

A Newsletter for Persons Interested in Yeast.

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

Associate Editors

Anna Kocková-Kratochvilová
Slovak Academy of Sciences
Bratislava, Czechoslovakia

Tadashi Hirano
Central Research Laboratory
The Jikei Univ. School of Medicine
3-25-8 Nishi-Shinbashi, Minato-ku
Tokyo 105, Japan

Richard Snow
Dept. of Genetics, Univ. of California
Davis, California 95616

G.G. Stewart
Labatt Breweries of Canada Ltd.
150 Simcoe Street
London, Ontario, Canada N6A 4M3

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Foreign Subscribers: It has come to our attention that mailing of the Yeast Newsletter by printed matter involves a 2-3 month delay in your receiving it. If you are not receiving the Yeast Newsletter by airmail (which takes approximately 2 weeks) and would like to, please let us know. An additional \$4 per year is required to cover postage and handling for this service.

Herman J. Phaff
Editor

NOTICE TO OUR READERS

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

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

H.J. Phaff
Editor

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

1. Centraalbureau voor Schimmelcultures, Julianalaan 67a, 2628 BC DELFT (Netherlands). Communicated by M. Th. Smith.

The following communication includes:

1. A list of recently acquired yeasts by the CBS.
2. A list of renamed strains.
3. One publication which is available now.

1. Recently acquired yeasts.

Candida atmosphaerica Santa Maria 7170, ex fruit body of Tyromyces ptychogaster on fallen Picea abies in Sweden, O. Constantinescu.

Candida deserticola Phaff et al 7121 = ATCC 58088, T, ex rotting cactus Stenocereus gummosus, 7122, ex rotting cactus Stenocereus hystrix, M. Miranda (Int. J. Syst. Bacteriol. 35:211-216, 1985).

Candida parapsilosis (Ashf.) Langeron & Talice 7154, 7155, 7156, 7157, resistant to chloramphenicol, erythromycin, paramomycin, mucidin, antimycin, oligomycin, CCCP (Eur. J. Biochem. 124:457-463, 1982, 135:367-371, 1984; Mol. Gen. Genetics 197:420-424, 1984, as S. cerevisiae).

Citeromyces matritensis Santa Maria 7151, ex concentrated sucrose solution from a Dutch factory, L. Rodrigues de Miranda.

Cryptococcus consortionis Vishniac 7159 = ATCC 56686, ex Antarctic soil, ATCC.

Cryptococcus friedmannii Vishniac 7160 = ATCC 56687, ex Antarctic soil, ATCC.

Cryptococcus socialis Vishniac 7158 = ATCC 56685, ex Antarctic soil, ATCC.

Hansenula arabitolgenes 7164 = 1FO 10109, ex jam in China, IFO.

Pichia deserticola Phaff et al 7119 = ATCC 58091, T. 7120, ex rotting cactus Opuntia phoeacantha, M. Miranda (Int. J. Syst. Bacteriol. 35:211-216, 1985).

Saccharomyces cerevisiae 7173, studies on catabolite repression (FEBS Lett. 109:271-274, 1980; Biochem. Biophys. Res. Commun. 100, 688-689, 103, 1981, 926-933, 1482-1488, 1982; Arch. Microbiol. 137, 104-108, 1984; J. Biol. Chem. 259, 7191-7197, 1984), W.A. Scheffers.

Saccharomyces dairensis 7188, ex silage in the Netherlands, W.J. Middelhoven.

Sterigmatomyces penicillatus Rodrigues de Miranda, 7152, ex woodland soil in South Africa, J.P. van der Walt.

2. Renamed strains.

Candida famata (Harrison) Meyer & Yarrow var. flareri (Ciferri & Redaelli) Nakase & Suzuki 1976, T, ex erythrematous lesion, P. Redaelli (was Candida famata var. famata, J. Gen. Appl. Microbiol. 31:71-86, 1985).

Candida saitoana Nakase & Suzuki 940 = JCM 1438, T, ex air, CLSMR (was Candida famata, J. Gen. Appl. Microbiol. 31:71-86, 1985).

Debaryomyces hansenii (Zopf) Lodder & Kreger-van Rij var. fabryi (Ota) Nakase & Suzuki 789 = IFO 0015 = JCM 2104, T, ex interdigital mycotic lesion, M. Ota; 792 = IFO 0794 (T, Eutorulopsis subglobosa Zach), ex nail of 12-year-old girl, F. Zach; 5230 = HUT 7005 = IFO 0037 = JCM 2207 (T, Debaryomyces fukuyamaensis Naganishi, K. Kodama; 6066 = ATCC 22262 = JCM 1441 = Ly 1425 = NRRL Y-7500 (T, Pichia adzetii Jacob), ex tanning fluid, F. Jacob (were Debaryomyces hansenii var. hansenii, J. Gen. Appl. Microbiol. 31:71-86, 1985).

Williopsis suaveolens (Kloecker) Naumov et al. 255, T, ex Danish soil, Oe. Winge; 1668 = NRRL Y-1715, 1669 = NRRL Y-1723, 1670 = NRRL Y-1725, ex soil, L. J. Wickerham (were Williopsis saturnus Mikrobiologiya 54, 239-244, 1985).

3. Publication

Smith, M. Th. & Poot, G.A. 1985 Conspecificity of Hanseniaspora nodinigri and Hanseniaspora vineae: comparison of DNA reassociation. Antonie van Leeuwenhoek 51:151-153.

* * *

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

The strains listed have been added to the ATCC since April 29, 1985. Complete information on these strains may be obtained upon request from the Mycology Department of ATCC.

Yeast Nos.	ATCC Nos.	Yeast	ATCC
<u>Apiotrichum futronensis</u>	58914	<u>Apiotrichum osmaldii</u>	58915
<u>Candida albicans</u>	60193	<u>Candida antillancae</u>	58887
<u>Candida bertae</u>	58889	<u>Candida bertae</u> var. <u>chiloensis</u>	58888
<u>Candida bondarzewiae</u>	58890	<u>Candida castrensis</u>	58891
<u>Candida coipomensis</u>	58904	<u>Candida drymisii</u>	58892
<u>Candida ernobii</u>	60118	<u>Candida etchellsii</u>	60119
<u>Candida friedrichii</u>	60120	<u>Candida futronensis</u>	58914
<u>Candida halophila</u>	60121	<u>Candida huempii</u>	58971
<u>Candida ingens</u>	60122	<u>Candida inositophila</u>	60200

<u>Candida jamata</u>	60229-60230	<u>Candida krissii</u>	60123
<u>Candida lactiscondensi</u>	60137	<u>Candida laureliae</u>	58893
<u>Candida llanguihuensis</u>	58894	<u>Candida lusitaniae</u>	60247
<u>Candida mogii</u>	60124	<u>Candida nodaensis</u>	60167
<u>Candida osornensis</u>	58895	<u>Candida parapsilosis</u>	58789-58791
<u>Candida petrohuensis</u>	58896	<u>Candida polymorpha</u>	60125
<u>Candida pseudointermedia</u>	60126	<u>Candida psychrophila</u>	60199
<u>Candida quercuum</u>	60127	<u>Candida railenensis</u>	58897
<u>Candida ralunensis</u>	58905	<u>Candida rugosa</u>	58964
<u>Candida santjacobensis</u>	58898	<u>Candida santamariae</u>	60128
<u>Candida sequanensis</u>	58862	<u>Candida shehatae</u>	58779-58780
<u>Candida sonorensis</u>	60129	<u>Candida sophiae-reginae</u>	58899
<u>Candida sorbophila</u>	60130	<u>Candida tenuis</u>	58781-58783
<u>Candida valida</u>	60221	<u>Candida versatilis</u>	60131
<u>Cryptococcus albidus</u>	60109	<u>Cryptococcus elinovii</u>	60132
<u>Cryptococcus neoformans</u> var. <u>neoformans</u>	60234	<u>Dekkera anomala</u>	58984-58985
<u>Hansenula anomala</u>	60231	<u>Hansenula saturnus</u>	58788
<u>Pichia scaptomyzae</u>	58900	<u>Pichia stipitis</u>	58784-58785
<u>Rhodotorula albidus</u>	60109	<u>Rhodotorula grinbergssii</u>	58901
<u>Rhodotorula minuta</u>	60108	<u>Rhodotorula nothofagi</u>	58902
<u>Saccharomyces cerevisiae</u>	60003, 60202, 60218-60220, 60222-60228, 60233, 60236, 60248-60251, 60264, 60290, 60307	<u>Saccharomyces diastaticus</u>	60270
<u>Saccharomycopsis fibuligera</u>	60235 58903	<u>Schizoblastosporion chiloensis</u>	
<u>Schizosaccharomyces pombe</u>	60232	<u>Schwanniomyces occidentalis</u>	60133
<u>Sporopachydermia cereana</u>	60319-60320	<u>Trichosporium heteromorphum</u>	58861
<u>Trichosporon figueirae</u>	60134	<u>Trichosporon sericeum</u>	60135

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- III. Agricultural and Food Research Council, Food Research Institute, National Collection of Yeast Cultures (NCYC), Colney Lane, Norwich NR4 7UA, UK. Communicated by Barbara Kirsop.

The NCYC has developed a computer assisted probabilistic yeast identification system based on that used for gram-negative bacteria by Lapage et al. (Journal of General Microbiology, 1973, 77, 291-315). The probabilistic matrix has been prepared from data in the 2nd and 3rd editions of "The Yeasts-A Taxonomic Study". Fifty tests have been used in the matrix, selected on the basis of reproducibility, independence and application to all species (470) in the data base.

The probabilistic system compares well with conventional systems and was demonstrated at the Society for General Microbiology symposium (April 1985) on "The use of computers in microbial systematics" using computer networking systems (JANET, AGRENET). Abstracts of the meeting will be published in Volume 5 of BINARY (SGM Computer Club Newsletter).

The NCYC plans to make this system available on-line towards the end of 1985 via PSS systems.

* * *

- IV. W.I. Golubev, All-Union Collection of Microorganisms, Inst. Biochem. and Physiol. of Microorganisms, USSR Academy of Sciences, Pushchino, Moscow region 142292 USSR. Communicated by W.I. Golubev.

The following are recent publications from our laboratory:

1. Golubev, W.I., 1984. Cryptococcus fuscescens sp. nov. and a diagnostic key to the nitrate-positive species of the genus Cryptococcus. J. gen appl. Microbiol., 30, 427-433.
2. Golubev, W.I., Tsiomenko, A.B., 1985. Killer character in ballistosporogenous yeast fungi of the genus Sporidiobolus Nyland - Doklady Akademii nauk SSSR (Proc. USSR Acad. Sci.) 282: N2, 425-428.
3. Golubev, W.I., 1985. The significance of D-glucuronate assimilation in yeast taxonomy. Abstr. VIIth. Symp. All-Union Microbiol. Soc./June, 1985, Alma-Ata/, I, 13.

* * *

- V. Microbiology Research Group, CSIR, P.O. Box 395, Pretoria 0001, South Africa. Communicated by J.P. van der Walt.

The following two papers have been published recently:

1. J.P. van der Walt and J.A. von Arx*. 1985. Use of ultrastructural characters in yeast systematics. *South African J. Sci.* 81:156-159.

*Het Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, The Netherlands.

Ultrastructural features of the cell wall, septa and ascospores provide definitive criteria for the natural classification of both perfect and imperfect yeasts. These characters permit the recognition of ascomycetous and basidiomycetous affinities without recourse of sexual stages, and the separation of the Ascomycetes from the Endomycetes and the Sporobolomycetaceae from the Filobasidiaceae.

* * *

2. J.P. van der Walt and J.A. von Arx. 1985. The Septal Ultrastructure of Hormoascus ambrosiae and the Emendation of the Genus Hormoascus. *System. Appl. Microbiol.* 6:90-92.

Summary

TEM studies show that the hyphae of Pichia ambrosiae have centrally-thickened septa, perforated by a plugged canal. The species is further characterized by erect ascophores with catenate asci and relatively small (3-4 μm), galeate ascospores. It is therefore reclassified in Hormoascus, together with Ambrosiozyma philentoma. The genus Hormoascus is emended to include both nitrate-positive and nitrate-negative species.

* * *

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

The following 4 manuscripts are in press:

1. Lachance, M.A., A. Luttikhuis, L.L., Anweiler, D.L. Paylor, and L.G. Olson. 1985. Assessment of intraspecific variation in Clavispora sp. by restriction mapping of tandemly repeated deoxyribonucleic acid. *Int. J. Syst. Bacteriol.* 35(4):462-466.

Abstract

Total deoxyribonucleic acids from 10 strains of an undescribed cactophilic species of Clavispora were subjected to restriction analyses with endonucleases Apa I, Eco RI, Kpn I, and Xho I. A 7.6 kb tandem repeated segment of the genome was mapped and found to possess both conserved and variable regions among the strains examined. A total of 8 possible endonuclease recognition sites were identified. Two strains were heterogeneous, and one of these shared one of its restriction maps with 3 other strains. In one instance, variation was correlated with a habitat difference. In addition, all strains possessed very large (up to 28 kb) repetitive Eco RI fragments. Preliminary results indicate that this yeast species shares parts of its conserved region with Clavispora lusitaniae,

but that major differences exist between the two species in their variable regions. The application of restriction mapping in taxonomic and other investigations of yeasts is discussed.

2. Sidenberg, D.G., and M.A. Lachance. 1986. Electrophoretic isoenzyme variation in Kluyveromyces populations and revision of Kluyveromyces marxianus (Hansen) van der Walt. Int. J. Syst. Bacteriol. 36(1).
3. Nigam, J.N., A. Margaritis, and M.A. Lachance. 1985. Aerobic fermentation of D-xylose to ethanol by Clavispora sp. Appl. Environ. Microbiol. 50(4).
4. Nigam, J.N., R.S. Ireland, A. Margaritis, and M.A. Lachance. 1985. Isolation and screening of yeasts that ferment D-xylose directly to ethanol. Appl. Environ. Microbiol. 50(6).

* * *

VII. Universita Degli Studi Di Perugia, Istituto Di Biologia Vegetale, Sezione di Microbiologia, Borgo XX Giugno, 74 - 06100 Perugia, Italy. Communicated by Ann Vaughan Martini.

The following represents recent publications from our Institute.

1. Ann Vaughan Martini and Cletus P. Kurtzman. 1985. Deoxyribonucleic acid relatedness among species of the genus Saccharomyces Sensu Stricto. Int. J. Syst. Bacteriol. 35:508-511.

Abstract

Twenty-four species assigned to the genus Saccharomyces sensu stricto were examined for deoxyribonucleic acid (DNA) relatedness. Results with type strains demonstrated the presence of four distinct species: Saccharomyces cerevisiae, S. bayanus, S. carlsbergensis and S. kluyveri. S. carlsbergensis NRRL Y-12693¹ showed intermediate relatedness between S. cerevisiae and S. bayanus and has a genome size approximately 1.5 times that of the last two species. These data suggest S. carlsbergensis to be a partial amphidiploid which may have arisen from a natural hybridization between S. cerevisiae and S. bayanus.

* * *

2. Rosini, G. 1985. Interaction between killer strains of Hansenula anomala var. anomala and Saccharomyces cerevisiae yeast species. Can. J. Microbiol. 31:300-302.

The cross-reaction between 6 killer strains of Saccharomyces cerevisiae and 41 killer strains of Hansenula anomala var. anomala was examined. Fifteen strains of Hansenula killed one or more cultures of S. cerevisiae. None of the killer strains of H. anomala var. anomala was killed by S. cerevisiae killer strains or by killer strains of the same species. In S. cerevisiae different killer toxin and immunity systems were represented. Intraspecific killing activity was not found among the 41 strains of H. anomala var. anomala.

* * *

3. Bertuccioli, M. and J. Rosi. 1984. Factors affecting the formation of by-products by Saccharomyces cerevisiae under simulated vinification conditions. Progress in Flavor Research. J. Adda (ed.) 1985 Elsevier Science Publishers B.V., Amsterdam.

Abstract

Numerous by-products which affect wine flavour are excreted in small amounts during alcoholic fermentation by yeast. In order to clarify the effect of lipids of grape must on the improvement of white wine aroma, the influence of fatty acids and triglycerides on the production of volatile compounds has been studied. The addition of lipids has a pronounced effect on yeast metabolism. Free fatty acids increased cell viability, decreased the production of ethyl and acetate esters, medium chain fatty acids and modified the fatty acids composition of yeast. Some considerations about the clarification procedure are also discussed.

* * *

4. F. Federici and M. Petruccioli. 1985. Growth and Polygalacturonase production by Aureobasidium pullulans on orange peel waste. M.A.N. 3:39-46.

Summary

An active strain of Aureobasidium pullulans was selected for the production of pectinase and protein from orange-processing wastes.

The organism grew well on orange peel-based substrates releasing high levels of polygalacturonase [poly (1,4- α -D galacturonide) glycanohydrolase EC 3.2.1.15] activity in the fermentation broth. The enzyme yield depended strictly upon medium composition: a contemporary presence of yeast extract (0.1%) and ammonium sulphate (0.2%) was, in fact, necessary for maximum enzyme production.

After fermentation, orange peel crude protein content increased ten to twelvefold (from less than 2% to about 20%) and, on the basis of its essential amino acid profile, the protein appeared to be of fair quality, equivalent at least to that of other yeasts.

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VIII. Section Levures, Bat. 405, Univ. Lyon I, 43 Bd 11 nov. 1918, 69622 Villeurbanne Cedex, France. Communicated by M.C. Pignal.

1. Recent Publications

- a) Poncet, S., Jacob, F.H., Berton, M.C. and Couble, A. 1985. Fermentation alcoolique de l'inuline par quelques souches de levures. Ann. Inst. Pasteur (Microbiol.) 136B:99-109.

During the last year, we worked on ethanol fermentation of inulin by some yeasts. Strains of 14 species of yeasts were studied on semi-synthetic medium and on Jerusalem artichoke juice. Among them, Kluyveromyces cicerisporus showed the best kinetic characteristics of fermentation. This strain appears to be a good yeast for industrial production of ethanol.

- b) Fiol, J.B. and Hmama, Z. A biochemical and immunological study of yeasts that produce true mycelium: Ambrosiozyma, Hormoascus and Hyphopichia (submitted to Mycopathologia in June 1985). Ambrosiozyma, yeasts capable of producing true mycelium with transversal septa that communicate by dolipores, are compared among themselves and with Hyphopichia. This study includes intracellular osidases, nitrite and nitrate reductases, GC content, the cytochrome spectra (a + a₃, b, c₁), coenzyme Q and serological comparison by immunodiffusion and immunoelectrophoresis with A. monospora and H. platypodis as references. The results show the division of the genus Ambrosiozyma into two groups and the analogy between H. platypodis and A. philentoma. A discussion concerning A. ambrosiae in relation to Ambrosiozyma and Pichia is given. The originality of Hy. burtonii is confirmed.
- c) Pignal, M.C., Lachaise, D. and Couturier, G. Les levures des figues et des drosophiles associées en forêt de Tai (Côte-d'Ivoire). Acta Ecologica/Oecologia Generalis 1985, 6:223-233.

A comparative analysis is made of the yeast microflora growing at the surface or within the cavity of tropical figs. Yeasts and bacteria were isolated for the first time from the gut of Lissocephala larvae suggesting that these specialist fruit flies can develop successfully within the cavity of immature receptacles by using as a resource the microflora introduced by the obligatory and species-specific pollinating fig wasp.

2. Research In Progress

- a) A student, A. Michel, will defend her thesis in December 1985 to obtain the degree of Doctor of Microbiology. Her thesis' title is: "Protein production by yeasts growing on raw whey."
- b) We have determined the cytochrome spectra of the Pichia complex; those of Hansenula spp. are being completed at this time (with M. Claisse, Centre of molecular genetics, L.P. CNRS ass. Univ. P. and M. Curie, Gif-sur-Yvette). At the same time we are determining quantitatively the respiratory ubiquinones of species of these two genera (with G. Billon-Grand). The results should give us a better understanding of the natural relationships among the vast domain Pichia-Hansenula.

* * *

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

Below follows the summary of a paper that has recently been accepted for publication by the Journal of Biological Chemistry "Dityrosine is a Prominent Component of the Yeast Ascospore Wall - A Proof for its Structure" by Peter Briza, Gunther Winkler, Hermann Kalchhauser, and Michael Breitenbach.

Summary

The yeast ascospore wall consists of four morphologically distinct layers. The hydrophobic surface layers are biogenically derived from the prospore wall and appear dark after OsO₄-staining. They seem to be responsible for the stability of the spores against attack by lytic enzymes. By amino acid analysis of acid hydrolysates of ascospore walls, two new peaks were detected, which were shown to be the racemic and meso-form, respectively, of dityrosine. The identity of this hitherto unknown component of the yeast ascospore wall with standard dityrosine was proven by ¹H-NMR and by mass-spectrometry. A ¹³C-NMR spectroscopic investigation of the structure of dityrosine confirmed that, in natural dityrosine, the biphenyl linkage is located ortho, ortho to the hydroxyl groups. Following digestion of the inner layers of isolated ascospore walls it was shown that dityrosine is very probably located only in the surface layers. The same conclusion was reached independently by an investigation of spores of a strain homozygous for the mutation gen1, which lack the outermost layers of the spore wall and were practically devoid of dityrosine. In sporulating yeast, L-tyrosine was readily incorporated into the dityrosine of the ascospore wall. Control experiments involving vegetative α/α cells, and nonsporulating α/α cells under sporulation conditions, showed that dityrosine is indeed sporulation-specific.

