

Y E A S T

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NOTICE TO OUR READERS

An increasing number of readers are taking advantage of electronic mail to send their Yeast Newsletter communications. Everyone who has access to E-mail is urged to explore its possibilities. Transmission times are very short, the text generally reaches its destination without errors, and the cost is low. As with any computer system, the initial learning steps may cause some anxiety, but the benefits are limitless. Address messages to All46@UWOCC1.BITNET. All E-mail messages will be acknowledged.

A diskette (5 1/4 inch DS-DD) containing MS-DOS compatible ASCII files also is a desirable medium. Your diskette will be returned with your next issue of the Yeast Newsletter.

We are experiencing difficulties with FAX transmissions, especially with dot matrix documents, which often arrive in a hardly legible state. Readers who must use FAX transmission are urged to send only documents typed with clear, large typeface. Please note the following new FAX number: (519)661-3935.

M. A. Lachance
Editor

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

The strains listed have been added to the ATCC recently. Complete information on these strains may be obtained upon request from the Mycology and Botany Department of ATCC.

Name	ATCC No.	Depositor & Strain	Significance & Reference
<u>Bensingtonia intermedia</u>	66425	T. Nakase, JCM 5291	Taxonomic purposes (J. Gen. Appl. Microbiol. <u>34</u> :433-437, 1988)
<u>Candida shehatae</u>	66604, 66611	NRRL, Y-12855, Y-12854	Fermentation of D-xylose to ethanol (Process Biochem. p. 21, 1989)
<u>Candida vini</u>	66816	M. Malfeito-Ferreira, ISA 1007	Wine spoilage yeast (Mycotaxon <u>36</u> :35-42, 1989)
<u>Cryptococcus clinovii</u>	66805	H.-J. Rehm, 1178	Degradation of phenol in mixed culture (Appl. Microbiol. Biotechnol. <u>30</u> :426-432, 1989)
<u>Hanseniaspora uvarum</u>	66807	F. Radler, 527	Killer toxin production (Arch. Microbiol. <u>149</u> :261-267, 1988)
<u>Kluyveromyces marxianus</u> var. <u>marxianus</u>	66613	D.A. Johnson, Yo 29	Phytopathogen causing soft rot of onion bulbs Plant Disease <u>73</u> :686, 1989)
<u>Pichia inositovora</u>	66612	NRRL, Y-12698	Genetic studies (Plasmid <u>21</u> :185-194, 1989)
<u>Pichia kluyveri</u>	66808- 66811	F. Radler, 392, 393, 394, 395	Killer toxin production (Arch. Microbiol. <u>149</u> :261-267, 1988)
<u>Pichia membranaefaciens</u>	66817	M. Malfeito-Ferreira, ISA 1030	Spoilage wine yeast (Mycotaxon <u>36</u> :35-42, 1989)
<u>Pichia stipitis</u>	66605, 66608	NRRL, Y-11542, Y-11543	Converting D-xylose to ethanol (Process Biochem. p.21, 1989)
<u>Rhodospiridium fluviale</u>	66477	J.W. Fell, ML 1580	Type strain (Mycologia <u>80</u> :560-564, 1988)
<u>Saccharomyces carlsbergensis</u>	66590	G.L. Hennebert, MUCL 28285	Brewery yeast (J. Inst. Brew. <u>94</u> :79-84, 1988)
<u>Saccharomyces cerevisiae</u>	66530	Y. Oshima, NA87-11A-D	Genetic studies (J.Mol.Biol. <u>182</u> :191-203, 1985)
<u>Saccharomyces cerevisiae</u>	66602- 66603	G. Braus, RH 1207, RH 1382	Genetic studies (Arch. Microbiol. <u>152</u> :263-268, 1989)
<u>Saccharomyces cerevisiae</u>	66818- 66820	M. Malfeito-Ferreira, ISA 1028, ISA 1063, ISA 1065	Commercial wine starter (Mycotaxon <u>36</u> :35-42, 1989)
<u>Saccharomyces cerevisiae</u>	20602	Phillips, et al. UCDE 694	Method for reducing fusel oil in alcoholic beverages and yeast strain useful in that method, U.S. Patent 4,374,859)
<u>Saccharomyces norbensis</u>	66812	G. Bambalov, MC-1	Sugar fermentation in olive and other effluents (Biological Wastes <u>27</u> :71-75, 1989)
<u>Saccharomyces oleaceus</u>	66813	G. Bambalov, MC-2	Sugar fermentation in olive and other effluents (Biological Wastes <u>27</u> :71-75, 1989)
<u>Saccharomyces oleaginosus</u>	66814	G. Bambalov, MC-5	Sugar fermentation in olive and other effluents (Biological Wastes <u>27</u> :71-75, 1989)
<u>Sporobolomyces ruberrimus</u> var. <u>albus</u>	66500, 66529	A. Shiraishi, No. 11, No. 10	Insensitive to antimycin A - a respiratory inhibitor of the cytochrome system (Agric. Biol. Chem. <u>50</u> :447-452.
<u>Tilletiopsis pallescens</u>	66528	A. Klecan	Severely inhibits growth of <u>Erysiphe graminis hordei</u> (Phytopathology - in press)
<u>Torulospira delbrueckii</u>	66821- 66824	M. Malfeito-Ferreira, ISA 1082, ISA 1102, ISA 1104, ISA 1105	Taxonomy (Mycotaxon <u>36</u> :35-42, 1989)
<u>Torulopsis</u> sp.	66815	G. Bambalov, MK-1	Sugar fermentation in olive effluents (Biological Wastes <u>27</u> :71-75, 1989)

Zygosaccharomyces bailii 66825- M. Malfeito-Ferreira, Wine spoilage yeast (Mycotaxon 36:35-42, 1989)
66826 ISA 1022, ISA 1100

II. National Collection of Yeast Cultures. Institute of Food Research, Colney Lane, Norwich NR4 7UA, United Kingdom. Communicated by P.J.H. Jackman.

NCYC 1990 Catalogue is now available both in printed form and as a text file on floppy disk together with a simple search program. The disk version is available for both IBM PCs and compatibles, and the Apple Macintosh. Please state disk format and size needed when ordering. All versions are £15 each + postage and packing from the National Collection of Yeast Cultures.

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

RECENT ACQUISITIONS

Ballistosporomyces ruber Nakase et al. 7512 = JCM 6884, T, ex dead leaf of Vitis ficifolia var. lobata on Mt. Fuji, Japan, T. Nakase (J. Gen. Appl. Microbiol. 35:389-309, 1989).

Ballistosporomyces xanthus Nakase et al. 7513 - JCM 6885, T, ex dead leaf of Acer rufinerve on Mt. Fuji, Japan, T. Nakase (J. Gen. Appl. Microbiol. 35:289-309, 1989).

Bensingtonia ciliata Ingold 7514 = CMI 291091, ex Auricularia auricula-judae var. lactae in UK, T. Boekhout (Ingold, Trans. Br. Mycol. Soc. 86:325-328, 1986).

Bullera alba (Hanna) Derx 7503 = IGC 4560, ex walnut leaf in Portugal, N. van Uden.

Candida odintsovi Bab'eva et al. 6026, T, 6025, 6194, ex sap of birch Betula verrucosa in USSR, I.P. Bab'eva (Mikrobiologiya 58:631-634, 1989).

Kluyveromyces lactis (Dombrowski) van der Walt 7505 - KR4, petite-positive strain with 300 kb deletion in the rDNA cluster, G.D. Clark-Walker (Galeotti & Clark-Walker, in Nagley et al. (eds.), Manipulation and Expression of Genes in Eukaryotes, Sydney: Academic Press 193, pp. 159-166. Maleszka & Clark Walker, Curr. Genet. 16:429-432, 1989. Hardy et al., Curr. Genet. 16:419-427, 1989); 7520, auxotrophic mutant of CBS 7505, requires adenine and lysine, G.D. Clark-Walker.

Pichia hangzhousana Lu & Li 7521, T, ex rotten pear in China, X. Lu (Acta Mycol. Sinica 8:251-255, 1989).

Pichia stipitis Pignal 7507, flocculent mutant of CBS 5773, D.R.J. Grootjen.

Sporobolomyces ruberrimus Yamasaki & Fujii var. ruberrimus 7500, ex atmosphere at. Fukuoka, Japan, A. Shiraishi.

Sporobolomyces ruberrimus Yamasaki & Fujii var. albus Yamasaki & Fujii 7501, T, ex CBS 7500, A. Shiraishi.

Trichosporon beigelii (Küchenm. & Rabenh.) Vuillemin 7506, ex cheese in the Netherlands, A.M. Jansen.

Yarrowia lipolytica (Wickerham et al.) van der Walt & von Arx 7504 - IFP29 = W29, US patent 3, 997, 399), production of citric & isocitric acids. Aconitase (EC 4.2.1.3), Treton & Heslot, Agric. Biol. Chem. 42: 1201-1206, 1978. Virus-like particles, Treton et al., Curr. Genet. 9:279-284, 1985. Cloning LYS5 gene for saccharopine dehydrogenase (EC 1.6.17), Xuan et al., Curr. Genet. 14:15-21, 1988. 7SL RNA genes, He et al., Current Genet. 16:347-350, 1989.

Zygozma arxii van der Walt et al. 7333, T, ex forest soil in South Africa, J.P. van der Walt (van der Walt et al., System. Appl. Microbiol. 12:288-290, 1989).

RE-IDENTIFIED STRAINS

Arthroascus schoenii (Nadson & Krasil'nikov) Bab'eva et al. 2556, 6423, 6449 (were A. javanensis) Smith et al., in press.

Pichia galoiformis Endo & Goto 763 (was Candida valida), 89% rDNA hybridization with type

Saccharomyces paradoxus Bachinskaya 7400 (was Saccaromyces douglasii) Naumov & Naumova, Koki. Acad. Nauk SSSR 311:975-976, 1990.

RE-NAMED SPECIES

Arxula adenivorans (Middelhoven et al.) van der Walt et al., A. van Leeuwenhoek 57:59-61, 1990 (was Trichosporon adeninovorans).

PUBLICATIONS:

1. van der Walt, J.P., M.Th. Smith & Y. Yamada. 1990. Arxula gen. nov. (Candidaceae), a new anamorphic, arthroconidial yeast genus. *Antonie van Leeuwenhoek* **57**:59-61.
2. van der Walt, J.P., M.Th. Smith, Y. Yamada & P.D.G. Richards. 1989. Zygozoma arxii sp.n. (Lipomycetaceae), a new species from Southern Africa. *System Appl. Microbiol.* **12**:288-290.

The following have been accepted:

3. Boekhout, T. 1990. Systematics of the genus Itersonilia Derx: a comparative phenetic study. *Mycological Research*.

A comparative study of the genus Itersonilia Derx (Basidiomycetes) was undertaken, including comparative morphology, nutritional physiology, septal ultrastructure, karyology and mating of yeast phases. Strains isolated from parsnip produce more chlamydospores than those from other sources, which frequently do not form chlamydospores at all. However, intergrading forms do occur. No species-specific differences are found in the nutritional physiology. Monokaryotic yeast phases sometimes differ physiologically from their parental strains, which are mostly dikaryotic. Yeast phases sometimes revert to monokaryotic hyphal phases with pseudoclamps. No karyogamy has been observed. The mating system seems to be tetrapolar. The presence of a relatively simple dolipore without parenthesomes points to a relationship with Tremellales. Because of 'interspecific' mating reactions the existence of separate species, I. perplexans, I. pyriformans and I. pastinacae is strongly questioned.

