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# Y E A S T

A Newsletter for Persons Interested in Yeast

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



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

The strains listed below have been added to the ATCC since October 30, 1986. Complete information on these strains may be obtained upon request from the Mycology and Botany Department of ATCC.

NEW YEAST STRAINS

| NAME | ATCC No. | Depositor & Strain | Significance & Ref. |
|---|---|---|---|
| Blastoschizomyces capitatus | 62963<br>62964 | R.C. Matthew, 533<br>534 | Human pathogen (J. Clin. Microbiol. 23: No. 2, 395-397, 1986). |
| Candida albicans | 62664 | H. Nakayama, KD 4900 | Accumulates 14-methylated sterols & unable to form hyphae (J. Med. Vet. Mycol. 24:327-336 1986). |
| Candida boidinii | 62807 | K. Karbaum, MH6 | Genetic Study (FEMS Microbiology Letters 10:133, 1981). |
| Candida milleri | 62655 | C.P. Kurtzman, NRRL Y-7246 | Make sour dough french bread U.S. Pat. 3,734, 743). |
| Candida valida | 62806 | K. Karbaum, H95 | Genetic study (FEBS Microbiology Letters 10:133, 1981). |
| Hansenula polymorpha | 62809 | K. Karbaum, MH20 | Genetic study (FEBS Microbiology Letters 10:133, 1981). |
| Pichia stipitis | 62970<br>62971 | D. Yarrow, CBS 5774<br>CBS 5775 | Environmental study (Appl. & Environ. Microbiology 52: No. 2, 320-324, 1986). |
| Saccharomyces bayanus | 62701<br>62702 | P. Romano, 6075<br>6151 | Flocculent strain dropped degree F5 to degree F3 after 3 years (Can. J. Microbiol. 30:36-39, 1984). |
| Saccharomyces cerevisiae | 62625 | J. Thorner, DA2100 | Genotype: MATa leu2-3, ura3-52 his 4 suc 2-D9 lys 2-D1; ura 3 (Molec & Cell |

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|---|---|---|---|
| | | | Biol. 6:2828-2838 1986). |
| Saccharomyces cerevisiae | 62693 | P. Romano, 7330 | Extremely flocculent (Can. J. Microbiol. 30: 36-39, 1984). |
| Saccharomyces cerevisiae | 62694 | P. Romano, 10278 | Used in winemaking without $SO_2$ addition and does not form $H_2S$ from sulfate (Ann. Microbiol. 34: 7-15, 1984). |
| Saccharomyces cerevisiae | 62695 | P. Romano,10278-1A | Diploid, ferments sugar (Appl. Environ. Microbiol. 50:1064-1067, 1985). |
| Saccharomyces cerevisiae | 62696<br>62697<br>62698 | P. Romano, 6213-1A<br>6213<br>6181 | Parental strain for crossing (Can. J. Microbiol. 30:36-39, 1984). |
| Saccharomyces cerevisiae | 62699 | P. Romano, 802 | Hybrid (ATCC 62695 x ATCC 62696) Flocculent and $H_2S$ producing (Appl. Environ. Microbiol. 50: 1064-1067, 1985). |
| Saccharomyces cerevisiae | 62700 | P. Romano, 404 | Able to make wine in presence of sulfur dioxide (Appl. Environ. Microbiol. 50: 1064-1067, 1985). |
| Saccharomyces cerevisiae | 62703 | P. Romano, 6007 | Flocculent in static culture (Can. J. Microbiol. 30:36-39, 1984). |
| Saccharomyces cerevisiae | 62906<br>62907 | D. Yarrow, CBS 6128<br>CBS 6131 | Active dried baker's yeast (U.S. Pat. 4,341, 871). |
| Saccharomyces cerevisiae | 62808 | K. Karbaum, H151 | Genetic study (FEBS Microbiology Letters 10:133, 1981). |
| Torulopsis candida | 62894 | G. St.-Germain, 6893 | Opportunistic pathogen (J. of Clinical Microbiol. 24:No. 5, 884, 1986). |
| Torulopsis domercquii | 62810 | K. Karbaum, MH1 | Genetic study (FEMS Microbiology |

| | | | |
|---|---|---|---|
| | | | Letters 10:133, 1981). |
| Trichosporon cutaneum | 62975 | M. Veenhuis, X4 | The compartmentation of the metabolism of ethylamine was studied in this yeast. Ethylamine was the sole source of energy, carbon & nitrogen in this study (Arch. Microbiol. 145: 39-50, 1986). |

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

LIST OF CULTURES

31st EDITION

1987

An up-to-date 31st edition of the CBS List of Cultures became available in April at the price of Hfl. 35,-- (Postage not included).

The new version has been extended with many new accessions and now lists a total of 24,150 strains, including 20,000 filamentous fungi, 3,200 yeasts and 950 actinomycetes. About 3,160 of these are type strains. Strains are listed with source, depositor, mating type and other properties in some cases.

The CBS collection comprises approximately 37,500 strains. Information on unlisted strains can be obtained on application.

The List of Cultures may be ordered from Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG BAARN, The Netherlands, List of Cultures Administrator.

PUBLICATIONS

1. de Hoog , G.S., Smith, M. Th., & Guého, E. 1986. A revision of the genus Geotrichum and its telemorphs. Studies in Mycology No. 29, 1-131; Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. HFL 45, - ISBN 90 73051 13 7.

2. Smith, M. Th. and Batenburg-van der Vegte, W.H. 1987. Additional information on the ultrastructure in the genus Sporothrix. J. Gen. Appl. Microbiol. 33, in press.

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

The following articles were recently published.

1. Makiko Hamamoto, Junta sugiyama, Shoji Goto, and Kazuo Komagata. 1986. Numercial taxonomy based on the electrophoretic mobility of enzymes in the genera Rhodosporidium, Cystofilobasidium, and Rhodotorula. J. Gen. Appl. Microbiol. 32: 89-99.

On the basis of the similarity values in the electrophoretic relative mobility of seven enzymes, the strains of the genera Rhodosporidium, Cystofilobasidium, and Rhodotorula were analyzed numerically. The strains with the ubiquinone Q-10 system were divided into six major clusters, the strains with the Q-9 system into two clusters, and the strains with the Q-8 system into major clusters. The strains of Rhodotorula glutinis were clearly separated into clusters. In the species of the genera Rhodosporidium and Cystofilobasidium, compatible mating-type strains were included in the same clusters. Furthermore, compatible mating-type strains of Rhodosporidium toruloides, Rhodosp. diobovatum, and Rhodosp. infirmominiatum, were divided into two subclusters, within the respective species.

2. Shoji Goto and Hideo Takami. 1986. Classification of Ascoideaceous yeasts based on the electrophoretic comparison of enzymes and co-enzymes Q systems. J. Gen. Appl. Microbiol. 32: 271-282.

Sixty one strains of ascoideaceous yeasts belonging to twenty species of nine genera were examined from a taxonomical viewpoint based on a study of the electrophoretic comparison of the enzymes and the coenzymes Q (Co-Q) systems. The fifty strains, excluding those yeasts in which the detected number of enzymes was 3 or less, were divided into 4 clusters and 2 subclusters by numerical analysis of the relative electrophoretic mobilities (Rm) of 8 enzymes. On the other hand, the Co-Q systems of the ascoideaceous yeasts were Q-7, Q-8, or Q-9. These Co-Q systems and the septal ultrastructures coincided with each other. It is possible to divide these yeasts into four groups on the basis of similarity value according to the Rm of 8 enzymes: Saccharomycopsis lipolytica, other Saccharomycopsis spp. and related genera, Hyphopichia burtonii and/or Stephanoascus ciferrii, and Ambrosiozyma and Hormoascus. Saccharomycopsis lipolytica should be excluded from the genus Saccharomycopsis and is assigned to the genus Yarrowia. Ambrosiozyma and Hormoascus are considered to be cogeneric. Our data revealed close taxonomic relationship in the numerical classification based on the Rm of enzymes, Co-Q systems, and the septal ultrastructure.

3. Shoji Goto, Junta Sugiyama, Makiko Hamamoto, and Kazuo Komagata. 1987. Saitoella, a new anamorph genus in the Cryptococcaceae to accommodate two Himalayan yeast isolates formerly identified as Rhodotorula glutinis. J. Gen. Appl. Microbiol. 33 : 75-85.

A description and two illustrations are presented of a new anamorph genus, Saitoella, in the Cryptococcaceae represented by a single species S. complicata sp. nov. to accommodate two yeast strains isolated from Himalayan soil. These were formerly identified as Rhodotorula glutinis (Fres.) (1970). The new genus can be distinguished from Cryptococcus, Rhodotorula, Phaffia, Sterigmatomyces, Fellomyces, and Sporobolomyces by a combination of characters. Saitoella is considered to be ascomycetous in the light of a negative Diazonium Blue B (DBB) reaction and a cell wall ultrastructure typical of ascomycetous yeasts; there is probably a close relationship between Saitoella and Taphrinales on the basis of the formation of carotenoid pigments, the absence of xylose in the cells, the DNA base composition in the "grey zone" (GC 51.5 mol%), a Q-10 ubiquinone system, and a negative DBB reaction.

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

Below follows an abstract of a recent publication:

Makkiko Hamamoto, Junta Sugiyama, and Kazuo Komagata. 1986. DNA Base Composition of Strains in the Genera Rhodosporidium, Cystofilobasidium, and Rhodotorula Determined by Reversed-Phase High-Performance Liquid Chromatography. J. Gen. Appl. Microbiol., 32, 215-223.

ABSTRACT

The DNA base composition of 74 strains in the genera Rhodosporidium, Cystofilobasidium, and Rhodotorula were determined by reversed-phase high-performance liquid chromatography (HPLC). The relative standard error of nucleoside analysis was less than 1%. The difference between the two values determined by the HPLC method and the $T_m$ method were less than 1% in three strains determined in this study and less than 2%, with a few exceptions, compared with reference data.

* * *

V. Department of Biology, Syracuse University, Syracuse, New York, 13244. Communicated by W.T. Starmer.

The following papers have been published or are in press:

1. Philip F. Ganter[1], William T. Starmer, Marc-Andre Lachance[1], and Herman J. Phaff[2]. 1986. Yeast Communities From Host Plants and Associated Drosophila in Southern Arizona: New Isolations and Analysis of the Relative Importance of Hosts and Vectors on Community Composition. Oecologia (Berlin) 70:386-392.

1. Department of Plant Sciences, University of Western Ontario, London, Ont., Canada N6A 5B7.
2. Department of Food Science and Technology, University of California, Davis, California 95616, USA.

ABSTRACT

The yeast communities from slime fluxes of three deciduous trees (Prosopis juliflora, Populus fremontii and Quercus emoryi) and the necroses of two cacti (Opuntia phaeacantha and Carnegiea gigantea) were surveyed in the region of Tucson, Arizona. In addition, the yeasts carried by dipterans associated with the fluxes or necroses (Drosophila carbonaria, D. brooksae, D. nigrospiracula, D. mettleri, and Aulacigaster leucopeza) were sampled. The results indicate that each host sampled had a distinct community of yeasts associated with it. The dipterans, which can act as vectors of the yeasts, deposited yeasts from other sources in addition to those found on their associated hosts. It is argued that host plant physiology is relatively more important than the activity of the vector in determining yeast community composition. Furthermore, the average number of yeast species per flux or necrosis is not different from the average number of yeast species per fly. It is hypothesized that the vector may affect the number of species per individual flux or rot, and that the number is lower than the rot or necrosis could potentially support.

* * *

2. William T. Starmer, Philip F. Ganter, Virginia Aberdeen, Marc-Andre Lachance[1] and Herman J. Phaff[2]. 1987. The Ecological Role of Killer Yeasts in Natural Communities of Yeasts. Submitted for publication.

1. Department of Plant Sciences, University of Western Ontario, London, Ontario, N6A 5B7, Canada.
2. Department of Food Science and Technology, University of California, Davis, California, 95616.

ABSTRACT

The killer phenomenon known in several yeast species was investigated in naturally occurring yeast communities. Yeast species from communities associated with the decaying stems and fruits of cactus and the slime fluxes of trees were studied for their production of killer toxins and their sensitivity to killer toxins produced by other yeasts. Yeast isolates found in decaying fruits showed the highest incidence of killing activity (30/112) while yeasts isolated from cactus necroses and tree fluxes showed significantly lower activity (62/691 and 11/140, respectively). Cross-reaction studies in general indicated that few yeast-yeast interactions occur within the same habitat at a particular time and locality but that killer-sensitive reactions occur more frequently across localities and habitats. The physical conditions that should be optimal for killer activity are found in fruit and young rots of Opuntia cladodes where the pH is low. The fruit habitat does appear to favor the establishment of killer species. An example of the importance that killer toxin can have is the natural distribution of the killer yeast Pichia kluyveri and the sensitive yeast Cryptococcus cereanus. Their distribution indicate that the toxin produced by P. kluyveri limits the occurrence of Cr. cereanus in fruit and Opuntia pads. In general most communities have only one major species that makes killer toxins. Sensitive strains are more widespread than killer strains and few species appear to be immune to all toxins. Genetic study of the killer yeast P. kluyveri indicates that

the mode of inheritance of killer toxin production is nuclear and not cytoplasmic as is found in Saccharomyces cerevisiae and Kluyveromyces lactis.

* * *

3. William T. Starmer, Herman J. Phaff[1], Jane M. Bowles[2] and Marc-Andre Lachance[2]. 1987. Yeasts Vectored by Insects Feeding on Decaying Saguaro Cactus. Submitted to South Western Naturalist.

1. University of California, Davis, California.
2. University of Western Ontario, London, Ontario, N6A 5B7.

ABSTRACT

We have conducted a study on insects found feeding on Carnegiea gigantea (saguaro) stem necroses in the vicinity of Tucson, Arizona, USA. After locating two decaying saguaro rots, the insects found attracted to and feeding on the decaying juices were captured and placed individually in petri dishes containing a complete yeast medium that had been acidified to inhibit bacterial growth. The insects were allowed access to the surface of the medium for 8-12 hours, after which they were removed and identified. Yeast and mold growth was observed over a span of 3-8 days. Yeast colonies were isolated, purified and identified. The results implicate Drosophila as one of the primary vectors of yeasts. Nereids also breed in rotting saguaro and show a similar vectoring profile to the drosophilids, i.e. abundant yeasts and few molds are transmitted. Muscoids, dolichopodids and other insects appear to carry incidental yeasts, while frequently carrying molds.

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

The following papers have been published or are in press since the listing in the Yeast Newsletter of June 1986 (Vol. 35 No. 1).

1. H.J. Phaff and W.T. Starmer. 1987. Yeasts associated with plants, insects, and soils. In A.H. Rose and J.S. Harrison (eds). The Yeasts, 2nd Ed. Vol. 1, pp. 123-180. Academic Press, London. (reprints not available).

2. C.P. Kurtzman and H.J. Phaff, 1987. Molecular taxonomy of yeasts. In A.H. Rose and J.S. Harrison (eds). The Yeasts, 2nd Ed., Vol. 1, pp. 63-94. Academic Press, London. (reprints not available).

3. H.J. Phaff, M. Miranda, W.T. Starmer, J. Tredick, and J.S.F. Barker. 1986. Clavispora opuntiae, a new heterothallic yeast occurring in necrotic tissue of Opuntia species. Int. J. Syst. Bacteriol. 36:372-379. (for abstract see YNL 35 No. 1).

4. W.T. Starmer, J.S.F. Barker, H.J. Phaff, and J.C. Fogleman. 1986. Adaptations of Drosophila and yeasts: their interactions with the volatile 2-propanol in the Cactus-Microorganism - Drosophila model system. Aust. J. Biol. Sci. 39:69-77. (for abstract see YNL 35 NO. 1).