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X. Laboratory of Microbiology and Cz. Collection of Yeasts, Institute of Chemistry of the Centre of Chemical Research of Slovak Academy of Sciences, 842 38 Bratislava, Dubravská Česta 9, CSSR.
Communicated by E. Sláviková.

1. Kocková-Kratochvilová, A., Sláviková, E. 1985. Classification principles for the identification of yeast-like species. *Biologia (Bratislava)*, 40:305-311.

Physiological features were applied for the construction of a code, characterizing individual species or strains. This code can characterize not only species, but also natural variability within the species. The code of species may be used together with a generic code (discussed in the previous paper) as the basis for elaboration identification key or in the Catalogue of Yeast Cultures using a computer with advantage.

* * *

2. Kocková-Kratochvilová, A. 1984. Classification principles for yeast-like genera. *Biologia (Bratislava)* 39:717-729.

In daily practice we meet with the necessity of identifying various organisms, which can be collected from nature and isolated in the same way as yeasts. Some of them appear to be yeast-like only in a certain phase of their life cycle. A large number of them have been described as new genera of yeast-like hyphal organisms. Thus, we included in this study 67 genera of yeasts, yeast-like organisms, hyphal yeast-like fungi of Endomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes. Thirty-one percent of these genera are Basidiomycetes, 39% of genera possess only a single species, 15% are big genera, 18% are hyphal fungi. The coding of generic phenotypes may serve for the computer program based on combinations and for identification of unknown genera. The code of a genus of yeasts and yeast-like organisms represents a shortened form of the precise description of a genus and may be used advantageously in Catalogues of Yeasts of individual collections. We shall operate with this code in our further papers.

* * *

3. Kovačovská, R., Sláviková, E. 1985. Optimization of conditions for assimilation tests of yeasts. *Kvas. prum.*, 31:204-206.

A significant factor for the determination of yeast species is the ability to assimilate certain carbon and nitrogen sources. The effects of pH, temperature, concentration of the inoculated suspension and that of substrates used for the assimilation were tested. The standard procedure for the determination of the yeast species is described.

* * *

4. Kocková-Kratochvilová, A., Sláviková, E., Breierová, E. 1984. Yeasts isolated from fruitbodies of mushrooms of the Lowland of Zahorie. *Česká Mykol.*, 38:218-229.

Yeasts appearing on the surface of fruitbodies of mushrooms were investigated from five localities of the Lowland of Zahorie in west Slovakia during the years 1981 and 1982. 224 fruitbodies were collected and 126 yeast strains isolated. 90 yeasts were identified using a shortened method of identification.

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5. Kocková-Kratochvilová, A., Švorcová, L., Breierová, E., Delgado, R. 1984. Taxonomic characteristics of yeasts isolated from bath facilities. *Česká Mykol.*, 38:11-20.

This paper deals with the taxonomy and identification of yeasts and yeast-like microorganisms isolated from West-Bohemian bath facilities. Twenty eight strains were isolated representing 11 genera grouped into seven groups: Bullera, Cryptococcus, Candida and Torulopsis, Trichosporon and Geotrichum, Hyphozyma, Rhodotorula, sporogenic yeasts.

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6. Kocková-Kratochvilová, A., Sláviková, E., Zemek, J., Kadlečiková, B., Kuniak, Ľ. 1985. Hydrolytic activity in the genus Schizosaccharomyces Lindner. Folia Microbiol. 30:443-451.

A total of 22 strains of known species of the genus Schizosaccharomyces Lindner were evaluated by numerical taxonomy based on conventional identification tests. The results of numerical taxonomy were supplemented by a determination of activity of extracellular hydrolytic enzymes, especially 1,3- β -D-glucanase, RNAase and DNAase. All strains tested were capable of utilizing the 1,4- α -D-glucan tamarind. Our study of life cycles of these organisms showed that extracellular hydrolytic enzymes were present mainly at the time of maturation of asci and release of their walls. Strains forming four-spored asci could be comprised in the single species Schizosaccharomyces pombe Lindner as two varieties: S. pombe var. pombe and S. pombe var. malidevorans. The two varieties differ in maltose fermentation. Urease is produced by all strains irrespective of the life cycle phase. A number of hydrolytic enzymes are not produced by species of the genus Schizosaccharomyces (e.g., amylolytic enzymes) despite the fact that oligomers of maltose are utilized. Species of the genus Schizosaccharomyces also lack xylanase, cellulases, mannanase, and are incapable of degrading carrageenan and acidic polysaccharides.

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- XI. Department of Microbiology, University of Glasgow, Garscube Estate, Bearsden, Glasgow. G61 1QH, U.K. Communicated by L. Julia Douglas.

The following papers have been published recently:

1. Douglas, L.J. (1985). Adhesion of pathogenic Candida species to host surfaces. Microbiological Sciences 2, 243-247 (A short review).
2. McCourtie, J. and Douglas, L.J. (1985). Extracellular polymer of Candida albicans: isolation, analysis and role in adhesion. Journal of General Microbiology 131, 495-503.

Summary

Extracellular polymeric material (EP) was isolated from culture supernatants of Candida albicans grown on carbon sources (50 mM-glucose, 500 mM-sucrose or 500 mM-galactose) known to promote yeast adhesion to different extents. Galactose-grown yeasts, which are the most adherent, produced more EP than sucrose-grown organisms, particularly after incubation for 5 d, while glucose-grown yeasts (the least adherent) gave the lowest yield. EP produced on all three carbon sources was of similar composition and contained carbohydrate (65 to 82%; mannose with some glucose), protein (7%), phosphorus (0.5%) and glucosamine (1.5%). Serological studies indicated that these EP preparations were

immunologically identical but that galactose-grown yeasts had more antigenic determinants than sucrose-grown organisms while glucose-grown yeasts had the fewest determinants. Antigenic differences were apparent between EP preparations of some strains of C. albicans. Pre-treatment of acrylic strips with EP to form a polymeric coating promoted yeast adhesion to the acrylic surface, but similar pretreatment of buccal epithelial cells with EP inhibited subsequent yeast adhesion. These results indicate that EP originates from the cell surface of C. albicans and that it contains the surface component(s), probably mannoprotein in nature, responsible for yeast adhesion.

3. McCourtie, J. & Douglas, L.J. (1985). Unequal distribution of adhesins within populations of Candida albicans. FEMS Microbiology Letters 27, 111-115.

Summary

The rate of adhesion of Candida albicans to acrylic surfaces in vitro was determined after growth of the yeast in defined media containing different sugars as the carbon source. Yeasts grown on 500 mM galactose adhered at a maximal, linear rate throughout the 60-min incubation period. Non-linear adhesion rates were observed with organisms grown on other carbon sources (500 mM sucrose, 50 mM glucose or 50 mM galactose) and from these cultures, populations of yeasts were isolated which showed increased adhesion and increased resistance to spheroplast formation. These results indicate that there is an unequal distribution of adhesins among cells in such cultures.

4. Critchley, I.A. & Douglas, L.J. (1985). Differential adhesion of pathogenic Candida species to epithelial and inert surfaces. FEMS Microbiology Letters 28, 199-203.

Summary

Growth in medium containing 500 mM galactose is known to promote the adhesion of Candida albicans to buccal epithelial cells or to acrylic in vitro. Of 5 other Candida species tested, only C. tropicalis (one strain) showed substantially increased adhesion to buccal cells (but not to acrylic) after growth under these conditions. A second strain of C. tropicalis as well as C. stellatoidea, C. parapsilosis, C. pseudotropicalis, C. guilliermondii and Saccharomyces cerevisiae showed little or no increased adhesion to either surface. However, after growth in medium containing 50 mM glucose, C. tropicalis and C. parapsilosis were significantly more adherent to acrylic than glucose-grown yeasts of the other species, including C. albicans. These results are discussed in relation to the colonization and infection potential of the pathogenic Candida species.

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- XII. Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL 60439, U.S.A. Communicated by H.E. Kubitschek.

The following is the abstract of a paper recently submitted to J. Bacteriol. for publication.

H.E. Kubitschek. Linear volume increase during the cell cycle of Schizosaccharomyces pombe.

Abstract

J.M. Mitchinson's classical determination of the mass and volume of individual cells of Schizosaccharomyces pombe during the division cycle showed that cell mass increased linearly during most of the cycle while increase in cell volume followed a biphasic growth pattern, with increase to a maximum cell volume occurring during the first 2/3 to 3/4 of the division cycle. A biphasic increase in cell volume requires that buoyant densities vary during the cycle if cell mass increase is to remain linear. On the other hand, our recent measurements of buoyant cell density in S. pombe support density invariance during the cycle. Given a linear increase in cell mass, buoyant density invariance would require that the increase in cell volume also be linear rather than biphasic. We tested this possibility by measuring lengths and widths of individual cells of S. pombe growing on agar squares on the warm stage (32°C) of a microscope (Zeiss, phase, 1000X). In contrast to other earlier reported procedures, ours were designed to minimize changes in culture media and osmolarity when cells were transferred from exponentially growing cultures to the agar.

Our results show that under constant (or nearly constant) growth conditions, the volumes of most cells increased linearly during the division cycle. When growth conditions under the microscope were altered, most or all cells increased in volume according to the well-established biphasic pattern. Furthermore, when growth was linear, both cell length and width increased linearly until formation of the cell plate. In contrast, when volume increased biphasically, length increased in a similar manner, while width quickly adjusted to a constant value during the cycle.

These results show that there is a second pattern of cell growth in S. pombe, other than that observed previously. Furthermore, the results indicate that linear increase in cell volume is the fundamental growth pattern during the cycle in steady-state cultures, and that relatively minor alterations in temperature, osmolarity, or composition of that growth medium are sufficient to induce biphasic growth.

These observations can resolve earlier conceptual difficulties concerning earlier observations on the growth of these cells. Linear increase in cell mass and cell volume are completely consistent with buoyant density invariance.

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XIII. Laboratory of Radiation Microbiology, The University of Tokyo, Bunkyo-ku, Tokyo, 113 Japan. Communicated by Masamichi Takagi.

A recent paper on Candida maltosa from our laboratory is given below.

Masamichi Takagi, Shinya Kawai, Yoshihiro Takata, Norihiro Tanaka, Michio Sunairi, Masazumi Miyazaki*, and Keiji Yano. 1985. Induction of

Cycloheximide Resistance in Candida maltosa by Modifying the Ribosomes. J. Gen. Appl. Microbiol., 31:267-275.

*Institute of Molecular Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464 Japan.

When Candida maltosa IAM12247 was grown in the presence of cycloheximide, the growth was repressed for a distinct period of time depending on the concentration of cycloheximide, then the growth recovered ("adapted"). The cell-free translation experiments using polyuridylic acid as mRNA indicated that the protein-synthesizing activity was altered in the cells adapted to resist cycloheximide. A reconstituted cell-free system was constructed consisting of ribosomes and soluble fraction (S-100), and it was concluded that ribosomal modification was induced during the cultivation of the cells in the presence of cycloheximide.

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XIV. Institute of Microbiology, Czechoslovak Academy of Sciences, Laboratory of Biochemistry and Physiology of Yeast, Department of Enzyme Engineering, Videnska 1083, 142 20 Prague 4, Czechoslovakia. Communicated by Cenek Novotny.

For the past several years we have been working on projects involving the regulation of sterol synthesis, the composition of yeast cell wall and the regulation of β -galactosidase synthesis. The following are abstracts of recent papers accepted for publication and patents from our laboratory.

1. A. Pichová, K. Beran, B. Běhalová and J. Zajiček. 1985. Ergosterol synthesis and population analysis of a fed-batch fermentation in Saccharomyces cerevisiae. Fol. Microbiol. 30:134-140.

Abstract

Saccharomyces cerevisiae with an increased content of ergosterol or $\Delta^{5,7}$ -sterols, growing on a molasses medium with a feed of ethanol and $(\text{NH}_4)_2\text{HPO}_4$, was analyzed as to the age of cell population. The analysis was done by centrifugation in a dextran gradient and by a fluorescent-microscopic technique. In the phase of batch fermentation at a mean specific growth rate of 0.22 h^{-1} daughter cells contained less than 1% ergosterol while the ergosterol content of mother cells depended on the time of cultivation, a maximum level (4%) being found after two generation times. In the fed-batch phase at a mean growth rate of 0.052 h^{-1} , both daughter and mother cells contained about the same amount of ergosterol (4.7-5.5%). Differences between daughter and mother cells are discussed in view of the relationship between the growth rate and the growth cycle.

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2. J. Votruba, B. Běhalová, and K. Beran. 1986. Batch cultivation of Saccharomyces cerevisiae with an increased content of $\Delta^{5,7}$ -sterols. Fol. Microbiol. 31, in press.

Abstract

Changes of physiological state were studied in a batch culture of *Saccharomyces cerevisiae*. Protein concentration and $\Delta^{5,7}$ -sterol content in biomass served as markers of these changes. Variations of these markers in relation to the concentrations of sources of carbon, nitrogen and phosphorus were examined. A mathematical model of batch cultivation was developed and identified. Effects of various technological parameters on increasing the proportion of $\Delta^{5,7}$ -sterols in biomass were evaluated by the method of parametric sensitivity.

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3. B. Běhalová, J. Votruba, A. Pichová and K. Beran. 1986. Fed-batch cultivation of *Saccharomyces cerevisiae* with an increased content of $\Delta^{5,7}$ -sterols. Fol. Microbiol. 31, in press.

Abstract

A mathematical model describing changes of the physiological state of culture was verified in a batch cultivation with the linear influx of nutrients. Protein concentration and $\Delta^{5,7}$ -sterol content in biomass were chosen as markers of these changes. The mathematical model was used to optimize $\Delta^{5,7}$ -sterol production in relation to the volume and composition of the inflowing growth medium.

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4. T. Řezanka, L. Doležalová, O. Vyhňálek and Č. Novotný. 1985. Effect of clomiphene on sterol and fatty acid content of *Saccharomyces cerevisiae*. Fol. Microbiol. 30, in press.

Abstract

Clomiphene regulated sterol and fatty acid production qualitatively and quantitatively. Sterol content decreased by a quarter, the amount of fatty acids produced was comparable with that without clomiphene. The treatment with clomiphene increased the proportion of sterols with methyl group in position 4α and unsaturated bond in position 24 or 24(28) but not in position 22. Short saturated and monoenic fatty acids prevailed while 2-hydroxy acids were not present.

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5. Č. Novotný and B. Sikyta. 1986. Inducible synthesis of β -galactosidase in *Kluyveromyces fragilis*. Fol. Microbiol. 31, in press.

Abstract

Lactose, D-galactose and L-arabinose induce the synthesis of β -galactosidase in *Kluyveromyces fragilis* CCY 51-1-1. Lactose is the best inducer with a maximum effect at 1 mmole per litre. The inducible synthesis of the enzyme in the glycerol-grown stationary-phase cells is triggered within 30 min after the addition of inducer, the full induction being achieved within the next 30 to 40 min.

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6. Č. Novotný, L. Doležalová, B. Běhalová and J. Zajiček. 1986. Accumulation pattern of $\Delta^{5,7}$ -sterols in Saccharomyces cerevisiae. Fol. Microbiol. 31, in press.

Abstract

Saccharomyces cerevisiae is characterized by accumulation of $\Delta^{5,7}$ -sterols during growth cycle. The differential rate of sterol synthesis gradually increased through the cultivation, an abrupt decrease of its value could be observed at 32 to 33 mg of $\Delta^{5,7}$ -sterols per g of biomass. The specific rate of sterol synthesis achieved a broad maximum when culture was growing. Both the rising sterol synthesis and declining growth were responsible for gradual increase of the differential rate of sterol synthesis which resulted in sterol accumulation in biomass. This gradual accumulation pattern was dominant both with fermentable and nonfermentable carbon sources.

Limited feeding with a fermentable carbon source had significantly greater negative impact on sterol accumulation than that with a nonfermentable carbon source. High concentrations of glucose in the medium (9 g/L) both delayed and decreased accumulation of $\Delta^{5,7}$ -sterols in Saccharomyces cerevisiae CCY 21-4-63.

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7. Č. Novotný, K. Beran, B. Běhalová, L. Doležalová and J. Zajiček. 1986. Effect of nitrogen on $\Delta^{5,7}$ -sterol synthesis in Saccharomyces cerevisiae.

Abstract

Effect of ammonium concentration in the medium on the $\Delta^{5,7}$ -sterol synthesis was examined. Higher concentrations of this nitrogen source decreased the sterol synthesis and accumulation in the growth cycle. A permanent supplying with ammonium resulted in proportional synthesis of $\Delta^{5,7}$ -sterols and biomass. The carbon/nitrogen molar ratios higher or equal to 40 allowed for the maximum accumulation of $\Delta^{5,7}$ -sterols in the growth cycle.

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8. B. Běhalová, Č. Novotný, J. Zajiček, J. Pásková, J. Latinák and M. Kožený. 1986. Sterols in various strains of Saccharomyces cerevisiae. Kvasny prumysl 32, in press (in Czech).

Abstract

The ability of increased ergosterol production was tested in various strains of Saccharomyces cerevisiae. The contents of ergosterol, $\Delta^{5,7}$ -sterols and total sterols changed in relation to used strain and cultivation conditions. The best producers were A22 and KBD, attaining about 2% ergosterol in dry matter on molasses medium with corn-steep liquor. The ratio of ergosterol and 24(28)-dehydroergosterol on the

synthetic Ol-son-Johnson medium had, in comparison with fermentation on molasses medium with corn-steep liquor, a twofold value.

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9. B. Běhalová and K. Beran. 1986. Autolysis of disintegrated cells of the yeast Saccharomyces cerevisiae. Acta Biotechnologica 2, in press.

Abstract

Optimum conditions for autolysis of disintegrated cells of Saccharomyces cerevisiae are at 50-53°C and pH 5.5; the process is terminated after 6 h. In the presence of sodium chloride (3-5%) the autolysis is complete after 5 h. The yield of autolysis of disintegrated yeast cells is about 70% of autolytic product per yeast dry weight. The product obtained after centrifugation, filtration and drying has very good sensoric and physical properties.

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10. A. Kotyk, P. Biely, O. Markovič, K. Beran and Z. Holan. 1986. Biochemical methods (in Czech), in Yeasts in research and practice (D. Vrana, ed.), Academia Publishing House, Prague, in press.
11. L. Kuniak, J. Zemek and Z. Holan. PV 5816-85. Tablets for the assay of β -glucanase (in Czech).
- L. Kuniak, J. Zemek and Z. Holan. PV 5619-85. A soluble chromolytic substrate for the assay of β -glucanase (in Czech).
- L. Kuniak, J. Zemek and Z. Holan. PV 5815-85. A chromolytic substrate for the assay of β -glucanase (in Czech).
- Z. Holan B. Sikyta and V. Palisa. PV 6615-84. A production of the particulate 1,3- β -D-glucan (in Czech).
- J. Pásková, B. Špaček, V. Hradec, B. Běhalová, Č. Novotný and J. Zajiček. PV3033-85. A Saccharomyces cerevisiae strain for production of ergosterol (in Czech).

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- XV. Alko Ltd., The Finnish State Alcohol Company, POB 350, SF-00101 Helsinki 10, Finland. Communicated by Matti Korhola.
1. We organized a satellite symposium "Commercially Feasible Alcohol Fermentation Systems" to GIAM VII (Global Impacts of Applied Microbiology) August 15th, 1985. Three aspects were dealt with: raw materials, micro-organisms, and fermentation technology. The specific topics were:
- Dr. Nick Coote (Tate and Lyle Ltd.)
The Impact of Raw Materials and Distillery Design: Maize and Molasses.

Dr. Matti Korhola (Alko Ltd.)
Distiller's Yeast.

Prof. Peter L. Rogers (University of New South Wales)
Commercial Potential of *Zymomonas* Process for Ethanol Production.

Dr. Carlos Rolz (ICAITI)
Ethanol from Sugar Cane: the Ex-Ferm Concept.

Prof. Naim Kosaric (University of Western Ontario)
New Technologies in Alcohol Fermentation.

Prof. Hermann Sahn (Kernforschungsanlage Julich GmbH)
Industrial Ethanol Production with *Zymomonas mobilis*.

The proceedings of the symposium will be published shortly as Volume 4 of the publication series by the Foundation for Biotechnical and Industrial Fermentation Research.

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2. The following abstract is from an academic dissertation at the Helsinki University, Department of Biochemistry. It is based on five papers. I J. Biol. Chem. 258, 2966-2972. II Anal. Biochem. 126, 139-143. III J. Biol. Chem. 259, 6964-6971. IV Biochem. J. 226, 897-900. V J. Cyc. Nucl. Prot. Phosph. Res. 10, 121-127.

Suoranta, Kari. Cyclic AMP phosphodiesterases of bakers' yeast. Research Laboratories of the Finnish State Alcohol Company, Alko Ltd. Helsinki, 1985.

Two cyclic AMP hydrolysing enzymes were purified to homogeneity from commercial bakers' yeast and their biochemical properties studied. Both were shown to be zinc metallo-enzymes.

The Mg-independent, high-K_m cyclic nucleotide phosphodiesterase with molecular weight of 43 kDa on SDS-PAGE and 88 kDa in sedimentation equilibrium, was purified from the soluble fraction of a yeast cell homogenate and was shown to contain about 2 atoms of zinc per 43 kDa. Removal of zinc resulted in loss of the enzymatic activity.