4. Smith, M.Th., M. Yamazaki & G.A. Poot. 1990. Dekkera, Brettanomyces and Eeniella: Electrophoretic comparison of enzymes and DNA-DNA homology. *Yeast* **6**.

The taxonomic status of various species of Dekkera, Brettanomyces and Eeniella was examined by electrophoretic comparison of enzymes, by deoxyribonucleic acid homology and by physiological characterization. These studies demonstrated that two teleomorphic Dekkera species D. anomala and D. bruxellensis (synonym D. intermedia), and four anamorphic Brettanomyces species, B. anomalus (synonym B. clausenii), B. bruxellensis (synonym B. abstinens), B. custersii, B. intermedius, B. lambicus), B. custerianus and B. naardenesis, can be recognized. The anamorphic genus Eeniella remained as a separate, monotypic taxon.

IV. Department of Agricultural Chemistry, Shizuoka University, Shizuoka 422, Japan. Communicated by Y. Yamada.

The following papers have been published recently.

1. Yamada, Y. & H. Kawasaki. 1989. The genus Phaffia is phylogenetically separate from the genus Cryptococcus (Cryptococcaceae). *Agric. Biol. Chem.* **53**:2845-2846.
2. Yamada, Y., H. Kawasaki¹, T. Nakase¹ & I. Banno². 1989. The phylogenetic relationship of the conidium-forming anamorphic yeast genera Sterigmatomyces, Kurtzmanomyces, Tsuchiyaea and Fellomyces, and the teleomorphic yeast genus Sterigmatosporidium on the basis of the partial sequences of 18S and 26S ribosomal ribonucleic acids. *Agric. Biol. Chem.* **53**:2993-3001.
¹Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan. ²Institute for Fermentation, Osaka, Jusohon-machi, Yodogawa-ku, Osaka 532, Japan

The eleven strains of conidium-forming yeasts classified in the basidiomycetous anamorphic genera Sterigmatomyces, Kurtzmanomyces, Tsuchiyaea and Fellomyces, and the teleomorphic genus Sterigmatosporidium were examined as to the partial sequences of 18S rRNA and 26S rRNA. The positions determined (in Saccharomyces cerevisiae) were 1451 through 1618 of 18S rRNA, and 1618 through 1835 and 470 through 626 of 26S rRNA. The partial sequence determination of positions 470 through 626 of 26S rRNA indicated that I. wingfieldii should be included in the group comprised of Fellomyces and Sterigmatosporidium species. However, the genera Sterigmatomyces, Kurtzmanomyces, Tsuchiyaea and Fellomyces constituted their own separate clusters as to the partial sequences of positions 1451 through 1618 of 18S rRNA and 1618 through 1835 of 26S rRNA. The classification of the conidium-forming yeasts in the four basidiomycetous anamorphic genera mentioned above was proved to be reasonable from the phylogenetic point of view.

3. Yamada, Y. & H. Kawasaki. 1989. The molecular phylogeny of the Q₀-equipped basidiomycetous yeast genera Mrakia Yamada et Komagata and Cystofilobasidium Oberwinkler et Bandoni based on the partial sequences of 18S and 26S ribosomal ribonucleic acids. *J. Gen. Appl. Microbiol.* **35**:173-183.

Ten strains of the Q₀-equipped basidiomycetous yeasts classified in the genera Mrakia and Cystofilobasidium were examined for the partial sequence determinations of 18S rRNA and 26S rRNA. The positions determined (in S. cerevisiae) were through 1,451 to 1,618 of 18S rRNA and through 493 to 624 and through 1,601 to 1,832 of 26S rRNA. The three determinations of the partial sequences of 18 rRNA and 26S rRNA divided the strains examined into two separate groups corresponding to the genera Mrakia and Cystofilobasidium. Group I contained the strains of M. frigida, M. gelida, M. nivalis, and M. stokesii, and group II contained the strains of C. bisporidii, C.

capitatum, and C. infirmominium. The inclusion of R. infirmominium in the genus Cystofilobasidium as C. infirmominium was proved to be phylogenetically reasonable. The data obtained indicate that the division of the Q₉-equipped basidiomycetous yeasts into the two genera mentioned above is more natural from the phylogenetic point of view. The utilization of the partial sequences of 18S rRNA and 26S rRNA is discussed, especially in the positions through 1,451 to 1,618 of 18S rRNA, for the classification of yeasts (and yeast-like fungi).

4. Yamada, Y., Y. Nakagawa & I. Banno¹. 1989. The phylogenetic relationship of the Q₉-equipped species of the heterobasidiomycetous yeast genera Rhodosporeidium and Leucosporidium based on the partial sequences of 18S and 26S ribosomal ribonucleic acids: the proposal of a new genus Kondoa. J. Gen. Appl. Microbiol. 35:377-385.

¹Institute for Fermentation, Osaka, Jusohon-machi, Yodogawa-ku, Osaka 532, Japan.

Eight strains of the Q₉-equipped heterobasidiomycetous yeast species, Rhodosporeidium malvinellum, Rhodosporeidium toruloides, and Leucosporidium scottii and the Q₉-equipped anamorphic yeast species, Rhodotorula lactosa were examined as to the partial sequence determinations of 18S rRNA and 26S rRNA. The positions determined were 1451 through 1618 of 18S rRNA and 492 through 625 of 26S rRNA. The two determinations of the partial sequences of 18S rRNA and 26S rRNA showed that the four species mentioned above constituted their own separate clusters. Rhodosporeidium malvinellum occupies a unique situation. The phylogenetic relationship between R. malvinellum and R. toruloides was more distant than that between R. toruloides and L. scottii. The data indicated that these three heterobasidiomycetous yeast species are distinguished from each other at the generic level. The new genus Kondoa was proposed for R. malvinellum.

V. Biotechnology R&D Division of the Soviet-Austrian Joint Venture "Vneshtreideinvest", USSR. Communicated by I. Krasilnikov.

RECOMBINANT YEAST HEPATITIS B VACCINE

The recombinant yeast Hepatitis B vaccine (RYHVacB) is indicated for active immunization against hepatitis B virus infection. RYHVacB may be used for vaccination (in the areas of high prevalence of hepatitis B) and for individual immunization (in subjects who are at high risk of infection: infants born to HBsAg-positive carrier mothers, health care professionals handling blood and other body fluids, users of addictive injectable drugs, homosexuals, etc., as in cases of AIDS). RYHVacB is a liquid pharmaceutical form in sterile ampoules, containing 1 ml (one dose) of vaccine:

- 20 µg of HBsAg (for adults), or 5 µg of HBsAg (for children to one year);
- 0.5 mg of aluminium hydroxide (as an adjuvant);
- 1 ml of H₂O with 0, 1 M NaCl; 0, 05 M Na₂HPO₄;
- 0.01% formaldehyde (as a preservative).

HBsAg (Hepatitis B virus surface antigen) is produced using recombinant DNA technique, that guarantees the safety of the vaccine since there is no risk of blood contaminants (HBV, HIV and other infectious agents of human blood and cells). HBsAg is synthesized in fast growing yeast cells (Saccharomyces cerevisiae) that are absolutely harmless for humans. The main stages of RYHVacB production: 1. Cultivation of HBsAg producing yeast cells; (final fermentation volume - 1-3 m³); 2. Desintegration of HBsAg containing cells; 3. HBsAg purification from yeast cell components; 4. Sterilization, filling, packaging.

Preclinical and clinical trials have shown that RYHVacB properties satisfy the WHO requirements for a recombinant hepatitis B vaccine. Dosage and Administration: The first injection - 1 ml; The second injection - 1 ml (a month later); The third injection - 1 ml (6 months from the first injection).

RYHVacB should be administered intramuscularly in the deltoid region. The protective level of specific antibodies against hepatitis B usually appears in 95% of vaccinees in a month after the third injection. RYHVacB is safe and effective vaccine with minimum of adverse reactions, typical for preparations of such kind. RYHVacB may be shipped both in ampoules and in bulk (in-bulk purchase will be discounted).

VI. Northern Regional Research Center, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois 61604. Communicated by C.P. Kurtzman.

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

1. Kurtzman, C.P. 1990. DNA relatedness among species of Sterigmatomyces and Fellomyces. Int. J. Syst. Bacteriol. 40:56-59.

Species assigned to the anamorphic yeast genera Sterigmatomyces, Fellomyces, Tsuchiyaea, and Kurtzmanomyces were compared for extent of nuclear DNA complementarity. The following organisms show little relatedness and are recognized as genetically distinct species: Sterigmatomyces halophilus, Sterigmatomyces elviae, and Sterigmatosporidium polymorphum. Sterigmatomyces indicus exhibited a high level of DNA relatedness with Sterigmatomyces halophilus and is considered a synonym of the latter species despite the difference in nitrate assimilation that led to the initial taxonomic separation of these taxa. The three taxa Sterigmatomyces aphidis, Trichosporon oryzae, and Sporobolomyces antarcticus showed moderate to high levels of DNA relatedness and are considered synonyms of Vanrija antarctica.

2. Guého, E.¹, C.P. Kurtzman, & S. W. Peterson. 1990. Phylogenetic relationships among species of Sterigmatomyces and Fellomyces as determined from partial rRNA sequences. Int. J. Syst. Bacteriol. **40**:60-65.
¹Institut Pasteur, Unité de Mycologie, 75724 Paris Cedex 15, France.

Sequence comparisons of selected regions from small (18S) and large (25S) subunit rRNAs were used to examine species relationships in the anamorphic yeast genera Sterigmatomyces, Fellomyces, Tsuchiyaee, and Kurtzmanomyces. On the basis of sequence similarity, the genus Sterigmatomyces is comprised of Sterigmatomyces halophilus and Sterigmatomyces elviae, while the genus Fellomyces contains three recognized species, Fellomyces fuzhouensis, Fellomyces penicillatus, and Fellomyces polyborus. Tsuchiyaee wingfieldii and Kurtzmanomyces nectairii and well separated from the other species which we examined. Comparisons with selected teleomorphs indicated that the genus Fellomyces is closely related to the genus Sterigmatosporidium, whereas the genus Sterigmatomyces exhibited somewhat closer relatedness with the genus Leucosporidium. Impacting on our estimates of relatedness was the finding that nucleotide substitution in the rRNA regions which we examined seems relatively constant only among closely related species.

3. Kurtzman, C.P. & C.J. Robnett. 1990. Phylogenetic relationships among species of Saccharomyces, Schizosaccharomyces, Debaryomyces, and Schwanniomyces determined from partial ribosomal RNA sequences. Yeast (accepted).