5. W.T. Starmer, P.F. Ganter, and H.J. Phaff. 1986. Quantum and continuous evolution of DNA base composition in the yeast genus Pichia. Evolution 40:1263-1274. (for abstract see YNL 35 No. 1).

6. H.J. Phaff, W.T. Starmer, J. Tredick-Kline, and V. Aberdeen. 1987. Pichia barkeri, a new species of yeast occurring in necrotic tissue of Opuntia stricta. Int. J. Syst. Bacteriol. (accepted for publication).


ABSTRACT

We describe Pichia barkeri, a new cactophilic species of yeast occurring in necrotic tissue of Opuntia stricta in New South Wales, Australia and on several islands in the Caribbean Sea. The new species is homothallic and appears to occur in nature in the haploid state. After conjugation between a mother cell and a bud four hat-shaped spores are produced that are rapidly released from the ascus. The range of guanine-plus-cytosine content for the nuclear deoxyribonucleic acids of 22 strains is 35.7 to 36.6 mol% (average, 36.1 mol%; standard deviation, 0.2 mol%). The type strain of P. barkeri is strain UCD-FST 83-994.3 (=ATCC 00000 = CBS 7256), isolated at Discovery Bay, Jamaica.


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7. G.B. Fuson, H.L. Presley, and H.J. Phaff. 1987. Deoxyribonucleic Acid Base Sequence Relatedness Among Members of the Yeast Genus Kluyveromyces. Int. J. Syst. Bacteriol. (acccepted for publication). (October Issue).


ABSTRACT

DNA base composition and DNA base sequence relatedness comparisons were employed for species delineation in Kluyveromyces. Base composition values separated the members of the genus into three groups. These were further subdivided by comparing base sequences, using DNA/DNA renaturation experiments. Two DNA homology groups were identified. The first group included K. marxianus, K. fragilis, K.bulgaricus, K. cicerisporus, K. wikenii, and three anamorphs (Candida kefyr, C. pseudotropicalis, and Torula cremoris); the members of this group exhibited > 90% DNA base sequence complementarity. The second group consisted of K. lactis, K.vanudenii, K. drosophilarum, and K. phaseolosporus; various pairs of these yeasts shared 64- 98% of their DNA sequences. The two groups were only distantly related to each other (< 15% DNA base sequence complementarity). The other species of Kluyveromyces appear to be unique, not being closely related to either of the two homology groups or to one another. Relationships deduced from comparisons of DNAs agreed well with those deduced by other workers form immunological comparisons of exo-β-glucanases and from isoenzyme analysis, but were only in partial agreement with a taxonomic arrangement based on mating studies. We propose

recognition of the following species: K. aestuarii, K. africanus, K. blattae, K. delphensis, K. dobzhanskii, K.lactis (syn. K.drosophilarum, K. phaseolosporus, and K. vanudenii), K. lodderi, K. marxianus (syn. K. bulgaricus, K. cicerisporus, K. fragilis, and K. wikenii), K. phaffii, K. polysporus, K. thermotolerans, K. waltii, and K. wickerhamii.

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

The following papers have been published recently:

1. Naumov, G.I. Comparative genetics of yeast XXIII. Unusual inheritance of toxin formation in Saccharomyces paradoxus Batschinskaia. Genetica, 1985, 21, No. 11, pp. 1794-1798 (in Russian), the abstract has appeared in Yeast Newsletter, 34, No. 1, pp. 77-78.

2. Naumov, G.I., Yurkevich, V.V. Operon-like systems in yeasts. Vestn. Mosk. Univ., ser. 16, Biologia, 1985, No. 3, pp. 40-42 (in Russian).

3. Babjeva, I.P., Vustin, M.M., Naumov, G.I., Vinovarova, M.E. Arthroascus schoenii comb. nov., a yeast-like organism. Mikrobiologia, 1985, 54, No. 5, pp. 724-729 (in Russian).

Abstract

Three new yeast cultures were isolated from oak exudate. They are similar to Endomyces schoenii described by G. Nadson and N. Krassilnikov. The yeasts can cross with the type strain of Arthroascus javanensis (Kloecker) von Arx, but differ from it in the character of sporulation and in genetic characteristics. A second species A. schoenii comb. nov. is created in the genus Arthroascus von Arx.

4. Kondratieva, V.I., Naumov, G.I., Rozenfeld, S.M., Kapitonova, O.N., Zhdanov, V.G. Hybridization and recombination in Eremothecium ashbyii. Cytology and Genetics (Kiev), 1986, 20, No. 4, pp. 287-291 (in Russian).

5. Naumov, G.I., Tyurina, L.V., Bur'yan, N.I., Skorikova, T.K. Composition of industrial populations of Saccharomyces cerevisiae yeast as affected by toxin formation. Biotechnologia, 1986, No. 4, pp. 28-34, (in Russian).

Abstract

Hybridization methods were developed for Eremothecium ashbyii (a riboflavine producer). The possibility of hybridization by joint incubation of spores of two auxotrophic mutants on minimal medium is demonstrated. The conditions for production, regeneration and fusion of E. ashbyii protoplasts are found. Auxotrophic recombinations are selected among ascosporous progeny of 29 hybrid

clones obtained by this method. The data which permit supposing meiotic sporulation in E. ashbyii are obtained. It is cytologically shown for the first time that spores contain two or more nuclei.

6. Golubev, V.I., Naumov, G.I., Bibikova, I.I., Blagodatskaya, V.M., Vustin, M.M., Nikitina, T.N., Buzurg-Zade, D.L., Gradova, N.B. A novel species assignment of hydrocarbon digesting strains of the genus Candida. Biotechnologia, 1986, No. 5, pp. 17-21 (in Russian).

7. Naumov, G.I., Kondratieva, V.I., Naumova, E.S. Hybridization of homothallic diplont and haplont yeasts. Biotechnologia, 1986, No. 6, pp. 33-36 (in Russian).

8. Naumov, G.I. Genetic differentiation and ecology of the yeast Saccharomyces paradoxus Batschinskaia. Dokl. Acad. Nauk SSSR, 1986, 291, No. 3, (in Russian).

Some of our recent studies are summarized below:

1. Naumov, G.I. Genosystematics of Zygofabospora Kudriavzev emend. G. Naumov. Molek. Genet., Mikrobiol. Virusol., 1986, No. 5, pp. 10-13 (in Russian).


ABSTRACT

The data on comparative study of genomes by genetic and molecular biology methods have permitted to identify yeasts of the genus Zygofabospora Kudriavzev emend. G. Naumov as a system of biological species. The genus is composed of hybridizable species mainly:

1. Z. marxiana (Hansen).
2. Z. aestuarii (Fell).
3. Z. delphensis (v.d. Walt et Tscheuschner).
4. Z. dobzhanskii (Shehata et al.).
5. Z. drosophilarum (Shehata et al.).
6. Z. lactis (Dombrowski)
7. Z. lodderi (v.d. Walt et Tscheuschner).
8. Z. phaseolospora (Shehata et al.).
9. Z. thermotolerans (Philippov).
10. Z. waltii (Kodama).
11. Z. wickerhamii (Phaff et al).


2. Naumov, G.I. Three sibling species of Saccharomyces sensu stricto.


ABSTRACT

The Saccharomyces yeasts have important scientific and applied significance, but only limited genofond of them - industrial and genetic laboratory strains - used now. All belong to the same biological species S. cerevisiae. Using hybridological analysis we demonstrated the existence in nature (tree exudates, soils et al.) of the biological species S. paradoxus Batschinskaia 1914 recently. S. cerevisiae var. tetrasporus (Bejerinck ex Dekker) Phaff et al. 1956 and S. cerevisiae var. terrestris Jensen 1967 are synonyms of S. paradoxus. Many strains of S. paradoxus we have isolated from oak exudates and revealed in All-Union Collection of

Microorganisms (VKM Y: 505, 506, 1697, 1704, 1707, 1708, 2472), CBS collection (CBS: 406, 432, 2980, 5829) and others.

Geneticists, biochemists, and molecular biologists have not studied the yeast S. paradoxus yet. There are important applications of this species in genetics and selection.

Using the yeasts VKM Y-1146 we detected by genetic methods a third biological species of Saccharomyces, that produces sterile hybrids with S. cerevisiae and S. paradoxus. The third species probably belongs to the group of strains revealed recently by low DNA homology with S. cerevisiae (Martini, Kurtzman, 1985).

The wild species of Saccharomyces sensu stricto constitute a new genofond for theoretical and biotechnological researchers.

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VIII. Levures - BAT. 405 - Univ. LYON 1, 43 Bd du 11 Nov. 1918, 69622 Villeurbanne Cedex - France. Communicated by M. C. Pignal.

Below follows news of our laboratory:

1. M.C. Pignal, C. Chararas & M. Bourgeay-Causse. Isolation and study of yeasts from the digestive tract of Ips sexdentatus, Coleoptera parasite of conifers. (in press in C.R. Acad. Sciences Paris).

We isolated several yeast strains from the digestive tract of adults caught in Pinus silvestris phloem, in Fontainebleau forest near Paris. These yeasts are identified as: Pichia bovis, P. rhodanensis, Hansenula holstii and Candida rhagii. The enzymatic equipment of these yeasts is studied and we show the presence of osidases which act on various oligosaccharides, heterosides and on pectin and starch; possibly, they complement the action of the insect enzymes in order to digest vegetable tissues. Moreover, we measure the amount of vitamins excreted in culture medium: it seems very likely that B-group vitamins, that Ips needs but cannot synthesize, are provided to the insect by the symbiotic yeasts.

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2. A. Michel, S. Poncet, F. Jacob & J. Perrier. Biomass composition of a Candida pseudotropicalis strain grown on crude sweet whey. This publication has been accepted by J. Sci. Food Agric. 1987, 39.

ABSTRACT

A yeast strain isolated from whey and named Candida LY 496 was grown continuously on crude sweet whey. The obtained biomass had a crude protein content of 47%. The amino-acid composition was determined by HPLC method. All essential amino-acids were present and among them the lysine was predominant (11.8%) The total nucleic acids of the biomass was 6.4%. Lipids represented 5% and the fatty acids 2% of the dry matter: 75% of the fatty acids were unsaturated, and linoleic acid constituted only 1% of the

total fatty acids. Niacin and vitamin B2 were the most abundant vitamins of the B group. Therefore Candida LY 496 biomass could be incorporated in animal food intakes.

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3. Participation at the International Symposium of Taxonomy, Ecology and Phylogeny of Yeasts and Yeast like fungi. Amersfoort, The Netherlands, 3-7 August 1987. Two posters. Genevieve Billon-Grand, Co-enzyme Q: Comparison between monokaryotic strains and corresponding fruit bodies.

We have tested two Basidiomycete mushrooms: Coprinus congregatus and Agrocybe segerita (monokaryotic strains, dikaryotic strains and fruit bodies). Furthermore, we have tested the cyanide-insensitive respiratory system in monokaryotic and dikaryotic mutant strains of the wild-type Agrocybe segerita.

Jean-Bernard Fiol, Ambrosiozyma, Hormoascus, Hyphopichia, yeast - like fungi and Hansenula and Pichia: biosystematic approach.

This paper appreciated the relationships between Ambrosiozyma, Hormoascus, Hyphopichia and Hansenula or Pichia which have close GC content (no more different than + or -0.5%).

Several genotypic approaches were used: intracellular osidases, nitrite and nitrate reductase, vitaminic requirement and cytochromic spectra in addition to co-enzyme Q system and GC content. Generally Pichia or Hansenula were unlike the Ascoideaceous yeasts by at least one character.

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

Recent communications.

Hagler, A.N. and D.G. Ahearn. 1987. Ecology of aquatic yeasts. In. A.H. Rose and J.S. Harrison (eds). The Yeasts, 2nd ed. Vol. 1. Academic Press, London, pp. 181-205.

Simmons, R.B. and D.G. Ahearn. 1987. Cell wall ultrastructure and DBB reaction of Sporopachydermia quercuum, Bullera tsugae and Malassezia spp. Mycologia 79: 38-43.

Abstract

Bullera tsugae and Malassezia spp., which demonstrated laminar cell walls, stained red to violet with diazonium blue B (DBB). Sporopachydermia quercuum, an Ascomycete which demonstrated two-layered cell walls, gave a

negative staining reaction. The finely laminar ultrastructure of the cell walls of Malassezia spp., which was similar to that of Filobasidiella neoformans, and the positive DBB reaction suggest that Malassezia is a basidiomycetous yeast.

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X. Department of Food Science, Louisiana State University, Baton Rouge, Louisiana 70803-4200. Communicated by Samuel P. Meyers.

Current yeast-related research in our laboratory involves a re-examination of the marsh yeast, Pichia spartinae, a rather "unique" sporogenous species found in striking concentrations associated with the salt marsh plant, Spartina alterniflora. The carbon assimilation pattern of P. spartinae suggests a possible commensal type of association with S. alterniflora. Two factors that have been reported contributing to "blooms" of unispecific yeast populations under natural in situ environmental conditions have included low pH and organic accumulation. I would welcome hearing from colleagues who have noted comparable uni-specific yeast blooms in asssociaton with viable, actively growing plant material, especially researchers with observations on such occurrences in aquatic ecosystems.

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XI. Department of General Microbiology and Biophysics, Faculty of Sciences, Charles University, 128 44 Prague 2, Vinicna 5, Czechoslovakia. Communicated by V. Vondrejs.

News of killer system research in our department.

The following papers have been recently published or completed for publication:

1. Špaček R., Vondrejs V.: 1986. Rapid method for estimation of killer toxin activity in Yeasts. Biotechnology Letters 8:701-706.

The procedure is based upon recognition of rhodamine B- stained, killed cells using a fluorescence microscope or by flow cytometer counting.

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2. Palková Z., Cvrčková F.: Method for estimation of Kluyveromyces lactis killer toxin activity. (paper in preparation).

The rhodamine B-method cannot be used for estimation of killer toxin activity which is not associated with functional damage to plasma membrane of sensitive cells (1). The killer toxin from K. lactis, however, inhibits the growth of sensitive culture and in this way protects the cells against membrane damage by nystatin treatment. The fraction of rhodamine B-unstained cells is a measure of the killer toxin activity.

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3. Janderová B., Davaasurengijn T., Bendová O. 1986. Hybrid strains of brewer's yeast obtained by protoplast fusion. Folia microbiol. 31:339-346.

Hybrid clones obtained by induced protoplast fusion of the polyploid killer strain S. uvarum P9-LK-12/1 and haploid strain S. diastaticus (CCY 21-49-9) acquired the ability to produce killer toxin and degrade dextrins in brewer's wort. Individual hybrid clones differ in many characteristics, very likely because different proportions of parental genomes in hybrids are present.

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4. Janderová B., Davaasurengijn T., Vondrejs V., Bendová O. 1986. A nex killer brewing yeast capable of degrading dextrin and starch. J. Basic. Microbiol. 26:000-000 (in press).

Hybrid strains able to ferment dextrins and to produce killer toxin were constructed by induced protoplast fusion of the brewing strain S. uvarum P9 with two different strains of Saccharomyces sp.: the killer strain S. cerevisiae T158C and strain S. diastaticus (CCY 21-49-9 a donor of dex genes). Hybrid strains utilise dextrins in fermented wort and are capable of eliminating heavy contaminations by sensitive strains of Saccharomyces sp.