The free bivalent cation-dependent, low-K_m cAMP phosphodiesterase was purified from the microsomal fraction of the homogenate. It contained about 1 atom of tightly bound zinc per 61 kDa. If the yeast was broken in the presence of a protease inhibitor, a product with only one band on SDS-PAGE, at 61 kDa, was found. If the inhibitor was omitted, the product had a slightly higher specific activity and showed two bands on SDS-PAGE, at 45 kDa and 17 kDa, although it had the same native molecular weight, same pI and similar amino acid composition Mg²⁺ as the free bivalent metal ion could be readily substituted by Mn²⁺ and Zn²⁺. In the presence of Mg²⁺ ions, the enzyme hydrolysed only the (Sp)-diastereoisomer of cyclic adenosinephosphorothioate but in the presence of Mn²⁺, Co²⁺, Zn²⁺ or Cd²⁺ both (Sp)- and (Rp)-isomers were hydrolysed.

During diauxic growth in 2% glucose batch culture the specific activity of the high-Km phosphodiesterase increased 20-fold throughout the 108 h cultivation, and that of the low K-m enzyme approximately doubled during glucose utilization but fell back to the initial level as the cells entered stationary phase. The specific activity also increased by even 230% when 84 h old yeast was provided with 2% glucose.

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3. Pirkko L. Liljeström. The nucleotide Sequence of the Yeast Mel1 gene. Nucleic Acids Research (in press).

The complete nucleotide sequence of the MEL1 gene of the yeast, Saccharomyces cerevisiae, encoding α -galactosidase was determined. The nucleotide sequence contains an open reading frame of 1413 bp encoding a protein of 471 amino acids. Comparison with the known N-terminal amino acid sequence of the mature secreted protein indicated that α -galactosidase is synthesized as a precursor with an N-terminal signal sequence of 18 amino acids. The general features of this signal peptide resemble those of other yeast signal peptides. Molecular weight of the mature α -galactosidase polypeptide deduced from the nucleotide sequence is 50.049 kd. The 5' regulatory region has sequences in common with other yeast genes regulated by the GAL4-protein.

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- XVI. Albert-Ludwigs-Universität Freiburg Im Breisgau, Biochemisches Institut, Hermann-Herder Str. 7, D-7800 Freiburg, West Germany.
Communicated by Helmut Holzer.

Below follow abstracts from recent publications from my laboratory which appeared in journals not widely available.

1. Helga Hinze¹ and Helmut Holzer^{1,2}. 1985. Effect of Sulfite or Nitrite on the ATP Content and the Carbohydrate Metabolism in Yeast. Zeitschrift Lebensmittel-Untersuchung und -Forschung 181:87-91.

¹Gesellschaft für Strahlen- und Umweltforschung, Abteilung für Enzymchemie, D-8042 Neuherberg (West Germany).

²Biochemisches Institut der Universität Freiburg.

Summary: Low concentrations of sulfite or nitrite (about 0.5 mmol) when applied at pH 3.6, caused a rapid and drastic decrease in the concentration of ATP of yeast cells. Under these conditions, alcoholic fermentation was inhibited by sulfite and to a lesser extent by nitrite. Ethanol consumption under aerobic conditions was shown to be more sensitive to nitrite than to sulfite. This indicates a higher sensitivity of respiratory processes to nitrite than to sulfite. Among 15 enzyme activities assayed in extracts from yeast cells after incubation with sulfite or nitrite, glyceraldehyde-3-phosphate dehydrogenase was shown to

be the most sensitive. Analysis of the steady-state concentrations of intermediates of alcoholic fermentation in intact yeast cells also implies inhibition by sulfite or nitrite of the glyceraldehyde-3-phosphate dehydrogenase step of fermentation. In contrast to nitrite, sulfite had an additional effect by accumulating the intracellular steady state concentration of glyceraldehyde-3-phosphate 10 to 100-fold over the concentration in the absence of sulfite. In vitro studies of the equilibrium catalyzed by triosephosphate isomerase or aldolase confirmed the postulated shift of equilibrium concentrations by a formation of a complex of glyceraldehyde-3-phosphate with sulfite.

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2. Matthias v. Herrath and Helmut Holzer. 1985. Oxidative Inactivation of Yeast Fructose-1,6-Bisphosphatase. In: "Intracellular Protein Catabolism", pp. 329-340, Alan R. Liss, Inc.

Summary: Active, non-phosphorylated fructose-1,6-bisphosphatase from yeast is partially inactivated by two different mixed-function oxidation systems: the ascorbate- $\text{FeCl}_3\text{-O}_2$ system described by Levine (Levine, R. (1983) J. Biol. Chem. 258, 11823-11827) and the NADH oxidase-NADH- $\text{FeCl}_3\text{-O}_2$ system described by Fucci et al. (Fucci, L., Oliver, C.N., Coon, M.J., and Stadtman, E.R. (1983) Proc. Natl. Acad. Sci. 80, 1521-1525). Fructose-2,6-bisphosphate (1 μM) or histidine (10 mM) partially protect from oxidative inactivation. The inactivation is characterized by the following changes in the kinetic properties of fructose-1,6-bisphosphatase: decrease of the ratio of activity at pH 8 to that at pH 9 and decrease of the ratio of activity with 10 to that with 2 mM Mn^{2+} . These changes of the kinetic properties are very similar to the ones previously observed following phosphorylation (Holzer, H. (1984) in "Enzyme regulation by reversible phosphorylation - further advances" (P. Cohen, ed.) pp. 143-154, Elsevier Science publishers) and limited proteolysis of fructose-1,6-bisphosphatase with yeast proteinase B (Pohlig, G., Schäfer, W., v. Herrath, M. and Holzer, H (1985) in "Current topics in cellular regulation" (S. Shaltiel and P. Boon Chock, eds.) Vol. 27, pp. 317-324.

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- XVII. Instituto de Quimica - Dept. Bioquimica, Universidade Federal do Rio de Janeiro, Centro do Tecnologia - Bloco A, Lab. 547 - IIha do Fundao, Cidade Universitaria - Rio de Janeiro, 21941 - RJ - Brazil, Communicated by Anita D. Panek.

Below follow abstracts of three studies from our Institute.

1. Gisela Dellamora-Ortiz, Claudio H. Ortiz and Anita D. Panek. Multiple Forms of Trehalase in Saccharomyces cerevisiae.

The apparent molecular weights of the cryptic and active forms of trehalase from Saccharomyces cerevisiae were determined after partial purification of the enzymes. Centrifugation on a glycerol density gradient indicated multiple forms of cryptic trehalase, with Mr of 320000, 160000, and 80000, which could correspond to tetramer, dimer and monomer of the inactive form of the enzyme. After activation of each of these forms by

cAMP-dependent protein kinase, only a single dimeric form of active trehalase, with a Mr of 160000, was observed. On DEAE-cellulose chromatography two peaks of active trehalase were obtained. Isoelectrofocusing on polyacrylamide gels also resulted in two separate activities of trehalase, which were both produced by phosphorylation (activation) of cryptic trehalase by cAMP-dependent protein kinase. On the other hand, cryptic trehalase can be phosphorylated by cAMP-independent protein kinases, without alteration of the inactive state of the enzyme. These findings indicate the existence of cryptic and active forms of trehalase which show different degrees of phosphorylation, indicating multiple phosphorylation sites for cAMP independent and dependent protein kinases.

This work was supported by grants from CNPq, FINEP and PADCT.

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2. Ana C. Panek, E. Bernardes and Anita D. Panek. Does Trehalose Play a Role in Yeast Cells Under Stress?

Stability and viability of yeast cells are important parameters in fermentation processes and in baker's yeast technology. Trehalose has been defined over the years, as a storage carbohydrate in Saccharomyces cerevisiae. We have studied the metabolism of this disaccharide in various conditions leading to stress. During growth of cells on glucose, trehalose accumulates only after the depletion of the carbon source. A requisite for high levels of the disaccharide, under these conditions, is the expression of a MAL gene. However, in response to nitrogen starvation yeast cells store trehalose in the presence of glucose. Spore formation is another response to stress. Diploid cells were constructed by mating various mutants. Wild type, as well as heterozygous diploids sporulate normally, whereas homozygous diploids for very low trehalose 6-phosphate activity (fdp/fdp) store insignificant amounts of trehalose and sporulation efficiency drops to 5%. Out of the very few spores obtained only an average of 16% germinates and no 4-spore tetrads are formed. Not even the high levels of glycogen found in such diploids can reverse the effect of a reduced trehalose synthase activity. On the other hand, when diploid cells homozygous for a defect in cAMP-dependent protein kinase were analyzed (glc1/glc1), glycogen accumulation was reduced to 40% and no spore formation could be detected whatsoever in spite of the presence of high trehalose levels. Therefore, we may conclude that trehalose affects sporulation and is an absolute requisite for germination while glycogen is essential for sporulation and does not replace trehalose in the germination step. This observation and the fact that no complete tetrads were formed led us to investigate the role of trehalose in guaranteeing viability of cells under the stress of nutrient deprivation. Diploid and haploid strains were starved for 4 to 6 weeks at 4°C, 26°C and 37°C because these are conditions which prevail during production and storage of baker's yeast, as well as, during the recycling of cells in the fermentation processes leading to alcohol formation. The results obtained indicate that strains with very low trehalose-6-phosphate synthase activity are far more fragile during starvation. On the other hand, high synthesising activity correlates well with longevity even in mutants with an elevated level of trehalase. Considering the well established role of trehalose during the formation of buds, its function in protecting cells during starvation and

the fact that the disaccharide can be found loosely bound to the yeast membrane, we are led to envisage the possibility that trehalose acts not only as a storage carbohydrate but also as a stabilizer of the cell membrane.

Presented at the Symposium on Anhydrous Biology held in Bellagio, Italy in October 1985.

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3. Ana C. Panek, Gisela Dellamora-Ortiz, Maria N. Tenan, Vania F. Paschoalin and Anita D. Panek. A New Gene Involved in Trehalose Metabolism.

Various genes involved in the regulation of metabolism of storage carbohydrates in yeast have been described. They have mostly been associated with the regulation of cyclic-AMP dependent protein kinase (GLC4, BCY1). Other genes associated with the protein dephosphorylation step of enzymes also affect trehalose metabolism (GLC1, FDP, CIF).

We have now identified a new group of dominant genes (TRD) involved in the regulation of trehalase activity. Strains harbouring a lesion in one of these genes exhibit very high levels of trehalase activity which cannot be further increased by cyclic-AMP. In these mutants trehalose-6-phosphate synthase activity is not affected and the phenotype for glycogen is normal. Therefore, *trd* mutants do not accumulate trehalose in non-proliferating conditions due to the loss of one of the regulatory mechanisms to which the enzyme trehalase is subject.

This work was supported by grants from CNPq, FINEP and PADCT.

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XVIII. Departamento Interfacultativo de Bioquímica, Universidad de Oviedo, 33071 Oviedo, Spain. Communicated by S. Ramos.

E. Valle, L. Bergillos, S. Gascón, F. Parra and S. Ramos. Trehalase activation in yeasts is mediated by an internal acidification. Submitted to Eur. J. Biochem.

Summary: It has been reported in yeasts that the addition of glucose, uncouplers and nystatin to cells grown in a sugar free medium causes trehalase activation and, it has been postulated that this activation might be mediated by the depolarization of the plasma membrane. In this article the values of membrane potential and pH gradient across the plasma membrane of *Saccharomyces cerevisiae* have been determined under the same condition in which trehalase is activated. Membrane potential was evaluated from the distribution of triphenylmethylphosphonium, and the pH gradient from the distribution of benzoic acid across the plasma membrane. When the effect of several agents on the two components of the electrochemical proton gradient across the plasma membrane of ethanol-grown yeast cells was studied, it was observed that under trehalase activation conditions: i) the addition of glucose activated trehalase and caused internal acidification of the cells, but had practically no effect on the membrane potential, ii) the addition of 200 mM KCl depolarized the cell membrane but did not affect

the internal pH or trehalase activity, iii) although CCCP depolarized the cells at external pH 6.0 and 7.0, it only activated trehalase at an external pH of 6.0, leading to the acidification of the internal medium at this pH, iv) nystatin caused an increase in the triphenylmethylphosphonium accumulation at external pH 6.0 and 7.0, but only activated trehalase at external pH 6.0, causing acidification of the cell interior at this pH, v) activation of trehalase was also observed when the internal acidification was caused by addition of a weak acid such as acetate. It is concluded that trehalase activation is mediated by an intracellular acidification and is independent of the membrane potential.

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A recent publication from this laboratory is:

S. Ramos, P. de la Peña, E. Valle, L. Bergillos, F. Parra & P.S. Lazo. 1985. Coupling of Protons and Potassium Gradients in Yeast, In: Environmental Regulation of Microbial Metabolism. (J.S. Kulaev, E.A. Dawes, & L.W. Tempest eds.) Academic Press.

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XIX. Zymology Department, School of Biological Sciences, University of Bath, Claverton Down, Bath, BA2 7AY, England. Communicated by Anthony H. Rose.

The following are summaries of two papers from this laboratory which will appear in the Journal of General Microbiology in early 1986.

1. Transport of Sulphur Dioxide by Saccharomyces cerevisiae by Malcolm Stratford and Anthony H. Rose.

Transport of SO_2 by Saccharomyces cerevisiae in a medium containing 100 mM glucose and 1 mM [^{35}S] sulphite was initially rapid. Net accumulation ceased after 5 min, but at this time [^{35}S] sulphite was still transported by the cells and could be washed out to an extent that depended on the wash volume. Pre-incubation in the absence of glucose, and omitting glucose from the reaction mixture, had no effect on initial velocity of sulphite accumulation, although it decreased the total amount accumulated. Initial velocity of accumulation was also unchanged when organisms were pre-incubated in the presence of 2-deoxy-D-glucose and this inhibitor was included in the reaction mixture. Initial velocity of sulphite accumulation decreased logarithmically as the pH value of the suspension was increased from 3.0 to 5.0; the decrease closely paralleled the decline in concentration of molecular SO_2 over the pH range. Woolf-Hofstee plots for accumulation of SO_2 , at pH 3.0 or 4.0, gave near vertical plots. Raising the temperature from 19°C to 39°C increased the initial velocity of SO_2 accumulation. The initial velocity of transport was not affected by pre-treatment of organisms with carbonyl cyanide *m*-chlorophenylhydrazone, DNP or iodoacetamide, but pre-treatment with 20 mM uranyl nitrate increased the initial velocity almost three-fold. It is concluded that SO_2 is transported into S. cerevisiae by simple diffusion.

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2. Ethanol Dissipates the Proton - Motive Force Across the Plasma Membrane of Saccharomyces cerevisiae by Charles P. Cartwright, Jean-Regis Juroszek, Michael J. Beavan, Fiona M.S. Ruby, Sonia M.F. DeMorais and Anthony H. Rose.

Populations of Saccharomyces cerevisiae NCYC 431, harvested after 16-h incubation from self-induced anaerobic cultures, were more tolerant to the inhibitory effect of ethanol on fermentation rate and viability than organisms harvested from 8-h cultures. Ethanol increased the rate of passive influx of protons into de-energized organisms at a rate which was greater with organisms from 8-h compared with 16-h cultures. Rates of passive influx of protons into spheroplasts were significantly greater than into intact organisms, although culture age did not affect rates of ethanol-induced influx of protons into spheroplasts. Ethanol retarded both the initial net rate of proton efflux and the final extent of acidification produced by suspensions of energized organisms, both effects being more pronounced with organisms from 8-h as compared with 16-h cultures. The magnitude of the proton-motive force (p) was decreased by ethanol in both energized and de-energized organisms. Although culture age did not affect the extent of ethanol-induced decrease in p in de-energized organisms, in energized organisms harvested from 8-h cultures ethanol produced a significantly greater decrease in p as compared with organisms from 16-h cultures. If the ability of ethanol to decrease the p value is important in its inhibitory effect on growth, it is suggested that some phenomenon other than proton uncoupling is involved.

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- XX. Instituto de Investigaciones Biomedicas. Facultad de Medicina de La Universidad Autonoma. Arzobispo Morcillo No4. Madrid 28029. Spain. Communicated by Juana M.Gancedo.

The following papers have been published recently:

1. Pilar Eraso, Maria J. Mazón and Juana M. Gancedo. 1984. Pitfalls in the measurement of membrane potential in yeast cells using tetraphenylphosphonium. Biochim. Biophys. Acta 778:516-520.

The uptake of the lipophilic cation tetraphenylphosphonium (Ph_4P^+) by Saccharomyces cerevisiae was measured using yeast grown on glucose and harvested either at the logarithmic or at the stationary phase of growth. When yeast was collected at the stationary phase, Ph_4P^+ uptake proceeded steadily during several hours until an equilibrium was reached. When yeast was collected in the logarithmic phase of growth, a biphasic uptake was observed. The second phase of uptake began when the glucose of the incubation medium had been exhausted. From experiments in the presence of cycloheximide or chloramphenicol it is concluded that the second phase of Ph_4P^+ uptake is dependent on the synthesis of some protein(s) represented by glucose but unrelated with the existence of functional mitochondria. The addition of compounds which collapse the membrane potential provokes an efflux from the yeast cells of the Ph_4P^+ accumulated both during the first phase and the second phase of uptake. It is concluded that accumulation of Ph_4P^+ in yeast cells is a complex process and that Ph_4P^+ cannot be used to give a quantitative measure of the yeast plasma membrane potential.

2. Pilar Eraso and Juana M. Gancedo. 1984. Catabolite repression in yeasts is not associated with low levels of cAMP. *Eur. J. Biochem.* 141:195-198.

The relationship between levels of cAMP and catabolite repression in yeasts has been investigated. Strains of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Kluyveromyces fragilis* were used. The yeasts were grown on different carbon sources to attain various degrees of repression. Galactose repressed as much as glucose, while maltose was less effective. Full derepression was achieved with ethanol.

The enzymes tested were fructose-bisphosphatase, malate dehydrogenase, glutamate dehydrogenase (NAD dependent), cytochrome oxidase and isocitrate lyase (this last enzyme was found to be absent in *Schizosaccharomyces*).

The levels of cAMP were 2-3 times higher in the repressed conditions than in the derepressed ones. It is therefore concluded that in yeasts catabolite repression is not mediated by a lowering of the intracellular concentration of cAMP.

3. Carlos Gancedo and Juana M. Gancedo. 1985. Phosphorylation of 3-O-methyl-D-glucose and catabolite repression in yeast. *Eur. J. Biochem.* 148:593-597.

The glucose analog, 3-O-methyl-D-glucose, inhibited growth of yeast on non-fermentable carbon sources. The sugar was phosphorylated by the yeast and also "in vitro" by a commercial preparation of yeast hexokinase. The chromatographic behaviour of the phosphorylated product was identical in both cases. This suggests that 3-O-methyl-D-glucose is phosphorylated to form 3-O-methyl-D-glucose 6-phosphate. The inhibition of the growth appears to be due to interference with the derepression of several enzymes necessary to grow on nonfermentable carbon sources. Spontaneous mutants whose growth was unaffected by 3-O-methyl-D-glucose were isolated. In these mutants there was no significant accumulation of the phosphorylated ester and the derepression of the enzymes tested was not affected by the glucose analog.

4. Juana M. Gancedo, Maria J. Mazón and Pilar Eraso. 1985. Biological roles of cAMP: similarities and differences between organisms. *TIBS* 10, 210-212.

The mode of action of cAMP is completely different in eukaryotes and in prokaryotes, although the proteins which bind cAMP in both cases probably have a common evolutionary origin. Some patterns of regulation through cAMP have been conserved among very different organisms but there are cases where the role of cAMP changes widely from one type of cell to the other. In particular, cAMP cannot be considered a universal "hunger" signal.

5. Carlos Gancedo and Marco A. Delgado. 1984. Isolation and characterization of a mutant from *Saccharomyces cerevisiae* lacking fructose 1, 6-bisphosphatase. *Eur. J. Biochem.* 139, 651-655.

Mutants lacking fructose 1, 6-bisphosphatase activity have been isolated from *Saccharomyces cerevisiae* and genetically purified. Mutants

were unable to grow on gluconeogenic carbon sources. Revertants that grew on glycerol have regained the fructose 1, 6-bisphosphatase activity, showing that there is no bypass in yeast for this enzymatic step. No significant differences were found in the growth of the mutants and the parental strains in other carbon sources. Other mutants lacking fructose-1, 6-bisphosphatase activity but pleiotropically affected in the derepression of several enzymes sensitive to catabolite repression were also isolated. All the mutants isolated were of nuclear origin and defined three complementation groups.

6. Francisco Portillo and Carlos Gancedo. 1984. Mode of action of miconazole on yeasts: inhibition of the mitochondrial ATPase. *Eur. J. Biochem.* 143, 273-276.

Miconazole ([1-{2-(2,3 dichlorophenyl)-2-(2,4 dichlorophenyl)methoxy] ethyl}-1 H-imidazole) completely inhibited growth of *Saccharomyces cerevisiae* and *Candida albicans* on glycerol at 10 μ M. 50 μ M miconazole was needed to achieve the same effect during growth on glucose. Miconazole inhibited competitively the mitochondrial ATPase of *S. cerevisiae* with a K_i of 1 μ M. F_1 activity of the enzyme was not affected. Mutants resistant to miconazole were isolated. The ATPase of these mutants was resistant to 10 μ M miconazole. Higher concentrations of miconazole inhibited the ATPase of the plasma membrane. The inhibition of the *S. cerevisiae* enzyme was competitive with a K_i of 50 μ M. The results point to the mitochondrial ATPase as the primary target of miconazole action at least during growth on non-fermentable carbon sources.