Species of the genera Saccharomyces, Schizosaccharomyces, Debaryomyces and Schwanniomyces were compared from their extent of divergence in three regions from small (18S) and large (25S) subunit ribosomal RNAs comprising a total of 900 nucleotides. With the exception of the closely related Saccharomyces bayanus and S. pastorianus, which appear to have identical sequences, all other species could be distinguished by nucleotide differences in a variable region of the large subunit, and genus-specific nucleotides were discernable in all three regions. The taxon D. tamarii differed markedly from other species and is excluded from Debaryomyces. By contrast, Schwanniomyces occidentalis showed few nucleotide differences with Debaryomyces, but species differences appear insufficient for dividing the genus. Some of the factors influencing estimates of phylogenetic distances from rRNA sequences are discussed.

4. Kurtzman, C.P. 1990. DNA relatedness among species of the genus Zygosaccharomyces. Yeast **6**:213-219.

Extent of nuclear DNA complementarity was determined for members of the genus Zygosaccharomyces. From these comparisons, nine species have been identified: Z. bailii, Z. bisporus, Z. cidri, Z. fermentati, Z. florentinus, Z. mellis, Z. microellipsoides, Z. mrakii, and Z. rouxii. Candida mogii, the proposed anamorph of Z. rouxii, showed low relatedness to all nine species. The recently described Saccharomyces astigiensis and S. albasitensis were conspecific with Z. fermentati, and S. placentae showed high relatedness with Z. rouxii. Growth tests were defined that allow recognition of all Zygosaccharomyces species.

5. (922) Proposal to conserve Cryptococcus (Fungi)

Cryptococcus Vuillemin, Rev. Gen. Sci. Pures Appl. **12**:741, 749. 1901, nom. cons. prop. T.: C. neoformans (Sanfelice) Vuillemin (Saccharomyces neoformans Sanfelice).

Cryptococcus Kutzing, Linnaea **8**:365. 1833, nom. rej. prop. [Fungi]
T.: C. molli Kutzing.

Proposed by: J. W. Fell, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 460 Rickenbacker Causeway, Miami, FL 33149, U.S.A.; C.P. Kurtzman, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, IL 61604, U.S.A.; K.J. Kwon-Chung, Clinical Mycology Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20982, U.S.A. Taxon **31**:151-156, February 1989.

6. Guého, E.¹, C.P. Kurtzman & S.W. Peterson. 1989. Evolutionary affinities of Heterobasidiomycetous yeasts estimated from 18S and 25S ribosomal RNA sequence divergence. System. Appl. Microbiol. **12**:230-236.
¹Institut Pasteur, Unité de Mycologie, 75724 Paris Cedex 15, France.

Phylogenetic relationships among heterobasidiomycetous yeasts, including anamorphic and teleomorphic taxa, have been compared from the sequence similarity of small (18S) and large (25S) subunit ribosomal RNA. Species examined were Cystofilobasidium capitatum, Filobasidiella neoformans, Filobasidium floriforme, Leucosporidium scottii, Malassezia furfur, M. pachydermatis, Phaffia rhodozyma, Rhodosporidium toruloide, Sporidiobolus johnsonii, Sterigmatosporidium polymorphum, Trichosporon beigelii, T. cutaneum and T. pullulans. The taxa cluster into three main groups. One group contains the non-teliospore forming genera Sterigmatosporidium, Filobasidiella, Filobasidium and the anamorphic species T. beigelii and T. cutaneum. The teliospore formers Leucosporidium, Rhodosporidium and Sporidiobolus, all of which have simple septal pores, cluster into a single group, despite the heterogeneity of carotenoid formation. By contrast, C. capitatum appears separate, not only from Rhodosporidium, but also from the Filobasidiaceae with which it shares a primitive dolipore septum. The uniqueness of the genus Malassezia among yeasts is confirmed, and one might predict from nucleotide sequence similarity that teleomorphs of those lipophilic organisms would form teliospores whereas Trichosporon beigelii and T. cutaneum appear related to the family Filobasidiaceae.

7. Kurtzman, C.P. 1990. Candida shehatae - genetic diversity and phylogenetic relationships with other xylose-fermenting yeasts. Antonie van Leeuwenhoek (in press).

The xylose-fermenting yeast Candida shehatae and Pichia stipitis were compared from extent of nuclear DNA complementarity and ribosomal RNA sequence similarity. Low levels of DNA relatedness confirmed that the two taxa are distinct biological species, but the similarity of rRNA sequences suggests that they only recently diverged. C. shehatae is comprised by three genetically divergent (ca. 50% DNA relatedness) subgroups that were accorded varietal status: C. shehatae var. shehatae, var. lignosa and var. insectosa. Estimates of phylogenetic distance from rRNA sequence similarity show C. shehatae and P. stipitis to be more closely related to Pachysolen tannophilus than to Saccharomyces cerevisiae, and that all of these budding yeasts are well separated from Schizosaccharomyces pombe.

VII. Department of Chemical Engineering, University of Melbourne, Parkville, Victoria, 3052, Australia. Communicated by N.B. Pamment.

The following is an abstract of a recently published paper.

1. Dasari, G., M. A. Worth, M. A. Connor & N. B. Pamment. 1990. Reasons for the apparent difference in the effects of produced and added ethanol on culture viability during rapid fermentations by Saccharomyces cerevisiae. Biotechnol. Bioeng. 35:109-122.

By feeding ethanol at various high rates to low cell density cultures of Saccharomyces cerevisiae it was shown that the sharp fall in viability when ethanol is produced during rapid fermentations is in part a direct consequence of the high rate of change of extracellular ethanol concentration. Nevertheless, the fall in viability in high cell density rapid fermentations which produced 98 g L⁻¹ ethanol in 3 h considerably exceeded that of control low cell density cultures to which ethanol was added at the same rate. This difference was shown to be not due to intracellular ethanol accumulation or to differences in glucose concentration between the cultures. The concentrations of a range of potentially toxic fatty acids, higher alcohols, and esters were measured during rapid fermentations, but when added at these concentrations to control cultures in the presence of ethanol they had no significant toxic effect. However, when rapid fermentations were conducted in rich medium containing 80 g L⁻¹ yeast extract, the apparent difference in toxicity of produced and added ethanol virtually disappeared. Magnesium was shown to be the component of yeast extract primarily responsible for this effect. The high rate of fall of viability when ethanol is rapidly produced is suggested to be partly due to the inability of the cells to adapt quickly enough to the rising ethanol concentration and partly to an increased demand for magnesium at higher ethanol concentrations which cannot be met in Mg-unsupplemented high cell density fermentations.

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

Summaries of recently accepted papers:

1. Minárik, E. 1990. Importance of biological stability of wine from the point of view of quality (in Slovak). Vinohrad 28(2).

Zygosaccharomyces bailii could hardly be found in long-term investigations on primary habitats (vine organs, grape must). In barreled wine prior to bottling and in bottled wine displaying cloud (haze) or refermentation the occurrence of Z. bailii was 55.5-64.5%, the representation in the yeast flora of wines 1.7-100%. Considering the chemo- and osmotolerance of Z. bailii, the only safe way to prevent wine clouding or refermentation, first of all in those containing residual sugar, may be EK or microfiltration of the wine prior to bottling regarded.

2. Minárik, E. & O. Jungová. 1990. Comparison of killer and neutral wine yeast strains (in Slovak). Vinohrad 28: in press.

Two active dry wine yeast preparations, the killer strain Benda K 158 and the neutral strain Benda N 156 have been tested in laboratory a pilot-plant grape must fermentation and compared with three Czechoslovak neutral strains (76/D, Bratislava 1, 2 v/f). Best results (fermentation rate, alcohol production, low volatile acid formation etc.) could be observed with the killer strain K 158.

IX. Biochemisches Institut der Universität Freiburg, Hermann-Herder-Strasse 7, D-7800 Freiburg, Federal Republic of Germany. Communicated by D. Wolf.

The following is an abstract of a recently published paper.

1. Teichert, U., B. Mechler, H. Müller, & D. H. Wolf. 1989. Lysosomal (vacuolar) proteinases of yeast are essential catalysts for protein degradation, differentiation, and cell survival. *J. Biol. Chem.* **264**:16037-16045.

Mutants deficient in the vacuolar (lysosomal) endopeptidases proteinase yscA and proteinase yscB of the yeast *Saccharomyces cerevisiae* exhibit a drastically reduced protein degradation rate under nutritional stress conditions. The differentiation process of sporulation is considerably disturbed by the absence of the two endopeptidases. Also under vegetative growth conditions and under conditions of false protein synthesis, the two vacuolar endopeptidases exhibit some effect on protein degradation, which is, however, much less pronounced as found under starvation conditions. Proteinase yscA deficiency leads to rapid cell death when glucose-grown cells starve for nitrogen or other nutrients. Whereas overall protein degradation is affected in the endopeptidase mutants, degradation of two distinct false proteins analyzed is not altered in the absence of proteinase yscA and proteinase yscB. Also catabolite inactivation and degradation of fructose-1,6-bisphosphatase is not affected to a greater extent in the endopeptidase-deficient strains.

X. Department of Biology, Japan's Women's University, 2-8-1, Mejirodai, Bunkyo-ku, Tokyo 112, Japan. Communicated by M. Osumi.

The following papers have been published recently.

1. Osumi, M., N. Yamada¹, H. Kobori, A. Taki¹, N. Natio¹, M. Baba¹ & T. Nagatani². 1989. Cell wall formation in regenerating protoplasts of *Schizosaccharomyces pombe*: study by high resolution, low voltage scanning electron microscopy. *J. Electron Microsc.* **38**:457-468.

¹Department of Electrical Engineering, Kogakuin University, 1-24-2, Nishishinjuku, Shinjuku-ku, Tokyo 163-91, Japan.

The ultrastructure of regenerating cell wall in *Schizosaccharomyces pombe* protoplasts was studied with a high resolution, low voltage scanning electron microscope (LVSEM). In contrast to the transmission electron microscopy, the LVSEM images give three-dimensional information on the cell wall regeneration in yeast protoplasts. We found that, after only a few minutes of incubation, the protoplasts began to show protuberances in a unipolar manner, and a fibrillar network was formed asymmetrically which covered the whole surface of the protoplasts after 5 hr. The network consisted of microfibrils about 8 to 10 nm wide, forming flat and wavy bundles of various widths and lengths, up to about 200 nm wide and 1 µm long, mainly made of yeast glucan. Free ends of microfibrils were seldom found. Interfibrillar spaces were progressively filled with granular particles and finally the complete cell wall was formed after 12 hr. The fibrillar network was destroyed by the digestion with β(1→3)-glucanase. When protoplasts were regenerating in the presence of aculeacin A, the fibrillar networks were not formed, resulting in incomplete cell wall formation. These observations suggest that β-glucan is the main component of the microfibrils and that it plays an important role in the formation of the cell wall in *S. pombe*. Key words = high resolution, low voltage scanning electron microscopy (HR-LVSEM): cell wall formation: protoplast reversion: yeast cell: *Schizosaccharomyces pombe*.

2. Osumi, M. 1989. Development of methods for observing of bacteria and fungi by electron microscopy. *J. Electron Microsc.* **38** Suppl.:S150-S155.
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3. Baba, M., N. Baba¹, Y. Ohsumi², K. Kanaya¹, & M. Osumi. 1989. Three-dimensional analysis of morphogenesis induced by mating pheromone a factor in *Saccharomyces cerevisiae*. *J. Cell Sci.* **94**:207-216.