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Two papers were presented at the 17th Conference of the Czechoslovak Microbiol. Soc., České Budějovice, 1986:

1. Cvrčková F., Vondrejs V.: An enrichment method for yeast mutants. Abstr. book O-43 in czech.

It was demonstrated that the treatment of mixed yeast cultures with killer toxin (K1) under proper conditions can be exploited for the isolation of auxotrophic mutants. The method is based on evidence that the killer toxin attack is dependent on the metabolic status of sensitive cells and is similar to the penicillin technique used in bacterial selections.

2. Zikánová B., Vondrejs V., Vernerová J., Bendová O.: Breeding of a hybrid industrial strain of S. uvarum P9-LK-12/1. Abstr. book O-44 in czech.

Improvement of growth characteristics of a new killer brewing strain was reached by continual cultivation under conditions simulating the industrial production of lager beer.

Two review papers have been completed:

1. Vondrejs V.: A killer system in yeasts: Applications to genetics and industry.

Submitted to Microbiol. Sciences. This minireview summarizes new killer toxin assays and preparation procedures, applications of killer toxin to selection of hybrid strains, enrichment of mutants, and application of killer systems to recombinant DNA technologies.

2. Palková Z., Vondrejs V.: Killer system in Kluyveromyces lactis: Applications to recombinant DNA technology. Posted to Biologicke listy (in czech). The killer system of K. lactis is characterized and attempts to use DNA plasmids to determine the killer phenotype are summarized.

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XII. Johannes Gutenberg-Universität Mainz, FB 21/Biologie, Institut für Mikrobiologie und Weinforschung, Universität - Postfach 3980, D-6500 Mainz, Fed. Republic of Germany. Communicated by F. Radler.

Below follows a summary of a recent publication from this institute.

Ferdinand Radler and Manfred Schmitt. 1987.
Killer toxins of yeasts: inhibitors of fermentation and their adsorption.
J. Food Protection (in press).

Abstract

The killer toxin (KT 28), a glycoprotein of Saccharomyces cerevisiae strain 28, was almost completely adsorbed by bentonite, when applied at a concentration of 1 g per liter. No significant differences were found between several types of bentonite. Killer toxin KT 28 is similarly adsorbed by intact yeast cells or by a commercial preparation of yeast cell walls that has been recommended to prevent stuck fermentations. An investigation of the cell wall fractions revealed that the toxin KT 28 was mainly adsorbed by mannan, that removed the toxin completely. The alkali-soluble and the alkali-insoluble β-1,3- and β-1,6-D-glucans lowered the toxin concentration to one tenth of the original amount. The killer toxin of the type K of S. cerevisiae was adsorbed much better by glucans than by mannan.

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XIII. Labatt Brewing Company Limited, 150 Simcoe Street, London, Ontario N6A 4M3. Communicated by G.G. Stewart.

Publications on Yeast from the Production Research Department, Labatt Brewing Company Limited for the past two years.

Bilinski, C.A., Innamorato, G., and Stewart, G.G. 1985.
Identification and characterization of antimicrobial activity in two yeast genera. Applied and Environmentla Microbiology. 50(5), 1330-1332.

Marmiroli, N. and Bilinski, C.A. 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.

Panchal, C.J., Meacher, C., Van Oostrom, J., and Stewart, G.G. 1985. Phenotypic expression of Kluyveromyces lactis killer toxin against Saccharomyces spp. Applied and Environmental Microbiology 50(2), 257-260.

Russell, I. and Stewart, G.G. 1985. Valuable techniques in the genetic manipulation of industrial yeast strains. Journal of the American Society of Brewing Chemists 43(2), 84-90.

Russell, I., Jones, R.M., and Stewart, G.G. 1985. The influence of cytoplasmic control on the expression of nuclear genes in brewing yeast strains. Proceedings of the European Brewing Convention Congress, Helsinki, 235-236.

Sills, A.M. and Stewart, G.G. 1985. Studies on cellobiose metabolism by yeasts. Developments in Industrial Microbiology 26, 527-534.

Stewart, G.G. 1985. New developments in ethanol fermentation. Journal of the American Society of Brewing Chemists 43(2), 61-65.

Stewart, G.G. and Russell, I. 1985. Tradition meets innovation in brewing. Bio/Technology 3, 791-798.

Stewart, G.G. and Russell, I. 1985. Modern brewing biotechnology. Comprehensive Biotechnology 335-381.

Stewart, G.G., Jones, R.M., and russell, I. 1985. The use of derepressed yeast mutants in the fermentation of brewery wort. Proceedings of the European Brewing Convention Congress, Helsinki 243-250.

Stewart, G.G., Panchal, C.J., Russell, I., and Sills, A.M. 1985. Studies on the use of genetically manipulated yeast strains in traditional fermentation processes. Biotechnology and Bioprocess Engineering. Proceedings of the 7th International Biotechnology Symposium, New Delhi, India. 485-487.

Stewart, G.G., Bilinski, C.A., Panchal, C.J., Russell, I., and Sills, A.M. 1985. Genetic manipulation of brewer's yeast strains. Microbiology, 367-374.

Whitney, G.K., Murray, C.R., Russell, I., and Stewart, G.G. 1985. Potential cost savings for fuel ethanol production by employing a novel hybrid yeast strain. Biotechnology Letters 8(5), 349-354.

Bilinski, C.A., Russell, I., and Stewart, G.G. 1986. Analysis of sporulation in brewer's yeast: induction of tetrad formation. Journal of the Institute of Brewing 92, 594-598.

Jones, R.M., Russell, I., and Stewart, G.G. 1986. The use of catabolite derepression as a means of improving the fermentation rate of brewing yeast strains. Journal of the American Society of Brewing Chemists 44(4), 161-166.

Panchal, C.J., Bilinski, C.A., Russell, I., and Stewart, G.G. 1986. Yeast stability in the brewing and industrial fermentation ethanol industries. CRC Critical Reviews in Biotechnology 4(3), 253-262.

Russell, I. 1986. Killer yeast identification. Journal of the American Society of Brewing Chemists 44(3), 123-125.

Russell, I., Crumplen, C.M., Jones, R.M., and Stewart, G.G. 1986. Efficiency of genetically engineered yeast in the production of ethanol from dextrinized cassava starch. Biotechnology Letters 8(3), 169-174.

Stewwart, G.G. and Russell, I. 1986. One hundred years of yeast research and development in the brewing industry. Journal of the Institute of Brewing 92: 537-558.

Bilinski, C.A., Russell, I., and Stewart, G.G. 1987. Applicability of yeast extracellular proteinases in brewing: physiological and biochemical aspects. Applied and Environmental Microbiology 53(3), 495-499.

D.R. Berry, I. Russell, and G.G. Stewart (Eds). 1987. Yeast Biotechnology. George Allen & Unwin. London, In press.

Slapack, G.E., Russell, I., and Stewart, G.G. 1987. Thermophilic Microorganisms for Ethanol Production. CRC Press.

G.G. Stewart, I. Russell, R.D. Klein, and R.R. Hiebsch (Eds). 1987. Biological Research of Industrial Yeast Strains. CRC Press.

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XIV. Botanisches Institut, Universität Düsseldorf, Universitätsstrasse 1, D-4000 Düsseldorf 1, Fed. Rep. Germany. Communicated by G. Michaelis.

Thomas Lisowsky, Eckhart Schweizer[1] and Georg Michaelis. 1987. A nuclear mutation affecting mitochondrial transcription in Saccharomyces cerevisiae. Eur. J. Biochem. (in press).

[1]Lehrstuhl für Biochemie der Universität Erlangen-Nürnberg.

Mitochondrial transcription was studied in a nuclear temperature-sensitive pet mutant of Saccharomcyes cerevisiae. The mitochondrial RNA levels in vivo and the in vitro transcriptional activities of isolated mitochondria were analysed. In comparison to the wild-type an overall reduction of mitochondrial gene expression together with a changed expression pattern was observed for the mutant, indicating a defect in mitochondrial RNA synthesis. These findings were supported by studies with a purified DNA-protein complex from yeast mitochondria. This complex was able to synthesize ribosomal and messenger RNAs in an in vitro system. Proteins from wild-type and mutant transcription complexes were tested for their DNA-binding abilities; one of the proteins identified in the wild-type had either lost this ability or was absent in the mutant.

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XV. Laboratoire de Génétique des Microorganismes, Centre de Biotechnologies Agro-Industrielles, Institut National Agronomique, 78850 Thiverval-Grignon, France. Communicated by H. Heslot and C. Gaillardin.

Our laboratory has recently moved to new facilities built on the second campus of the Institut National Agronomique, 40 km westwards of Paris. Our phone number now is: 30-54-45-10 ext. 1245.

Abstracts of our last published papers follow below:

1. -P. Fournier, C. Gaillardin, M.A. Persuy, J. Klootwijk, H. van Heerikhuizen. 1986. Heterogeneity in the ribosomal family of the yeast Yarrowia lipolytica: genomic organization and segregation studies. Gene 42:273-282.

The cloned r-DNA units of Y. lipolytica (van Heerikhuizen et al., 39 (1985) 213-222) and their restriction fragments have been used to probe blots of genomic DNA of this yeast. Wild type and laboratory strains were shown to contain 2 to 5 types of repeated units, each strain displaying a specific pattern. By comparing their restriction patterns, we could localize the differences between units within their spacer region. Tetrad analysis strongly suggested a clustered organization of each type of repeats as well as the occurrence of meiotic exchanges within the r-DNA family. Chromosome loss was induced by benomyl and allowed to map several r-DNA clusters on the same chromosome. All those results indicate that the Y. lipolytica r-DNA gene family is quite different from other yeasts.

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2. -B. Treton, M.T. Le Dall, H. Heslot. 9186. In vitro RNA synthesis by viruses isolated from the yeast Yarrowia lipolytica. Biochimie 68:991-999.

Viruses isolated from the yeast Y. lipolytica possess a DNA-independent RNA polymerase activity which is inhibited by ethidium bromide and by sodium pyrophosphate but not by actinomycin D. RNA synthesis is maximum at pH 8.0 and at 30°C. Newly synthesized RNA molecules are largely released from the particles, are single stranded and are able to hybridize with denatured viral genomic RNA.

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3. -C. Gaillardin, A.M. Ribet. 1987. LEU2 directed expression of β-galactosidase activity and phleomycin resistance in Yarrowia lipolytica. Curr. Genet. 11:369-375.

The nucleotide sequence of a 968 bp DNA fragment spanning the promotor and the 5'upstream sequence of the LEU2 coding sequence of the yeast Y. lipolytica has been determined. A LEU2: :lacZ gene fuxion has been constructed and expressed in transformed yeast cells, showing that as few as 232 bp of the LEU2 promotor were sufficient to direct gene expression. In order to develop new markers for transformation of this yeast, the LEU2 initiation codon was destroyed by in vitro mutagenesis and replaced by a cloning site. A gene conferring phleomycin resistance in E. coli was attached to the LEU2 promotor and shown to be efficiently expressed in yeast: direct selection of phleomycin resistant transformants was possible.

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4. -B. Kudla. 1987. Construction of an expression vector for the yeast Schizosaccharomyces pombe. Ph.D. thesis, Paris XI Orsay (in French).

Using a promotor-probe vector based on lacZ, genomic fragments of the fission yeast were screened for the presence of strong promotors. Transformants displaying high levels of β-galactosidase were detected on X-Gal plates. In one of them β-galactosidase amounted to 5% of the total protein synthesized in exponential phase; the corresponding insert was sequenced and typical promotor sequences (called 54/1) were observed. Deletion analysis of 54/1 revealed a complex regulatory region upstream of the TATA box. After recloning of the chromosomal locus, the homologous transcript directed by 54/1 was identified and compared to the heterologous 54/1: :lacZ transcript. Both transcripts were made at similar rates, with steady state levels of 0.3% vs. 0.4% of total mRNAs per copy of 54/1, and half-lifes of 11 min. vs. 8 min. The amount of β-galactosidase transcript increased linearly with the copy number of 54/1: :lacZ over a range from 1 to 15.

This promotor has been used to direct expression in S. pombe of other heterologous genes: XylE from Pseudomonas putida, a gene from Tn5 confering resistance to antibiotics of the bleomycin family, and a Bacillus licheniformis gene coding for a thermostable alpha-amylase.

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XVI. ALKO LTD, The Finnish State Alcohol Company, POB 350, SF-00101 HELSINKI, FINLAND. Communicated by Matti Korhola.

Below follows a list of our work published since the November 1986 Fall Issue of the Yeast Newsletter.

1. Roy S. Tubb. 1987. Gene Technology for Industrial Yeasts. Cambridge Prize Lecture. J. Inst. Brew., (March-April) 93:91-96.

ABSTRACT

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

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2. R.S. Tubb, P.L. Liljeström, T. Torkkeli and M. Korhola, Melibiase (MEL) genes in brewing and distilling yeasts.....298-303. Proceedings of the Second Aviemore Conference on Malting, Brewing and Distilling, held at the Aviemore Centre, Aviemore, Inverness-Shire, 19-23 May, 1986. Edited by I. Campbell and F. G. Priest (Heriot-Watt University, Edinburgh). Published by the Institute of Brewing, London.


ABSTRACT

The ability to secrete an α-galactosidase (melibiase) and, thereby, to ferment or assimilate the disaccharide melibiose has been widely used to differentiate strains of Saccharomyces uvarum or S. carlsbergensis ($Mel^+$) from S. cerevisiae ($Mel^-$). The use of an agar medium incorporating 5-bromo-4-chloro-3-indolyl-α-D-galactoside to differentiate α-galactosidase-positive strains of yeast, has now been demonstrated. This sensitive and convenient test is applicable to brewery quality control, particularly for detecting cross-contamination in breweries which use both ale ($Mel^-$) and lager ($Mel^+$) strains.

Cloned MEL1 DNA has been used as a molecular probe to show that a number of different MEL genes exist within the genus Saccharomyces. Brewing lager strains form a unique group in which the $Mel^+$ characteristic is conferred by a gene which is not closely related to MEL1.

Using recombinant DNA techniques, the MEL1 gene has been introduced into a brewery ale strain. The MEL1 gene itself provides a selectable marker for transformation of industrial yeast strains. Stable transformants which have the MEL1 gene integrated into chromosomal DNA have been obtained. Genetic manipulation of α-galactosidase production in brewing yeasts should (1) increase the scope and sensitivity of a method for estimating the level of pasteurisation a beer has received and (11) provide the opportunity of producing α-galactosidase as a by-product of the brewing process.

Therefore, genetic studies on the $Mel^+$ characteristic of yeast are being applied in a number of ways to the differentiation and construction of yeast strains of industrial interest.


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3. R. S. Tubb and J. R. M. Hammond. 1987. Yeast Genetics. In: Prest, E. G. and Campbell, I. (eds.) Brewing Microbiology. Barking: Elsevier Applied Science Publishers Ltd, p. 47-82. Chapter 3. 1. Introduction...2. Genetic Features of Industrial Yeasts...2.1. Life Cycle and sporulation...2.2. Chromosomes, ploidy and genetic stability...2.3. Extrachromosomal elements...3. Targets for Strain Development...3.1. The need for strain development...3.2. The required yeast strains for brewing and distilling...3.3. Specfic targets for yeast genetics in brewing and distilling...4. Genetic Techniques for Analysis and

Development of Strains...4.1. Mutation and selection...4.2. Hybridization...4.3. Rare mating and single-chromosome transfer...4.4. Spheroplastfusion...4.5. Transformation and recombinant DNA methods...5. Code...References.

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

ABSTRACT

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

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XVII. Arbeitsgruppe Genetik, Institut für Mikrobiologie, Technical University of Darmstadt, D-6100, Federal Republic of Germany. Communicated by F.K. Zimmermann.