7. Francisco Portillo and Carlos Gancedo. 1985. Mitochondrial resistance to miconazole in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 199, 495-499.

One mutant of mitochondrial origin resistant to miconazole has been isolated and characterized in *S. cerevisiae*. The mutation is linked to the locus *ol1*, the structural gene for subunit 9 at ATPase on mitochondrial DNA. Miconazole inhibited the mitochondrial ATPase on mitochondrial DNA. Miconazole inhibited the mitochondrial ATPase of the wild type while the enzyme of the resistant mutant was insensitive to his effect. Levels of ATP decreased to one-third of the control in the wild type in the presence of miconazole, while they were unaffected in the mutant.

8. Rosa M. P. Castellanos and Maria J. Mazón. 1985. Identification of phosphotyrosine in yeast proteins and of a protein tyrosine kinase associated with the plasma membrane. *J. Biol. Chem.* 260, 8240-8242.

³²P Phosphotyrosine was detected in a hydrolysate of yeast proteins after "in vivo" labeling with ³²P phosphoric acid. The phosphoamino acid was present in cells exponentially growing on glucose as well as in cells that had reached the stationary phase of growth. Also, a plasma membrane preparation was shown to phosphorylate casein on tyrosine residues.

9. Francisco Portillo and Maria J. Mazón, Activation of yeast plasma membrane ATPase by phorbol ester. *FEBS Letters.* In press.

Addition of 12-O-tetradecanoylphorbol-13-acetate (TPA) to yeast cells produces a two fold activation of the plasma membrane ATPase. The activation is reversible and time and dose dependent. The activated enzyme shows an increased affinity for its substrate, ATP, and its optimum pH is shifted to a more alkaline range. These changes are similar to those observed in the reported activation by glucose. Upon incubation of yeast cells with ^{32}P i an incorporation of radioactivity in a membrane polypeptide of 105 Kd is observed after addition of either glucose or TPA.

10. Jose M. Guijarro and Rosario Lagunas. 1984. Saccharomyces cerevisiae does not accumulate ethanol against a concentration gradient. Journal of Bacteriol. 160, 874-878.

It has been reported that yeast cells accumulate ethanol against a concentration gradient. We initiated a study of the mechanism involved in this phenomenon. However, we found that this accumulation does not occur and the ethanol permeates the yeast cell plasma membrane by simple diffusion. The following evidence supports this conclusion. (i) Uptake and outflow of ethanol in yeast cells followed first-order kinetics and were insensitive to the presence of structural analogs of ethanol, to drastic pH changes, and to the action of reagents of amino and thiol groups. These results strongly suggest that ethanol permeates the yeast cell plasma membrane without involvement of any carrier. (ii) The outflow rate of ethanol seems greater than the ability of this organism to produce ethanol, indicating that intracellular accumulation of ethanol is not possible. (iii) The intracellular concentration of ethanol found was similar to the concentration in culture media in all tested conditions. With the available information, it is difficult to ascertain the reasons for the discrepancy between our results and those previously reported by other authors. However, the inadequacy of the cell-sampling procedure and of the chromatographic conditions used by those authors suggests that the discrepancy may be due to artifacts in the measurements of ethanol.

11. Rosario Lagunas and Eulalia Moreno. 1985. The calculation of cellular parameters from the turbidity of yeast cultures may give rise to important errors. FEMS Microbiol. Letters. 29, 335-337.

It is shown that cellular parameters of the yeast culture (e.g. intracellular volume, cellular dry weight, protein content, number of cells, and turbidity) are differently influenced by metabolic changes. Therefore, the cellular parameters change independantly of each other. It is hence concluded that whenever quantitation is required, the values of these parameters should be measured independently and not calculated from the turbidity of the cultures or other parameters, as is often done.

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

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

1. P.M. Bruinenberg, J.P. van Dijken, J.G.Kuenen and W.A. Scheffers. 1985. Critical parameters in the isolation of mitochondria from Candida utilis grown in continuous culture. Journal of General Microbiology 131:1035-1042.
2. P.M. Bruinenberg, J.P. van Dijken, J.G. Kuenen and W.A. Scheffers. 1985. Oxidation of NADH and NADPH by mitochondria from the yeast Candida utilis. Journal of General Microbiology 131:1043-1051.
3. P.M. Bruinenberg, R.Jonker, J.P. van Dijken and W.A. Scheffers. 1985. Utilization of formate as an additional energy source by glucose-limited chemostat cultures of Candida utilis CBS 621 and Saccharomyces cerevisiae CBS 8066. Evidence for the absence of transhydrogenase activity in yeasts. Archives of Microbiology 142:302-306.

The following papers have been accepted for publication:

4. P.M. Bruinenberg, J.P. van Dijken and W.A. Scheffers. A radiorespirometric study on the contribution of the hexose monophosphate pathway to glucose metabolism in Candida utilis CBS 621 grown in glucose-limited chemostat cultures. Journal of General Microbiology, in press.

Abstract

A radiorespirometric study of glucose metabolism in Candida utilis CBS 621 was performed using glucose-limited chemostat cultures growing at a dilution rate of 0.1 h^{-1} with ammonium or nitrate as the nitrogen source. From a steady-state analysis of $^{14}\text{CO}_2$ yields from (1- ^{14}C)- and (6- ^{14}C)-glucose supplied in the medium feed it appeared that during growth with nitrate the flow of glucose through the hexose monophosphate (HMP) pathway was much higher than during growth with ammonium as the nitrogen source. The same phenomenon was apparent from an analysis of the rate of $^{14}\text{CO}_2$ production after administration of small amounts of labeled glucose to samples withdrawn from steady-state cultures. Additionally, these experiments revealed not only that the initial fraction of glucose-6-phosphate routed into the HMP pathway increases, but also that recycling of hexose phosphate via this pathway increases when nitrate is used as the nitrogen source. From a quantitative analysis of the results it is concluded that the contribution of the HMP pathway to glucose metabolism is close to the theoretical minimum required to cover the NADPH requirement for biosynthesis.

5. C. Verduyn, J. Frank, J.P. van Dijken and W.A. Scheffers. Multiple forms of xylose reductase in Pachysolen tannophilus CBS 4044. FEMS Microbiology Letters, in press.

Abstract

Cell-free extracts of xylose-grown Pachysolen tannophilus exhibited xylose reductase activity with both NADPH and NADH. The ratio of the NADPH- and NADH-dependent activities varied with growth conditions. Affinity chromatography of cell-free extracts resulted in a separation of

two xylose reductases. One was active with both NADPH and NADH, the other was specific for NADPH. Apart from this coenzyme specificity the two enzymes also differed in their affinities for xylose and NADPH. The role of the two enzymes in xylose metabolism is discussed in relation to attempts to use Pachysolen tannophilus for the alcoholic fermentation of wood sugars.

Dr. P. M. Bruinenberg has completed his Ph.D. Thesis at the Delft University of Technology. His dissertation is:

6. P.M. Bruinenberg. The NADP(H) Redox Couple in Yeast Metabolism: Fundamental and Applied Aspects. 129 pp. (1985).

Contents:

- I. Introduction
- II. A theoretical analysis of NADPH production and consumption in yeasts.
- III. An enzymic analysis of NADPH production and consumption in Candida utilis CBS 621.
- IV. Utilization of formate as an additional energy source by glucose-limited chemostat cultures of Candida utilis CBS 621 and Saccharomyces cerevisiae CBS 8066. Evidence for the absence of transhydrogenase activity in yeasts.
- V. Critical parameters in the isolation of mitochondria from Candida utilis.
- VI. Oxidation of NADH and HADPH by mitochondria from the yeast Candida utilis.
- VII. A radiorespirometric study on the contribution of the hexose monophosphate pathway to glucose metabolism of Candida utilis CBS 621 grown in glucose-limited chemostat cultures.
- VIII. A comparative radiorespirometric study of glucose metabolism in yeasts.
- IX. The role of redox balances in the anaerobic fermentation of xylose by yeasts.
- X. NADH-aldose reductase: the key to anaerobic alcoholic fermentation of xylose by yeasts.
- XI. Summary.

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XXII. National Research Council, Canada, Division of Biological Sciences, Ottawa, Canada K1A 0R6. Communicated by G.B. Calleja.

Below are abstracts of recent publications from our laboratory.

1. G.B. Calleja, B. Atkinson, D.R. Garrod, P. Hirsch, D. Jenkins, B.F. Johnson, H. Reichenbach, A.H. Rose, B. Schink, B. Vincent, and P.A. Wilderer. 1984. Aggregation: group report. In: Microbial Adhesion and Aggregation (ed. K.C. Marshall) pp. 303-321. Dahlem Konferenzen 1984. Berlin: Springer-Verlag.

Abstract

The report covers the following topics: structures and functions, methods of characterizing an aggregate, mechanisms of movement and of contact, cell-cell communication, abiotic environmental influences, and the implications of being aggregated or dispersed (to the cell and to the experimenter and industry). Microbial systems discussed include flocculation of brewing yeast, aggregation in cellular slime moulds, aggregation in myxobacteria, sex-directed flocculation of fission yeast, artificially provoked flocculation of industrially important microorganisms (brewing yeast, Aspergillus niger, actinomycetes, etc.), sludge-floc formation in waste treatment, and a variety of naturally occurring microcolonies and consortia, including rosettes or stars found in a variety of bacterial species.

2. C.V. Lusena, C.C. Champagne, and G.B. Calleja. 1985. Secretion and export of amylolytic activities in Schwanniomyces alluvius. Can. J. Biochem. Cell Biol. 63:366-371.

We define secretion as the passage from the cytoplasm, across the cell membrane, to the periplasmic space. By contrast, export is the passage across the cell wall into the medium. Operationally we distinguish the two processes by comparing the molecular species in the medium, in whole cells, and in spheroplasts. Two techniques make the task possible: complete spheroplast preparation and detection of activities in bands obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The capability of Schwanniomyces alluvius to export α -amylase during stationary phase gradually increased with continual successive transfers from a slant culture to a liquid medium containing starch until a maximum was reached. Only cells which had developed full capability to export α -amylase were used in these studies. About 1 h after the end of the log phase of growth, α -amylase and glucoamylase start to be exported above constitutive levels and a concentration 10 times the constitutive level is reached 3 h later. Electrophoretic results show that at least three active molecular species of α -amylase appear in the cytoplasm at the end of log phase and that the smaller component (52 000 daltons) is secreted into the periplasm 0.5 h later and starts to be exported 1 h after that. The sequence of events suggests that the larger species are precursors of the 52 000 dalton molecules. Amylolytic activities in the cytoplasm and periplasm in late log phase are not detectable.

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XXIII. Institute of Genetics, University of Parma, Borgo Carissimi 10, I-43100 Parma, Italy. Communicated by Iliana Ferrero.

Below follow summaries of papers published, or in press, or submitted to journals dealing with yeast research, since our last posting of May 16, 1984.

1. Nelson Marmioli. 1985. Sporulation and mitochondrial activity in the dimorphic yeast Endomycopsis capsularis. *Current Genetics* 9:587-595.

Summary. E. capsularis transferred to sporulation medium undergoes two different differentiation processes: formation of a true mycelium and sporulation. Formation of a true mycelium, which occurs during mitotic division, can occur in the absence of 1st respiration and in the presence of a very low level of mitochondrial translation. Sporulation occurring within both specialized and unspecialized structures of the mycelium, which involves meiosis, requires a full level of both mitochondrial protein synthesis and 1st respiration.

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2. Nelson Marmioli and Carl A. Bilinski*. 1985. Partial Restoration of Meiosis in an Apomictic Strain of Saccharomyces cerevisiae: A Model System for Investigation of Nucleomitochondrial Interactions during Sporulation. *Yeast* 1:39-47.

*Production Research Department, Labatt Brewing Co., Ltd, London, Ontario, Canada.

In an apomictic strain of Saccharomyces cerevisiae (ATCC 4117-H2) which undergoes a single nuclear division during sporulation and consequently forms asci containing two uninucleate diploid spores, a study was undertaken to investigate the effects of cultivation in three presporulation media (YPA; YNB; SMM) on nuclear division and ascosporeogenesis in sporulation medium. Comparison of effects of presporulation culture in these media on the number of spores formed per ascus showed that a marked induction (30 ± 4.3 percent) of three- and four-spored asci could occur in sporulation medium following cultivation in a defined YNB medium supplemented with a 1 percent solution of vitamins and containing decreased ammonium sulphate and increased glucose levels. Experiments in which the concentrations of glucose and of ammonium sulphate were varied simultaneously indicated that the initial presporulation carbon to nitrogen source ratio is an important factor in determining tetrad formation in sporulation medium. Nuclear staining demonstrated two classes of asci: binucleate (one- and two-spored) and tetranucleate (three- and four-spored). Genetic evidence and data concerning effects of inclusion in sporulation medium of a meiotic inhibitor (glucose) indicated spores in tetrads were haploid rather than diploid. This ability to condition a significant number of cells for meiotic rather than apomictic differentiation made possible investigation of effects of mitochondrial inhibitors on both developmental processes simultaneously. It was found possible to selectively inhibit meiotic development by inclusion in sporulation medium of appropriate concentrations of specific inhibitors. Moreover, the data suggest meiotic sporulation is more strictly dependent than apomictic sporulation on mitochondrial function.

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3. Nelson Marmioli, Filomeno Tedeschi*, Giorgio Truzzi**, Corrado Ferrari* and Pier-Paolo Puglisi. 1985. Relationship between

Growth Inhibition and Mitochondrial Function in Petite-Negative Yeasts. I Effects of Antibiotics and Dyes upon Pathogenic and Non-Pathogenic Candida Species.

*Institute of Morbid Anatomy, University of Parma, **Suzzara Civic Hospital, Mantova, Italy.

Antibiotics and dyes which preclude growth of Saccharomyces cerevisiae in media containing oxidizable carbon sources arrested the growth of Candida albicans, Candida tropicalis and Candida utilis even in glucose medium. The growth in the presence of sub-inhibitory concentrations of the various antibiotics and dyes determined a reduction in the cell survival but with no accumulation of respiratory deficient mutants. Under these culture conditions, the total respiration declined leaving a residual antimycin A-resistant - hydroxamate-sensitive O_2 uptake, and the amount of the respiratory cytochromes aa3 and b synthesized was reduced. SDS gel electrophoresis of soluble proteins prepared from the antibiotic-treated cells showed some bands in the MW range 92-100 K, which became faint after the cells were grown in the presence of some mitochondrial inhibitors. The ultrastructural analysis of these cells evidenced disappearance of the mitochondrial cristae and their replacement by unfolded membranes.

The data obtained suggest that the petite negative trait of Candida could depend on the non-viability or on the very low viability of those cells which have lost their mitochondrial function.

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4. Nelson Marmioli, Filomeno Tedeschi², Maria-Agnese Sabatini², Giorgio Truzzi³, Corrado Ferrari² and Pier-Paolo Puglisi. 1985. Relationship between Growth Inhibition and Mitochondrial Function in Petite-Negative Yeasts. II. Effects of Central Nervous System Drugs upon Pathogenic and Non-Pathogenic Candida Species.

²Institute of Morbid Anatomy, University of Parma, Italy.

³Suzzara Civic Hospital, Mantova, Italy.

Six nervous system drugs which inhibited vegetative reproduction of S. cerevisiae arrested also mitotic division of C. utilis, C. albicans and C. tropicalis. Chlorpromazine and chlorpheniramine which proved to be the most effective, affected respiration and cytochrome biosynthesis. Electrophoretic bands with MW \approx 100 K were faint in silver-stained electrophoregrams of proteins from cells grown in the presence of a sub-inhibitory concentration of chlorpromazine.

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5. Marmioli, N., Tedeschi, F.¹, Ferrari, C.¹, and Puglisi, P.P. 1985. Sporulation of Saccharomyces cerevisiae in the Absence of Mitochondrial Translation is Arrested Before Completion of Meiosis I. Ultrastructural Analysis of the Nuclear and Cytosolic Variations. *Microbiologica* 8:233-246.

¹Institute of Morbid Anatomy, University of Parma - 43100 Parma, Italy.

Summary. Upon transfer in sporulation medium, diploid cells of the yeast Saccharomyces cerevisiae undergo a series of developmental events which lead to meiosis and ascospore formation. When the inhibitor of mitochondrial protein synthesis, erythromycin, was added to the sporulation medium within the first 2-4 h after the transfer, the meiotic development was arrested between intragenic and intergenic recombination (Marmioli et al., 1983). The ultrastructural analysis reported here has shown that in the absence of mitochondrial protein synthesis, cells formed axial cores (leptotene), synaptonemal complex and polycomplexes (zygotene-pachytene) and the sometimes two spindle pole bodies which remained side-by-side on the nuclear envelope (diplotene-diakinesis) are formed, but after this the cells did not complete the first meiotic division. The ultrastructural analysis also evidenced that the mitochondrial internal architecture was maintained in the erythromycin treated sporulation culture. The nucleolus of cells exposed to the sporulation medium in the presence of erythromycin became condensed, cap-shaped with associated dense bodies and laid on the nuclear envelope as the typical diplotene nucleolus. The inhibition of sporulation by erythromycin was only partial when the drug was added 4 h after the transfer to the sporulation medium and normal sporulation was re-established when the drug was added after 8 h and thereafter.

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6. Already reported as 'in press' in our posting of May 16th, 1984 (Yeast Newsletter 33: p. 76).

Lodi, T., Viola, A.M., Rossi, C. and Ferrero, I. 1985. Antimycin A- and hydroxamate-insensitive respiration in yeasts. Antonie van Leeuwenhoek 51:57-64.

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7. I. Ferrero, R. Rambaldelli, A.M. Genga, C. Donnini, and P.P. Puglisi. 1984. ALG/alg: a single gene controlling the utilization of lactate in the presence of antimycin in the yeast Saccharomyces cerevisiae. Current Genetics 8:407-411. (see Yeast Newsletter 33: p. 75 for abstract).

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8. Already reported with a different title in our posting of May 16, 1984 (see Yeast Newsletter 33: p. 77 for abstract).

N. Marmioli and T. Lodi. 1984. Modification of nuclear gene expression by inhibition of mitochondrial translation during sporulation in MAT α /MAT α diploids of Saccharomyces cerevisiae. Mol Gen Genet 198:69-74.

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9. Claudia Donnini, Nadia Artoni, Lorella Bonelli, and Nelson Marmioli. 1985. Germination of Saccharomyces cerevisiae Ascospores in the Presence of Fermentable and Non-Fermentable Substrates: Comparison of Landmark Events and Macromolecular Synthesis. Submitted to Journal of Bacteriology.

Abstract. Germination of *S. cerevisiae* ascospores can occur in the presence of non-fermentable carbon sources: glycerol, ethanol, acetate, pyruvate, succinate, since they maintain a functional respiratory chain during sporulation. During germination on acetate, nuclear division, DNA, RNA, and protein synthesis occurred at a lower rate than on glucose and the whole process proceeded slowly. Appearance and curtailment of individual proteins were observed during germination on glucose and similar modifications were found early during germination on acetate, even though differences between acetate and glucose became evident during the outgrowth phase. Mitochondrial inhibitors did not affect germination and protein synthesis on glucose but did arrest germination and protein synthesis on acetate and other non-fermentable substrates.

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10. Anna Maria Viola, Tiziana Bortesi, Rita Pizzigoni, P.P. Puglisi, Paola Goffrini and Iliana Ferrero. 1985. The Respiratory Activities of Four *Hansenula* Species. Accepted by Antonie van Leeuwenhoek.

Abstract. The respiratory activities and the cytochrome spectra from four species belonging to the genus *Hansenula* have been analysed. The results obtained and described in this paper show that: *H. glucozyma* possesses only the primary, antimycin A-sensitive respiration, *H. anomala* and *H. californica* possess primary and secondary (salicyl-hydroxamate-sensitive) respirations, whereas *H. saturnus* possesses three respiratory activities (AA-sensitive, SHAM-sensitive, and AA+SHAM-insensitive), as schematically represented in Scheme 1. The respiratory activity of *H. glucozyma* is glucose-repressible, whereas the activities of the other three species are not. In addition, antimycin A (AA) and/or erythromycin (ERY) in the culture media differently inhibit(s) the growth of the four species and regulate(s) the respiratory pathway(s) in the species analysed.

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11. A.A. Algeri, P. Goffrini, L. Piana, E. Cavazzoni, I. Ferrero. 1985. The Genetic System Controlling the Growth on Glucose and Antimycin A and the Glucose-Catabolite Repression in *Kluyveromyces lactis*. I. The AGG/agg gene. Submitted to *Current Genetics*.

Summary

By means of genetic and biochemical techniques it has been determined the existence in *Kluyveromyces lactis* of nuclear gene AGG the dominant allele of which controls two phenotypes; i.e., growth on glucose and antimycin A and catabolite repression of the nucleo-cytoplasmic enzymes β -galactosidase and NAD-glutamate dehydrogenase.

On the other hand, catabolite repression of both enzymes has been observed also in the presence of the recessive agg allele, suggesting the existence of a complex genetic system for the regulation of catabolite repression in petite-negative yeast *K. lactis*, to which the AGG gene belongs.