¹Department of Electrical Engineering, Kogakuin University, 1-24-2, Nishishinjuku, Shinjuku-ku, Tokyo 163-91, Japan. ²Department of Biology, College of Arts and Sciences, University of Tokyo, 3-8-1, Komaba, Meguro-ku, Tokyo 153, Japan.

Ultrastructural analyses of cytoplasmic changes in *Saccharomyces cerevisiae* X2180-1A (MATa) that had been treated with a factor were performed by using the freeze-substitution fixation method. After a factor treatment, cells exhibited a pointed projection, which is a unique pattern of oriented cell surface growth. The relationship between projection formation and intracellular organelles was examined using serial thin sections and computer-aided three-dimensional reconstructions. Using these analyses membrane vesicles and other organelles were detected, and studies on their dynamic structural reorganization became feasible. Production of membrane vesicles (average 65 nm in diameter) was induced upon exposure of the cells to a factor before projection emergence. The total number of membrane vesicles increased at the early stage and decreased at the late stage of projection formation. Three-dimensional analysis indicated that the vesicles were at first dispersed throughout the cell, then accumulated at the site where the projection formed. Morphological changes and multiplication of the Golgi body were seen during the process of projection formation. Other intracellular organelles (nucleus, vacuole, rough endoplasmic reticulum and mitochondria) were also rearranged, showing a polar organization of the cytoplasm during projection formation.

XI. University of Guelph, Department of Environmental Biology, Guelph, Ontario, Canada N1G 2W1. Communicated by H. Lee.

The following papers have been published recently.

1. Schneider, H., H. Lee, M. de F.S. Barbosa, C.P. Kubicek & A.P. James. 1989. Physiological properties of a mutant of Pachysolen tannophilus deficient in NADPH-dependent D-xylose reductase. Appl. Environ. Microbiol. **55**:2877-2881.

A D-xylose reductase mutant of Pachysolen tannophilus was isolated on the basis of its poor growth on D-xylose but normal growth on xylitol and D-glucose. Fractionation of cell extracts indicated that the mutant was deficient in D-xylose reductase activity that used NADPH exclusively as a cofactor, but not in activity that used both NADH and NADPH. Mutant cultures grown on D-xylose as the sole source exhibited some properties that would be desired in improved strains. Growth rate, growth yield, and D-xylose consumption rate of the mutant were less sensitive than those of the wild type to changes in aeration rate. D-xylose was utilized more efficiently in that less of a by-product, xylitol, was produced. In addition, under low aeration condition, more ethanol was produced. A disadvantage was a relatively slow rate of D-xylose utilization.

2. Barbosa, M., de F.S., H. Lee & D.L. Collins-Thompson. 1990. Additive effects of alcohols, their acidic by-products, and temperature on the yeast Pachysolen tannophilus. Appl. Environ. Microbiol. **56**:545-550.

The effects of alcohols on the growth and fermentation of the yeast Pachysolen tannophilus were investigated at both 30 and 35°C. Addition of alcohols to the culture medium decreased both the growth rate and the final cell yield in a dose-dependent manner, and this decrease was more severe at 35°C. The concentration for 50% growth rate inhibition decreased as the chain length of the alcohol increased. In fermentations using a high initial cell density, production of acids was always observed when the medium was supplemented with alcohols. Supplementation of the culture medium with a short-chain alcohol plus the corresponding acid was shown to exert an additive deleterious effect on fermentation, and this effect increased with temperature. Production of acids was associated with the presence of alcohol dehydrogenase activity in cell extracts.

3. Lee, H. & B.G. Fisher. 1990. Unusual fructose utilization by Pichia stipitis and its potential application. J. Ferment. Bioengin. **69**:79-82.

The pentose-fermenting yeast Pichia stipitis was recently found to metabolize D-fructose poorly in the presence of D-xylose. In a survey of strains from 17 other Pichia species growing in a xylose/fructose mixture, only P. abadiae displayed a pattern of sugar utilization similar to P. stipitis in that the pentose was consumed preferentially over D-fructose. However, poor or incomplete D-fructose utilization was found with several species. The question was raised whether such a property may be useful for D-fructose enrichment in a glucose/fructose mixture. Among the species which utilized D-fructose incompletely, P. stipitis strains showed the best potential in this regard, as it rapidly consumed D-glucose with the production of ethanol, while D-fructose remained unused. Cell extracts of P. stipitis possessed D-fructose-phosphorylating activity. Therefore, the site responsible for poor D-fructose metabolism was postulated to be D-fructose transport.

XII. Department of Genetics, University of Warsaw, 00-478 Warszawa, Al. Ujazdowskie 4, Poland. Communicated by R. Maleszka.

Present Address: Molecular and Population Genetics, RSBS, ANU, Canberra, Australia.

We are employing molecular techniques in order to characterize a pentose fermenting yeast, Pachysolen tannophilus. A number of genes have been cloned and one of them, encoding the ornithine carbamoyltransferase (OTC), is now sequenced. Four unidentified genes involved in the metabolism of D-xylose (XIN for xylose inducible) have been cloned by differential hybridization with cDNA probes. The chromosomal assignments of the cloned genes were obtained by pulsed field gel electrophoresis (PFGE). We are now sequencing one of the xylose inducible genes and attempting to identify its precise role in the catabolism of D-xylose.

Relevant publications:

1. Skrzypek, M., P. Borsuk & R. Maleszka. 1990. Cloning and sequencing of the ornithine carbamoyltransferase gene from Pachysolen tannophilus. Yeast **6**:141-148.
 2. Maleszka, R. & M. Skrzypek. 1990. Assignment of cloned genes to electrophoretically separated chromosomes of the yeast Pachysolen tannophilus. FEMS Microbiol. Lett. **69** (in press).
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**XIII. Molecular Genetics Group, Research School of Biological Sciences,
Australian National University, P.O. Box 475, Canberra ACT 2601,
Australia. Communicated by G.D. Clark-Walker.**

As well as investigating aspects of mitochondrial genome structure, function, replication and evolution in various yeasts, our interest is also focusing on nuclear genes. Using pulsed field gel electrophoresis we are investigating the copy number of ribosomal DNA and consequences of altering the number of repeats for growth parameters. Strains deleted for rDNA can be obtained by UV mutagenesis. Such mutants grow slower than parental strains and show a correlation between growth rate and rDNA copy number. The following papers have been published recently or have been submitted for publication:

1. Clark-Walker, G.D. 1989. In vivo rearrangement of mitochondrial DNA in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 86:8847-8851.
2. Hardy, C.M., C.L. Galeotti & G.D. Clark-Walker. 1989. Deletions and rearrangements in Kluyveromyces lactis mitochondrial DNA. Curr.Genet. 16:429-432.
3. Maleszka, R. & G.D. Clark-Walker. 1989. A petite positive strain of Kluyveromyces lactis has a 300kb deletion in the rDNA cluster. Curr.Genet. 16:429-432.
4. Maleszka, R. & G.D. Clark-Walker. 1990. Sequence of the gene for the cytoplasmic ribosomal RNA small subunit from Kluyveromyces lactis. Nucl.Acids Res. 18:1889.
5. Wong, O.C. & G.D. Clark-Walker. 1990. Sequence of the gene for the cytoplasmic ribosomal RNA small subunit from Candida (Torulopsis) glabrata. Nucl. Acids res. 18:1888.
6. Skelly P.J. & G.D. Clark-Walker. 1990. Conversion at large intergenic regions of mitochondrial DNA in Saccharomyces cerevisiae. Mol.Cell. Biol. 10:1530-1537.
7. Hardy, C.M. & G.D. Clark-Walker. 1990. Nucleotide sequence of the cytochrome oxidase subunit 2 and val-tRNA genes and surrounding sequences from Kluyveromyces lactis K8 mitochondrial DNA. Yeast (in press).
8. Maleszka, R. & G.D. Clark-Walker. 1990. Magnification of the rDNA cluster in Kluyveromyces lactis. Mol. Gen. Genet. (submitted).

XIV. ALKO Ltd., The Finnish State Alcohol Company, POB 350, SF-00101 Helsinki, Finland. Communicated by M. Korhola.

The following paper, whose abstract appeared in the last issue, has now been published.

1. Londesborough, J. 1989. Purification of a Ca^{2+} /calmodulin-dependent protein kinase from baker's yeast. J. Gen. Microbiol. 135:3373-3383.

XV. Departamento de Microbiologia, Instituto de Fisiologia, Celular, U.N.A.M., A.P. 70-242, Del Coyoacan, Mexico 20, D.F., C.P. 04510 Mexico. Communicated by A. Brunner.

The following is an abstract of a recently published paper.

1. Brunner, A. & R. Coria. 1989. Cloning and sequencing of the gene for apocytochrome b of the yeast Kluyveromyces lactis Strains WM27 (NRRL Y-17066) and WM37 (NRRL Y-1140). Yeast 5:209-218.

The apocytochrome b genes from two strains of the yeast Kluyveromyces lactis, have been isolated and sequenced. The coding sequences in strains WM27 (NRRL Y-17066) and WM37 (NRRL Y-1140) were identical but the upstream noncoding regions were slightly different. The sequences demonstrated the presence of a continuous open reading frame with no introns. The amino acid sequence, derived from the coding stand, showed 82% homology to the apocytochrome b of Saccharomyces cerevisiae strain D273-10B and only 58% homology to the protein from Schizosaccharomyces pombe strain 50. CUN and CGN codon families were absent from the K. lactis gene. Codon usage was very similar to that of other mitochondrial genomes with mostly U or A in the third position. There were two unusual features. All threonines were coded by ACA(U) and all arginines by AGA.

XVI. University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, Alberta, Canada. Communicated by L. Sigler.

The following is an abstract of a recently published paper.

1. Sigler, L. 1990. Occurrence of a yeast-like synanamorph in the fungicolous coelomycete Eleutheromyces subulatus. Cryptogamic Botany (in press).

The *in vitro* morphology of the fungicolous coelomycete Eleutheromyces subulatus is described and illustrated. The yeast-like synanamorph is compared to Hyphozyma.

XVII. Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331-6601. Communicated by A. Bakalinsky.

The following papers are recently published or in press.

1. Bakalinsky, A.T. & R. Snow. 1990. Conversion of wine strains of Saccharomyces cerevisiae to heterothallism. Appl. Env. Micro. 56:849-857.
 2. Bakalinsky, A.T. & R. Snow. 1990. The chromosomal constitution of wine strains of Saccharomyces cerevisiae. Yeast 6: (in press).
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XVIII. Institut für Mikrobiologie und Weinforschung, Johannes Gutenberg - Universität Mainz. Communicated by F. Radler.

The following papers have been published recently.