The following are summaries of the activities of our yeast genetics group:

1. Stefan Hohmann: Sucrose fermentation in a mutant with a deletion of the SUC2 gene.

All strains of Saccharomyces cerevisiae carry either a silent or an active allele of SUC2. The silent suc0 allele reverts to an active form which codes for an active invertase at frequencies of about $1 \times 10^{-8}$. The SUC2 gene was deleted from a maltose-negative strain using a cloned fragment of the SUC2 gene (Hohmann and Zimmermann, Curr. Genet. 11, 217-225, 1986). The deletion was confirmed by Southern analysis. Cells of the deletion mutants were mutagenized with EMS and mutants selected for growth on sucrose on a medium containing antimycin A, an inhibitor of respiration which allows only for a utilization of sucrose via an efficient glycolysis. These mutants did not grow on raffinose, a substrate for invertase, maltose or α-methyl glucoside. However, enzyme assays definitely showed that α-glucosidase was present in all mutants. Maltose does not inhibit growth as found with the hex2 mutants which are inhibited because of an uncontrolled maltose uptake (Entian: Molec. Gen. Genet. 179, 169-175, 1980). The mutants fall in different phenotypic groups and some were shown not to be allelic by crossing and tetrad analysis.

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2. Ine Schaaff and Jürgen Heinisch: The effects of increased levels of various glycolytic enzymes on the rate of alcohol production in Saccharomyces cerevisiae.

The rate of the glycolytic flux in yeast is considered to be regulated at the phosphofructokinase and pyruvate kinase reaction steps. It has been impossible to enhance the activity of glycolytic enzymes by classical genetic or biochemical methods. The availability in our group of various glycolytic enzyme genes in multicopy vector YEp13 allowed to artificially increase individual enzyme activities and study their effects on the rate of ethanol production in one and the same strain. An 8.7 fold increase in phosphoglucose isomerase, 4 fold in phosphofructokinase (PFK1 and PFK2 simultaneously present), 5 fold in phosphoglycerate kinase, 8 fold in phosphoglyceromutase and 5 fold in pyruvate kinase did not affect the rate of ethanol formation nor did it speed up the increase in the rate of ethanol formation after a shift from non-fermentable carbon sources to glucose. A plasmid simultanously containing PFK1, PFK2 and PYK1 was transformed into a pyruvatekinase point mutant. The enzyme activities were increased 3 fold for phosphofructokinase and 5.8 fold for pyruvate kinase, but the rate of ethanol formation remained at wild type level.

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3. Carmen Jäger: One of the two structural genes of phosphofructokinase PFK1 on a multicopy plamid restores glycolysis in a pfk2 disruption and byp1 double mutant.

Yeast strains with a disrupted gene PFK1 or disrupted gene PFK2 have no soluble phosphofructokinase but are still able to form ethanol from glucose (Heinisch: Curr. Genet. 11, 227-234, 1986). Double mutants with defects in both genes or one of the PFK genes and one of the additional so-called BYP genes cannot form ethanol from glucose (Breitenbach-Schmitt et al.: Molec. gen Genet. 195, 530-535 and 536-540, 1984). A haploid double mutant carrying a disrupted PFK2 and a point mutant allele of BYP1 unable to grow on glucose media was transformed with a yeast gene pool in multicopy vector YEp13 and transformants selected for growth on glucose media. Two of these transformants contained plasmids with overlapping sequences which turned out to be PFK1. There was no detectable phosphofructokinase activity in centrifuged crude extracts. The restoration of glycolytic activity could be caused by a compensation of the pfk2 defect by an over-production of the PFK1 gene product.

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4. Marion Michaelis: Deletion of the PYK1 gene coding for pyruvate kinase of Saccharomyces cerevisiae.

The 5'-flanking region and most of the coding region of the PYK1 gene was replaced by the LEU2 gene and the deletion confirmed by Southern analysis. Northern analysis showed that there was no PYK1 transcript formed under conditions under which a transcript of the yeast actin gene was readily detected. Polyacrylamide gel electrophoresis confirmed that a major band corresponding to yeast pyruvate kinase was absent. Unexpectedly, a very intense band of a 23 kdal protein appeared instead. Enzyme assays for pyruvate kinase activity were performed using crude extracts from the deletion mutant using lactate dehydrogenase and NADH as an ancillary enzyme system to follow the formation of pyruvate from phosphoenol pyruvate and ADP. There was a residual activity of about 0.5% of that found in wildtype using the same assay. However, this activity was

not influenced by the presence or absence of fructose-1, 6-bisphosphate, the allosteric activator of wild type pyruvate kinase. This deletion mutant will be used to select secondary mutations which open a bypass of the pyruvate kinase reaction.

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5. F. K. Zimmermann: Induction of chromosomal malsegregation by aprotic polar solvents in yeast strain D61.M.

It has been shown that a large number of aprotic polar solvents can be potent inducers of chromosomal malsegregation (Mayer and Goin, Mutation Res. 187, 21-30 and 31-36, 1987; F. K. Zimmermann et al.: Mutation Res. 141, 15-18; 149, 339-351, 1985; 150, 203-210, 1985; 163, 23-31, 1986). Certain solvents only killed yeast cells but did not induce chromosomal malsegregation. As shown by Mayer and Goin, combinations of different aneuploidy inducing agents give potentiating effects at concentration levels at which each of the individual agents is almost completely inactive. We exploited this principle using propionitrile, a strong inducer of chromosomal malsegregation, at concentrations which barely induced chromosomal malsegregation, with various concentrations of other polar aprotic solvents which were only marginally active or negative in the test for induction of aneuploidy. Strong effects in combination treatments were observed for ethyl propionate, n-propyl acetate, isopropyl acetate, methyl n-butyrate, methyl iso-butyrate but only a borderline response was obtained with amyl propionate (raspberry flavor ester). Also negative in this type of assay was benzyl acetate (peach aroma ester). Certain ketones, some of which are known neurotoxic agents, were tested and found to be strong inducers of chromosomal malsegregation in combination treatments: 2-pentanone, 2-hexanone, 3-hexanone and 2, 5-hexanedione. Naturally occurring l-(-)- and d-(+)- carvone (used as flavoring agents in certain spirits) and angelicalactone were also positive. Industrially used solvents are mostly mixtures containing some of the chemicals positive in the yeast chromosomal malsegregation assay.

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XVIII. Experimental Oral Biology Unit, Faculty of Dentistry, University of Otago, Box 647, Dunedin, New Zealand. Communicated by Max Shepherd.

The following are abstracts of recent publications and completed projects and dissertations in my laboratory.

1. Jenkinson, H.F. and Shepherd, M.G. A. Mutant of Candida albicans Deficient in β-N-Acetylglucosaminidase (Chitobiase). J. Gen. Microbiol. (in press) 1987.

ABSTRACT

A mutant of Candida albicans strain ATCC 10261 was isolated which was defective in the production of β-N-acetylglucosaminidase (chitobiase). The mutant strain grew normally in minimal medium supplemented with either glucose or N-acetyl-D-glucosamine as carbon and energy source, and the cells formed germ-tubes at 37°C when induced to do so with N-acetyl-D-glucosamine. However, unlike the wild-type parent strain, the mutant

strain did not utilize N,N'-diacetylchitobiose for growth. The mutant and parent strains had similar growth rates on glucose or N-acetylglucosamine, similar rates of uptake of these sugars and similar rates of $^{14}C$-labelled amino acids incorporation. The chitobiase mutant did, however, produce 53-85% more chitin than the wild-type strain. No reversion of the mutant phenotype was observed following induction of mitotic recombination with ultraviolet light suggesting that the mutant (chi$^-$) allele was carried homozygously in the chitobiase-deficient mutant. Although the chitobiase deficient mutant was pathogenic, it was not as virulent as the wild-type strain.

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2. Jenkinson, H.F., Schep, G.P. and Shepherd, M.G. Cloning and Characterization of the LEU2 Gene and Autonomously Replicating Sequence (ARS) Elements from Candida albicans.

ABSTRACT

Candida albicans chromosomal DNA fragments generated by HindIII digestion were cloned into the HindIII site of plasmid pBR322. A recombinant plasmid containing 10.9 kb passenger DNA was isolated (pMK100) that complemented the leuB6 mutation in Escherichia coli. The complementing activity was localized by transposon (Tn1000) mutagenesis and subcloned as a 2.2 kb EcoRI-BamHI fragment, together with a yeast 2 μm origin of replication, on to pBR322. The resultant plasmid (pmK152) containing the C. albicans LEU2 gene transformed a Saccharomyces cerevisiae leu2 mutant to Leu$^+$. A derivative of pMK152 lacking the yeast origin of replication was then used to isolate further fragments of C. albicans DNA that would promote autonomous replication of the otherwise non-replicative plasmid in S. cerevisiae. Three separate EcoRI fragments of C. albicans DNA were isolated, each containing an autonomously replicating sequence (ARS) element. The fragments did not show extensive homology in Southern hybridizations. Two of the cloned fragments contained sequences that hybridized to single sites in the C. albicans genome, and that were unique to C. albicans DNA. The third fragment, localized to a 1.2 kb sequence of DNA, hybridized to several sites in the C. albicans genome, and also with sites in the genomic DNAs isolated from a number of other fungi.

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3. Rudee Surarit Ph.D. Dissertation Entitled "Control of Morphogenesis in Candida albicans. One study was on the effects of azole and polyene antifungals on the plasma membrane enzymes of Candida albicans.

ABSTRACT

There was extensive (>75%) inhibition of the plasma membrane enzymes ATPase, glucan synthase, adenyl cyclase, 5' -nucleotidase, and 3', 5' -cyclic phosphodiesterase assayed in situ. Purified plasma membrane was prepared from yeast cells of C. albicans by two different methods: Concanavalin A stabilization and coating of spheroplasts with silica microbeads. In the purified plasma membrane vesicles the adenyl cyclase and phosphodiesterase were extensively inhibited by the antifungal drugs; variable inhibition was observed with ATPase (50-100%). The 3', 5' -cyclic

phosphodiesterase of the plasma membrane purified by the microbeads method was completely inhibited by all of the antifungals and there was partial inhibition of ATPase (25-75%) and adenyl cyclase (30-90%).

A second area of study was on the structure of the β-glucans in the cell wall. The alkali-insoluble fraction of the wall contained both glucan and chitin. When this material was fractionated by specific enzymic hydrolysis followed by Bio Gel p.30 column chromatography, together with chemical analysis by methylation, acid hydrolysis and g.l.c. - m.s, evidence was obtained for a covalent linkage between the β-1-6 glucan fraction and chitin. The g.l.c. mass spec data indicated that the chitin was linked to β-1-6 glucan from the sixth position of the GlcNAc residue to the one position of the β-1-6 glucan chain.

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4. Broom, M.F. Ph.D. Dissertation entitled "Changes in Cell Envelope Glycoproteins During Germ-tube Formation in C. albicans.

ABSTRACT

Germ-tube formation by C. albicans ATCC 10261 induced by N-acetylglucosamine or glucose resulted in the appearance of a 43kDA protein in a cell envelope fraction. The protein increased quantitatively in the cell envelope during the emergence of the germ-tube and the amount of the 43kDA protein in the envelope fraction reflected the efficiency of the morphogenesis. The 43kDa protein was shown to have a surface location by lactoperoxidase catalysed iodination of the germ-tube cells and by protease digestion of the same cells. Lectin binding to the 43kDa protein demonstrated that this protein contains carbohydrate. Tunicamycin inhibited germ-tube formation in C. albicans and morphogenesis appeared to be more sensitive to this antibiotic than yeast phase growth. Tunicamycin also prevented the appearance of the 43kDa protein in the cell envelope fraction: under tunicamycin inhibition of germ-tube formation a new protein of 39kDa molecular weight appeared in the cell envelope. The 39kDa protein was also shown to have a surface location by iodination and protease digestion of the tunicamycin-treated cells. The 39kDa protein did not bind concanavalin A and wheatgerm lectin suggesting a lack of carbohydrate moieties on this protein. Endoglycosidase H digestion of the 43kDa protein resulted in the formation of a protein band with identical mobility to the 39kDa protein in SDS polyacrylamide gels. Peptide mapping of the 43kDa protein from germ-tube cells and the 39kDa protein from tunicamycin-treated cells suggested that these proteins are homologous. A number of other strains of C. albicans also secreted to the cell envelope a protein with molecular weight of 43kDa during germ-tube formation. These strains secreted a 39kDa protein to the cell envelope when germ-tube formation was inhibited by tunicamycin.

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6. Holmes, A. and Shepherd, M.G. Proline Induced Germ-tube Formation in Candida albicans; Role of Proline Uptake and Nitrogen Metabolism.

ABSTRACT

Proline induced germ-tube formation and cell-cell aggregation in three strains of Candida albicans was completely inhibited when the pH of the medium was 5.0 or lower, whereas GlcNAc-induced morphogenesis was unaffected even at pH 4.5. The pH sensitivity of proline-induced germtube formation was not caused by a modulation of uptake, which was unchanged (0.5 nmol mg dry weight$^{-1}$ m$^{-1}$) over the pH range 4.5 to 6.5. Furthermore, decreasing L-proline uptake with the competitive inhibitor D-proline did not inhibit germ-tube formation. D-proline itself cannot act as a mediator of morphogenesis.

There was a single uptake system for proline which was specific, constitutive and subject to ammonnium repression.

Cultures deprived of nitrogen in the presence of glucose were derepressed for proline uptake but the yeast-mycelial transition could not be effected by either proline or GlcNAc. The inhibition of morphogenesis was reversed when the nitrogen starvation was relieved by the addition of either ammonium ions, or the amino acids proline, arginine, glutamine, asparagine or ornithine.

These results indicate that the nitrogen status of the cells is critical for the morphogenesis of C. albicans.

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XIX. Division of Infectious Diseases, Department of Medicine, Harbor-UCLA Medical Center, 1000 West Carson Street, Bldg. E-5, Torrance, CA 90509. Communicated by Marjorie Crandall.

The following is an abstract of a paper presented at the American Society for Microbiology Conference: The biology and pathogenicity of Candida albicans, May 14-16, 1987, Palm Springs, California (see section on Meetings).

An EIA for Candida albicans Proteinase.
M. Crandall, A. Nelson, and D. Sustarsic.
Diagnostic Products Corp., Los Angeles, and
Harbor-UCLA Medical Center, Torrance, California.

The acidic proteinase produced by C. albicans is an extracellular enzyme with an optimum at pH 4. The enzyme in culture supernatants was assayed using hide powder azure as substrate, and it was found that all C. albicans isolates tested (46 out of 46) produced this proteinase.

Mouse monoclonal antibodies specific for purfiied C. albicans acidic proteinase antigen (CAPA) were produced and used in a two-site "sandwich" enzyme immunoassay (EIA). The EIA could detect 100 picograms of CAPA and was 200X more sensitive than the assay for proteinase activity.

Our monoclonal EIA is a modification of the rabbit antibody EIA of Ruchel and Boning (1983). In contrast to their polyclonal antibodies, our anti-CAPA monoclonals do not cross react with acidic proteinases from other Candida species. We obtained evidence for the production of this secreted proteinase in vaginal candidiasis. Both CAPA and anti-CAPA antibodies were

detected in vaginal swabs from Monilia patients. These human antibodies against proteinase were of the IgE class as well as other immunoglobulin classes. Our antigen capture EIA may be incorporated into a rapid diagnostic test for Candida vaginitis. This test would be an attractive alternative to vaginal smears and swab cultures.