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XXIV. Institute of Microbiology, Czechoslovak Academy of Sciences,
Videňská 1083, CS-142 20 Prague 4, Czechoslovakia. Communicated
by J. Vorišek.

The following is an abstract of my recent work. Its publication is in press:

Ultracytochemical localization of X-prolyl-dipeptidyl (amino)-peptidase in microglobules and endoplasmic membranes accumulated in pep4-3 mutants of *Saccharomyces cerevisiae*. Histochemistry (in press)

Summary: The ultracytochemical localization of X-prolyl-dipeptidyl(amino)peptidase (DPP) activity was studied in a late exponential culture of a haploid (α) wild-type strain of *Saccharomyces cerevisiae* and its pep4-3 mutant. Yeast cells were fixed for 20 min in cold 1% glutaraldehyde buffered with 50 mM TES buffer to pH 7.0 and then incubated for 80 min with 1.2 mM L-alanyl-L-proline-4-methoxy-2-naphthylamide (Ala-Pro-MNA) or Lys-Pro-MNA as cytochemical substrates plus 0.06% hexazonium p-rosaniline (HPR) buffered with 160 mM cacodylate to pH 7.0. The osmiophilic azoindoxyl complex was formed by coupling HPR with MNA liberated by DPP activity and was then osmicated during an overnight post-fixation of cells in cold 1% OsO₄. In the wild-type strain, conspicuous deposits of DPP reaction product were observed in vacuolar membranes. When compared with the parent strain, the pep4-3 mutant cells were enriched in endoplasmic reticulum (ER), cytoplasmic lipoprotein, and microcompartments: membranous vesicles and microglobules. In the mutant, DPP reaction product was found in about 50% of non-vacuolated cells at the following sites: the nuclear envelope, polar layers of ER sheets and of membranous vesicles (diameter, 40-90 nm), the surface or the lumen of these vesicles, the cytoplasmic membrane (under some bud scars) and the periplasmic space. The largest amount of reaction product was found in microglobules (diameter, 20-50 nm) that were mainly observed in the cytoplasmic matrix but were also present in nuclei (nucleoli) and mitochondria. These microglobules had a single-line boundary and appeared to be composed of lipoprotein. The surface ultrastructure of sectioned microglobules in the cytoplasmic matrix was similar to that of the coated vesicles found in mammalian cells. Only sparse amounts of DPP reaction product were seen in budding yeast. In all pep4-3 cells with electron-lucent vacuoles, the reaction product was confined to the vacuolar membranes (i.e., homologous to the ER), microglobules and the periplasmic space. Polysaccharides with free vic-groups were shown by the cytochemical reaction to be present on the surface of ER membranes, in microglobules, in the periplasmic space and in the cell wall. Our cytochemical results indicate that microglobules participate in the exocytosis of both DPP and glycoproteins, as well as, revealing new features of vacuolar morphogenesis in yeast.

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XXV. Biochemisch Laboratorium, Vrije Universiteit de Boelelaan 1083,
1081 HV Amsterdam, The Netherlands. Communicated by J. Klootwijk.

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

1. Annemarie E. Kempers-Veenstra, Wouter Musters, Albert Dekker, Jacobus Klootwijk and Rudi J. Planta. Deletion mapping of the yeast Pol I promoter. *Current Genetics*, in press.

Abstract

Deletions in the promoter region for the 37S pre-rRNA operon in yeast were constructed and analysed in vivo using an artificial ribosomal minigene present on an extrachromosomal yeast vector. Sequences required for correct transcription initiation were found to be located between positions -192 and +15 relative to the start; a 5'-deletion down to position -133 reduces the transcription yield of the minigene at least five-fold.

To allow detection of transcription of the minigene in isolated nuclei of yeast transformed with a minigene-bearing plasmid we attempted to increase the minigene copy number. The transcription yield in vivo appeared not to be proportional to the copy number but was found to be greatly enhanced when two or three minigenes are present in tandem. α -Amanitin sensitivity of transcription of these minigenes in isolated nuclei proved that RNA polymerase I is responsible for their transcription.

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2. Elisabetta Balzi, Attilio DiPietro, Andre Goffeau, Harm van Heerikhuizen and Jacobus Klootwijk. The RNA polymerase I initiation site and the external transcribed spacer of the fission yeast Schizosaccharomyces pombe ribosomal RNA genes. *Gene*, in press.

Abstract

A 5.45-kb fragment containing the 5' end of the ribosomal RNA transcriptional unit from the fission yeast Schizosaccharomyces pombe was cloned in the yeast-*E. coli* shuttle vector YEp13. The transcription initiation start point was mapped by R looping and S1 nuclease protection. The sequence of the entire external transcribed spacer (ETS) and its flanking regions was determined. Comparison of the sequence around the transcription start point with those of four budding yeasts (*Saccharomycetoideae*) reveals a consensus sequence from position -9 to -4 from the start. This sequence is likely to be an important element of the promoter for yeast RNA polymerase I. Comparison of all known RNA polymerase I promoter sequences reveals a strong bias for nucleotides at several positions between -16 and +10. These nucleotides may have a critical role in the transcription initiation process. The *S. pombe* ETS, which comprises 1361 bp, is significantly longer than those of the budding yeasts and lacks any significant sequence homology with the *Saccharomyces cerevisiae* ETS. R-loop analysis reveals a putative processing site within the ETS of *S. pombe*.

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3. Harm van Heerikhuizen, Adrie Ykema, Jacobus Klootwijk, Claude Gaillardin, Christine Ballas and Philippe Fournier. Heterogeneity

in the ribosomal DNA family of the yeast Yarrowia lipolytica.
Molecular cloning and analysis of two different repeated units.
Gene, in press.

Abstract

Southern blotting of DNA from the ascomycetous yeast Yarrowia lipolytica revealed two major size classes of DNA units coding for rRNAs, which differ in length by about 1000 bp. We have cloned an rDNA unit of each size class. R-looping experiments revealed that the ribosomal genes of both units are uninterrupted; subsequent heteroduplex analysis showed that the size difference between both units is located within the nontranscribed spacer. Sequence analysis revealed that a major part of these spacers consists of a complex pattern of repetitions in periodicities of up to about 150 bp and that the difference between both rDNA units are located mainly in this repetitive region. Apart from a different length of the repetitive regions, both rDNA units also reveal extended microheterogeneity within their homologous parts. Furthermore, no gene for 5S rRNA was observed in the spacer region. Therefore the organization of the spacer of Yarrowia rDNA is clearly different from that of Saccharomyces cerevisiae.

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4. L.P. Woudt, J.J. van den Heuvel, M.M.C. van Raamsdonk-Duin, W.H. Mager and R.J. Planta. Correct removal by splicing of a Neurospora intron in yeast. Nucleic Acids Research, in press.

Processing of intron-containing nuclear messenger RNAs in yeast requires an internal conserved sequence (ICS) element, UACUAAC. Similar elements

(ug CU_G^AAC) have been identified in sequences interrupting nuclear genes of the related ascomycete Neurospora crassa. To examine the structural splicing requirements in yeast, we constructed hybrid genes containing the intron of the Neurospora histone H3 gene and cloned them into high copy number yeast vectors. Subsequently we analyzed the RNAs transcribed in yeast from the fusion genes by Northern analysis and primer extended sequencing. It turned out that the Neurospora intron, which contains the sequence element UGCUAAC, can be removed, though very inefficiently, provided that it is located near the 5'-end of the primary transcript. This proves that an A at the second position of the ICS is no absolute requirement for splicing in yeast. In addition, the results indicate that the yeast splicing machinery is intron-position dependent.

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- XXVI. Laboratory of Genetics, Institut National Agronomique, 16 Rue Claude Bernard, 75231 Paris Cedex 05, France. Communicated by H. Heslot.

The following are abstracts of papers from our laboratory that were recently published or are in press.

1. Abstract of a paper accepted for publication in Gene.

H. van Heerikhuizen, A. Ykema, J. Klotwijk, C. Gaillardin, C. Ballas and P. Fournier. "Heterogeneity in the chromosomal DNA family of the yeast Yarrowia lipolytica; I. Molecular cloning and analysis of two different repeated units".

Southern blotting of DNA from the ascomyceteous yeast Yarrowia lipolytica (strain 15901.4) revealed two major size classes of ribosomal DNA units which differ in length by about 1000 bp. We have cloned an rDNA unit of each size class. R-looping experiments revealed that the ribosomal genes of both units are uninterrupted; subsequent heteroduplex analysis showed that the size difference between both units is located within the nontranscribed spacer. Sequence analysis revealed that a major part of these spacers consists of a complex pattern of repetitions in periodicities of up to about 150 bp and that the differences between both rDNA units are located mainly in this repetitive region. Apart from a different length of the repetitive regions, both rDNA units also reveal extended microheterogeneity within their homologous spacer parts. Furthermore, no gene for 5s rDNA was observed in the spacer region. Therefore the organization of the spacer of Yarrowia rDNA is clearly different from that of Saccharomyces cerevisiae.

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2. Abstract of a paper submitted for publication in Gene:

P. Fournier, C. Gaillardin, M. A. Persuy, J. Klotwijk, H. van Heerikhuizen. "Heterogeneity in the ribosomal DNA family of the yeast Yarrowia lipolytica: II. Genomic organization and segregation studies".

The cloned rDNA units of Yarrowia lipolytica (van Heerikhuizen et al. 1985) and their restriction fragments have been used to probe blots of genomic DNA of this yeast. Wild-type and laboratory strains contained 2 to 5 types of repeated units, each strain displaying a specific pattern. Tetrad analysis strongly suggested a clustered organization of each type of repeat. Chromosome loss experiments showed that several rDNA clusters are on the same chromosome. Possible occurrence of meiotic exchanges in the rDNA is discussed.

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3. C. Gaillardin, A. M. Ribet, H. Heslot. "Integrative transformation of yeast Yarrowia lipolytica".

An EcoRI shotgun of Yarrowia lipolytica DNA was inserted into the plasmid YIp333 which carries the LYS2 gene of S. cerevisiae. The resulting plasmid pool was transformed in both S. cerevisiae and Y. lipolytica. Whereas numerous replicating plasmids could be isolated from the S. cerevisiae LYS⁺ transformants, all transformants of Y. lipolytica so far analyzed were found to result from integrative transformation. This occurred at a frequency of 1 to 10 transformants per ug of input DNA. Cotransformation occurred at high frequency and resulted in tandem integration of 2 to 10 copies of the incoming DNA. Structural and segregational stability of the transforming DNA were both high.

4. Abstract of a paper in preparation:

P. Fournier, H. Heslot, R. Goursot, A. Dedieu, S. D. Ehrlich.
"Behaviour of a Staphylococcus aureus plasmid in the yeast
Schizosaccharomyces pombe".

The plasmid pC194, isolated from Staphylococcus aureus, is able to replicate in Bacillus subtilis and Escherichia coli. Goursot et al. (Nature, 1982, 298, 488-490) have shown that this plasmid is able to replicate autonomously in Saccharomyces cerevisiae. Subsequent analysis revealed that this property was due to the presence of two copies of the S. cerevisiae ars consensus on pC194. Here we have investigated if this plasmid, associated with the URA3 gene of S. cerevisiae was able to replicate in Schizosaccharomyces pombe. We found that it behaved as an autonomously replicating plasmid, mitotically unstable but structurally stable, in this yeast. Deletions of the S. cerevisiae ars consensus did not alter this behavior. This is in accordance with the fact that S. cerevisiae ars is not functional in S. pombe, as suggested by our previous studies (Gaillardin et al. Current Genetics, 1983, 7, 245-253). Homology search for consensus sequence between the various deletions and cloned S. pombe ars allowed to tentatively define what could be an ars consensus for this yeast.

5. Abstract of a paper submitted for publication in Biochimie:

J. M. Beckerich, E. Pommies, C. Faivre, M. Lambert and H. Heslot. "Estimation of compartmentation of lysine inside the cells of Yarrowia lipolytica."

Summary. We studied the compartmentation of lysine in vivo by the tracer method of Subramanian et al. (J. Bacteriol. 115, (1973) 284-290). To calculate the size of the different lysine pools and lysine fluxes inside the cell, a mathematical model was designed allowing quantitative estimation of these data from experiment measures. This technique was applied to a lysine accumulating strain 15901.7 and to its low lysine pool derivatives ply-1, ply-4 and ply-9 which were selected for their low polyphosphate pools. In the mother strain 15901.7, it appeared that lysine was stored mainly in a non-cytoplasmic compartment. In the mutants, the efflux from this compartment was higher and accounted for the higher percentage of lysine in the cytoplasm of these strains. The simulation with the calculated parameters fitted very well the experimental curves. This was a good clue for the pertinance of the model. the biological significance of these results are discussed.

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XXVII. Centre National de la Recherche Scientifique, Laboratoire d'Enzymologie, 91190 Gif Sur Yvette, France. Communicated by J. Schwencke.

The following is the summary of a paper of our group which has been recently published.

Jaime F. Angulo, Jaime Schwencke, Patrice L. Moreau, Ethel Moustacchi* and Raymond Devoret. 1985. A yeast protein analogous to *Escherichia coli* RecA protein whose cellular level is enhanced after UV irradiation. *Mol. Gen. Genet.* 201:20-24.

*Institut Curie-Biologie, 75231 Paris Cedex 05, France.

Abstract

In *Saccharomyces cerevisiae*, a protein was recognized by polyclonal antibodies raised against homogeneous *Escherichia coli* K12 RecA protein. The cellular level of the yeast protein called RecAsc (MW 44 kDa, pI 6.3) was transiently enhanced after UV-irradiation. Protease inhibitors were required to minimize degradation of the RecAsc protein during cell lysis. The RecAsc protein exhibited similar basal levels and similar kinetics of increase after UV-irradiation in DNA-repair proficient (RAD^+) strains carrying mitochondrial DNA or not (ρ^0). This was also true for the following DNA-repair deficient (rad^-) strains: *rad2-6 rad6-1 rad52-1*, a triple mutant blocked in three major repair pathways; *rad6-Δ*, a mutant containing an integrative deletion in a gene playing a central role in mutagenesis; and *pso2-1*, a mutant that exhibits a reduced rate of mutagenesis and recombination after exposure to DNA cross-linking agents.

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XXVIII. Rutgers Medical School, Dept. of Microbiology, Busch Campus, Piscataway, New Jersey 08854. Communicated by M.J. Leibowitz.

Below follow summaries of recent work in our laboratory.

1. Lee A. Weinstein and Michael J. Leibowitz. 5S RNA and tRNA-like Molecules are Associated with Killer Virus dsRNA of Yeast. *J. Gen. Virol.* (in press).

Summary

A population of oligonucleotides co-purifies with the double-stranded (ds) RNA genomic segments of the killer virus of *Saccharomyces cerevisiae* during electrophoresis through agarose gels. These smaller RNA molecules must be separated from the viral genome in order to determine the structure of the dsRNA molecules. Sequence analysis of these isolated oligonucleotides shows the population to contain tRNA-like molecules, as well as 5S RNA, which are presumably encoded by the host cell genome.

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2. Ernest M. Hannig and Michael J. Leibowitz. 1985. Structure and expression of the M_2 genomic segment of a type 2 killer virus of yeast. *Nucleic Acids Res.* 13:4379-4400.

Abstract

The M_2 double-stranded (ds) RNA species encodes toxin and resistance functions in *Saccharomyces cerevisiae* strains with the K_2 killer specificity. RNA sequence analysis reveals the presence of a large open

reading frame on the larger heat-cleavage product of M_2 dsRNA, which is translated in vitro to yield a 28 kd polypeptide as a major product. The postulated translation initiator AUG triplet is located within a stem and loop structure near the 5' terminus of the positive strand, which also contains plausible 18S and 5.8S ribosomal RNA binding sites. These features may serve to regulate the translation of the K_2 toxin precursor. The M_1 (from type 1 yeast killers) and M_2 dsRNA species lack extensive sequence homology, although specific features are shared, which may represent structural elements required for gene expression and replication.

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3. Ernest M. Hannig, Michael J. Leibowitz and Reed B. Wickner[†]. 1985. On the Mechanism of Exclusion of M_2 Double-stranded RNA by L-A-E Double-stranded RNA in Saccharomyces cerevisiae. Yeast 1:57-65.

[†]Section on Genetics of Simple Eukaryotes, Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Building 4, Room 116, Bethesda, Maryland 20205, U.S.A.

L-A-E double-stranded RNA (dsRNA), when introduced into cells carrying L-A-H and M_2 dsRNAs, does not eliminate the L-A-H dsRNA, but (i) L-A-E does lower the copy number of L-A-H dramatically and (ii) L-A-E eliminates M_2 dsRNA from the cell. That these two effects of L-A-E are related is shown by the fact that mutants of a strain carrying L-A-H and M_2 selected for their resistance to exclusion of M_2 by L-A-E [effect (ii)] have an altered L-A-H whose copy number is not lowered by L-A-E [effect (i)]. Although the L-A in K_1 strains (L-A-HN in all cases examined) differs significantly both genetically and physically from the L-A in the K_2 strain studied (L-A-H), the L-A-HN from the K_1 strains can maintain M_2 dsRNA, and the L-A-H from the K_2 strains can maintain M_1 dsRNA.

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XXIX. Institute for Genetics and Selection of Industrial Microorganisms, Moscow 113545, USSR. Communicated by G.I. Naumov.

1. Naumov, G.I. Genetic control of toxin formation in Saccharomyces paradoxus CBS 5829 (in press).

Abstract

We have found an unusual type of yeast killer - K_3^+ . According to the latest reidentification this strain (CBS 5829) belongs to S. paradoxus Batschinskaia. K_3^+ yeasts kill killers of two types (K_1^+ , K_2^+), but does not kill sensitive yeasts to the latter. The sensibility to the K_3^+ toxin is determined by the mitochondrial genome and plasmid (KIL-k2).

Adenine deficiency of ade mutants of strain CBS 5829 results in suppression of toxin formation. Influence of ADE prototrophy on toxin formation was proved by white ADE reversions. Using the collection of soil Saccharomyces (Jensen, 1967) producing fertile hybrids with strain CBS 5829 we have determined genetic control of K_3^+ toxin production. New soil

strains did not kill the $K2^+$ killer (M-437) and sensitive yeasts (SBY 2576) and were resistant to the $K3^+$ toxin. Their hybrids with auxotrophic strain CBS 5829 (*ade*) have the K^+ phenotype and monogenic tetrad segregation $2K^+ : 2K^-$. The K^+ property cosegregated with *ADE* prototrophy. This data confirmed suppressive influence of *ade* allele on the toxin formation and allowed the cytoplasmic control of the $K3^+$ phenotype of strain CBS 5829 to be determined. All of the prototrophic segregants, contrary to the prototrophic parents, had the K^+ property. When cells of strain CBS 5829 were treated with ethidium bromide or acriflavine they lost K^+ and ρ^+ properties simultaneously. Therefore, new determinant (*KIL-k3*) is probably located in the mitochondrial genome. No ds RNA and plasmid DNA were detected by electrophoresis in strain CBS 5829.

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The following papers have been published recently:

1. Naumov, G.I., Bashkirova, E.V. On the identification of α -methylglucoside genes in the yeast *Saccharomyces cerevisiae*. Dokl. Akad. Nauk SSSR, 1984, 279, No 6, pp. 1496-1499 (in Russian).
2. Naumov, G.I., Bashkirova, E.V. Comparative genetics of yeast XXII. Detection of α -methylglucoside fermentation by *MAL6^{C2}* and *mal_x* alleles in offsprings of *Saccharomyces cerevisiae* N.C.Y.C. 74. *Genetika*, 1984, 20, No 9, pp. 1472-1478 (in Russian).
3. Shchurov, M.N., Naumov, G.I. Hybridological study of the yeasts *Hansenula anomala* var. *anomala* of different origin. *Biol. Nauki.*, 1984, No 11, pp. 82-87 (in Russian).
4. Vustin, M.M., Naumov, G.I. Anomalous meiosis in hybrids of yeast species of the genus *Williopsis*. *Biol. Nauki*, 1984, No 7, pp. 88-91 (in Russian).
5. Naumov, G.I., Vustin, M.M., Babjeva, I.P. Genetic identification of two species of yeast-like fungi in the genus *Arthroascus* von Arx. Dokl. Acad. Nauk SSSR, 1985, 281, No 6, pp. 1463-1465 (in Russian).
6. Naumov, G.I., Vustin, M.M., Babjeva, I.P., Reshetova, I.S. Addition to the genotaxonomy of the genera *Williopsis* and *Zygowilliopsis*. *Mikrobiologia*, 1985, 54, No 2, pp. 239-244 (in Russian).
7. Naumov, G.I., Shchurov, M.N. Genetic taxonomy of *Hansenula anomala* and *Hansenula subpelliculosa*. *Mikrobiologia*, 1985, 54, No 3, pp. 441-445 (in Russian).

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XXX. Department of Biophysics and Medical Physics, University of California, Berkeley, California 94720⁺ and Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow, G1 1XW, Scotland⁺. Communicated by R.K. Mortimer⁺ and J.R. Johnston⁺.

Recent studies from our laboratories are summarized below:

1. Robert K. Mortimer and John R. Johnston. 1986. Genealogy of Principal Strains of the Yeast Genetic Stock Center. Genetics (in press).