1. Radler, F. & C. Knoll. 1988. Formation of killer toxin by apiculate yeasts and interference with fermentation. Vitis 27:111-132. (In German)

Several strains of the frequently occurring apiculate yeast (Hanseniaspora uvarum) form a killer toxin (a protein) which inhibits a sensitive wine yeast (Saccharomyces cerevisiae). This toxin was partially concentrated by lyophilization and precipitation with ethanol. The toxin activity was determined with the agar diffusion method. The optimum of activity was observed at pH 4, but the killer toxin is also active at pH 3.5, the pH of grape must. The presence of apiculate killer yeasts slowed the fermentation of a sensitive wine yeast S. cerevisiae 381). In mixed cultures of the killer strain H. uvarum 470 with S. cerevisiae the maximum inhibition of the latter was at 20°C and at pH 3.5. Within 2d about 95% of the sensitive yeast cells were killed. Several days later the wine yeast resumed fermentation. The killer toxin delayed the normal fermentation by 10-20 d. Depending on the ratio of cell numbers between the sensitive wine yeast and the apiculate killer yeast, the fermentation was suppressed for up to 15 d. When the cell number of wine yeasts was less than 0.01% of the killer yeast cells (H. uvarum 470) all sensitive cells were killed. Even at an inoculum at 10⁶ cells/ml of S. cerevisiae a delay in the normal fermentation was observed when the sensitive yeast was added 2 d after the apiculate killer strain. By adding bentonite at a concentration of 0.01% of the interference of killer toxin with fermentation was prevented. The delay of normal fermentation which was caused by killer strains of H. uvarum was not only observed in laboratory media but also in experiments with grape must.

2. Herzberger, E. & F. Radler. 1988. How hexoses and inhibitors influence the malate transport system in Zygosaccharomyces bailii. Arch. Microbiol. 150:37-41.

When grown in fructose or glucose the cells of Zygosaccharomyces bailii were physiologically different. Only the glucose grown cells (glucose cells) possessed an additional transport system for glucose and malate. Experiments with transport mutants had led to the assumption that malate and glucose were transported by one carrier, but further experiments proved the existence of two separate carrier systems. Glucose was taken up by carriers with high and low affinity. Malate was only transported by an uptake system and it was not liberated by starved malate-loaded cells, probably due to the low affinity of the intracellular anion to the carrier. The uptake of malate was inhibited by fructose, glucose, mannose, and 2-DOG but not by non-metabolisable analogues of glucose. The interference of malate transport by glucose, mannose or 2-DOG was prevented by 2,4-dinitrophenol, probably by inhibiting the sugar phosphorylation by hexokinase. Preincubation of glucose-cells with metabolisable hexoses promoted the subsequent malate transport in a sugar free environment. Preincubation of glucose-cells with 2-DOG, but not with 2-DOG/2,4-DNP, decreased the subsequent malate transport. The existence of two separate transport systems for glucose and malate was demonstrated with specific inhibitors: malate transport was inhibited by sodium fluoride and glucose transport by uranyl nitrate. A model has been discussed that might explain the interference of hexoses with malate uptake in Z. bailii.

3. Pfeiffer, P., F. Radler, G. Caspritz & H. Hänel. 1988. Effect of a killer toxin of yeast on eucaryotic systems. Appl. Environ. Microbiol. 54:1068-1069.

The Saccharomyces cerevisiae killer toxin KT 28, which inhibits sensitive yeasts, was shown to have no effect on several pathogenic fungi or on the protozoan Trichomonas vaginalis. At concentrations of about 0.1 mg/ml, a partial inhibition of the skin pathogenic fungi Trichophyton rubrum and Microsporum canis was observed at pH 6.5. No pharmacological activity was detected in various tests with several animal organs.

4. Schmitt, M. & F. Radler. 1988. Molecular structure of the cell wall receptor for killer toxin KT28 in Saccharomyces cerevisiae. J. Bacteriol. 170:2192-2196.

The adsorption of the yeast killer toxin KT28 to susceptible cells of Saccharomyces cerevisiae was prevented by concanavalin A, which blocks the mannoprotein receptor. Certain mannoprotein mutants of S. cerevisiae that lack definite structures in the mannan of their cell walls were found to be resistant to KT28, whereas the wild-type yeast from which the mutants were derived was susceptible. Isolated mannoprotein from a resistant mutant was unable to adsorb killer toxin. By comparing the resistances of different mannoprotein mutants, information about the molecular structure of the receptor was obtained. At least two mannose residues have to be present in the side chains of the outer chain of the cell wall mannan, whereas the phosphodiester-linked mannose group is not essential for binding and the subsequent action of killer toxin KT28.

5. Herzberger, E., R. Kapol, P. Pfeiffer & F. Radler. 1989. Degradation of diols and formation of ethylene glycol by different yeast species. Z. Lebensm. Unters. Forsch. 188:309-313 (in German).

The degradation of diols and the formation of ethylene glycol by yeasts of different genera were investigated. Diols, with the exception of diethylene glycol, were metabolized under aerobic conditions by species of the genera Hansenula, Candida, Pichia and Rhodotorula. Certain yeasts formed ethylene glycol in a synthetic medium or in grape must and this formation was greatly increased by ethanolamine. If 400 mg/l ethanolamine was added to the synthetic medium three strains of the series Zygosaccharomyces bailii, Torulopsis ernobii, and Kluuyveromyces veronae produced 70-210 mg ethylene glycol/l. Anaerobically, only 7-30 mg ethylene glycol/l was formed in the synthetic medium or in grape must. The aerobic formation of ethylene glycol increased with the amount of ethanolamine present in the medium, whereas the reduced formation of ethylene glycol from anaerobic cultures was not affected by ethanolamine. The strain Z. bailii 429 (a yeast species that is also found in wine) formed the largest amount of ethylene glycol, which amounted to more than half of the ethanolamine consumed. Hydroxylamine, an inhibitor of aminoxidase, prevented the formation of ethylene glycol from ethanolamine. Therefore, it is assumed that yeasts produce ethylene glycol from ethanolamine via glycolaldehyde. Under aerobic as well as under anaerobic conditions strains of Saccharomyces cerevisiae formed only small amounts of ethylene glycol.

6. Schmitt, M. & F. Radler. 1989. Purification of yeast killer toxin KT28 by receptor-mediated affinity chromatography. J. Chromatography 469:448-452.

This note describes how the specific binding of killer toxin KT28 to immobilized mannoprotein was utilized to develop a simple and effective method for the purification of this killer toxin, via a receptor-mediated affinity chromatographic technique.

7. Schmitt, M., M. Brendel, R. Schwarz¹ & F. Radler. 1989. Inhibition of DNA synthesis of Saccharomyces cerevisiae by yeast killer toxin KT28. J. Gen. Microbiol. 135:1529-1535.

¹Institut für Mikrobiologie der Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, D-6000 Frankfurt am Main, FRG.

Treatment of sensitive cells of Saccharomyces cerevisiae with killer toxin KT28 affected cell viability after 2 h; the effect was dependent upon the availability of a utilizable energy source. Treatment led to an interruption of cell growth. The mother cells contained nuclear DNA, whereas their daughter buds did not. Using a killer-toxin-sensitive thymidine auxotroph of S. cerevisiae carrying a temperature-sensitive thymidylate uptake mutation, it was shown that the incorporation of dTMP at the permissive temperature was inhibited within 30 min of the addition of KT28. When cells labelled at the permissive temperature were incubated at the restrictive temperature, the level of radioactivity declined in the absence but not in the presence of KT28. No other effects of KT28 were observed within 2 h of its addition, and it is concluded that the inhibition of DNA synthesis is an early effect of the action of KT28.

8. Herzberger, E. & F. Radler. 1989. Purification of plasma membranes from different cells types of Zygosaccharomyces bailii. J. Microbiol. Methods 10:199-205.

Zygosaccharomyces bailii produced physiologically different cells when grown on glucose as compared with fructose. Only the glucose grown cells metabolised glucose and malate, due to the induction of a transport system by glucose. From both cell types, protoplasts were produced by enzymatic hydrolysis of the cell wall. Cell membranes were isolated by treatment with cationic silica microbeads. The membrane proteins were extracted with SDS and separated by SDS-PAGE and isoelectric focusing. The membranes from glucose grown cells contained several proteins which were absent from the membranes of fructose grown cells. Two of the proteins had a similar isoelectric point and M_r values of 37000 and 38000. A further protein with a lower isoelectric point showed an M_r of 44000. These proteins may form part of the inducible carrier system for glucose and malate.

XIX. Microbiologie physiologique et appliquée - Levures, Université Lyon I, Bât. 405 - 43 Bd du 11 novembre 1918, F-69622 Villeurbanne Cedex, France. Communicated by M.C. Pignal.

The following results have been or will be published.

1. Poncet, S., R. Montrocher & A. Couble. Electrophorèse des protéines cellulaires de souches de levure de la vinification. Communication au 2è congrès de la Société Française de Microbiologie, Strasbourg.

Cell proteins of 12 strains of wine yeast were analysed by denaturing polyacrylamide gel electrophoresis (SDS-PAGE). The resulting profiles comprised a great variety of bands differing by their position as well as their intensity. Among about 40 bands identified, 2/3 were common to every strain, some appearing to be major on the basis of their intensity. Discrimination between the strains was based on the remaining bands which represented peptides smaller than 20 kd or larger than 55 kd with reference to external standards. This technique allows the identification of individual strains. It confirms the synonymy of *S. cerevisiae* and *S. bayanus* and thus can contribute to yeast chemotaxonomy.

2. Billon-Grand, G. 1989. A new ascosporegenous yeast genus: *Yamadazyma* gen. nov. Mycotaxon 35:201-204.

The genus *Pichia* is very heterogeneous as the coenzyme Q system: 67 species are characterized by a coenzyme Q7, 4 species by a coenzyme Q8 and 20 by a coenzyme Q9. We propose the new ascosporegenous genus *Yamadazyma* for those species equipped with the coenzyme Q9 and with hat-shaped ascospores.

3. Billon-Grand, G. 1989. Influence on minor peaks of coenzymes Q of the glucose concentration in the culture medium, the stage of the growth cycle, and the duration of the coenzyme Q extraction: required conditions for determining the minor coenzymes Q. J. Gen. Appl. Microbiol. 35:261-268.

An HPLC method has been developed to quantify and compare the relative amounts of minor and major coenzymes Q within and between different yeast species and under different cultural conditions. Four control yeast species were studied: *Pichia rhodanensis* (Q7), *Pichia methanolica* (Q7 and Q8), *Pichia inositovora* (Q9) and *Rhodotorula glutinis* (Q10). The glucose concentration in the culture medium moderately affected the minor peaks of coenzyme Q as did the duration of the coenzyme Q extraction. But the culture age was the main influence on the minor and major coenzymes Q. A careful standardization of growth conditions is required to obtain significant and comparative results from one species to another.

4. Fiol, J.B. & M.L. Claisse. 1990. Spectrophotometric analysis of yeasts: cytochrome spectra of some Q7 *Pichia*. J. Gen. Appl. Microbiol. (in press).

Whole-cell pastes of *Pichia*, equipped with the coenzyme Q7, were spectrophotometrically analysed (38 species). The low-temperature absorption cytochrome spectra showed a great diversity of response. The typical α 1 peak of cytochrome c (545-546.5 nm) might be complemented in a few species by a less pronounced α 2 peak (544-545 nm). The cytochrome oxidase peak (A + a3) was rarely typical and symmetric and showed two noticeable peculiarities: an unsymmetrical peak with the main absorption range from 597.5 to 602.5 nm and a shoulder at the lower or upper wavelength, and a double peak of cytochrome oxidase (596-598.5 nm) - (605-607 nm) or (593-594 nm) - (601-602 nm) rarely seen elsewhere in the yeasts. These characteristics, associated with GC contents, led to the identification of 3 main groups in the genus *Pichia* concerning at least 35 species.