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XX. Instituto de Quimica, Universidade Federal do Rio de Janeiro. Ilha do fundão - caixa postal 1573, Rio de Janeiro, Brasil. Communicated by Anita D. Panek.

The following papers have recently been published:

1. A.C. Panek, P.S. de Araujo, V. Moura Neto and A.D. Panek. 1987. Regulation of the trehalose-6-phosphate synthase complex in Saccharomyces. Current Genetics 11: (in press).

ABSTRACT

Trehalose-6-phosphate synthase is another example of an enzyme of carbohydrate metabolism, in Saccharomyces, which could be regulated by interconversion of forms. Deactivation was mediated both in vivo and in vitro by a cyclic AMP-dependent protein kinase. Reversibility of this process was obtained by a phosphatase treatment leading to an increase in activity. The phosphorylated, less active form of the enzyme proved to be more susceptible to activation by ATP.Mg. Mutants with well defined lesions in the cyclic AMP-dependent protein kinase system were used to corroborate our findings of a possible regulatory mechanism of trehalose-6-phosphate synthase activity by interconversion of forms.

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2. Dulce E. Oliveira, Manuel Arrese, Getacew Kidane, Anita Panek and James R. Mattoon. 1986. Trehalose and Maltose metabolism in yeast transformed by a MAL4 regulatory gene cloned from a constitutive donor strain. Current Genetics 11:97-106.

ABSTRACT

A 6.8 kb fragment of DNA containing the regulatory sequence MAL4p has been cloned from a genomic library prepared from Saccharomyces cerevisiae strain 1403-7A which ferments maltose constitutively. The library was prepared by ligation of 5-20 kb Sau3AI restriction fragments of total yeast DNA into the BamH1 restriction site of shuttle vector YEp13. A restriction map of the cloned fragment indicates that it encompasses a 2.6 kb segment which closely resembles the regulatory MAL6 gene previously identified (Needleman et al. 1984). The hybrid plasmid, p(MAL4p)4, could transform maltose-nonfermenting strains which contain cryptic α-glucosidase and maltose permease genes (malp MALg), but could not transform strains containing a functional regulatory sequence and a defective maltase-permease region (MAlp malg). A correlated absence of maltase and permease DNA from the cloned fragment was indicated by the restriction map. Although the cloned DNA fragment was derived from a constitutive strain, maltose fermentation and α-glucosidase formation by yeast transformed with p(MAL4p)4 was largely inducible by maltose and sensitive to catabolite

repression. Moreover, the active trehalose accumulation pattern (TAC(+) phenotype) linked to the complete MAL4 locus in strain 1403-7A and other constitutive MAL strains (Oliveira et al. 1981b) was not found in p(MAL4p)4 transformants. It may be concluded that constitutivity of maltose fermentation and the associated active trehalose accumulation are not merely consequences of a cis-dominant mutation causing constitutive formation of the MALp regulatory product. Moreover, constitutivity may not be caused solely by a mutation within the structural region of the MALp gene.

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XXI. Albert-Ludwigs-Universität, Biochemische Institut, D-7800 Freiburg I. Br., Hermann-Herder-Str. 7, West Germany. Communicated by Claudio Purwin.

Below follow two abstracts which summarize our recent work about the metabolism of Fructose-2, 6-bisphosphate in the yeast Saccharomyces cerevisiae.

1. Claudio Purwin[1], Martina Laux[1] and Helmut Holzer[1,2]. 1987. Fructose-2-phosphate, an intermediate of the dephosphorylation of fructose-2, 6-bisphosphate with a purified yeast enzyme. Eur. J. Biochem. 164, 27-30.

A fructose-2, 6-bisphosphate dephosphorylating enzyme was 3000-fold purified to electrophoretic homogeneity from Saccharomyces cerevisiae. Half-maximal activity was obtained at pH 6.0 with 6 µM fructose-2, 6-bisphosphate and 0.15 mM $Mg^{2+}$. On incubation for 90 min with fructose-2, 6-bisphosphate, about 80% of the substrate appears with an almost linear time dependence as fructose. In the first 30 min a substance accumulates to about 40% of the consumed fructose-2, 6-bisphosphate which forms free fructose on mild acid treatment. formation of fructose-6-phosphate was negligible. The mild-acid-labile intermediate was identified as fructose-2-phosphate by comparative ion-exchange chromatography with authentic fructose-2-phosphate synthesized from fructose-1-phosphate (Pontis, H.G. & Fischer, C.L. (1963) Biochem. J. 89, 452-459). The data suggest the reaction sequence fructose-2, 6-bisphosphate ⟶ fructose-2-phosphate ⟶ fructose. The designation fructose-2, 6-bisphosphate 6-phosphohydrolase is proposed for the enzyme described here.

2. Claudio Purwin[1], Martina Laux[1] and Helmut Holzer[1,2]. 1987. fructofuranose-2-phosphate is the product of dephosphorylation of fructose-2, 6-bisphosphate. Eur. J. Biochem., in press.

Using comparative ion exchange chromatography on Dowex 1 x 4 the product of dephosphorylation of fructose-2, 6-bisphosphate with purified yeast fructose-2, 6-bisphosphate 6-phosphohydrolase, was shown to be identical to the furanose form of fructose-2-phosphate prepared by chemical synthesis according to Pontis and Fischer (Biochem. J. 89, 452-459 (1963)). As expected for the furanose form of fructose-2-phosphate, the enzymatically formed product consumes 1 mole of periodate per mole fructose-2-phosphate, whereas the chemically synthesized pyranose form consumes 2 moles of periodate. In addition, it is shown that the enzymatic product behaves identically to the furanose, not the pyranose form of fructose-2-phosphate in hydrolysis of the ester bond at pH 4.0 and 37°C as

described previously for the chemically synthesized compounds (Pontis and Fischer, 1963).

[1]Biochemisches Institut, Universität Freiburg, Hermann-Herder-Str. 7, D-7800 Freiburg, West Germany.
[2]Gesellschaft für Strahlen- und Umweltforschung, Geschäftsführung, Ingolstadter Landstr. 1, D-8042 Neuherberg, West Germany.

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XXII. Albert-Ludwigs-Universität, Biochemisches Institut, D-7800 Freiburg 1. BR., Hermann-Herder-Str. 7. Federal Republic of Germany. Communicated by Dieter H. Wolf.

Below follows four communications on our research, which have either recently appeared or are in press.

1. Nieves Garcia-Alvarez, Ulrich Teichert and Dieter H. Wolf. 1987. Proteinase yscD Mutants of Yeast. Isolation and Characterization. Eur. J. Biochem. 163:339-346.

ABSTRACT

Mutants of the yeast Saccharomyces cerevisiae devoid of proteinase yscD activity were isolated by screening for the inability of mutagenzied cells to hydrolyze Ac-Ala-Ala-Pro-Ala-β-naphthylamide in situ. One of the selected mutants bears a thermolabile activity pointing to the gene called **PRD1** as being the structural gene for proteinase yscD. All mutants isolated fell into one complementation group. The defect segregates 2:2 in meiotic tetrads indicating a single gene mutation which was shown to be recessive. Diploids heterozygous for **PRD1** display gene dosage. The absence of proteinase yscD did not affect mitotic growth under rich or poor growth conditions, neither mating nor ascopore formation. Also growth of mutant cells after a nutritional shift-down was not altered. Inactivation of enzymes tested which are subject to carbon-catabolite inactivation, a process proposed to be of proteolytic nature, is not affected by the absence of proteinase yscD. Protein degradation rate in growing cells, in cells under differentiation- or heat-shock conditions, showed no obvious alteration in the absence of proteinase yscD activity. Also inactivation of α-factor pheromone was not affected by proteinase yscD absence. Normal growth of mutant cells on glycerol indicate that the enzyme is not involved in any vital event in mitochondrial biogenesis.

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2. Paz Suarez Rendueles and Dieter H. Wolf. 1987. Identification of the Structural Gene for Dipeptidyl Aminopeptidase yscV (DAP1) in Saccharomyces cerevisiae. J. Bacteriol. (in press).

ABSTRACT

Mutants of Saccharomyces cerevisiae lacking dipeptidyl aminopeptidase yscV were isolated from a strain already defective in dipeptidyl aminopeptidase yscIV, an enzyme with overlapping substrate specificity. The mutants were identified by an in situ technique using the chromogenic

substrate Ala-Pro-4-methoxy-β-naphthylamide to screen colonies for the absence of the enzyme. One of the selected mutants bore a thermolabile activity which is the consequence of a structural gene mutation. Fifty-three mutants analyzed fell into one complementation group that corresponded to the structural gene of the enzyme called **DAP1**. The defect segregated 2:2 in meiotic tetrads indicating a single chromosomal gene mutation which was shown to be recessive. Diploids heterozygous for **DAP1** display gene dosage. The absence of dipeptidyl aminopeptidase yscV or the combined loss of both dipeptidyl aminopeptidases yscIV and yscV did not affect mitotic growth under rich or poor growth conditions. In contrast to the dipeptidyl aminopeptidase yscIV lesion (ste13) which leads to α-sterility because strains secrete uncompletely processed forms of the α-factor pheromone, the dipeptidyl aminopeptidase yscV lesion does not affect mating, and strains produce fully active α-factor pheromone. Also growth of mutant cells on a non-fermentable carbon source such as glycerol was not affected. A nutritional shift-down did not lead to either increased lag phase nor reduced growth rate in dipeptidyl aminopeptidase yscV mutants or in multiple proteinase-deficient mutants. The differentiation process of sporulation was not disturbed by the absence of the two dipeptidyl aminopeptidases yscIV and yscV. Double mutants were still able to use the tripeptide Ala-Pro-Ala as sole nitrogen source.

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3. Jean-Claude Wagner, Claudia Escher and Dieter H. Wolf. 1987. Some Characteristics of Hormone (pheromone) Processing Enzymes in Yeast. FEBS-Letters (in press).


ABSTRACT

The **KEX2** gene encoded, membrane-bound $Ca^{2+}$-dependent thiol-endoproteinase - proteinase yscF (Achstetter, T., and Wolf, D.H., 1985, EMBO J. 4, 173-177) - responsible for processing of the precursor protein of the sex pheromone α-factor of the yeast Saccharomyces cerevisiae was solubilized from the membraneous fraction and partially purified. Gel filtration revealed an apparent molecular weight of the native protein of around 150,000. $Ca^{2+}$ concentration for half-maximal activity was in the micromolar range and concentration of the substrate Cbz-Tyr-Lys-Arg-4-nitroanilide for half-maximal velocity was 0.05 mM. The enzyme able to cleave basic amino acids from the carboxyterminus of peptides and probably involved in final maturation of the α-factor peptides generated by proteinase yscF is membrane associated, is active at neutral pH and responds strongly to the serine proteinase inhibitor phenylmethylsulfonyl fluoride (PMSF) as well as to -SH group blocking agents.


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4. Bernd Mechler, Hanne Müller and Dieter H. Wolf. 1987. Maturation of Vacuolar (lysosomal) Enzymes in Yeast: Proteinase yscA and Proteinase yscB are Catalysts of the Processing and Activation Event of Carboxypeptidase yscY. EMBO J. (in press).

ABSTRACT

Studies were performed to unravel the activation and maturation mechanism of vacuolar (lysosomal) proteinases in Saccharomyces cerevisiae. In vivo and in vitro studies show that proteinase yscA and proteinase yscB are involved in the activation and processing event of pro-carboxypeptidase yscY. Processing and activation of pro-carboxypeptidase yscY by proteinase yscA depends on an additional factor contained in the vacuolar fraction. Comparable activation can be mimicked by sodium polyphosphate. Optimum pH for processing by this proteinase yscA-triggered event is 5. The proteinase yscA-triggered maturation process of pro-carboxypeptidase yscY leads to an intermediate molecular weight form of the enzynme which is, however, fully active. Proteinase yscB transfers the intermediate molecular weight form or the original precursor to the apparently authentic, mature and active carboxypeptidase yscY. An activation and maturation scheme is devised.

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XXIII. Ruhr-Universität Bochum, Institut für Physiologische Chemie D-4630 Bochum 1, Federal Republic of Germany. Communicated by Richard Betz and Wolfgang Duntze.

Research in Progress: 1987.

Structure of a-factor from S. cerevisiae

We have recently shown that the mating hormone from haploid a cells is a hydrophobic dodekapeptide containing a carboxyterminal S-alkylated cysteine residue. Using NMR spectroscopy of native a-factor we have now been able to identify the hydrophobic substituent of the cysteine as a farnesyl residue. In addition the NMR spectra show that a factor contains a carboxymethyl group. A-factor thus belongs to the group of farnesyl peptides several of which have been recently detected as mating peptides of various fungal species.

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XXIV. University of Vienna, Department of Microbiology and Genetics, Althanstrasse 14, A-109- Vienna, Austria. Communicated by Michael Breitenbach.

Recently we moved to the Department of Microbiology and Genetics of Vienna University. Following are two abstracts of papers now being written in our group.

Chemical composition of the outer layers of the yeast ascospore wall. 2. Characterization of a D,L-Dityrosine-containing macromolecule. P. Briza, A. Ellinger[1], G. Winker[2], M. Breitenbach.

ABSTRACT

The outermost layer of yeast ascospore walls appears thin and very osmiophilic in electron microscopy. It confers resistance to lytic enzymes and is impermeable even to small molecules like primulin. This layer

contains dityrosine (P. Briza et al., J. Biol. Chem., 261, 4288-4294 (1986) as well as the larger part of the spore wall protein.

We are presenting here a partial characterisation of the macromolecular structure containing dityrosine. This macromolecule seems to be very highly crosslinked. Part of its dityrosine appears to be D,L already in vivo. The D-configuration of part of the alpha-C-atoms of dityrosine could contribute to the spores resistance against lytic enzymes.

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Chemical composition of the outer layers of the yeast ascospore wall. 3. The second layer from the surface consists of chitosan. P. Briza, A. Ellinger[1], G. Winkler[2], M. Breitenbach.


ABSTRACT

In two preceding papers we have reported the occurrence, optical configuration and macromolecular linkage of dityrosine in yeast ascospore walls. A dityrosine-containing macromolecule constitutes the outermost layer of these walls. In electron microscopy, a second surface layer can be seen which is also spore-specific. In intact spores, it is shielded from staining with fluorescent dyes by the impermeable outermost layer. However, in purified spore walls the second layer is brightly stained with primulin, and hydrolyzates of the same preparations contain about 10% glucosamine relative to spore wall dry weight. The spore wall material staining with primulin is resistant to chitinase, but readily degraded by treatment with $HNO_2$. Acetylation prior to $HNO_2$ treatment completely prohibits its degradation. A partial acid hydrolyzate of spore walls contains predominantly soluble poly-β-(1,4) glucosamine, as determined by $^{13}C$-NMR spectroscopy. By these criteria, the second layer from the surface of yeast ascospore walls is chitosan.



[1]Institut f. Elektronenmikroskopie und Ultrastrukturforschung, Schwarzspanierstr. 5,
[2]Institut f. Virologie, Kinderspitalgasse 15, Universität Wien, A-1090 WIEN.


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XXV. University of Glasgow, Biotechnology Unit, Institute of Genetics, Church Street, Glasgow, G11 5JS, Scotland. Communicated by Alistair J.P. Brown.

The abstracts below represent recent publications from my group, and reflect our interest in mRNA translation and degradation in yeast.

1. T. Chinnappan Santiago. Ian J. Purvis, Andrew J. E. Bettany and Alistair J.P. Brown. 1986. The Relationship Between mRNA Stability and Length in Saccharomyces cerevisiae. Nucleic Acids Research 14(21):8347-8360.