Abstract

We have constructed a genealogy of strain S288C, from which many of the mutant and segregant strains currently used in studies on the genetics and molecular biology of Saccharomyces cerevisiae have been derived. We have determined that its six progenitor strains were EM93, EM126, NRRL YB-210 and the three baking strains Yeast Foam, FLD and LK. We have estimated that approximately 88% of the gene pool of S288C is contributed by strain EM93. The principal ancestral genotypes were those of segregant strains EM93-1C and EM93-3B initially distributed by C.C. Lindegren to several laboratories. We have analysed an isolate of a lyophilized culture of strain EM93 and determined its genotype as MAT α /MAT α SUC2/SUC2 GAL2/gal2 MAL/MAL mel/mel CUP1/cup1 FLO1/flo1. Strain EM93 is therefore the probable origin of genes SUC2, gal2, CUP1 and flo1 of S288C. We give details of the current availability of several of the progenitor strains and propose that this genealogy should be of assistance in elucidating the origins of several types of genetic and molecular heterogeneities in Saccharomyces.

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2. Karyotyping of Laboratory and Industrial Strains of Saccharomyces and of other Yeasts by Orthogonal-Field Alternating Gel Electrophoresis. J.R. Johnston and R.K. Mortimer.

Abstract XIth ISSY Meeting, Lisbon, Portugal, March 17-21, 1986.

The method of OFAGE allows separation and sizing of the chromosomes of Saccharomyces (Carle and Olson, PNAS 82, 3756). We have subjected 32 strains of yeast to analysis by this technique as follows: (1) 7 strains of S. cerevisiae related to X2180-1A (control); (2) NRRL type strains of S. cerevisiae, S. uvarum, S. bayanus, S. carlsbergensis and S. kluyveri (C. Kurtzman, pers. comm.); (3) 13 commercial strains, comprising 6 brewing, 3 distilling, 2 baking and 2 wine yeasts; (4) selected strains of C. utilis, C. albicans, H. wingei, K. lactis, P. canadensis and Schw. occidentalis. All but one of the Saccharomyces strains showed the chromosome band pattern typical of X2180-1A, i.e., many bands within the range 200-1600 kb. However, there is extensive chromosome polymorphism among strains and many strains show unique band patterns. The type strains of the species S. cerevisiae, S. bayanus, S. uvarum and S. carlsbergensis give different karyotypes, most likely because of this polymorphism. The yeasts other than Saccharomyces gave a distinctly different result, that of a few bands representative of larger chromosomes. These yeasts produced bands in the appr. size range 1000-1700 kb. The numbers of bands seen in this range were: C. utilis, 1; C. albicans, 4; H. wingei, 2; K. lactis, 3; P. canadensis, 3; Schw. occidentalis, 4. S. kluyveri showed 3 bands in this range; it apparently belongs to a genus other than Saccharomyces. Y. lipolytica also gave the latter pattern (J. Bassel, pers. comm.); S. pombe and L. lipofer gave no bands. Many of the commercial strains of

Saccharomyces produce numbers and sizes of bands consistent with their diploid, polyploid or aneuploid nature. The relative intensities (from densitometer profiles) of certain bands indicate differences in the number of copies of particular chromosomes carried by specific strains. These profiles may provide a convenient means of karyotyping and identifying particular strains. Experiments to assign bands to known Saccharomyces chromosomes by hybridization with gene probes are in progress.

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3. C. Rebecca Contopoulou, Vincent Cook and Robert K. Mortimer. Analysis of DNA Double Strand Breakage and Repair Using Orthogonal Field Alternating Gel Electrophoresis. In preparation.

Abstract

Orthogonal Field Alternating Gel Electrophoresis (OFAGE) allows separation of DNA molecules in the size range of <100 kb to >3000 kb. These sizes encompass the chromosome sizes of the genome of Saccharomyces cerevisiae. Using this technique, we have found that yeast cells exposed to x-rays generate a smear of DNA fragments corresponding to the products of random, double strand breaks, and that the bands corresponding to unbroken chromosomes decrease in intensity in direct proportion to chromosome size. If exposed wild type cells are permitted time to repair (5 hrs @ 30°C on YPD), the unbroken fragments partially disappear and the chromosome bands reappear at near normal intensity. In certain radiation-sensitive mutants (rad51, rad52, rad54, and rad55), the fragment smear appears following x-ray exposure but no repair of broken chromosomes occurs. In fact, degradation of the fragments occurs; this could appear as partial repair using other procedures.

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4. Jeffery A. Hodgson, David R. Berry and John R. Johnston. 1986. Discrimination by heat and proteinase treatments between flocculent phenotypes conferred on Saccharomyces cerevisiae by the genes FL01 and FL05. J. Gen. Microbiol. (in press).

Summary

The effects of elevated temperature and of digestion with a variety of proteinases on the floc-forming ability of flocculent strains of S. cerevisiae, both genetically defined (FL01 and FL05) laboratory and genetically undefined brewing strains have been determined. This has permitted classification of the flocculent phenotypes of these strains according to criteria other than quantitative grading of flocculence. The flocculent phenotypes conferred by both the FL01 and FL05 genes were found to be irreversibly lost upon pronase, proteinase K, trypsin, and 2-mercaptoethanol treatments. However, the floc-forming ability of cells of the FL01 strain ABXL-1D was destroyed by chymotrypsin digestion and was stable to incubation at 70°C, whereas the floc-forming ability of cells of the FL05 strain ABXR-11A was resistant to the action of chymotrypsin and was heat labile. Tetrad analysis of a cross of these FL01 and FL05 strains indicated that the chymotrypsin and heat sensitivity phenotypes were FLO-gene determined. It appears that expression of the FL01 and FL05 genes

leads to the production of different and characteristic cell-wall proteins underlying their respective flocculent phenotypes.

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XXXI. Universidade de São Paulo, Instituto de Química, Cidade Universitária, Caixa Postal, 20.780, São Paulo, Brasil.
Communicated by Ana Clara Schenberg Frascino.

Below follow summaries of 3 recently completed research topics from our group.

1. Stable Yeast Transformants Secreting Mouse Pancreatic α -amylase: A.C. Schenberg-Frascino, S. Astolfi Filho*, E.V. Galembeck and J.B. Faria.

*Biological Sciences Institute, Universidade de Brasília, Brasília, DF, Brazil.

Mouse pancreatic α -amylase cDNA was isolated from the bacterial plasmid pCEPa15 (1) through HpaII digestion. HindIII ends were generated in the fragments resulting from Ba131 treatment of the HpaII cDNA containing fragment. On the other hand, plasmid p69A, which carries the entire MF α 1 gene coding for the *S. cerevisiae* α mating factor(2), was digested to completion with HindIII and ligated again to remove part of the MF α 1 coding region. This plasmid was linearized with HindIII and then ligated to the α -amylase cDNA fragments. Therefore, we constructed gene fusions consisting of the yeast α -factor promoter and leader region (signal peptide, pro-segment and first spacer peptide coding region) and mouse pancreatic α -amylase cDNA containing fragments. 200 ampicillin resistant bacterial transformants were colony-hybridized to a 32 P-labeled mouse pancreatic α -amylase DNA probe and 46 positive clones were identified. The plasmid DNA pool from these transformants was used to transform α leu2 *S. cerevisiae* strains. Out of 600 LEU⁺ transformants which were analysed on starch containing media, 16 were found to produce haloes of starch hydrolysis after iodine staining of the plates. Upon relaxation of selective pressure, 2 transformants were selected which had integrated plasmid information into the chromosomal DNA of the host cell, as shown by Southern analysis and by meiotic analysis following crosses to LEU2 tester strains. These transformants are extremely stable regarding α -amylase activity (6 halo⁻ among 1824 colonies). Indirect immunofluorescence assays showed that the transformants were surrounded by mouse pancreatic α -amylase cross reacting material. The stable transformants are able to degrade all of the starch after 48h of growth in liquid synthetic medium containing 1% starch. The α -amylase secreted into the medium after 48h was purified by affinity column chromatography and the specific activity was found to be 235U/mg protein. Studies are in progress to determine the molecular nature of the secreted biologically active product. (Supported by CNPq).

(1) Tosi et al., EMBO J. 3: 2809 (1984).

(2) Kurjan, J. and I. Herskowitz, Cell 30: 933 (1982).

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2. Isolation of *Trypanosoma cruzi* DNA Fragments which Function as ARS Elements in *Saccharomyces cerevisiae*. Teixeira, S.M.R.¹, Schenberg Frascino, A.C., Galembek, E.V., Azevedo, M.O.¹ and S. Astolfi Filho¹, ¹Biological Sciences Institute, Universidade de Brasilia, Brasilia, DF.

Genomic banks from *Trypanosoma cruzi* whole cell DNA and kinetoplast DNA were constructed in the Yp328 plasmid, which was obtained by inserting the yeast URA3 gene into the Hind III site of pBR328. The Yp328 vector is by itself unable to be maintained in yeast since it can neither replicate as an extrachromosomal element nor integrate in the chromosome of *ura3-52* yeast hosts. Using this system, sequences from kinetoplast and nuclear DNA were isolated which enable the vector to replicate autonomously in *Saccharomyces cerevisiae*. The cloned DNA fragments fit the description of ARS previously isolated from other eukaryotic organisms.

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3. Mutants of *Saccharomyces cerevisiae* sensitive to 4', 5' monofunctional furocoumarins photoaddition.

Carmen B. Querol, Marcelo R. Vasconcellos, Maria Cristina Garcia, Joao Antonio P. Henriques.

Departamento de Fisiologia, Farmacologia e Biofisica, Instituto de Biociencias, UFRGS, Porto Alegre, Brasil, RS.

The photochemical reaction of 8-Methoxypsoralen (8-MOP) with DNA occurs in two stages. The first involves breakage of the 3, 4 carbon or 4', 5' carbon double-bond to form cyclic addition products with pyrimidine bases. A proportion (25-30%) of all adducts are of the 4', 5' adduct type, which are able to use another photon to react via 3', 4' double-bond with pyrimidine on the opposite strand of DNA to form a crosslink. Both lesions induce lethal, mutagenic and recombinogenic effects in procaryotic and eucaryotic systems. Recently, mutants sensitive to photoaddition of furocoumarins derivatives (*pso*) have been isolated in yeast (Henriques and Moustachi Genetics, 95: 273-284, 1980). Only three of these mutants (*psol-1*, *pso2-1*, *pso3-1*) were analysed in detail. In this work, we describe the phenotypic and genotypic characterization of three new *pso* mutants (43(19), 14(02), 44(20)). They segregate in meiosis like mendelian genes, are recessive and complement each other, as well as the already characterized *pso* mutants. These three mutants are specifically sensitive to photoaddition of monofunctional agent 3-carbethoxypsoralen (3-CPs), which induces only 4', 5' adducts. The dose modifying factor for these mutants when compared to the wild-type strain is about 3.0. However, mutants 14(02) and 44(20) demonstrate a cross-sensitivity to 254 nm UV radiation and to monofunctional nitrogen mustard, respectively. The comparison of exponentially growing cells to stationary phase cells demonstrates that for all mutants the defects in repair capacity of 4', 5' adducts concerns G1 phase cells. The existence of 14(02) mutant suggests that in the wild-type cells the repair of both UV-lesions and 4', 5' adducts share common step(s). The existence of 44(20) mutant indicates that there is a repair process in the wild-type cells that is able to recognize the monofunctional lesions of different molecular nature. The reverse mutation induced by photoaddition of 8-MOP and 3-CPs is increased in the three mutants in

comparison to the wild-type isogenic strain. This implies that the products of the genes of these mutants control an error-free repair process.

Supported by CNPq, CNPq-PIG IV, FAPERGS, PROPESP-UFRGS

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XXXII. Department of Molecular Biology, Odense University, Campusvej 55, DK-5230 Odense M, Denmark. Communicated by Jørgen Friis.

Below follows the abstract of a recently published paper.

Kay Gulløv and Jørgen Friis. 1985. Maintenance and copy number control of ARS1 plasmids in Saccharomyces cerevisiae - Evidence of a mating type effect. Current Genetics 10:21-27.

Summary

Following mating of a and α isogenic haploids we observe that the frequency of plasmid bearing cells, during selective growth, increases three fold. By examining the mitotic stability, the frequency of plasmid bearing cells during the cell cycle and the copy number of ARS1 plasmids in isogenic haploid and diploid cells, we show that the apparent stability of circular ARS1 plasmids in a/ α cells is largely due to a diminished copy number in these cells. This observation is fully comprehensible with the model for plasmid segregation as presented by Murray and Szostak (1983). In order to account for the differences in copy numbers, α and α/α isogenic strains were compared. Likewise a number of mating type nonspecific sterile mutants were compared with the parental Ste⁺ strain. It seems that a diminished copy number is established when the MAT₁/MAT₂ regulatory system (Klar et al., 1981) is switched on, since the effect is observed in Sir⁻ strains only.

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XXXIII. Instituto de Biotecnologia - Universidade de Caxias do Sul - Caixa Postal No. 1352, 95.001, Caxias do Sul, RS, Brasil. Communicated by Juan L. Carrau.

The following are summaries of work going on in our Institute.

1. Carrau, J.L., Azevedo, J.L., and Campbell, D. MB6TC, a new strain for wine making obtained by the fusion of Montrachet (Saccharomyces cerevisiae) and Benda I (Schizosaccharomyces pombe).

Abstract

We have used new methods to recover and stabilise fusion products of industrial yeast strains, substituting the previous introduction of nutritional requirements of the parental strains by the development of special media that permit to recover the fusion products. It has been possible to obtain a genetically stable intergeneric fusion product. The new strain, MB6TC, preserves the capacity of decomposing L-malic acid as in

Schizosaccharomyces and has a growth rate twice that of the parental strain.

It is expected that the new strain will be useful for the control of L-malic acid excess at the vinification of juices obtained from unripened grapes in cold years.

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2. Dillon, A.J.P. and Tavares, F.C.A.
Mitotic recombination in the arg4 locus of Saccharomyces cerevisiae.

Three new alleles of the arg4 - locus (arginine biosynthesis) of Saccharomyces cerevisiae, arg4-66, arg4-93 and arg4-80, were isolated in order to study intragenic mitotic recombination events. Arginine-less media were used to observe mitotic recombination between three heteroallelic pairs: the IG 100 (arg4-66/arg4-93), IG 200 (arg4-93/arg4-80) and IG 300 (arg4-66/arg4-80). In two other heteroallelic pairs, IG 400 (arg4-80/arg4-41) and IG 500 (arg4-93/arg4-41) where arg4-80 and arg4-93 were also paired with arg4-41, an allele with known position in the complementation map. Ultraviolet light in sublethal doses showed non-linear kinetics curves in IG 100 and IG 200 and linear kinetics curves in IG 300, IG 400 and IG 500.

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

The following are summaries of papers accepted for publication in 1985/1986:

1. E. Minárik, V. Kubalova, Z. Šilhárová: Further knowledge on the influence of quantity of yeast inoculum and the inactivator prepared from Botrytis cinerea on the fermentation of musts containing inhibitors (in Slovak). Kvasny prumysl (Prague).

Musts with high sugar concentration or containing inhibitors, e.g., pesticide residues, may be more profoundly and rapidly fermented by using yeast starter amounts, particularly in the presence of the activator from Botrytis cinerea. 5-6% yeast starter (by volume) with a concentration of 10^7 - 10^8 cells/ml and 150-200 mg.l⁻¹ of the activator added prior to fermentation start are recommended. Higher alcohol levels and lower volatile acid concentrations may be achieved in the wines even under unfavourable fermentation conditions.

* * *

2. E. Minárik, Z. Šilhárová, K. Pruša: Yeasts and yeast-like microorganisms of bulk and bottled wines (in Slovak). Kvasny prumysl (Prague).

Ecological studies on yeasts of bulk wines stored in barrels, tanks and cisterns originating from different South Moravian wineries confirmed

the dominance of Saccharomyces oviformis and S. cerevisiae in the yeast flora. In bottled (shelf) wines the osmotolerant and chemoresistant S. bailii var. bailii prevails. The importance of strict principles of hygiene and sanitary measures in the wineries as well as the necessity of sterile bottling of wines containing residual sugar is emphasized.

* * *

3. O. Jungová, E. Minárik: Ecology of yeasts and yeast-like microorganisms of grape vine (in German). Mitteilungen Klosterneuburg (Austria) 1986 (in press).

The yeast flora of different vine organs and unripe berries were investigated during the vegetation period 1980-1982 in two vine regions of Czechoslovakia. The only species identified on vine organs in 1980-1981 was Aureobasidium pullulans. The occurrence of Candida pulcherrima, Kloeckera apiculata and Rhodotorula sp. could be sporadically detected only in the most favourable vintage 1982. No influence of the vine variety on the occurrence and representation of yeasts and yeast-like microorganisms was observed.

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- XXXV. Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Canada, S7N 0W0. Communicated by W.M. (Mike) Ingledew.

I am pleased to announce that Dr. G.P. Casey will be joining our department as an Assistant Professor in May, 1986. Dr. Casey has been working at the Carlsberg Laboratory in Copenhagen on a NATO Postdoctorate Fellowship since fall 1984. He will become an integral part of the Food Biotechnology Group and is expected to continue work in the area of Biotechnology as it relates to the food industry.

* * *

The following papers are in press:

1. Gregory P. Casey, E.C.-H. Chen and W.M. Ingledew. High Gravity Brewing: Production of High Levels of Ethanol Without Excessive Concentrations of Esters and Fusel Alcohols. J. Amer. Soc. Brew. Chem. 43(4): (in press). 1985.

Abstract: The influence of nutritional supplementation and the degree of anaerobiosis on the production of esters and fusel alcohols in very high gravity brewing was examined. Although fermentation in semi-anaerobic conditions led to a drastic reduction in ester production, acceptable ester levels were found under anaerobic conditions where worts were supplemented with lipids and assimilable nitrogen. Such fermentations were rapid, and resultant beers may be organoleptically acceptable to the consumer.

* * *

2. M.L. Kalmokoff and W.M. Ingledew

Evaluation of Ethanol Tolerance in Selected Saccharomyces strains. J. Am. Soc. Brew. Chem. 43(4): (in press). 1985.

Abstract: Methodologies for the determination of ethanol tolerance were compared using four industrial yeasts. Ethanol was found to reduce the rates of growth and of fermentation in a linear fashion. Growth was completely inhibited near 12% ethanol (v/v) whereas only a 50% reduction in fermentative ability occurred at this level. Ethanol was also found to reduce cell viability but only at levels which had completely suppressed growth; the rates of viability loss were strain dependent.

The ability of each strain to produce ethanol was assessed using 30° Plato wort under various nutritional conditions. The strains differed in attenuation and ethanol production, both of which were influenced by nutritional conditions.

Differences in ethanol tolerance found using methods based on growth, viability loss and batch culture performance all may reflect differences in the nutritional requirements and growth history of the yeast strain and the conditions of fermentation not just ethanol tolerance. The method measuring inhibition of fermentative ability may be the best index of ethanol tolerance, as this characteristic is not influenced by nutritional factors or the culture growth phase. It also more closely reflects the upper limits of ethanol production reported in brewing and sake yeasts.

Yeasts within the genus Saccharomyces do not differ extensively in terms of their inherent ability to tolerate ethanol.

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XXXVI. Massey University, Department of Microbiology and Genetics, Palmerston North, New Zealand. Communicated by Roy J. Thornton.

The following research is taking place in my laboratory.

1. Dr. Susan B. Rodriguez (U.G.C. Postdoctoral Research Fellow) has continued her study of malate utilisation by yeasts.
 - (a) A yeast which has accelerated utilisation of malic acid has been identified using the indicator medium reported previously (Yeast Newsletter Vol. 33 #2) and is now under provisional patent.
 - (b) The following is an abstract of a paper submitted for publication:

A pH indicator medium was developed to differentiate yeast of varying malate-utilising capabilities. Representatives of five genera of yeast (Schizosaccharomyces, Zygosaccharomyces, Saccharomyces, Pichia and Pachysolen) covering a broad range of malate utilisation as predicted by the indicator medium, were selected for analysis of malate utilisation by HPLC. In the presence of glucose, Schizosaccharomyces malidevorans was the most efficient malate-utilising yeast. Two yeasts, Pichia stipitis and Pachysolen tannophilus could grow on malate as sole carbon source. Their utilisation of malate in the presence of glucose was greatly repressed. The results of HPLC analysis confirmed the observations made from the indicator medium.

2. Mr. R. Ravji, Dr. S.B. Rodriguez and I have been studying the production of glycerol by four common grape moulds when grown on both free run and pressed white and red grape juices under different conditions of aerobiosis. HPLC was used to follow sugar utilisation and glycerol production over a period of a month. The glycerol concentrations of the grape juices and the dry weight of mycelia have been determined. A paper is in preparation.
3. Miss R. Munster (graduate student) is comparing the production of glycerol by yeasts when grown in grape juice under conditions of (a) constant temperature and (b) constant sugar utilisation. She is also extending a hybridisation programme aimed at developing yeast strains which produce high glycerol levels, e.g. >11 g/L during grape juice fermentation. The effect of SO₂ levels on glycerol formation by yeast is also being investigated.
4. Miss Susan Hornblow (graduate student) is investigating factors affecting cell death and autolysis. The cell death and autolysis of a number of haploid strains descended from a triploid champagne yeast has been studied over a period of months. Haploid strains which died and autolysed relatively rapidly have been hybridised and the cell death and autolytic characters of the diploid strains are now being studied.
5. Mr. N. Wedlock (Ph.D. candidate) is studying Pachysolen tannophilus with a view to improving its ability to ferment pentose sugars from wood hydrolysates for the production of ethanol. A mutant unable to grow on ethanol has been back-crossed with a strain that produces enhanced levels of ethanol and the segregants from these crosses are currently being examined for ethanol production. A series of polyploid strains have been constructed and their ability to ferment pentoses are also being examined. The genetical basis of "flocculent" strains is being investigated and current indications are that the flocculent phenotype is probably under the control of more than one dominant gene.
6. Dr. Susan B. Rodriguez has been appointed Assistant Professor in the Department of Food Science and Technology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, U.S.A. and will take up her appointment in December 1985.
7. I am taking a long overdue sabbatical leave and will be working in Dr. Rodriguez' laboratory (above address) from January through December 1986. I would be pleased to be contacted there by any yeast scientist.

