophys., Rikkyo Univ., [4)] Sch. Med., Tokai Univ., [5)] Coll. Arts & Sci., Univ. Tokyo). Induction of mitotic gene conversion in Saccharomyces cerevisiae irradiated with monochromatic soft X-rays at the resonance absorption of phosphorus.

10. D. Keszenman-Pereyra and K. Hieda (Dept. Phys., Rikkyo Univ.). Repair of UV-irradiated transforming single- and double-stranded DNA in Saccharomyces cerevisiae.

11. A. Yasui (Res. Inst. Tuberculosis and Cancer, Yohoku Univ.). Structure and function of photoreactivation repair gene of Saccharomyces cerevisiae.

12. T. Tachikawa, T. Miyakawa, E. Tsuchiya and S. Fukui (Dept. Ferment. Tech., Hiroshima Univ.). Mating pheromone-induced rapid and transient raise of cellular Ca^{2+} in yeasts.

13. Y. Azuma, Y. Oka, T. Miyakawa, E. Tsuchiya and S. Fukui (Dept. Ferment. Tech., Hiroshima Univ.). Auto-regulated Ca^{2+}/calmodulin-dependent protein kinase: A possible signal transducer of mating pheromone in yeasts.

14. Y. Ohsumi (Dept. of Biol., Faclt. of Sci., Univ. of Tokyo). Morphological Change Induced by α factor.

15. H. Fujimura (Develop. Repro. Biol. Cent.). DAC1, a gene essential for mating pheromone response in Saccharomyces cerevisiae.

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17. M. Tsuboi, H. Kobayashi and T. Takahashi (Dept. Biotechnol., Fukuyama Univ.). On the sporulation ability of industrial yeasts.

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21. Y. Iino, K. Furuhata, Y. Watanabe and M. Yamamoto (Inst. Med. Sci., Tokyo Univ.). Cloning and characterization of a gene involved in the regulation of life cycle in Schizosaccharomyces pombe.

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28. K. Iwama, T. Nagasu, Y. Ikeda (EISAI Co., Ltd.). Isolation of the temperature sensitive mutant deficient in mannose outer chain elongation.

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51. Yukimasa Azuma, Junpei Ishiguro and Masazumi Miyazaki* (Dept. Biol., Facul. Sci., Konan Univ., *Inst. Mol. Biol., Sch. Sci., Nagoya Univ.,). Changes in ribosomal properties in an adenine-requiring mutant strain of the yeast, K. lactis:II. Changes in ribosomal properties, intracellular ATP contents and proteinase B activities.

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85. Y. Shirahige, M. Yamamoto and B. Ono (Fac. of Pharm. Sci., Okayama Univ.). Regulation of cysteine biosynthetic pathways in S. cerevisiae.

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XXXVII. Brief News Items:

1. J.P. van der Walt retired at the end of 1986 from the Microbiology Research Group of the South African Council for Scientific and Industrial Research, Pretoria, S.A.

2. W. Alexander Scheffers of the Delft University of Technology, The Netherlands was appointed by the Royal Netherlands Academy of Sciences as a member of the Supervisory Commission of the Centraalbureau voor Schimmelcultures (CBS Culture Collection, Baarn-Delft).

3. Jerome Birnbaum has left his position at the Merck Institute for Therapeutic Research and was appointed Senior Vice President for Infectious Diseases at the Pharmaceutical Research and Development Division, Bristol-Myers Company, 5 Research Parkway, P.O. Box 5100, Wallingford, CT 06492.

4. Candida News - Newsletter No. 1, August 1987:

Candida News is published quarterly from the Experimental Oral Biology Unit, Faculty of Dentistry, University of Otago, P.O. Box 647, Dunedin, New Zealand. Editor: Maxwell G. Shepherd. Subscription Payments:

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5. Position Available

Director of the French National Yeast Collection to be recruited during the first semester of 1988. The Director tasks will be: 1) To set up the collection with his collaborators, 2) To do research on yeast molecular taxonomy. The collection will be established on the campus of INAPG, close to the laboratory of yeast genetics (H. Heslot, C. Gaillardin).

Information can be obtained by writing to the Institut National de la Recherche Agronomique (147 Rue de L'Université, 75341 Paris Cedex 07, France - TEL: 42.75.90.00). Application must be made before February 12, 1988.

H. Heslot

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