ABSTRACT

A rapid and convenient procedure has been developed for the measurement of mRNA half-life in S. cerevisiae using the transcriptional inhibitor, 1,10-phenanthroline. A range of half-lives from 6.6 ± 0.67 minutes to over 100 minutes, relative to the stability of the 18S rRNA control, has been obtained for fifteen mRNAs. They include the pyruvate kinase and actin mRNAs, as well as 13 randomly picked mRNAs of unknown function. The mRNAs clearly fall into two populations when their lengths and half-lives are analysed; one population is considerably more stable than the other when mRNAs of similar length are compared. Also, within each population, there is an inverse relationship between mRNA length and half-life. These results suggest that mRNA length and at least one additional factor strongly influence mRNA stability in yeast.

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2. T. Chinnappan Santiago[+], Andrew J.E. Bettany, Ian J. Purvis and Alistair J.P. Brown. 1987. Messenger RNA Stability in Saccharomyces cerevisiae: the influence of translation and poly(A) tail length. Nucleic Acids Research 15(6):2417-2429.

ABSTRACT

A comparison between the half-lives of 10 specific yeast mRNAs and their distribution within polysomes (fractionated on sucrose density gradients) was used to test the relationship between mRNA translation and degradation in the eukaryote Saccharomyces cerevisiae. Although the mRNAs vary in their distribution across the same polysome gradients, there is no obvious correlation between the stability of an mRNA and the number of ribosomes it carries in vivo. This suggests that ribosomal protection against nucleolytic attack is not a major factor in determining the stability of an mRNA in yeast. The relative lengths of the poly(A) tails of 9 yeast mRNAs were analysed using thermal elution from poly(U)-Sepharose. No dramatic differences in poly(A) tail length were observed amongst the mRNAs which could account for their wide ranging half-lives. Minor differences were consistent with shortening of the poly(A) tail as an mRNA ages.

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3. Ian J. Purvis, Andrew J.E. Bettany, T. Chinnanppan Santiago, John R. Coggins, Kenneth Duncan, Robert Eason, and Alistar J.P. Brown. 1987. The Efficiency of Folding of Some Proteins is Increased by Controlled Rates of Translation in Vivo A Hypothesis. J. Mol. Biol. 193:413-417.

ABSTRACT

We propose that the way in which some proteins fold is affected by the rates at which regions of their polypeptide chains are translated in vivo. Furthermore, we suggest that their gene sequences have evolved to control the rate of translational elongation such that the synthesis of defined portions of their polypeptide chains is separated temporally. We stress that many proteins are capable of folding efficiently into their native conformations without the help of differential translation rates.

For these proteins the amino acid sequence does indeed contain all the information needed for the polypeptide chain to fold correctly (even in vitro, after denaturation). However, other proteins clearly do not fold efficiently into their native conformation in vitro. We argue that the efficiency of folding of these problematic proteins in vivo may be improved by controlled synthesis of the nascent polypeptide.

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XXVI. Swiss Federal Institute of Technology, ETH- Honggerberg, 8093 Zürich, Switzerland. Communicated by O. Käppeli.

The following papers were recently published by the yeast group at the Institute of Biotechnology.

On regulation of sugar metabolism:

Käppeli, O. 1986. Regulation of carbon metabolism in Saccharomyces cerevisiae and related yeasts. Adv. Microbial Physiol., 28, 181-209.

Sonnleitner, B. and Käppeli, O. 1986. Growth of Saccharomyces cerevisiae is controlled by its limited respiratory capacity: Formulation and verification of a hypothesis. Biotechnol. Bio-eng., 28, 927-937.

Käppeli, O. and Sonnleitner, B. 1986. Regulation of sugar metabolism in Saccharomyces - type yeasts: Experimental and conceptual considerations. CRC Crit. Rev. Biotechnol., 4, 299-325.

Arreguin de Lorencez, M. and Käppeli, O. 1987. Regulation of gluconeogenic enzymes during the cell cycle of Saccharomyces cerevisiae growing in a chemostat. J. Gen. Microbiol., in press.

On cytochrome P-450 of yeasts:

Sanglard, D., Käppeli, O. and Fiechter, A. 1986. The distinction of different types of cytochromes P-450 from the yeasts Candida tropicalis and Saccharomyces uvarum. Arch. Biochem. Biophys., 251, 276-286.

Käppeli, O. 1986. Cytochromes P-450 of yeasts. Microbiol. Rev., 50, 244-258.

*I have left the yeast group and joined an engineering company but I still maintain my yeast projects at the Institute of Biotechnology.

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XXVII. Institute of Biotechnology - ETH-Honggerberg, 8093 Zürich, Switzerland. Communicated by A. Fiechter.

Below follow several recent publications from this Institute:

1. F. Meussdoerffer and A. Fiechter. Effect of Mitochondrial Cytochromes and Haem Content on Cytochrome P-450 in Saccharomyces cerevisiae. J. of Gen. Microbiol. 132:2187-2193, 1986.

2. M.S.A. Leisola, B. Schmidt and A. Fiechter. Enzymatic Determination of Veratryl Alcohol. Anal. Biochem. 155:108-111, 1986.

3. L. Kätterer, H. Allemann, O. Käppeli, A. Fiechter. Transient Responses of Continuously Growing Yeast Cultures to Dilution Rate Shifts: A Sensitive Means to Analyze Biology and the Performance of Equipment. Biotechnol. Bioeng. 28:146-150, 1986.

4. H. Gruninger and A. Fiechter. A Novel, Highly Termostable D-xylanase. Enzyme Microb. Technol., 8:309-314, 1986.

5. A. Fiechter, O. Käppeli and F. Meussdoerffer. Batch and Continuous Culture. In: The Yeasts, Vol. 2, 1986.

6. I. Adler and A. Fiechter. Valuation of Bioreactors for Low Viscous Media and High Oxygen Transfer Demand. Bioprocess Engineering 1:51-59, 1986.

## Doctoral Dissertations

Biologische Leistungsfahigkeit Des Torus-Bioreaktors Unter Variation Der Mediumseigenschaften. 1987. By Urs André Krebser.

Leistungsfähigkeit eines Propellerschlaufenreaktors Mit Niedrig-und hochviskosen Biologischen Testsystemen. 1986. By Ulrike Hermine Rutte-Müller.

Characterization of the Respirative and Respiro-Fermentative Glucose Metabolism in Saccharomyces cerevisiae. 1986. By María Mónica Arreguin Görz de Lorencez.

## Summary

The metabolism of glucose respiratively assimilating cells of Saccharomyces cerevisia was investigated and compared with that of glucose respiro-fermentatively and ethanol utilizing cells. Continuous culture technique was employed to study the different metabolic states, since glucose respirative metabolism can be observed in feed controlled cultivation systems only.

Early observations of the glucose metabolism of yeast indicated that the key gluconeogenetic enzymes (FBPase and PEPCK), MDH and CAT are strongly repressed in the presence of glucose in batch culture. The levels of these enzymes were found to be highest in ethanol growing cells and lowest in cells growing respiro-fermentatively. Cells with respirative glucose metabolism exhibited activities in the range between these two extremes. This effect was ascribed to induction by ethanol, which is produced and consumed in some phases of the cell cycle. The higher expression of such enzymes means that the metabolic states of glucose respiratively degrading cells and ethanol assimilating cells are similar. The degree of expression of these enzymes did not depend on the presence of glucose but rather depends on metabolic rates.

Early studies of m- and c-MDH isoenzymes in batch culture indicated that c-MDH was inactivated by glucose. These isoenzymes were studied in the different metabolic states. The results suggested that both enzymes are present under aerobic conditions.

The cytochrome content of ethanol utilizing cells was slightly higher than that of cells consuming glucose respiratively. The relation between cellular content of mitochondria and cytochromes was determined. Ethanol utilizing cells had significantly more mitochondrial protein as compared to cells grown on glucose respiratively at the same dilution rate. The portion of mitochondrial protein remained constant under glucose respirative and respiro-fermentative conditions.

The effect of ethanol production on the respiratory capacity of the cells was investigated. Ethanol produced by the cells had a strong inhibitory effect on the oxygen uptake rate when the ethanol concentration was higher than 10 g $l^{-1}$.

The strain of S. cerevisiae used in this work had the tendency to synchronize spontaneously during oxidative glucose metabolism. The study of the cell cycle contributed some evidence to the overflow hypothesis. This mechnism has been proposed to explain ethanol production. Ethanol formation coincided with the bud emergence. Before ethanol is produced, the pyruvate levels increased which is in agreement with the overflow hypothesis. The variation of the gluconeogenetic enzymes activities was parallel to the changes in ethanol concentration. The addition of ethanol to synchronous culture as pulse or substrate shift from glucose to ethanol did not produce an uniform pattern in the variation of the enzyme activities studied. This suggests different regulation mechanisms. It was concluded that ethanol induced the synthesis of gluconeogenetic enzymes in the respirative metabolism whereas the role of pyruvate was not significant. The activities determined in asynchronously growing cells represented and average value of the activities measured in synchronous culture. This indicated that synchronous culture yields a more differentiated picture of enzyme regulation than asynchronous culture.

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XXVIII. Instituto de Fisiologia Celular, Universidad Nacional Autonoma de Mexico, Apartado postal 70-600 04510 Mexico, D.F. Communicated by Antonio Peña.

The following summaries are from papers that are in the process of publication in different journals.

1. Jorge Ramirez, Martha Calahorra, and Antonio Peña. 1987. Variations on the "Dilution" Method for Reconstituting Cytochrome c Oxidase into Membrane Vesicles. Analytical Biochemistry 162 (in press).

A method for the rapid incorporation of cytochrome c oxidase into membranes has been developed. This method essentially consists of obtaining a preparation of the enzyme in which it is isolated and then dissolving it in a medium containing 0.5% of the detergent Tween 20, which gives a final concentration of 0.0125% after reconstitution. These studies revealed an optimal ratio of 1µg of enzyme to 5 mg of phospholipids. A

similar optimal ratio was found when the amount of protein was varied. The optimum temperature was found to be 30°C. Without a peak value being reached, it was found that the best reconstitution was obtained at pH 7.0-8.0. When measurements were performed either with a fluorescent cyanine ($DiSC_3(3)$) or by the uptake of tetraphenylphosphonium, it was found that the enzyme, with cytochrome c added to the outside, was capable of generating a membrane potential that was negative inside. Using the same procedure, the enzyme could also be reconstituted into vesicles of yeast plasma membrane. The procedure, then, seems adequate for incorporating cytochrome c oxidase into different kinds of membrane vesicles.

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2. Antonio Peña, J. Pablo Pardo, and Jorge Ramirez. 1987. Early Metabolic Effects and Mechanism of Ammonium Transport in Yeast. Arch. Biochem. Biophys. 253(2), in press.

Studies were performed to define the effects and mechanism of $NH_4^+$ transport in yeast. The following results were obtained. Glucose was a better facilitator than ethanol-$H_2O_2$ for ammonium transport; low concentrations of uncouplers or respiratory inhibitors could inhibit the transport with ethanol as the substrate. With glucose, respiratory inhibitors showed only small inhibitory effects, and only high concentrations of azide or trifluoromethoxy carbonylcyanide phenylhydrazone could inhibit ammonium transport. Ammonium in the free state could be concentrated approximately 200 fold by the cells. Also, the addition of ammonium produced (a) stimulation of both respiration and fermentation; (b) an increased rate of $H^+$ extrusion and an alkalinization of the interior of the cell; (c) a decrease of the membrane potential, as monitored by fluorescent cyanine; (d) an immediate decrease of the levels of ATP and an increase of ADP, which may account for the stimulation of both fermentation and respiration; and (e) an increase of the levels of inorganic phosphate. Ammonium was found to inhibit $^{86}Rb^+$ transport much less than $K^+$. Also, while $K^+$ produced a competitive type of inhibition, that produced by $NH_4^+$ was of the noncompetitive type. From the distribution ratio of ammonium and the pH gradient, an electrochemical potential gradient of around -180 mV was calculated. The results indicate that ammonium is transported in yeast by a mechanism similar to that of monovalent alkaline cations, driven by a membrane potential. The immediate metabolic effects of this cation seem to be due to an increased $[H^+]$ATPase, to which its transport is coupled. However, the carriers seem to be different. The transport system studied in this work was that of low affinity.

* * *

3. Martha Calahorra, Jorge Ramirez, S. Monica Clemente and Antonio Peña. 1987. Electrochemical potential and ion transport in vesicles of yeast plasma membrane. Biochim. Biophys. Acta (in press).

Vesicles from yeast plasma membrane were prepared according to Franzusoff and Cirillo (1983) J. Biol. Chem. 258, 3608), with slight modifications. When Mg-ATP was added, this preparation was able to generate a membrane potential, that was sensitive to inhibitors of the yeast $H^+$-ATPase and uncouplers, and could be decreased by the addition of

permeant anions, as measured by the fluorescence changes of the dye oxonol V. The addition of ATP could also generate a pH gradient, detectable by the fluorescence changes of the monitor aminochloromethoxyacridine. This gradient was sensitive to inhibitors of ATPase and uncouplers, and could be increased by the addition of permeant anions to the incubation mixture. When the vesicles were loaded with KCl, an increased rate of $K^+$ efflux was produced upon the addition of ATP. Cytochrome oxidase from bovine heart could be reconstituted into the vesicles and was shown to generate a membrane potential difference, negative inside, evidenced by the fluorescence quenching of the cyanide dipropylthiacarbocyanine and the uptake of tetraphenylphosphonium. Besides, in these vesicels, $K^+$ and $Rb^+$, but not $Na^+$ or $NH_4^+$ could decrease the quenching of fluorescence and the uptake of tetraphenylphosphonium produced when the electron-donor system was present. In the vesicles in which cytochrome oxidase was incorporated, upon the addition of cytochrome c and ascorbate, the uptake of $^{86}Rb^+$ could be demonstrated also. This uptake was found to be saturable and inhibited by $K^+$, and to a lesser degree by $Na^+$. The results obtained indicate that these vesicles are reasonably sealed and capable of generating and maintaining a membrane potential. The membrane potential could be used to drive ions across the membrane of the vesicles, indicating the presence and functionality of the monovalent cation carrier. The vesicles, in general terms seem to be suitable for studying transport of ions and metabolites in yeast.

* * *

XXIX. Ecole Nationale Supérieure Agronomique De Montpellier, Chaire De Génétique et de Microbiologie, Place Pierre Viala, 34060 Montpellier Cedex, France. Communicated by P. Galzy.

Below follows a list of recent articles from my laboratory:

1. Leclerc M., Ghemardin P., Arnaud A., Ratomahenina R., Galzy P., Gerbaud C. and Raynal A. 1986.

Biosynthesis regulation of the β-glucosidase produced by a yeast strain transformed by genetic engineering. Arch. Microbiol. 146:115-117.

2. Leclerc M., Chemardin P., Arnaud A., Ratomahenina R. and Galzy P. 1987.

Exocellular β-glucosidase inducibility in a mutant strain of Candida wickerhamii derepressed for endocellular β-glucosidase production. Mircen Journal (GB) 3:87-90.

3. Laborbe J.M., Rieu Y., Ratomahenina R., Montet D., Pina M., J. Graille, et Galzy P. 1987.

Essai de multiplication de cellules de Geotrichum candidum Link CBS 178.53 sur pâte de neutralisation. Oléagineux 42:(n° 2) 83-86.

4. Poinsot C., Moulin G., Claisee, M. and P. Galzy. 1987.

Isolation and characterization of a Schwanniomyces castellii Capriotti respiratory deficient mutant. Antonie Van Leeuwenhoek, 53: (in press).