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XXXVII. Ecole Nationale Supérieure Agronomique de Montpellier, Chaire de Génétique et de Microbiologie, 34060 Montpellier Cedex.
Communicated by P. Galzy.

The following summarizes publications of the last two years from my laboratory.

- S. Louis-Eugene, R. Ratomahenina and P. Galzy. 1984.
Reduction enzymatique du diacétyle et de l'acétoïne par une souche de S. uvarum Beijerinck.
Z. Allg. Mikrobiol. 24: 151-159.
- B. Blondin, P. Gondé, R. Ratomahenina, A. Arnaud and P. Galzy. 1984.
A study of cyanide-insensitive respiration in the genus Dekkera and Brettanomyces.
Microbiol. Immunol. (JPN) 28, 637-644.
- P. Gondé, B. Blondin, M. LeClerc, R. Ratomahenina, A. Arnaud and P. Galzy. 1984.
Fermentation of cellodextrins by different yeast strains.
Appl. Environ. Microbiol. (USA), 48: 265-269.
- P. Gondé, B. Blondin, R. Ratomahenina, A. Arnaud and P. Galzy. 1984.
Cellobiose fermentation by several yeast strains.
Third European Congress on Biotechnology (Verlag Chemie - Dechema): Basic organic chemicals - Raw Materials - Bioprocesses (DEU). 2:15-20.
- A.M. Bajon, J.P. Guiraud et P. Galzy. 1984.
Isolation of an inulinase derepressed mutant of Pichia polymorpha for the production of fructose.
Biotechnol. Bioeng. 26:128.
- J. Sanchez, J.P. Guiraud et P. Galzy. 1984.
A study of the polygalacturonase activity of several yeast strains isolated from cocoa.
E.J. Appl. Microbiol. Biotechnol. 20:262.
- J.P. Guiraud, N. Chabbert et P. Galzy. 1984.
Alcohol from Jerusalem artichoke: Influence of the extract and of fermentation conditions.
Proc. Third Eur. Congr. Biotechnol. 2, 197.
- R. Ravelomanana, J.P. Guiraud, J.C. Vincent et P. Galzy. 1984.
Etude de la flore de levures de la fermentation traditionnelle du cacao a Madagascar.
Rev. Ferm. Ind. Aliment. 39, 103.
- C. Novotny, J.L. Fuentes, Ph. Carré, Hélène Boze, G. Moulin and P. Galzy. 1983.
Homothalism expression in Kluyveromyces lactis.
Heredity 51, 549-559.
- G. Moulin and P. Galzy. 1984.
Whey a potential substrate for biotechnology.
In: Biotechnology and Genetic Engineering Reviews (Russel G.E. Ed.)
1: 348-374. Intercept Limited Newcastle.
- G. Moulin, Hélène Boze and P. Galzy. 1984.
Inhibition of alcoholic fermentation.

In: Biotechnology and Genetic Engineering Reviews (Russel G.E. Ed.)
2: 365-382. Intercept limited Newcastle.

N. Chabbert, J.P. Guiraud, M. Arnoux et P. Galzy. 1985.
Productivity and fermentability of different Jerusalem artichoke cultivars.
Biomass 6.

J. Sanchez, G. Daguene, J.P. Guiraud, J.C. Vincent et P. Galzy. 1985.
A study of the yeast flora and the effect of pure culture seeding during
the fermentation of cocoa beans.
Lebens. Wissent. Technol. 18: 69.

N. Chabbert, J.P. Guiraud et P. Galzy. 1985.
Protein production potential in the ethanol production process from
Jerusalem artichoke.
Biotechnol. Letters 7: 443.

M. LeClerc, P. Gonde, A. Arnaud, R. Ratomahenina, P. Galzy and M. Nicolas.
1984. The enzyme systems in a strain of Candida wickerhamii Meyer and
Yarrow participating in the hydrolysis of cellodextrins.
J. Gen. Appl. Microbiol. (JPN) 30, 509-521.

M. LeClerc, P. Gonde, R. Ratomahenina, A. Arnaud and P. Galzy. 1984.
Fermentation of xylose and cellobiose by yeasts.
Vith International Symposium on yeasts. Montpellier (France) July 9-13.

P. Gonde, M. LeClerc, R. Ratomahenina, A. Arnaud and P. Galzy. 1984.
Selection of yeast strains for cellobiose and cellodextrins alcoholic
fermentation.
Vith International Symposium on yeasts. Montpellier (France) July 9-13.

B. Blondin, P. Gondé, R. Ratomahenina, A. Arnaud and P. Galzy. 1984.
Cyanide resistant respiration in the genus Dekkera and Brettanomyces.
12th International Conference of Yeast Genetics and Molecular Biology
Edinburgh, Scotland, U.K. 16-21 September, 1984.

D. Montet, R. Ratomahenina, M. Pina, J. Graille and P. Galzy. 1985.
Purification and characterisation of a lipase from Candida curvata Lodder
and Kreger van Rij.
Fette Seifen, Anstrichmittel 87.

J.M. Muderhwa, R. Ratomahenina, M. Pina, J. Graille and P. Galzy. 1985.
Purification and properties of the lipase from Candida deformans (Zach).
JAOCs, 62, n° 6.

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XXXVIII. A.U. Ziraat Fakultesi, Tarimsal Biyoteknoloji Bilim Dalı, Ankara,
Turkey. Communicated by M. Hilmi Pamir.

The following provides information on two research projects carried out in
our laboratory.

1. A Study on Yeast Strains Isolated from Wines Obtained from the
Nevshehir District.

(Has been published in turkish by Prof. I. Shahin)

In this study 26 yeast strains isolated from 9 wine samples were investigated morphologically and physiologically. The characters of these yeasts agree with those of Saccharomyces bayanus Saccardo according to Lodder's taxonomy (1970). Therefore, all 26 strains have been determined as S. bayanus. These yeasts play a part especially in making dry and also sweet wines from grapes with high sugar content because they can produce a great deal of alcohol (18% v/v) and also Sherry flower.

* * *

2. A Study on Microbial Protein (SCP) production from crude petroleum and refinery waste waters by some microorganisms and on reducing the biochemical oxygen demand (B.O.D.) of Refinery Waste Waters.

This study will be published by Dönmez S. and Pamir M.H.

The results obtained from the study that was carried out on the waste waters of the IPRAS Refinery are summarized as follows.

We found that petroleum oils of the waste waters of the IPRAS Refinery are one of the most important polluting agents. They are susceptible to biological degradation. Therefore, instead of discharging these wastes into the sea without pre-treatment, as currently done, it is necessary for the wastes to be treated biologically to prevent their polluting effects or at least to minimize them.

The A.P.I. Separator currently used in the refinery for taking out petroleum oil from the waste waters discharged into the sea, has no significant effect and so we found that the amount of petroleum oil in the waste waters discharged is higher than acceptable values.

The toxic substances, Hg and Cd, contained in the waste waters discharged into the sea retain their harmful effects for a long time. But the substances in our samples were not in a sufficient concentration to be deleterious for microorganisms and their quantities were normal according to the "Legislations on Water Products".

The legislation on water products consider only the subjects related to maritime products. But they do not apply to the most important problem of environmental pollution. Therefore, this legislation must contain also some regulations on environmental pollution.

The yeasts, Rh. glutinis var. glutinis and C. tropicalis isolated and identified from the waste waters, give a more remarkable biomass production than the yeasts, C. guilliermondii var. guilliermondii, C. membranaefaciens No. 1 and 2, Endomycopsis lipolytica isolated and identified from the same waste water. In general, we found that the yeasts could utilize partially C₁₀-C₁₄ n-paraffins, and the other hydrocarbons completely.

The waste waters of the refinery have an important influence on the pollution problem of the Marmara Sea. The importance of these results obtained during this study is that it will enable us to purify the waste

waters by biological treatment or at least to minimize their polluting effects or to purify them by producing single cell protein.

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XXXIX. Meetings

1. International Commission on Yeasts (ICY)

Below follow the minutes of the ICY Meeting that was held on November 7, 1985 in Varna, Bulgaria, during the Xth ISSY meeting organized by our colleague, Pr. Venkov.

Members present or represented at the meeting: P. Galzy, France; P. Venkov, Bulgaria; L. Stateva, Bulgaria; H. Weber, GDR; G. Shavlovsky, USSR; O. Bendova, CSSR representing Mrs. Kockova; E. Novak, Hungary; C. Panchal, Canada representing Dr. Stewart and Dr. Russel; B. Johnson, Canada; R. De Mot, Belgium representing Dr. Verachttert; J. Szopa, Poland; D. Vrana, CSSR; T. Deak, Hungary; G. Moulin, France representing Pr. Bastide.

The Commission paid homage to our regretted colleague Dr. Suomalainen.

According to Dr. Rankine's suggestion who left the Commission, it was decided that he be replaced by Dr. G.H. Fleet for the representation of Australia.

According to Dr. Venkov's proposal, the Commission has accepted Mrs. L. Stateva, Secretary of the Xth ISSY Meeting, as a new member representing Bulgaria.

Pr. Rose proposed Dr. Dawes, Dr. Douglas and himself for the representation of Great Britain.

The Commission called back to mind that the existing British Delegation is composed of Dr. Rose, Dr. Dawes and Dr. Spencer; consequently, it is not necessary to provide for a fourth member for one country.

Our colleague of the German Democratic Republic (GDR) represented by Pr. Weber announced the departure of Dr. Koch. Dr. Koch has left the GDR in order to live in the USA. The commission calls back to mind that no scientific member of the organization has ever been dismissed from the Commission.

Consequently, Dr. Koch will remain a member of the Commission and will be in the US delegation. The GDR delegation will propose a new member to the Commission for completing its representation.

Pr. Verachttert confirms his hope to organize at Louvain, Belgium, a symposium on the topic: "Yeast Technology-Relationship to alcoholic fermentation."

The Commission proposed to organize it in 1989. It was decided in the Meeting of Montpellier (1984) that a symposium on "Taxonomy of Yeast" would be held in 1989 or 1990. The Commission hopes it will be held in 1990.

During the meeting, Dr. Bendova, representing Dr. Kockova proposes to organize this symposium at Smolnice in Czechoslovakia. These two points will be discussed and a decision will be taken at the Xith ISSY meeting of Lisbon in 1986.

Pr. Venkov and Pr. Novak suggested that the Commission organize "teaching sessions" for young research workers. The Commission agrees with the proposition and asks all members to think about it.

The Xith ISSY has been held in excellent conditions. The Commission thanks Dr. Venkov and his colleagues and congratulates him for this realization.

Professor P. Galzy
President
Ecole Nationale Supérieure
Agronomique de Montpellier
Chaire de Génétique et de

Microbiologie

2. EMBO Workshop on Proteinases and Biological Control in Yeast.
September 21-25, 1986, Freiburg, Fed. Rep. of Germany.

Topics Include:

Proteinases, characteristics and function
Extra- and intravacuolar protein degradation under different metabolic conditions during the life cycle
Mechanisms of protein recognition for degradation
Mechanisms of protein uptake into the vacuole
Proteolysis and proteinases in secretion and transport
Proteolysis and proteinases in the biogenesis of cell organelles
Proteolysis and proteinases in pheromone maturation
Proteolysis and proteinases in biotechnology

Speakers are:

A. Brake (USA), K. Breddam (Denmark), P. Bohni (USA), A. Ciechanover (Israel), S. Emr (USA), A. Hinnen (Switzerland), H. Holzer (FRG), E.W. Jones (USA), G. Kawasaki (USA), V. Luzikov (USSR), G. Michaelis (FRG), H. Riezman (Switzerland), G. Schatz (Switzerland), J. Schwencke (France), T. Stevens (USA), M.P. Suarez-Rendueles (Spain), J. Thorner (USA), A. Varshavsky (USA), D.H. Wolf (FRG).

The total number of participants will be limited to 50. The workshop is directed primarily towards scientists with serious interest in the field of proteinases and cellular control. Applicants are invited to present short contributions. Applications should be sent to the organizers and be accompanied by a short C.V., a description of current research and a summary of the contribution. The registration-subsistence fee is DM 780,-. Some grants in aid will be available to cover part of the registration-subsistence.

Organizer: Dieter H. Wolf
Co-organizer: Matthias Müller

Biochemisches Institut der Universität Freiburg, Hermann-Herder-Strasse 7,
D-7800, Freiburg, FRG.

Closing date for applications: May 1, 1986.

3. EMBO Workshop Viruses of Fungi and Simple Eukaryotes August 24-29, 1986, Jerusalem, Israel.

Objective: To promote communication among those studying viruses of taxonomically diverse simple eukaryotes, as model systems for molecular studies, as tools for biotechnology, and as economically important modulators of microbial pathogenicity. The relationships of these viruses to those infecting higher eukaryotes will also be considered.

Main Topics:

- I. Molecular Biology of Fungal Double-Stranded RNA Viruses
- II. Retrovirus-Like Transposable Genetic Elements of Yeast
- III. DNA Viruses of Fungi and Algae
- IV. Fungal Virus in Agriculture, Biotechnology and Medicine
- V. Relationships to Viruses of Higher Eukaryotes

Each topic will be reviewed by a limited number of speakers to be followed by open discussion. All participants (not to exceed 60) should present talks or posters and will be encouraged to actively participate in discussions.

Invited Speakers (partial list):

J. Adler	H. Fukuhara
J.D. Boeke	D. Nuss
K.A. Bostian	A.J. Shatkin
J.A. Bruenn	J. Van Etten
K.W. Buck	R.B. Wickner
H. Bussey	

Applicants active in research on these or related viruses should submit a preliminary abstract of their proposed presentation and a brief description of their interests.

Total costs, including registration fee and full board: approximately U.S. \$300.00. Limited financial aid will be available.

Applications (to arrive before April 15), requests for further information and suggestions should be sent to either of the organizers:

Dr. Yigal Koltin (Chairman)
chairman)

Department of Microbiology
University of Tel-Aviv
Tel-Aviv, Ramat-Aviv
Israel
Telephone #03-420988

or

Dr. Michael J. Leibowitz (Co-

Department of Microbiology
UMDNJ-Rutgers Medical School
P.O. Box 101
Piscataway, New Jersey 08854, U.S.A.
(201) 463-4795

4. Intensive Workshop on Regulation of Metabolism in Yeasts
(For information only - application deadline is past)

Workshop Leaders:

C. Gancedo. Instituto de Investigaciones Biomedicas, C.S.I.C., Facultad de Medicina de la Universidad Autonoma. Madrid, Spain.

K.D. Entian. Physiologisch-chemisches Institut. Universität Tübingen. Federal Republic of Germany.

R. Hütter. P. Niederberger. Mikrobiologisches Institut. Eidgenössische Technische Hochschule. Zürich, Switzerland.

P. Labbe. Laboratoire Biochimie des Porphyrines. Université Paris VII. France.

Date: 10-21 March, 1986.

Location: During its first week the workshop will take place in the Gulbenkian Institute of Science which is located in Oeiras about 20 Km west of Lisbon at walking distance from the Atlantic Ocean. During its second week the workshop leaders and the participants will attend the XIth International Specialized Symposium on Yeasts (XI ISSY) on "Regulation of Transport and Metabolism in Yeasts. Basic and Biotechnological Aspects". The XI ISSY will be held in the Headquarters of the Calouste Gulbenkian Foundation in Lisbon.

Participants: The number will be limited to twenty. Participants may be accepted from any part of the world based on merit only. Candidates should have previous experience in a related field and be in a position to contribute a seminar paper to the workshop as well as a poster to the XI ISSY.

Financial aspects: The workshop is financed by the Calouste Gulbenkian Foundation and no fee is charged to the participants with respect to the first week. The fee for the second week (XI ISSY) must be paid by the participants however. Amount and mode of payment will be indicated in the Second Announcement of the XI ISSY which in due time will be sent to the candidates for participation in the workshop. Travel and living expenses are the responsibility of the participants. Lunch may be taken at the Gulbenkian Institute of Science (Monday through Friday during the first week) at a subsidized price.

Housing: Successful 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 are available free of charge. Applicants interested in such a room should indicate this on their application-for-admission form.

Professor N. van Uden
Department of Teaching,
Gulbenkian Institute of Science
Apartado 14, 2781 Oeiras Codex
Portugal

5. The 9th meeting of the British Yeast Group was held at the University of Glasgow on September 10-11, 1985, and was attended by more than 100 participants. The following papers were presented:

Plasmids of *Zygosaccharomyces bailii*

D.A. MacKenzie, B.M. Pearson and M.H.J. Keenan
(Food Research Institute, Norwich)

Characterisation of novel plasmids in *Zygosaccharomyces bailii*

K. Painting (Food Research Institute, Norwich)

The effect of leucine on the maintenance of plasmids in a *leu* strain of *Saccharomyces cerevisiae* grown in continuous culture

C.A. Caulcott, M. Rhodes, E. Fintan Walton and Yarranton
(Celltech Ltd., Slough)

Expression of foreign genes in yeast

G.T. Yarranton, L. Mynett, N. Dathan and E. Fintan
Walton (Celltech Ltd., Slough)

Regulation of gene expression and secretion of foreign proteins in yeast

E. Fintan Walton, N. Dathan, L. Mynett and G. Yarranton
(Celltech Ltd., Slough)

Mutagenesis

B.J. Kilbey (Genetics Dept., University of Edinburgh)

Catabolite repression of sterol biosynthesis in *Saccharomyces cerevisiae*

J.M. Haslam (Biochemistry Dept., University of Liverpool)

The effects of high carbon dioxide pressure on yeast growth and division

W.B. Lumsden (Brewing & Biol. Sci. Dept., Heriot-Watt University)

Transport of the beta-subunit of ATP synthase into mitochondria

G. Reid and M. Lloyd (Microbiology Dept., University of Edinburgh)

Biotin biosynthesis of yeasts

B.M. Pearson, D.A. MacKenzie and M.H.J. Keenan
(Food Research Institute, Norwich)

Synergistic effects of weak-acid preservatives and pH on *Zygosaccharomyces bailii*

M. Cole and M.H.J. Keenan (Food Research Institute, Norwich)

The regulation of flavin biosynthesis in the methylotrophic yeast *Hansenula polymorpha*

A.G. Brooke, L. Dijkhuizen and W. Harder (Rijksuniversiteit Groningen, Netherlands)

A novel proteinase from *Candida albicans*: its proposed role in activation of zymogenic chitin synthase

G.J. McDougall and G.W. Gooday (Microbiology Dept., University of Aberdeen)

The hungry plasmodium in *Candida* germ tubes

N.A.R. Gow and G.W. Gooday (Microbiology Dept., University of Aberdeen)

Mechanism of adherence of Candida albicans to epithelial cells
I.A. Critchley and L.J. Douglas (Microbiology Dept., University of Glasgow)

The effect of antifungal agents on the adherence of Candida species to denture acrylic
J. McCourtie, T.W. MacFarlane and L.P. Samaranayake (Glasgow University Dental School)

The effect of oral bacteria on the growth and adhesion of Candida albicans
K.H. Fearn, T.W. MacFarlane and L.J. Douglas (Microbiology Dept., and Glasgow Dental School, University of Glasgow)

Mannan biosynthesis in Candida albicans and its inhibition
K. Barrett-Bee and J. Lees (I.C.I. Pharmaceuticals Division, Alderley Park)

The effect of growth rate on secretion of alpha-factor from Saccharomyces cerevisiae
B.W. Smith, E. Fintan Walton, G. Yarranton and C.A. Caulcott (Celltech Ltd., Slough)

Regulation of the biosynthesis of organoleptic compounds
D.R. Berry and H. Chamberlain (Applied Microbiology Div., University of Strathclyde)

Amino acid transport in Hansenula polymorpha
S. Dunn and J.R. Woodward (Biotechnology Unit, University of Leeds)

Initiation of sporulation in Saccharomyces cerevisiae
J.L. Doull, I.W. Dawes and D.J. Roy (Microbiology Dept., University of Edinburgh)

Messenger RNA structure and function in yeast
A.J.P. Brown, T.C. Santiago, A.J.E. Bettany and I.J. Purvis (Genetics Dept., University of Glasgow)

Transformation of the methylotrophic yeast Hansenula polymorpha
P.E. Sudbery, M.A. Gleeson and S. Otori (Genetics Dept., University of Sheffield)

The mapping of a gene conferring flocculence in Saccharomyces cerevisiae
P. de Zoysa and J.R. Johnston (Applied Microbiology Div., University of Strathclyde)

Improved vectors for transforming brewers' yeast
A. Ramage, C. Perry and P. Meaden (Brewing Research Foundation, Nutfield)

Foreign gene expression in brewing yeast
B.A. Cantwell, G. Brazil, J.C. Hurley and D.J. McConnell (Genetics Dept., Trinity College, Dublin)

6. The Eighteenth Annual Meeting of the Yeast Genetics and Molecular Biology Society of Japan was held from September 18 to 20, 1985 at the Noguchi Hideyo Memorial Hall in Tokyo. The following eighty

topics were presented and discussed in eleven Sessions: Session I, Plasmids, Mitochondria and Extrachromosomal Factors (topic 1-10); II, Cell Structure and Function (11-17); III, Mutants, Chromosomal Map (18-22); IV, Biosynthesis, Metabolism and Physiology (23-30); V, Transcription and Regulation (31-44); VI Replication, Partition (45-51); VII, Translation (52); VIII, Secretion, Active Transport (53, 54); IX, Application, Techniques (55,56); X, Repair and Recombination (57-60); XI, Sporulation, Mating and Life Cycle (61-80). The abstracts of these talks will be published in Japanese in "Yeast Genetics and Molecular Biology News, Japan" at the end of 1985. Communicated by Masayuki Yamamoto, Laboratory of Molecular Genetics, Institute of Medical Science, University of Tokyo, P.O. Takanawa, Tokyo 108, Japan.