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

New publications: the following are to be published in August this year.

1. Barnett J.A., R.W. Payne¹ & D. Yarrow². Photomicrographs by L. Barnett. Yeasts: Characteristics and Identification. Second Edition (completely revised)

¹Statistics Department, Rothamsted Experimental Station, Harpenden, England ²Yeast Division, Centraalbureau voor Schimmelcultures, Delft, Netherlands

This new edition includes: (a) Descriptions of 597 species, each with information on (i) the results of 91 tests, (ii) growth temperatures, (iii) the effect of cycloheximide on growth, (iv) DNA base composition, (v) the morphology of the cells, (vi) sexual reproduction. (b) 850 photomicrographs, about 200 of which show ascospores. (c) nearly 4000 published yeast names listed with known current synonyms. (d) about 1700 references given in full and checked individually for accuracy. (e) 18 identification keys. (f) tables with minimal listed of tests for identifying each species. (g) register of specific epithets giving the genera to which they have belonged. (h) an account of laboratory methods for identifying yeasts. (i) a description of the way yeasts are classified.

297 x 210 mm About 1000 pages and 850 photomicrographs. ISBN 0-521-35056-5. Price about £125.00 net. To be published in August 1990 by: Cambridge University Press, The Edinburgh Building, Shaftesbury Road, Cambridge CB2 2RU, UK; or Cambridge University Press, American Branch, 32 East 57th Street, New York, NY 10022, USA.

2. Barnett, J.A., R.W. Payne & D. Yarrow. August 1990. Yeast Identification: PC Program - Version 2.

Based on data (updated and revised) from their book, *Yeasts: Characteristics and Identification*, Second Edition (1990). This program (i) identifies yeasts; (ii) selects yeasts with chosen characteristics; (iii) is for use in industry, medical mycology and research; (iv) is available for IBM PC and compatibles with MS/PC-DOS; (v) simplifies the process of identifying yeast and reduces time-consuming searches through identification keys or descriptions of species. After entering the results of tests and observations into the computer, lists can be obtained of the following. (1) All species with a matching set of characteristics. (2) Yeasts with characteristics that most nearly match the entered set, together with a statement of the characteristic(s) that differ. (3) Further tests necessary to complete the identification.

With the program you can also (i) allow for mistakes in the test-results and (ii) display the description of a selected species, (iii) select any yeasts with particular characteristics. Results can be either entered from the keyboard or read from a file.

Improvements to the previous version, published in 1987, include (a) the ability to list the differences between 2 species or between an unknown and any other species and (b) the calculation of probabilities of identification. Price £125.00 or \$210 (US); 10% discount if payment made simultaneously with order. 50% discount for registered users of version 1 (1987) who order before November 1990.

Direct enquires to Dr. J.A. Barnett, 36 Le Strange Close, Norwich NR2 3PW, UK.

XXI. Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6. Communicated by B.F. Johnson.

The following papers have been published since my last communication to the Yeast Newsletter.

1. Tsai, C.S.¹, A.J. Avelledo¹, I.J. McDonald & B.F. Johnson. 1987. Diauxic growth of the fission yeast *Schizosaccharomyces pombe* in mixtures of D-glucose and ethanol or acetate. *Can. J. Microbiol.* **33**:593-597.
¹Department of Chemistry, Carleton University, Ottawa, Ontario, Canada K1A 0R6

The fission yeast *Schizosaccharomyces pombe* was unable to utilize ethanol or acetate as its sole carbon source for growth. However, ethanol and acetate were utilized in the presence of D-glucose during diauxic growth. No mutants capable of utilizing ethanol or acetate as sole carbon source were isolated from cultures grown in glucose together with ethanol or acetate. Low concentrations of acetate facilitated growth with glucose, whereas high concentrations of ethanol or acetate were inhibitory. Growing cells readily took up [1-¹⁴C] ethanol and [1-¹⁴C] acetate. The exogenous [1-¹⁴C] acetate was initially incorporated into biomacromolecules which were subsequently catabolized.

2. Johnson, B.F., T. Walker, M. Miyata¹, H. Miyata² & G.B. Calleja. 1987. Sexual co-flocculation and asexual self-flocculation of heterothallic fission-yeast cells (*Schizosaccharomyces pombe*). *Can. J. Microbiol.* **33**:684-688.
¹Gifu Pharmaceutical University, 6-1, Mitahora-higashi 5 chome, Gifu 502, Japan. ²Department of Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464, Japan.

Culturing conditions have been found that engender flocculation of strains 975h⁺ and 972h⁻ of the fission yeast *Schizosaccharomyces pombe* Lindner. Both strains separately exhibit asexual self-flocculation and together, sexual co-flocculation. Flocculation is glucose repressible, but that repression is leaky, because self-flocculation can occur during the late logarithmic phase of growth before either of the C or N sources are depleted.

3. Sowden, L.C. & T. Walker. 1988. A procedure for the study by scanning electron microscopy of flocculating cells of the fission yeast *Schizosaccharomyces pombe*. *Can. J. Microbiol.* **34**:577-582.

We have investigated a variety of preparative and dehydrating technics in order to examine, by scanning electron microscopy, the cell surface morphology of the fission yeast *Schizosaccharomyces pombe*, during asexual and sexual flocculation. We found that the loss of the flocculum material surrounding the cell wall was a problem common to many technics. Observations ranged from cells that were covered in varying amounts of hairs, hairlike threads, or a mucilaginous coat to cells so devoid of surface material as to show only well-defined scar regions. The loss of flocculum material is particularly severe with different dehydrating procedures. The method found to provide the best preservation of the cell coat and to produce the most consistent results was double fixation by glutaraldehyde-osmium tetroxide followed by splash-freeze lyophilization. Floccs unfixed, fixed with glutaraldehyde, or stained with uranyl sulfate, and dehydrated followed by critical-point drying or solvent substitution were found to be less satisfactory in the preservation of the cell coat for visualization by scanning electron microscopy.

4. Johnson, B.F., T. Walker, G.B. Calleja & V.L. Seligy. 1988. Sexual co-flocculation and asexual self-flocculation in budding and fission yeasts: experimental establishment of a fundamental difference. *Can. J. Microbiol.* **34**:1105-1107.

Heavily flocculated yeasts were resuspended in a variety of sugar syrups and solutions. Asexual *Saccharomyces* floccs remained patent in water and in 50% galactose but dispersed in 50% mannose but dispersed in 50% galactose and 10 mM EDTA. Sexual *Schizosaccharomyces* floccs (homothallic NCYC 132, or heterothallic h⁻ and h⁺ combined) remained patent in water, in 50% galactose, in 50% mannose, and in 10 mM EDTA. Dispersal of asexual floccs in sugar syrups and EDTA suggests that asexual flocculation is mediated by protein - carbohydrate

lectinlike interaction stabilized by Ca^{2+} . Failure of the sexually flocculent fission yeasts to disperse in any of these diagnostic solutions suggests that sexual flocculation is mediated by protein-protein interactions in which Ca^{2+} is irrelevant.

5. Miyata, H.¹, M. Miyata² & B.F. Johnson. 1988. Pseudo-experimental growth in length of the fission yeasts, Schizosaccharomyces pombe. Can. J. Microbiol. **34**:1338-1343.

¹Department of Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464, Japan. ²Gifu Pharmaceutical University, 6-1, Mitahora-higashi 5 chome, Gifu 502, Japan.

The growth patterns of individual cells of the fission yeast (Schizosaccharomyces pombe wild-type, cells, strain 972 h⁻; cells exposed to hydroxyurea; ad cdc mutants, 11-123, 2-33) were investigated by time-lapse photomicrography. Wild-type cells showed one, two, or three linear-growth segments followed by a constant-length stage. Cells with two segments were most frequent. Hydroxyurea cells that divided as oversized cells (about three times the birth length) had three linear-growth segments in a cycle. Mutant cdc11-123 cells did not divide but had a constant-length stage separating the cycles; both the first and second cycles consisted of two linear-growth segments, and cells were oversized at the second constant-length stage (about 3.5 times the birth length). Elongating cdc2-33 cells that did not divide and were oversized (about five times the birth length) while under observation, showed four linear-growth segments. Cells of all strains showed 30 to 40% increase in growth rate at the rate-change point and maintained approximate exponential (pseudo-exponential) growth. We conclude that the normal growth pattern of individual fission-yeast cells is the pseudo-exponential pattern.

6. Johnson, B.F., M. Miyata¹ & H. Miyata.² 1989. Morphogenesis of Fission Yeasts. Chapter 9 of reference 7, below.

¹Gifu Pharmaceutical University, 6-1, Mitahora-higashi 5 chome, Gifu 502, Japan. ²Department of Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464, Japan.

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7. Nasim, A., P. Young¹ & B.F. Johnson (eds.). 1989. Molecular Biology of the Fission Yeast. Academic Press, Inc. 469 pp. ¹Department of Biology, Queen's University, Kingston, Ontario, Canada

8. Tsai, C.S.¹, K.P. Mitton¹ & B.F. Johnson. 1989. Acetate assimilation by the fission yeast, Schizosaccharomyces pombe. Biochem. Cell Biol. **67**:464-467.

¹Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada K1S 5B6

The fission yeast Schizosaccharomyces pombe utilizes acetate at subinhibitory concentrations in the presence of D-glucose. The nonionized form of acetate is preferentially utilized, oxidized to ¹⁴C₂, and assimilated into lipids and proteins. Acetyl CoA synthetase activity greatly increases in the yeast cells growth in media containing acetate. However, glyoxylate cycle enzymes are not detectable in Schizosaccharomyces pombe. [1-¹⁴C] Acetate is incorporated into sterols, sterol esters, neutral lipids, and phospholipids. Assimilation of [1-¹⁴C] acetate into the peptide structure of proteins was confirmed by a proteolytic digestion experiment.

9. Johnson, B.F., L.C. Sowden & T. Walker. 1989. Use of electron microscopy to characterize the surfaces of flocculent and nonflocculent yeasts cells. Can. J. Microbiol. **35**:1081-1086.

The surfaces of flocculent and nonflocculent yeast cells have been examined by electron microscopy. Nonextractive preparative procedures for scanning electron microscopy allow comparison in which sharp or softened images of surface details (scars, etc.) are the criteria for relative abundance of flocculum material. Asexually flocculent budding-yeast cells cannot be distinguished from nonflocculent budding-yeast cells in scanning electron micrographs because the scar details of both are well resolved, being hard and sharp. On the other hand, flocculent fission-yeast cells are readily distinguished from nonflocculent cells because fission scars are mostly soft or obscured on flocculent cells, but sharp on nonflocculent cells. Sexually and asexually flocculent fission-yeast cells cannot be distinguished from one another as both are heavily clad in "mucilaginous" or "hairy" coverings. Examination of lightly extracted and heavily extracted flocculent fission-yeast cells by transmission electron microscopy provides micrographs consistent with the scanning electron micrographs.