5. Poinsot, C., Boze Hélène, Moulin G. and P. Galzy. 1986.

Respiratory pathway in Schwanniomyces castellii. Biology of the Cell. 58:65-70.

6. Boze, Hélène, Moulin G. and P. Galzy. 1987.

Uptake of galactose and lactose by Kluyveromyces lactis. Biochemical characteristics and attempted genetical analysis. J. Gen. Microbiol. 133:15-23.

7. Boze, Hélène, Nicol D., Moulin, G. and P. Galzy. 1987.

The roles of genes LAC1 and LAC2 on the biosynthesis of the lactose metabolism enzymes by Kluyveromyces lactis. Acta Microbiologica Hungarica, 34:73-83.

* * *

XXX. National Research Council Canada, Plant Biotechnology Institute, 110 Gymnasium Road, University Campus, Saskatoon S7N 0W9, Saskatchewan. Communicated by J.W.D. Grootwassink.

The following paper has appeared since the last issue of the Newsletter.

J.A. Brodsky and J.W.D. Grootwassink. 1986. Development and Evaluation of Whole-Cell Yeast Lactase for Use in Dairy Processing. J. Food Science 51(4):897-903.

Abstract

A low-cost industrial grade lactase was developed for one-time use in dairy products. The preparation contained the entire biomass of a selected hyperproducing strain of the yeast Kluyveromyces fragilis. Intracellular lactase was made freely accessible to its substrate by permeabilization of the cell membrane with food compatible reagents. In 0.1M phosphate plus 0.4% methyl paraben, permeabilization was complete in 0.5-1 hr at 50°C, with 90% activity recovery from 180 g/L of cells. Whole-cell lactase contained no viable cells and was free of proteolytic activity. Its pH optimum of 5.6-6.0 proved suitable for lactose hydrolysis concurrent with cottage cheese fermentation. Hydrolyzed whey was used in ice cream and bakers' yeast production.

* * *

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

The following are summaries of papers submitted for publication:

1. E. Minárik and O. Jungová: Undesirable biological activity of yeasts in bottled wines (in Solovak). Vinohrad (Bratislava) 25:1987 (in press).

Saccharomyces oviformis Osterwalder (=S. cerevisiae Hansen) and Zygosaccharomyces bailii (Lindner) Guilliermond are the most frequently occurring yeast species causing secondary fermentation and/or turbidity in bottled grape wines with residual sugar. Owing to comparatively high tolerance of Z. bailii to authorized preservatives in wine making (sulphur dioxide, sorbic acid) only total elimination of allyeasts from the wine by sterile bottling (membrane or EK-filtration) provided reliable results and stable wines.

* * *

2. E. Minárik, O. Jungová, Z. Šilhárová, F. Nemeček: Possibilities of increasing yeast activity in secondary fermentation of wines with residual sugar (in Slovak). Kvasny prumysl (Prague) 33:1987 (in press).

Some sorbents (yeast cell wall preparations, microcrystalline cellulose) and the activator of the hyphal fungus Botrytis cinerea show a stimulating influence not only on the ferementation of grape must under unfavorable fermentation conditions but also partly on secondary fermentation of wines with residual sugar-. Technological aspects are briefly discussed.

* * *

XXXII. Department of Microbiology CBSH G.B. Pant University of Agriculture and Technology, Pantnagar 263145 Distt. Nainital. India. Communicated by R.S. Rana.

Below follow abstract of a Masters thesis from the above institution:

Solid State Alcohol Fermentation of Sugarbeet. Manoj Kumar and R.S. Rana. Thesis, Master of Science (Microbiology) G.B. Pant University of Agriculture & Technology, Pantnagar. 103 pp, 1987.


ABSTRACT

1. Total extractable sugar in sugarbeet on dry weight basis was 70% equivalent to 15% of fresh weight of sugarbeet. The ash and nitrogen contents were 0.64% and 0.11%, respectively on fresh weight basis.

2. The ethanol yield in case of S. cerevisiae CDRI NTG was 5.5% (W/W) with fermentation efficiency 68.7% while it was 5.8% (W/W) with fermentation efficiency of 72.4% in S. cerevisiae NCIM 3187. The fermentation was completed in 20 hr. Maximum ethanol yield was obtained from 50 g of sugarbeet cossettes impregnated with 100 ml water.

3. The pH optima of S. cerevisiae CDRI NTG for ethanol production was 5.0 and for S. cerevisiae NCIM 3187, 4.5. In solid state fermentation nitrogen supplementation did not affect ethanol yield. An increase in inoculum size from 1.2 to 2.0 g dry weight of yeast/100 g of sugarbeet reduced the fermentation time by 4 to 5 hr in S.cerevisiae CDRI NTG and NCIM 3187, without affecting the ethanol yield.

4. Silage from sugarbeet tope contained 12.8% crude protein, and 63% nitrogen free extractives. Silage from stillage of all three strains of S. cerevisiae (CDRI, CDRI NTG and NCIM 3187) contained 8 to 9% crude protein and 58 to 61% nitrogen-free extractives.

The cost of ethanol production from sugarbeet based on the laboratory data of solid stage fermentation was calculated to be Rs. 2.40 per litre (95% ethanol) excluding the excise duty.

* * *

XXXIII. Department of Biochemistry, University of Kansas Medical Center, Kansas City, Kansas 66103. Communicated by Wilfred N. Arnold.

## A Small, Sturdy and Durable Rotator for the Continuous Agitation of Yeasts in Liquid Cultures.

Several observations from this and other laboratories indicate that the history of the inoculum influences the status of batch-produced yeast cells. Accordingly, our practice is to maintain small, liquid cultures of the working strain as a steady source of inoculum. By using two cultures, with different but regular transfer dates, cells are on hand twice a week to serve as uniform inoculum for a series of larger cultures.

Some years ago we were dissatisfied with both cost and performance of commercially available shakers and rotators that might be applied to the continuous but gentle agitation of a small number of growth flasks. The following apparatus and method met our requirements.

We elected to use a heavy-duty, in-line gearmotor that was designed for a long life in an industrial setting. Such a motor is offered by Dayton Electric Mfg. Co., Chicago, and has these specifications: 6.0 RPM, 1/40 Horse Power (18.65 Watts), overall length 25 cm, weight 4 kg, model number 3M 327. The shaft was connected to a platform-disc (2 cm x 15 cm diam.) via a standard bushing and a square key. Two spring-clamps for the flasks were lined up on the platform, each centered 5.5 cm from the shaft. The assembly was mounted on a board (2.5 x 14 x 30 cm) and bolted to a wall at about 6° off true vertical. Incidently, the gearmotor can be operated in any position so that other angles are permissible. The whole unit can be assembled for about $150 at current prices.

For the culture vessel we prefer a screw-capped Erlenmeyer flask (300 ml) with a nephelometer tube attached as a side-arm. Such a flask is offered by Bellco Glass Inc., Vineland N.J., as catalog number 2574-14133. Our standard volume of medium is 40 ml per flask. A glass marble (16 mm diam.) is added to each flask before sterilization by autoclaving.

The contents of the flasks are well stirred because of the geometry of rotation plus the sweep of the marble. We have obtained "smooth" cultures with both yeasts and yeastlike organisms with various degrees of filamentation. For our purposes room temperature operation has been convenient and satisfactory. The device is set up in a corner that has low traffic but is well served with conditioned air (23°C). The normal running temperature of the motor itself is about 35°C but this has no significant effect on the temperature of the flasks. A switch can be incorporated into

the power line but we operate our unit continuously; the slow rate of rotation permits exchange of flasks while running. The gearmotor is permanently lubricated and requires no maintenance. Our unit has given trouble-free performance for five years and continues in operation. Fabrication is straight-forward but we would be happy to supply a photograph if further detail is required.

* * *

## XXXIV. New Books

Yeast Biotechnology

Edited by D.R. Berry, G.G. Stewart[1] & I. Russell

Publishers - Unwin Hyman Limited, Hemel Hempstead, England. To be published late 1987.

This book will provide a comprehensive analysis of techniques and processes fundamental to the manufacture of industrial products from yeast. Concentrating largely, but not exclusively, on Saccharomyces cerevisiae, the book deals with yeast biotechnology in both the traditional industries of brewing, distilling and baking, and in the new industrial applications which require the same techniques of handling, manipulation and growth. Yeast Biotechnology is organized into sections based on areas of technology which are fundamental to all yeast industries. The first sections are concerned with the identification, maintenance and genetic manipulation of yeast; these are followed by the technology of yeast growth, product formation and processing. Thus, the book will be equally relevant to the traditional industries as to those involved with such newer processes as fuel alcohol production and the genetic engineering of pharmaceutical products.

* * *

[1]Graham G. Stewart, Ph.D., D.Sc., is Director of Technical Affairs for the Labatt Brewing Company, London, Ontario, Canada. He received his B.Sc. Hons. Microbiology and B.Sc. Hons. biochemistry degrees from the University of Wales, his Ph.D. in Microbial Biochemistry from Bath University and a D.Sc. from Bath University in 1983. Between 1967 and 1969, Dr. Stewart lectured in Biochemistry at the Portsmouth College of Technology. he joined the labatt Brewing Company in 1969 and has worked, published and lectured extensively on the biochemistry of yeast.

Dr. Stewart, as Chairman of the International Commission for Yeasts, organized the Vth International symposium for Yeasts held in London, Ontario in July, 1980. He is presently Treasurer of the International Union of Microbiological Societies, Vice-Chairman of the International Commission for Yeasts and Secretary of the Biotechnology Commission of IUPAC. He is co-editor of the CRC Press journal, Critical Reviews in Biotechnology, is on the editorial board of the Journal of Food and Microbiology and of Biotechnology Letters, as well as being on the advisory board of Microbiological Sciences. In addition to editing a number of books, Dr. Stewart has published over 150 original papers, patents, and reviews.

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## XXXV. Obituaries

In memoriam of Dr. Jacomina Lodder

On February 2, Dr. J. Lodder died at her home at Bennekom, the Netherlands. She was a distinguished yeast taxonomist, who in a number of authoritative monographs has made major contributions to yeast taxonomy.

Dr. Lodder was born at Schiedam in 1905. After taking her degree in biology at Leyden University in 1930, she was appointed at the "Centraalbureau voor Schimmelcultures" in 1932 and worked in the yeast collection which was housed in the Laboratory of Microbiology of the Technical University at Delft. The collection was under the direction of Prof. Dr. A.J. Kluyver who took the initiative for a taxonomic study of all yeast species of which cultures were available. The study resulted in three monographs. The first one, "Die sporogenen Hefen" (1931), was written by N.M. Stelling-Dekker. The second one, "Die anaskosporogenen Hefen, I. Halfte", was Dr. Lodder's thesis. It appeared in 1934 and was awarded a "cum laude". The third monograph, "die anaskosporogenen Hefen, II. Halfte", by H.A. diddens and J. Lodder, was published in 1942. The three volumes formed a solid basis for future developments and thus had a great impact on yeast taxonomy, creating order in a partly chaotic system and defining species by a number of features determined with simple, standardized tests. Stelling-Dekker used, apart from morphological critera, fermentation of sugars and utilization of nitrate for differentiation. Lodder extended the number of physiological tests with the assimilation of sugars. This resulted in an improved system and the number of species was considerably reduced.

Lodder left the "C.B.S." in 1938 and was employed by the Dutch Gist- en Spiritus Fabriek at Delft till her retirement in 1965. She started her work there with a period in the Carlsberg Laboratory with Winge in Copenhagen where she learned to isolate ascospores with a micromanipulator and to perform crossings with the isolated spores. Lodder's research at the yeast and alcohol factory has led to a number of patents. Her work also included supervision of the yeast culture collection.

After the war, a revision of the three monographs mentioned above had become desirable. Lodder obtained permission from the management of the yeast factory to devote all her time to this work. With the then assistant at the yeast collection of the "C.B.S." as a coworker this enterprise resulted in a new monograph "The Yeasts, a taxonomic study" (J. Lodder and N.J.W. Kreger-van Rij, 1952). The book supplied an urgent need and was very well received.

About the same time, L.J. Wickerham in the U.S.A. published some new ideas concerning yeast taxonomy involving the extension of the number of carbon compounds tested for assimilation reactions. Data obtained in this way afforded an improved differentation of species. Wickerham also introduced a new phylogenetic theory. In 1956, Lodder went to work first with Wickerham in Peoria and then with H. Roman, yeast geneticist in Seattle to become more familiar with new ideas and methods.

The developments after 1952 made a revision of "The Yeasts" advisable. This time it was to be a combined effort of 13 authors under

the editorship of Lodder. The second edition of "The Yeasts" appeared in 1970. It contained a number of new genera and species and the standard description of the species included many more features. For instance, data on homo- and heterothallism in ascosporogenous yeasts and on the life cycle of basidiomycetous yeasts were new. The physiological data were also more elaborate. Lodder, in her chapter on General Classification, referred to the first studies on DNA in yeasts, i.e., the determination of base composition as promising for the future of yeast taxonomy. She was always open to new ideas about classification and use of new methods, although she was reluctant to express an opinion on phylogenetic theories. She was a meticulous editor. In spite of her authority and fame in the yeast domain, Mia Lodder was very unpretentious. People meeting her, were captivated by her kindness and charm and she was a true friend. Mia was also a good teacher and I am thankful and proud to have learned so much from her.

N.J.W. Kreger-van Rij

* * *

In memoriam of Emil M. Mrak.

Emil M. Mrak died on April 9, 1987, at the age of 85.

Born in San Francisco and raised on a prune ranch in the Santa Clara Valley, Emil Marcel Mrak devoted his life and career to the University of California. He received his Bachelor of Science, Master of Science, and Doctor of Philosophy degrees from the University of California, Berkeley and joined the faculty at Berkeley in 1937. He became Chair of the Department of Food Science in 1948 and moved to the Davis campus in 1951, with most of the department staff.

In 1959, he was chosen chancellor at Davis, a position he held for 10 years until his retirement, in 1969. During that time the campus experienced its most rapid growth and development.

He was widely recognized for his research on yeast systematics and ecology. Two yeast species have been named after Mrak: Williopsis mrakii (Wickerham) Naumov and Vustin (Syn. Hansenula mrakii Wickerham) and Zygosaccharomyces mrakii Capriotti. His earlier work involved preservation of fruits by drying, and he later focused on world food problems, particularly in developing nations, and environmental issues.

Following his retirement, he served on several national commissions, including the U.S. Presidential Mission to the Latin American Republics, led by Nelson Rockefeller, in 1969. He received numerous international and national awards, medals and degrees, and was the first to receive the Institute of Food Technologists' three awards: Nicholas Appert Medal, Babcock-Hart Award and International Award.

The administration building at Davis was named Emil M. Mrak Hall in 1969.

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## XXXVI. Meetings

### 1. XVIth International Congress of Genetics, Toronto, Ontario, Canada, August 20-27, 1988.

The theme of the next International Congress of Genetics, to be held at the new Metropolitan Toronto Convention Centre, will be Genetics and the Unity of Biology. Invited and proffered papers will be presented on the structure, behavior, evolution, pathology and engineering of genomes at all levels of biological and phylogenetic organization. In addition, there will be sessions on historical, philosophical and social aspects of genetics. A large exhibition of scientific equipment and books, a varied social program and several satellite symposia and workshops on specialized topics are being planned. To place your name on the mailing list for Congress circulars, please write to:

Mr. Laurier Forget
Office of Conference Services
National Research Council
Ottawa, Canada K1A 0R6

Note: "If you have any intention of attending the Congress you must complete and return the Reply Form contained in the First Announcement of the Congress. Because of the high printing and mailing cost of the registration package, it will be sent only to those who have returned the Reply Form. If you have not received a First Announcement, please write directly to Mr. Laurier Forget, Office of Conference Services, National Research Council, Ottawa, Ontario, Canada, K1A 0R6 (telephone (613) 993-9009); he will then put your name on the final mailing list for the registration package. Having merely received a copy of the First Announcement does not mean that your name is on the final mailing list. Please do this as soon as possible so that the organizers can estimate attendance as accurately as possible".