1. H. Fujimura, F. Hishinuma and N. Gunge (Mitsubishi-Kasei Insti. Life Sic.). Stability of pGKL2-ARS plasmid in yeasts
2. K. Kitada and F. Hishinuma (Mitsubishi-Kasei Institute of Life Sciences) Linear plasmid found in Saccharomyces kluyveri cells.
3. I. Utatsu, A. Utsunomiya and A. Toh-e (Dept. Ferment. Technol., Hiroshima Univ.) Structure and function of plasmid pSB3.
4. Y. Kaisho, H. Araki and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Functional Region of Yeast Plasmid pSR1 - Analysis of Transcriptional Region.
5. H. Matsuzaki, H. Araki and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Functional region of yeast plasmid pSR1 - Intramolecular recombination site.
6. H. Araki, A. Jearnpipatkul and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Functional region of yeast plasmid pSR1-Initiation region of DNA replication and stability of plasmid propagation.
7. S. Kawai, I. Shibuya, M. Takagi and K. Yano (Dept. Agricult. Chemist., Univ. of Tokyo)
Isolation and utilization of an ARS site of Candida maltosa.
8. M. Takagi, S. Kawai, I. Shibuya and K. Yano (Dept. Agricult. Chemist., Univ. of Tokyo).
Isolation and utilization of cycloheximide-resistant gene of Candida maltosa.
9. M. Takeda and M.G. Douglas* (Yamagata Univ., Sch. Med., *Univ. of Texas Health Science Center at San Antonio). Intracellular Delivery and Import of F₁-ATPase β Subunit to the Mitochondria in Yeast.
10. A. Toh-e and Y. Sahashi (Dept. Ferment. Technol., Hiroshima Univ.) Structure and function of the pet18 gene.
11. K. Takeo (Dept. Antibiotics, Res. Inst. Chemobiodynamics, Chiba Univ.). Regional ultrastructural characteristics of the plasma membrane of Schizosaccharomyces pombe.

12. M. Yamaguchi, T. Hirano, A. Tanaka⁺ and T. Tachibana⁺. (Central Res. Lab., Jikei Univ. Sch. Med. and ⁺Ultrastructural Sec., Tokyo Metrop. Inst. Med. Sci.). Electron microscopy of surface antigens of yeast protoplasts.
13. T. Hirano and M. Yamaguchi (Central Res. Lab., Jikei Univ. School of Medicine). Ultrastructure and function of the yeast cell envelope.
14. E. Tsuchiya and S. Fukui (Dept. Ferment. Tech., Hiroshima Univ.) Isolation and biochemical characterization of the yeast nuclear-matrix.
15. T. Yata, E. Tsuchiya, T. Miyakawa and S. Fukui (Dept. Ferment. Technol., Hiroshima Univ.). Characterization of DNA fragments tightly associated to the yeast nuclear-matrix.
16. M. Baba, N. Baba*, K. Kanaya* and M. Osumi (Dept. Biol., Japan Women's Univ., *Dept. Electr. Eng., Kogakuin Univ.). 3-dimensional observation of the intracellular structure in yeast, Saccharomyces cerevisiae.
17. T. Yamazaki and H. Nonomura (Dept. Ferment. Technol., Yamanashi Univ.). Use of KCl in yeast protoplast formation process and sorbitol in the regeneration, as osmotic stabilizer.
18. Y. Adachi, H. Ohkura and M. Yanagida (Dept. Biophys., Kyoto Univ.). Isolation of Schizosaccharomyces pombe cold sensitive mutants that show abnormal chromatin region.
19. H. Oh-ue and B. Ono (Fac. Pharm. Sci., Okayama Univ.). Genetic characterization of inorganic mercury resistant mutants of S. cerevisiae.
20. F. Miyamoto (Dept. Biol., Wakayama Univ.). Studies on the localization of genetic factors inducing hyper suppressive petites in yeast.
21. K. Ushio, Y. Nakata (Research laboratory of Higashimaru Shoyu Co. Ltd). Salt-tolerant deficient mutants of Zygosaccharomyces rouxii.
22. K. Kitamoto, K. Yoshizawa, Y. Ohsumi* and Y. Anraku* (Natl. Res. Inst. Brewing, Dept. Biol., Fac. Sci., Univ. Tokyo). Characterization of mutants defective in vacuolar function of Saccharomyces cerevisiae.
23. K. Okazaki*, T. Takechi, N. Kambara, S. Fukui* and T. Kamiryo (Fac. Integ. Arts Sci & Fac. Technol., Hiroshima Univ.). Structure of Candida tropicalis genes for peroxisomal protein.
24. B. Ono, H. Ohue and A. Nanjoh (fac. Pharm. Sci., Okayama Univ.). Isolation of DNA clones that complement the cys2 mutation in S. cerevisiae.
25. B. Ono, E. Sakamoto and K. Yamaguchi (Fac. Pharm. Sci.). Role of the tyrosine transport system in inorganic mercury sensitive strains of S. cerevisiae.

26. Y. Hayashi, Chiaki W. Nakagawa and A. Murasugi (Inst. for Developmental Res., Aichi Colony). Analysis of Inductive Syntheses of Cd-Binding Peptides with the use of Cycloheximide and Actinomycin D in Fission Yeast.
27. Y. Ohya, I. Uno*, T. Ishikawa*, and Y. Anraku (Dept. Biol., Fac. Sci, *Inst. Appl. Microbiol., Univ. Tokyo). Isolation and molecular properties of yeast calmodulin.
28. T. Yoshihisa, Y. Ohsumi and Y. Anraku (Dept. Biol., Fac. Sci., Univ. Tokyo). Solubilization and Partial Purification of α -Mannosidase-Marker Enzyme of Yeast Vacuole.
29. T. Hikiji, K. Kiyono, A. Ohota and I. Shibuya (Dept. Biochem., Saitama Univ.). Disruption of PSS gene responsible for phosphatidylterine synthase activity in Saccharomyces cerevisiae.
30. T. Kamihara, T. Takata, H. Kikutsugi and I. Nakamura (Dept. Indust. Chem., Kyoto Univ.). Filamentous growth of Candida tropicalis caused by diacylglycerol and calcium ionophore.
31. D.Y. Shin, *K. Matsumoto, **H. Iida, I. Uno and T. Ishikawa (App. Microbiol. Ins. Tokyo Univ. *DNAX Ins. **Harvard Univ.). Regulation of Heat shock response by cAMP.
32. H. Mitsuzawa, I. Uno, K. Tanaka*, T. Oshima*, K. Matsumoto** and T. Ishikawa (Inst. Appl. Microbiol. Univ. Tokyo, *Suntory Inst. Biomed. Res., **DNAX Res. Inst. Mol. Cell. Biol.). Reconstitution of the GTP-dependent adenylate cyclase from products of the yeast CYR1 and RAS2 genes in Escherichia coli.
33. Y. Suzuki, Y. Nogi and T. Fukasawa (Lab. Mol. Genetics, Keio Univ. Sch. Med.). Cloning of the Saccharomyces cerevisiae regulatory gene GAL 11.
34. Y. Nogi and T. Fukasawa (Lab. Mol. Genetics, Keio Univ. Sch. Med.). An analysis of functional domain in GAL80 gene by targeted deletion.
35. M. Tajima*, Y. Nogi and T. Fukasawa (Lab. Mole. Genet., Keio Uni. School of Medicine, *Shiseido Lab.). A sequence in pBR322 that enhances transcription of yeast gene.
36. J. Kuromitsu, N. Ogawa and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Structure and transcription of the PH02 and PH04 gene in Saccharomyces cerevisiae.
37. K. Yoshida and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Structure and transcriptional regulation of PH081 gene of Saccharomyces cerevisiae.
38. Y. Kaneko, I. Banno, A. Toh-e* and Y. Oshima** (IFO, *Dept. Ferment. Technol., Hiroshima Univ., **Dept. Ferment. Technol., Osaka Univ.). The nucleotide sequence of the PH08 gene encoding repressible alkaline phosphatase of Saccharomyces cerevisiae.

39. K. Nishiwaki, N. Hayashi, S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Analysis of 5' upstream region of the HIS5 gene in Saccharomyces cerevisiae.
40. Y.I. Hwang, H. Araki, S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Construction of a promoter probing vector in Saccharomyces cerevisiae.
41. P. Lund and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo) Expression of the ADH promoter in Schizosaccharomyces pombe.
42. Y. Adachi, O. Niwa and M. Yanagida (Dept. Biophys., Kyoto Univ.). Expressions of essential and non-essential -tubulin genes in fission yeast.
43. S. Matsumoto and M. Yanagida (Dept. Biophysics, Kyoto Univ.). Histone Gene Organization and Cell Cycle Specific Transcription in Fission Yeast.
44. N. Itoh, K. Mizumoto and Y. Kaziro (Inst. Med. Sci. Univ. Tokyo). Structure and reaction mechanism of mRNA capping enzyme from yeast.
45. Y. Nakaseko, O. Niwa and M. Yanagida (Dept. Biophys., Kyoto Univ.). Organization of centromeric DNA sequences in fission yeast.
46. T. Uemura, Y. Adachi, H. Ohokura, T. Hirano and M. Yanagida (Dept. Biophys., Kyoto Univ.). Chromosome segregation is uncoupled with spindle dynamics in fission yeast type II DNA topoisomerase mutants.
47. T. Hirano, Y. Hiraoka and M. Yanagida (Dept. Biophysics, Kyoto Univ.). NUC2 CUT1 CUT2; genes controlling spindle elongation and chromosome segregation.
48. T. Matsumoto, O. Niwa and M. Yanagida (Dept. Biophys. Kyoto Univ.). Organization of fragments cloned from the mini-chromosome Ch¹⁶ in the fission yeast.
49. O. Niwa, T. Matsumoto and M. Yanagida (Dept. Biophys. Kyoto Univ.) Genetic behavior of the mini-chromosome Ch¹⁶ in the fission yeast.
50. S. Nakade, Y. Shimada*, S. Harashima and Y. Oshima (Dept. Ferment. Technol., Osaka Univ., *Osaka Municipal Technical Research Institute). Polymerization of the ARS1 plasmids.
51. S. Miyamoto, Y. Ohya, Y. Ohsumi and Y. Anraku (Dept. Biol., Fac. Sci., Univ. Tokyo). Cloning and DNA sequence of the CLS4 (CDC24) gene in the yeast Saccharomyces cerevisiae.
52. M. Uritani and M. Miyazaki (Inst. Molec. Biol., Nagoya Univ.). Participation of the polypeptide elongation factor, EF-3 in the stimulatory effect of ATP on poly(Phe) synthesis reaction in yeast.
53. E. Uchida, Y. Ohsumi and Y. Anraku (Dept. Biol., Fac. Sci., Tokyo Univ.). H⁺-ATPase from vacuolar membranes of Saccharomyces cerevisiae.

54. T. Mizunaga, M. Izawa and Y. Maruyama (Dept. Agric. Chem., Univ. of Tokyo). Is addition of mannan chains required for secretion of repressible acid phosphatase in Saccharomyces cerevisiae?
55. K. Sakai (Centr. Res. Lab., Asahi Brew. Ltd.). Transformation of the yeast Saccharomyces carlsbergensis.
56. M. Osumi, N. Yamada*, K. Koga**, M. Okazaki** (Dept. Biol., *Institute of Electron Microscope, Japan Women's Univ., **Lab. of Alc. Beverages and Fermentation Technology, Suntory Ltd.). Immobilized living yeast cells and calcium alginate gel.
57. S. Nakai (Health Res. Found.) Induction of sister-chromatid recombination (SCR) in cell-cycle mutants of yeast, S. cerevisiae.
58. I. Machida and T. Saeki (Div. Genet., Natl. Inst. Dad. Sci.). Intergenic recombination induction by near-UV and far-UV light in repair defective mutants of Saccharomyces cerevisiae.
59. K. Tano-Ikai, K. Tano, T. Ohnishi and K. Nozu (Dept. Biology, Nara Medical University) Repair of UV-irradiated plasmid DNA in excision repair deficient mutants of Saccharomyces cerevisiae.
60. K. Nakagawa, J. Hashikawa, O. Makino, M. Iwabuchi, T. Shibata, and T. Ando (Department of Microbiology, Riken Institute, Saitama 351-01). Structure and function of a yeast site-specific endo-deoxyribonuclease Sce I: A study with monoclonal antibodies.
61. A. Hirata, K. Tanaka* and C. Shimoda** (Inst. Appl. Microbiol., Univ. Tokyo, *Lab. Med. Mycol., Inst. Dis. Mech. Con., Nagoya Univ. Sch. Med., and **Dept. Biol., Fac. Sci., Osaka City Univ.). The fine structure of ascospore development in the fission Yeast by freeze substitution.
62. C. Shimoda and K. Kitamura (Dept. Biol., Fac. Sci., Osaka City Univ.). Genes essential for the initiation of meiosis in the fission yeast Schizosaccharomyces pombe.
63. H. Fujioka, C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.). Functional region of the mating-type gene (mat2-P) responsible for meiosis in the fission yeast Schizosaccharomyces pombe.
64. M. Kishida and C. Shimoda (Dept. Biol., Fac. Sci., Osaka City Univ.). Transcriptional regulation of the meiotic and sporulation genes in the fission yeast, Schizosaccharomyces pombe.
65. Y. Watanabe, Y. Iino, J. Sakaguchi-Inoue and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo) Structural analysis of the mei2 gene encoding a positive factor for the initiation of meiosis.
66. Y. Fukui, Y. Kaziro, and M. Yamamoto* (Dept. Chem., Inst. of Med. Science, Tokyo Univ., *Lab. of Mol. Genetics, Inst. of Med. Science, Tokyo Univ.). Role of a ras homologue in Schizosaccharomyces pombe.

67. Y. Iino and M. Yamamoto (Inst. Med. Sci., Univ. Tokyo). Genetic analysis of mating inhibition by cAMP in Schizosaccharomyces pombe.
68. Y. Ohsumi and Y. Anraku (Dept. of Biol., Facult. of Sci., Univ. of Tokyo). Specific induction of Ca²⁺ transport activity in MATa cells of S. cerevisiae by a mating pheromone, alpha factor.
69. T. Tachikawa, R. Akada, T. Miyakawa, E. Tsuchiya and S. Fukui (Dept. Ferment. Tech., Hiroshima Univ.). Change in Ca²⁺ metabolism as a signal for mating pheromon-induced sexual differentiation in heterobasidiomycetous yeast.
70. M. Kaji, Y.K. Jeong, T. Miyakawa, E. Tsuchiya, S. Fukui (Dept. Ferment. Tech., Hiroshima Univ.). The trigger reaction for sexual differentiation by rhodotorucine A, farnesylpeptide mating pheromone of Rhodospordium toruloides.
71. S. Hasegawa, I. Banno[†] and N. Yanagishima (Biol. Inst., Nagoya Univ., Inst. [†]Ferment., Osaka). Secreted and wall-bound forms of the agglutination substance in the yeast Issatchenkia scutulata var. scutulata.
72. K. Suzuki and N. Yanagishima (Nagoya Univ., Biol. Inst.). Genetic characterization of α -mating-type specific gene responsible for sexual agglutination in Saccharomyces cerevisiae.
73. K. Tachibana, H. Miyata and N. Yanagishima (Dept. Biol., Nagoya Univ.). Prelude for the initiation of zygote formation in Saccharomyces cerevisiae.
74. T. Hisatomi, N. Yanagishima, H. Tanaka*, A. Sakurai* and I. Banno** (Biol. Inst., Nagoya Univ., *Inst. Phys. Chem. Res., **Inst. Ferment., Osaka). Sexual differentiation of Saccharomyces exiguus.
75. K. Kusumoto, N. Ikeda, S. Harashima, and Y. Oshima (Dept. Ferment. Technol., Osaka Univ.). Control system of mating types of Saccharomyces cerevisiae.
76. H. Tanaka, M. Tsuboi and T. Oshima* (Dept. Biol., Fac. Sci., Osaka City Univ., *Suntry Inst. Biomed. Res.). Expression of the sporulation gene, spoT7, in S. cerevisiae.
77. M. Tsuboi, H. Maruo and H. Tanaka (Dept. Biol., Fac. Sci., Osaka City Univ.). Genetic analysis of mutations affecting expression of sporulation gene, spoT7 in S. cerevisiae.
78. H. Tamaki (Doshisha Women's College). Genetic analysis of intergeneric hybrids by protoplast fusion in yeasts.
79. J. Akimaru and H. Agui (Biotechnology laboratory, Takarazuka Research Center, Sumitomo Chemical Co. Ltd.). Cloning of α -factor gene from S. cerevisiae.
80. S. Yamano, A. Toh-e, and K. Matsumoto (Dept. Ferment. Technol., Hiroshima Univ., DNAX). Cloning of the BCY1 gene of Saccharomyces cerevisiae.

7. Announcements and Call for Papers
Yeast Genetics and Molecular Biology Meeting, June 3-8, 1986
55th Annual Meeting, Genetics Society of America, June 8-10, 1986
University of Illinois, Urbana-Champaign

Abstract Receipt Deadlines
February 21 for GSA abstracts
March 7 for Yeast abstracts

There will be three genetics meetings at the University of Illinois, Urbana-Champaign, during the summer of 1986.

June 1-5: Extrachromosomal Elements in Lower Eukaryotes. For further information, contact Dr. I.C. Gunsalus, Organizer, Department of Biochemistry, 420 Roger Adams Laboratory, University of Illinois, Urbana, Illinois 61801 (217) 333-2010.

For more information about the Yeast/GSA meetings, please contact the GSA Administrative Office, 15501-B Monona Drive, Derwood, Maryland 20855. (301) 762-1424.

In Addition: The 13th International Conference on Yeast Genetics and Molecular Biology will be held August 31-September 5, 1986, in Banff, Alberta, Canada. For further information, contact Dr. R.C. von Borstel, Chairman, Organizing Committee, Department of Genetics, University of Alberta, Edmonton, Alberta T6G 2E9, Canada (403) 432-5381.

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XL. Brief News Items

1. The Yeasts, A Taxonomic Study
Fourth Edition

C.P. Kurtzman and J.W. Fell have agreed to serve as editors for the fourth edition of The Yeasts, A Taxonomic Study. Publication is planned for 1990. The editors would be pleased to receive any comments concerning format or coverage that would increase the usefulness of this new edition. Comments may be directed to either editor:

Dr. C.P. Kurtzman
Northern Regional Research Center
1815 N. University St.
Peoria, Illinois 61604

Dr. J.W. Fell
Rosenstiel School of Marine
and Atmospheric Science
Miami, Florida 33149

2. Published recently: Early research on fermentation - a story of missed opportunities. F. Schlenk, Trends in Biochemical Sciences, Vol. 10, 252-254 (1985).

F. Schlenk
University of Illinois
Dept. of Biol. Sciences
Box 4348
Chicago, IL 60680

3. Change of address:
Dr. Norio Gunge
The Kumamoto Institute of Technology
Applied Microbial Technology
Ikeda 4-22-1, Kumamoto 860, Japan

(Former address is: Mitsubishi-Kasei Institute of Life Sciences,
Minamiooya,
Machida-shi, Tokyo)

4. Change of address:
Dr. Jeremy Thorner
Department of Biochemistry
Room 401, Biochemistry Building
University of California
Berkeley, California 94720

(Former address is: Dept. of Bacteriology, University of California,
Berkeley)

5. Biotechnology Monographs
Volume 1
Single Cell Protein -
Israel Goldberg, 1985
* * *
Springer-Verlag, Berlin
Heidelberg Platz
D-1000 Berlin 33
West Germany
DM 136, --/U.S. \$49.50

6. Biotechnology Series: Biology of Industrial Microorganisms (A.L.
Demain and N.A. Solomon, eds) 1985. The Benjamin/Cummings Publ.
Co., Menlo Park, California 573 pp.

7. Yeast Molecular Genetics

The Department of Biology invites applications for a tenure track
Assistant Professorship effective August 20, 1986. Applications, including
an outline of research plans and the name of three references should be
submitted to: Dr. P. Dennis Smith, Yeast Molecular Genetics Committee,
Department of Biology, Southern Methodist University, Dallas, Texas 75275.