10. Yoo, B.Y.¹, B.F. Johnson & G.B. Calleja. 1990. Ultrastructure of the ascospore walls of Schizosaccharomyces pombe during examination. *Mycologia* 82:43-47.

¹Biology Department, University of New Brunswick, Fredericton, N.B. E3B 5A3

At onset of germination, spores of S. pombe show localized swelling, the first vestige of germ-tube extension by the spore wall. As germination progresses, the original spore wall becomes thinned at the site of extension and is medially replaced by a new germinative layer. Germ tube growth is not erumpent. Cytology of germination of other spores is compared.

XXII. Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa. Communicated by J.C. du Preez.

The following papers have recently appeared or are in press:

1. van Zyl, C., B.A. Prior, S.G. Kilian & J.L.F. Kock. 1989. D-xylose utilization by Saccharomyces cerevisiae. *J. Gen. Microbiol.* 135:2791-2798.
2. van Eck, J.H., B.A. Prior & E.V. Brandt. 1989. Accumulation of polyhydroxy alcohols by Hansenula anomala in response to water stress. *J. Gen. Microbiol.* 135:3505-3513.
3. Cottrell, M. & J.L.F. Kock. 1989. The yeast family Lipomycetaceae Novak et Zsolt emend. van der Walt et al. and the genus Myxozyma van der Walt et al. 1. A historical account of its delimitation and 2. The taxonomic relevance of cellular long - chain fatty acid composition and other phenotypic characters. *System. Appl. Microbiol.* 12:291-305.
4. Pearson, T., J.L.F. Kock & S.G. Kilian. The value of carbon compound mixtures in the identification of the yeast genus Kluyveromyces. *System. Appl. Microbiol.* (in press).

A yeast identification procedure based on the utilization of carbon compounds present in mixtures was developed. Mixtures of carbon compounds were inoculated with 21 yeast strains, representing 11 Kluyveromyces species, and the remaining carbon sources were determined by high performance liquid chromatography (HPLC) after incubation. This method was compared to the well-known conventional method where singly offered compounds in liquid media are used for characterization. In some cases, carbon sources which were not utilized according to the conventional system disappeared in mixtures while carbon compounds which were assimilated according to the conventional system were not utilized in mixtures. Disappearance from and formation of compounds in a mixture containing D-glucose, D-glycerol, D-raffinose, D-ribose and D-xylose during growth of K. phaffii showed that xylose and raffinose disappeared from the medium and were possibly converted into xylitol and melibiose, respectively. Care should therefore be taken before direct comparisons between the conventional assimilation method, which is based on conversion of sugars into biomass, and the disappearance of carbon sources from mixtures are made. The results show that the technique can be used to identify most of the Kluyveromyces strains tested.

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

The following abstracts are from recent publications from our laboratories:

1. Simmons, R.B. & E. Guého. 1990. A new species of Malassezia. *Mycol. Res.* 94: (in press).

Malassezia sympodialis sp. nov. was isolated from the auditory tract of a healthy human male and from the scalp of an Acquired Immune Deficiency (AIDS) patient suffering from tinea capitis. The yeast is characterized by a pronounced lipophily, unilateral, percurrent or sympodial budding, an irregular, corrugated cell wall ultrastructure, and a G+C content of 54%.

2. Mahrous, M., T.J. Lott, S.A. Meyer, A.D. Sawant & D.G. Ahearn. 1990. Electrophoretic karyotyping of typical and atypical C. albicans. *J. Clin. Microbiol.* 28:876-881.

Electrophoretic karyotypes of atypical isolates of Candida albicans, e.g., strains that were germ tube negative, failed to express proteinase activity, demonstrated low virulence for mice, formed hyperchlamydoconidia, produced hyperhyphae, or were sucrose negative (including the type strain of Candida stellatoidea), were compared with those of typical C. albicans. Karyotypes of whole-cell DNA of classical C. albicans examined with transverse alternating-field electrophoresis under specific conditions were composed of seven DNA bands with a specific migration pattern. Certain atypical strains and representatives of the three serotypes of C. stellatoidea produced discrete karyotypes with 5 to 10 bands. All isolates demonstrated a significant degree of DNA relatedness, suggesting their conspecificity. Densitometric tracings of DNA bands provided an objective and standardized method for comparing bands within the gels.

3. Simmons, R.B. 1989. Comparison of chitin localization in Saccharomyces cerevisiae, Cryptococcus neoformans, and Malassezia spp. Mycol. Res. 94: 551-553.

Wheat germ agglutinin conjugated to colloidal gold was reacted with chitin in whole cell sections of representative ascomycetous and basidiomycetous yeasts. In Saccharomyces cerevisiae chitin was concentrated throughout the bud scar region and was of negligible levels in other regions of the wall. In contrast, chitin was distributed evenly throughout the cell wall of Cryptococcus neoformans and persistent bud scars were not observed. Isolated of Malassezia spp. had obvious bud scars with chitin restricted to the collarette portion of the scar.

4. Sawant, A.D., A.T. Abdelal & D.G. Ahearn. 1989. Purification and characterization of the anti-Candida toxin of Pichia anomala WC65. Antimicrobial Agents and Chemotherapy 33:48-52.

Pichia anomala WC 65 secretes a toxin that is inhibitory to a variety of yeasts, including strains of the animal pathogen Candida albicans. The toxin was purified to homogeneity by ultrafiltration, ethanol precipitation, ion-exchange chromatography with a Mono Q column, and gel permeation chromatography with a Superose 12 column. The toxin had a molecular weight of 83,300 as determined by electrophoresis on sodium dodecyl sulfate-polyacrylamide gradient gels and a molecular weight of 85,290 as determined by gel permeation chromatography. The isoelectric point of the toxin was pH 5.0. The toxin was stable between pH 2.0 and 5.0. Chemical analysis of the purified toxin indicated that the toxin was a glycoprotein composed of about 86% protein and 14% carbohydrate. At high concentrations, the toxin showed a tendency of aggregate, with low of biological activity against C. albicans, Pichia bimundalis, and Saccharomyces ludwigii. Purified toxin expressed killing activity against C. albicans in contrast to the static activity of the crude toxin expressed killing activity against C. albicans in contrast to the static activity of the crude toxin.

5. Su, C.S., S.A. Meyer & M.W. Reeves. 1990. Genetic and biochemical characterization of some physiologically similar Candida species. In: Abstracts of the 90th Annual Meeting of the American Society for Microbiology, p. 248. Poster presented May 15, 1990.

Species identification and delimitation of physiologically similar yeasts are often difficult. It is well known that some species can not be separated on the basis of a single or even a few physiological differences. Nuclear DNA (nuDNA) reassociation, mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (rflps) and multilocus enzyme mobility typing singly and in concert can reconcile strain identity and distinction. These three methods showed significant differences between strains of C. parapsilosis and its once proposed anamorph, Lodderomyces elongisporus, as well as between strains of C. albicans and C. tropicalis. On the other hand, nearly identical results were observed between C. tropicalis, sucrose negative C. tropicalis and C. paratropicalis (type strain) and between sucrose negative C. albicans and the type strain of C. albicans, C. lusitanae and its teleomorph Clavispora lusitanae showed slightly more variability in certain enzyme mobilities. Polymorphisms of mtDNA among different strains of C. maltosa did exist and enzyme mobility patterns showed some differences. Strains can be separated into two groups. The results of mtDNA rflps and multilocus enzyme typing were in agreement with nuDNA relatedness, plus they showed where variation existed among strains. These genetic and biochemical methods demonstrated their usefulness in distinguishing species and denoting the degree of variation within a species.

**XXIV. VTT Biotechnical Laboratory, Tietotie 2, SF-02150 Espoo, Finland.
Communicated by M.-L. Suihko.**

The following papers/works have been published or accepted for publication during the last year:

1. Suihko, M.-L., M. Penttilä, H. Sone, S. Home, K. Blomqvist, J. Tanaka, T. Inoue & J. Knowles. 1989. Pilot-brewing with α -acetolactate decarboxylase active yeasts. Proc. EBC Congress, Zurich 483-490.

In production of quality beer a lengthy lagering period is necessary for reduction of diacetyl. However, the excretion of α -acetolactate can be avoided if a gene encoding α -acetolactate decarboxylase is inserted into the yeast genome. Beer was produced in pilot scales (50 μ l) using recombinant brewing yeast strains expressing different α -acetolactate decarboxylase genes. With the best strains the total diacetyl contents in the green beers were already below the taste threshold level. The total production time can thus be significantly reduced with no detectable change in the fermentation performance or in the flavour of the final product.

2. Kronlöf, J., T. Härkönen, P. Hartwall, S. Home & M. Linko. 1989. Main fermentation with immobilized yeast. Proc. EBC Congress, Zurich, 355-362.

The continuous immobilized system has been investigated in the main fermentation (also called primary fermentation). The yeast was immobilized by adsorption on DEAE-cellulose granules. The effects of column design, wort composition and yeast condition on the fermentation rate and on flavour formation were investigated. Production of acetate esters was suppressed during the initial growth stage, but sufficient concentrations were formed later. Diacetyl concentrations were comparable with those in batch fermentation. The advantages of the system are evident. A rapid fermentation with a volumetric productivity more than 6-fold compared with that of a conventional batch process is possible. Savings in sanitation, transfers etc. can also be achieved. Further advantages can be envisaged when using an immobilized α -acetolactate decarboxylase active yeast strain.

3. Lankinen, P. 1989. Comparison of brewer's yeasts in pilot brewing and characterization by gel electrophoresis. M.Sc. Thesis, University of Helsinki, Department of Microbiology, 75 p. (in Finnish)

The brewing properties of seven industrial brewer's yeast strains and seven recombinant brewer's yeast strains, containing the gene coding for endo- β -1,4-glucanase, were compared in pilot brewing trials. The karyotype of strains was determined by gel electrophoresis. One of the industrial strains had different brewing properties and probably also a different karyotype. Integration in the recombinant strains had occurred as intended in a prespecified locus/chromosome.

4. Suihko, M.-L. 1989. VTT Collection of Industrial Microorganisms. Catalogue of strains, 2nd ed., VTT Offsetpaino, Espoo. 124 p.

The VTT Collection of Industrial Microorganisms now contains about 330 yeasts, 410 filamentous fungi and 400 bacteria. In the yeast collection there are 56 baker's yeast strains and 100 bottom fermenting brewer's yeast strains, six of which are at present in industrial use. Depending on the pure culture equipment available in the different breweries or baker's yeast factories, pure cultures are delivered weekly, monthly or twice per year. The culture is delivered as an agar slant or a yeast suspension of 5l. In addition, there are top fermenting brewer's yeasts, distiller's yeasts, wine yeasts, sour dough yeasts, fodder yeasts and other strains in the VTT collection.

5. Suihko, M.-L., S. Home & M. Linko. 1990. Testing of brewer's yeast strains using different industrial worts. *Mallas ja Olut* 1:5-16. (in Finnish, English and Swedish summary).

In order to understand better the relationship between brewing properties of yeast and the composition of wort, five different industrial brewer's yeast strains have been tested in five worts containing different amounts of nutrients. The nutrient content of wort, e.g. amounts of amino acids, minerals, vitamins and fatty acids had a strong effect on the growth of yeast and thus on its brewing properties. The effect was similar with all five strains.