#### Organization and Sponsorship

The Congress is sponsored by the International Genetics Federation, the Genetics Society of Canada, the National Research Council of Canada, the Royal Society of Canada and the Biological Council of Canada, under the auspirces of the International Council of Scientific Unions, and organized by a committee consisting of the following:

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Scientific Program

The scientific program will emphasize the most recent and exciting developments in genetics. It will consist of symposia, workshops and posters grouped into four main divisions as follows:

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| I. Genes and Chromosomes | III. Populations and Evolution |
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2. 3rd Trilateral Conference On Yeasts, Czechoslovakia, Hungary and East Germany, was held in Smolenice April 6-10, 1987. Communicated by A. Kockova-Kratochvilova, and E. Slavikova.

SECTION I

GENETIC MANIPULATIONS

LECTURES

Vondrejs, V., Janderová, B., Bendová, O.: Some Recent Advances in Yeast Genetic manipulations.
Novák, B., László, E., Mitchison, J.M.: A Cytoplasmatic Clock in the Fission Yeast Cell Cycle.
Weber, H., Kurischko, C.: Sexuality in the Yeast Yarrowia lipolytica.
Barth, G.: Regulation of Isocitrate Lyase in the Yeast Yarrowia lipolytica.
Šilhánková, L., Ruml, T.: Characterization of Complementary Genes Controlling the Over-production of Thiamine in Mutants of Saccharomyces cerevisiae.
Brantl, S., Lang, H.: Expression of the Surface of the Bovine Leukemia Virus in the Yeast S. cerevisiae.

Kunze, G.: Manipulation of the substrate utilization in Yeasts by Means of DNA Technics.

POSTERS

Becher, D., Bőtcher, F.: Transformation in Pichia guilliermondii.
Brozmanová, J., Vlčková, V., Kováčová, V.: Introduction of the Plasmid Containing Rec A Gene From Escherichia coli into Saccharomyces cerevisiae.
Cvrčková, P., Pálková, Z., Hahnová, Z.: Estimation of Activity of the Kluyveromyces lactis Killer Toxin.
Cvrčková, F., Vondrejs, V.: An Enrichment Method for Yeast Mutants.
Janatová, I.: Induction and Isolation of Auxotrophic Mutants of the Methylotrophic Yeast Candida boidinii.
Janderová, B., Bendová, O., Vondrejs, V.: Analysis of the Progeny of a Hybrid Obtained by Protoplast Fusion.
Klinner, U., Böttcher, F.: Mitotic Segregation in Growing Yeast Cultures.
Maráz, A.: Analysis of Somatic Hybrids of Saccharomyces cerevisiae and Candida (Torulopsis) glabrata.
Pálková, Z.: Killing Action of Kluyveromyces lactis On Some Strains of Bacteria.
Pálková, Z.: Construction of the Secretion Vector for the Yeast Saccharomyces cerevisiae.
Ruml, T., Šilhánková, L.: Mapping of a Gene Controlling Sensitivity to Oxythiamine in Saccharomyces cerevisiae.
Sipiczki, M., Bódi, Zs.: Increased Mitotic Recombination in cdc17⁻ Mutants of Schizosaccharomyces pombe var. pombe.
Šubík, J.: Physical mapping of the Cloned DNA Sequences to Specific Yeast Chromosomes.
Sulo, P.: Production of Yeast Hybrids by Protoplast Fusion.
Ulehlová, M., Šilhánková, L.: Mutagenic Effects of Herbicides on Saccharomyces cerevisiae.

## SECTION II

## MEMBRANE FUNCTIONS

LECTURES

Kotyk, A.: Effect of Cell Suspension Density on Transport in Yeast.
Novak, E.K.: Data Contradictory to the Membrane Pore Inducing Effect of the Polyenic Macrolide Antifungals.
Sigler, K.: Generation and Regulation of Protonmotive Force in Yeast.

POSTERS

Opekarová, M., Sigler, K.: Uptake of Leucine by Yeast Plasma Membrane Vesicles.
Ruml, T., Šilhánková, L., Rauch, P.: The Irreversibility of Thiamine Uptake in Saccharomyces cerevisiae.
Sychrová, H., Kotyk, A.: Transport of L-leucine in Schizosaccharomyecs pombe.
Vořišek, J., Martinková, J., Kalachová, L.: Cytochemical Evidence for the Participation of Coated Microglobules in the Secretory Pathway for Glycoproteins in Yeast.

## SECTION III

## EXO-AND INTRACELLULAR STRUCTURES OF YEAST CELLS

### LECTURES

Hašek, M.: Cytoskeleton in Yeast.
Streiblová, E.,: The Cytoskeleton and Control of the Yeast Cell Cycle.
Svobodá, A.: Yeast Exocytosis and Endocytosis.

### POSTERS

Kogan, G., Pavliak, V., Masler, L., Šandula, J., Alföldi, J.: New Structure of Candida krusei cell-wall mannan.
Kopecká, M., Gabriel, M., Venkov, P.V.: Ultrastructure and Cell Wall Formation of Osmotically Fragile Mutant of Saccharomyces cerevisiae VY 1160.

## SECTION IV

## BIOTECHNOLOGY

### LECTURES

Votruba, J., Vraná, D., Havlík, I., Sobotka, M.: Manipulation of Cultivation Conditions to Control the Physiological Functions in Yeasts.
Babel, W.: Consequences of the Simultaneous Utilization of Physiologically Similar Substrates by Microorganisms.
Malík, F., Minárik, E., Vojteková, G.: Pure Wine Yeasts in Czechoslovak Wine Making.
Basařová, G., Rychtera, M.: New Methods for Modification of Technological Properties of Brewer's and Distiller's Yeasts.
Biely, P.: New Abilities of Yeasts Related to Bioconversion of Plant Polysaccharides.
Straube, G.: Degradation of Hydroxybenzenes by Candida tropicalis HP 15.
Navara, A.: Effect of Yeast Cells on $NO_2^-$ and $NO_3^-$ Content in Grape Must of V. vinifera.
Klinner, U., Böttcher, F.: Genetic Manipulation of Imperfect Yeasts.

### POSTERS

Běhalová, B., Pásková, J., Machek, F.: Perspective of Ergosterol Production From Industrial Yeast.
Cseh, Z.: Characterization of Malolactic Strains With Their Isoenzymes.
Dercová, K., Augustin, J.: Effect of Formaldehyde on the Level of Cytochrome in Yeasts and Yeast-like Microorganisms.
Doležalová, L., Musil, P., Novotný, C.: Synthesis and Properties of Lipase in Candida rugosa.
Doušová, M.: Biosorption.
Kärgel, E., Schmidt, E.H., Schunck, W.H.., Riege, P., and Müller, H.G.: Immunological Investigation of the Occurence of Multiple Forms of Cytochrome P-450 in the Alkane-metabolizing Yeast Candida maltosa.
Kovács, B., Deak, P., Soyom, L., Pandi, F., Kovács, C.S.B.: Dried Wine Yeasts.
Mauersberger, S., Wiedmann, B., Schunck, W.H., Kärgel, E., Sharysehv, A.A., and Müller, H.G.: Regulation of Cytochrome P-450 Biosynthesis in alkane-

assimilating yeast.
Novotný, C., Novotná, J., Novák, M.: A study of the regulation of sterol synthesis in a chemostat.
Pólós, V.: Fatty Acid Desaturase Activity of Wine Yeasts.
Šmogrovičová, D., Augustin, J.: Metabolism of $^{14}$C-acetate in Yeasts Under Different Cultivation Conditions.
Ujcová, E., Machek, F., Ludvik, J., Švejcar, V., Pruša, K., and Malik, F.: The Use of Immobilized Yeast Cultures for the Production of Sparkling Wine.
Vojtková-Lepšiková, A., Machová, E., Kossaczká, Z.: D-Xylose Metabolism by Yeasts.
Vršanská, M., Biely, P.: New Simple Procedure for Purification of Yeast Xylanase.

## SECTION V

## TAXONOMY, ECOLOGY AND PRESERVATION OF THE GENE POOL

### LECTURES

Kocková-Kratochvilová, A.: Recent Taxonomy of Yeasts and Yeast-like Organisms.
Novák, E.K., Takáts, A., Deák, T.: Theoretical and Practical Value of the Physiological Data of Yeasts Identification Systems.
Jungová, O., Minárik, E.: Ecology of Vineyard Yeasts.
Deák, T.: Microecological Factors Underlying the Activity of Yeasts in Foods.

### POSTERS

Breierová, E.: Cryopreservation of Yeasts.
Flemming, Ch.: A New Method for Identification of Differentation of Yeast Strains.
Slávíková, E., Grabińska-Loniewska, A.: The Yeasts and Yeast-like Microorganisms in the Denitrification Unit Biocenosis.
Stollárová, V.: Composition of Natural Populations of Yeasts and Yeast-like Microorganisms in the Area of the Construction of the Atomic Power Plant at Mochovce.
Tomšiková, A.,Vraná, D.: Serotypes of *C. albicans*, Their Growth Characteristics, Adherence Capability and Virulance.
Černáková, M.: Microbial Degradation of the Lignin-cellulose Complex.
Daňková, R., Pichová, A., Streiblová, E., Nečas, O.: Structure of RAS Mutants of *Schizosaccharomyces pombe*.
Daňková, R., Hašek, J., Streiblová, E.: Dynamics of Actin and Tubulin Patterns During the *Saccharomycodes ludwigii* Cell Cycle.
Dobolyi, Cs., Péter, G., Kecskés, M.: Yeast Population on the Aerial Surfaces of Several Agricultural Plants.
Halász, A., Kassim, M., Szalma-Pfeiffer, I.: Protease Activity of *Saccharomyces*,*Candida*, and *Rhodotorula* Yeasts.
Havelková, M., Brieštanská, J.: Polyploid Nuclei and Morphology of Cytoskeletal Apparatus in Growing Yeast Protoplasts.
Kuncová, T., Hodný, Z., Nečas, O.: Enhancement of Cryoresistance of Yeasts by Clonal Selection.
Mátrai, B., Halász, A.: Identification of Yeasts by PAGE Technique.
Pavliak, V., Šandula, J., Paulovičová, E.: Comparative Studies of Pathogenic *Candida* Antigens by Precipitin Methods and Enzyme Linked

Immunosorbent Assay (ELISA).
Svobodá, A., Nečas, O.: Morphology of the Golgi Apparatus in Yeast.

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3. ASM Conference: The Biology and Pathogenicity of Candida albicans.

The ASM Conference on Biology and Pathogenicity of Candida albicans was held 14-16 May 1987 at Annenberge Center for Health Sciences, Palm Springs, California.

The conference focused on three broad areas that have received considerable attention: (i) basic biology including morphogenesis, genetics and cell surface structure; (ii) host-parasite interactions including adherence, cell-mediated immunity, phagocytic function, and humoral factors; and (iii) diagnosis and treatment. The organizing committee consists of Richard Calderone (Georgetown University), LaJean Chaffin (Texas Tech University Health Sciences Center), John Edwards (Harbor/UCLA Medical Center), and Dexter Howard (UCLA).

For further information, please write to Meetings Department, American Society for Microbiology, 1913 I Street NW, Washington, DC 20006. USA.

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4. X11$^{TH}$ International Specialized Symposium On Yeast: Genetics of Non-conventional Yeasts, September 13-19, 1987, Weimar German Democratic Republic (See also Yeast Newsletter 35 No 1).

For recent details write to: Dr. H. Weber, Central Institute of Microbiology and Experimental Therapy of the Academy of Sciences of the GDR, JENA, 6900, German Democratic Republic.

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5. Royal Netherlands Academy of Arts and Sciences, CENTRAALBUREAU VOOR SCHIMMELCULTURES, P.O. Box 273,3740 ag baarn, The Netherlands. The Expanding Realm of Yeast-like Fungi.

About 70 participants from 18 countries have registered. There still is room for additional participants.

The symposium will be held at the Hotel Amersfoort, a modern conference center about 50 kilometers from Amsterdam. The town has excellent highway and railroad connections to all parts of the Netherlands. During the summer in this country the so-called Holland Festival will take place, which stands for a large series of cultural events at major Dutch cities, particularly at Europa's 1987 cultural capital Amsterdam. In our last circular, to be published early July 1987, and during the symposium, we will present you with more detailed recreational facilities in the Netherlands.

A detailed, definitive programme will also be published in July. The main programme deviates only slightly from the Preliminary Programme as mailed earlier. During evening hours a number of workshops will be

organized on automated identification and data-handling. In all cases a short plenary introduction will be given, whereafter demonstrations will take place in adjacent rooms.

We have invited a number of commercial firms, to display their relevant products. This exhibition will change every day, so that in all about 15 firms will be represented.

For the poster sessions each contribuant will be granted maximally a 1 x 2 m board. Material for display, such as sellotape or velcro, will be available. The posters will be displayed during the entire symposium week.

Each speaker may contribute 10 to 15 pages in print to the Proceedings, to be published by Elsevier. The deadline for sending in full papers is July 31, 1987, but we urgently request you to send your manuscript well in advance. This will greatly speed up typing and editing, so that the Proceedings can be out within a relatively short period after the symposium. Authors will be granted a 30% discount on all Elsevier publications.

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6. 3rd Coordination Workshop on Microbial Protoplasts in Cell Biology, Genetics and Biotechnology, May 11 to May 14, 1988, Debrecen, Hungary.

INVITATION

The Coordinating Centre of the CMEA Programme IV-3-2-4 and the Department of Genetics, L.K. University are pleased to announce the 3rd Workshop on Microbial Protoplasts in Cell Biology, Genetics and Biotechnology to be held in Hungary in 1988. The aim of the meeting is to bring together the members of the cooperation agreement, but it is also open to non-members interested in the relevant topics. We sincerely hope that the Workshop will provide an excellent opportunity to obtain an overview of the recent developments in the field both within and outside the CMEA countries and thus we encourage your interest and participation.

TENTATIVE PROGRAMME

The areas to be covered by the meeting will be broadly similar to those presented at previous workshops:

- cell envelope
- protoplast techniques in cytology and physiology
- somatic hybridization (protoplast fusion)
- cell engineering
- cloning and genetic transformation
- strain improvement and biotechnological applications

Each session will consist of invited review lectures followed by shorter talks and poster discussions which will reflect current developments in similar fields. Ample time will be given to extensive discussions and every participant will have the opportunity to present her/his results in the form of a poster or a short oral communication. The abstracts will be published in Progress Report, the newsletter of the CMEA

Programme IV-3-2-4. The official language of the Workshop will be English.

Registration

Those wishing to receive further information are requested to write to: M. Sipiczki, Department of Genetics, L.K. University, H-4010 Debrecen, P.O. Box 56, Hungary.

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XXXVII. Brief News Items

Dear Colleagues:

I have relocated from the University of Rochester School of Medicine, Department of Radiation Biology and Biophysics, Rochester, New York 14642. My new address is:

City University of New York Medical School
Department of Microbiology
Science/Robert Marshak Building
138th Street at Convent Avenue
New York, New York 10031
Office: (212) 690-6926
Laboratory: (212) 690-5478

Carol Wood Moore, Ph.D.

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