6. Suihko, M.-L., K. Blomqvist, M. Penttilä, R. Gisler & J. Knowles. 1990. Recombinant brewer's yeast strains suitable for accelerated brewing. *J. Biotechnol.* 14:285-300.

Four brewer's yeast strains carrying the α -ald gene of *Klebsiella terrigena* (ex. *Aerobacter aerogenes*) or of *Enterobacter aerogenes* on autonomously replicating plasmids were constructed. The α -ald genes were linked either to the ADC1 promoter or to the PGK1 promoter of yeast *Saccharomyces cerevisiae*. In pilot scale brewing (50 μ l) with three of these recombinant yeasts the formation of diacetyl in beer was so low during fermentation that lagering was not required. All other brewing properties of the strains were unaffected and the quality of finished beers was as good as that of finished beer prepared with the control strain. The total process time of beer production could therefore be reduced to 2 weeks, in contrast to about 5 weeks required in the conventional process.

7. Aarnio, T.H. & M.-L. Suihko. 1990. Electrofusion of an industrial baker's yeast strain with a sour dough yeast. *Appl. Biochem. Biotechnol.* (in press).

A method for hybridization of yeast protoplasts of *Saccharomyces cerevisiae* and *Candida holmii* by electrofusion was optimized. The hybrids were screened on maltose-acetate agar plates. The average fusion frequency was 1.1×10^{-3} . Two hybrids of 132 collected from selection plates were found to be stable over 15 sequential shake flask cultivations. However, the strains reverted during production of baker's yeast in a laboratory scale process limiting the industrial process.

8. Aarnio, T.H., M.-L. Suihko & V. Kauppinen. 1990. Isolation of acetic acid-tolerant baker's yeast variants in a turbidostat. *Appl. Biochem. Biotechnol.* (in press).

A commercial baker's yeast was subjected to selection in a continuous turbidostat cultivation with increasing concentration of acetic acid. The final acetic acid content in fresh medium was 0.6% or 0.18% v/v. Two of seven selected variants were stable over 15 sequential shake flask cultivations without selection pressure. After laboratory scale production of baker's yeast one of the variants also showed increased acetic acid tolerance in sour dough. The overall raising power (ml CO₂/h) in sour dough was improved 36%.

XXV. Department of Microbiology and Enzymology, Faculty of Chemical Engineering and Materials Science, Delft University of Technology, Julianalaan 67, 2628 BC, Delft, The Netherlands. Communicated by W.A. Scheffers.

The following is an abstract of a recently defended thesis.

1. Postma, E. 1990. Regulation of sugar transport in yeasts; a continuous culture study. Ph.D. Thesis, Delft University of Technology. 173 pp.

This thesis deals with a study on the influence of transport on the physiology and ecology of yeasts. The work has concentrated on two forms of transport encountered in yeasts, namely proton-glucose symport and glucose uniport. Furthermore, special attention has been paid to the control of metabolic fluxes under various growth conditions in relation to sugar transport. For this purpose, continuous cultures were used. Only this

method of cultivation allows a quantitative description of the kinetics of transport in relation of the kinetics of growth. The methods for the determination of these kinetics and other relevant procedures have been described in Chapter 2. In Chapter 3 the kinetics of glucose transport in the Crabtree-negative yeast Candida utilis were studied in continuous culture. Three different affinity constants for glucose transport were found that differed an order of magnitude: 25, 190 and 2000 μM , respectively. The presence of these transport systems depended on the growth rate and the environmental glucose concentration. Under all growth conditions the capacity of the different transport systems was tuned to the extracellular sugar concentration. When Michaelis-Menten kinetics were assumed for the transport process, a close correlation was observed between kinetics of transport and kinetics of growth. In Chapter 4 the observations with C. utilis were extended to the yeast Saccharomyces cerevisiae. In contrast to C. utilis this yeast did not follow classical Monod kinetics. The residual glucose concentration in the culture was independent of the dilution rate up to 0.39 h^{-1} . At higher growth rates the data, residual substrate concentration versus growth rate, could be fitted with the Monod equation. However, at these high growth rates the products formed by the yeast influenced the overall growth affinity constant. As with C. utilis the flux of glucose through the transport systems matched the flux of glucose required to maintain the imposed growth rate (Chapter 4). Overflow metabolism at the pyruvate level, the branching point between oxidative and fermentative metabolism, was studied in detail (Chapter 5). It was confirmed that respiration is a rate-limiting step in S. cerevisiae at high growth rates. However, the physiology of this yeast is influenced by the production of acetate at intermediate growth rates. The production of acetate results in spillage of ATP as a consequence of futile cycling of the acid. Therefore the maximal oxidation rate is reached at a growth rate lower than μ_{max} . The production of acetate is a consequence of another rate-limiting step, namely the capacity of acetylCoA synthetase. It is concluded that the "pyruvate oxidation bypass", involving acetaldehyde, acetate and acetylCoA as intermediates, is operative at all growth rates. The flux via the bypass is controlled by three parameters: 1. Pyruvate decarboxylase capacity. 2. Affinity constant of the pyruvate decarboxylase for pyruvate, that is modified by the cytosolic phosphate concentration. 3. Internal pyruvate concentration. A clear difference observed between the two yeasts growing under glucose-limited conditions was the residual substrate concentration in the culture which was 25-fold higher with S. cerevisiae than with C. utilis. It was suggested that this difference should be related to different modes of transport present in these yeasts. In Chapter 6, therefore, eight different yeasts were investigated. It was established that: 1. Low residual glucose concentrations in chemostats are typical for yeasts that exhibit proton-glucose symport. With yeasts possessing facilitated diffusion transporters for glucose, relatively high glucose concentrations were encountered. 2. Yeast possessing glucose/proton symport have a Crabtree-negative phenotype and those possessing facilitated diffusion for glucose have a Crabtree-positive phenotype. 3. In all yeasts, the rate of glucose consumption by glucose-limited steady-state cultures, as calculated from the growth yield and the growth rate, was equal to the rate of glucose transport, predicted on the basis of the estimated transport kinetics. After exposure to excess glucose, glucose consumption increased. However, in all yeasts less than was expected on the basis of transport kinetics. This indicates that both in Crabtree-negative and Crabtree-positive yeasts the flux at the start of the pulse is clearly not only controlled by transport, and thus that the flux through glycolysis cannot be predicted on the basis of transport kinetics alone. Under conditions of low sugar supply Crabtree-negative yeasts, which have a high affinity for glucose, can be expected to have competitive advantage over Crabtree-positive yeasts, which have a low affinity of glucose. Indeed, the results presented in Chapter 7 show that under aerobic glucose-limited conditions C. utilis rapidly outcompetes S. cerevisiae in chemostat cultures. It is concluded that the kinetics of glucose transport play an important role in the competition, since they determine to a large extent the growth kinetics. Although in Crabtree-positive yeasts glucose appears to be exclusively transported by facilitated diffusion, proton-sugar symport can occur in these organisms, for example in the case of maltose. S. cerevisiae seems not well adapted to this mode of transport. Sudden, exposure of maltose-limited cells to maltose excess leads to cell death. Apparently the mechanisms that regulate the rate of entry via proton-sugar symport in Crabtree-negative yeasts are absent or insufficient in the Crabtree-positive yeast S. cerevisiae (Chapter 8). In transport assays with whole cells the interference of metabolism cannot be excluded, even when a very short assay time (Chapter 2) is used. Attempts were therefore made to study transport in isolated plasma membrane vesicles. In Chapter 9 it is reported that plasma membrane vesicles of C. utilis can be isolated which, when properly energized, show uphill transport of solutes such as glucose and amino acids. However, the accumulation factors obtained were disappointingly low. Moreover, for unknown reasons only two out of six membrane preparations exhibited active transport, and only at a low rate. Further work is therefore required to establish the kinetics of glucose transport in vivo.

The following are abstracts of papers published recently.

2. Postma, E., A. Kuiper, W.F. Tomasouw, W.A. Scheffers & J.P. van Dijken. 1989. Competition for glucose between the yeasts Saccharomyces cerevisiae and Candida utilis. Appl. Env. Microbiol. 55:3214-3220.

The competition between the yeasts Saccharomyces cerevisiae CBS 8066 and Candida utilis CBS 621 for glucose was studied in sugar-limited chemostat cultures. Under aerobic conditions, C. utilis always successfully competed against S. cerevisiae. Only under anaerobic conditions did S. cerevisiae become the dominant species. The rationale behind these observations probably is that under aerobic glucose-limited conditions, high-affinity glucose/proton symporters are present in C. utilis, whereas in S. cerevisiae, glucose transport occurs via facilitated diffusion with low-affinity carriers. Our results explain the frequent occurrence of infections of Crabtree-negative yeasts during baker's yeast production.

3. Postma, E., C. Verduyn, A. Kuiper, W.A. Scheffers & J.P. van Dijken. 1990. Substrate-accelerated death of Saccharomyces cerevisiae CBS 8066 under maltose stress. Yeast 6:149-158.

When Saccharomyces cerevisiae CBS 8066 was grown under maltose limitation, two enzymes specific for maltose utilization were present: a maltose carrier, and the maltose-hydrolysing α -glucosidase. The role of these two enzymes in the physiology of S. cerevisiae was investigated in a comparative study in which Candida utilis CBS

621 was used as a reference organism. Maltose pulses to maltose-limited chemostat culture of *S. cerevisiae* resulted in 'substrate-accelerated death'. This was evident from: (1) enhanced protein release from cells; (2) excretion of glucose into the medium; (3) decreased viability. These effects were specific with respect to both substrate and organisms: pulses of glucose to maltose-limited cultures of *S. cerevisiae* did not result in cell death, neither did maltose pulses to maltose-limited cultures of *C. utilis*. The maltose-accelerated death of *S. cerevisiae* is most likely explained in terms of an uncontrolled uptake of maltose into the cell, resulting in an osmotic burst. Our results also provide evidence that the aerobic alcoholic fermentation that occurs after pulsing sugars to sugar-limited cultures of *S. cerevisiae* (short-term Crabtree effect) cannot solely be explained in terms of the mechanism of sugar transport. Both glucose and maltose pulses to maltose-limited cultures triggered aerobic alcohol formation. However, glucose transport by *S. cerevisiae* occurs via facilitated diffusion, whereas maltose entry into this yeast is mediated by a maltose/proton symport system.

4. Steensma, H.Y., P. de Jonge, A. Kaptein & D.B. Kaback. 1989. Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: localization of a repeated sequence containing an acid phosphatase gene near a telomere of chromosome I and chromosome VIII. *Current Genetics* 16:131-137.

A 17 kb region from near the right end of chromosome I of *Saccharomyces cerevisiae* was isolated on recombinant (λ) bacteriophages. This region contained the PHO11 gene which was located only 3.4 kb from the right end of the chromosome. We found that this region also was repeated approximately 13 kb from the end of the chromosome VIII DNA molecule. The chromosome VIII sequence appears to be a previously unnamed acid phosphatase gene that we propose to call PHO12. Thus, similar to the repeated SUC, MAL, X and Y' sequences, some members of the repeated acid phosphatase gene family also appear near the termini of yeasts chromosomes.

5. van Urk, H., W.S.L. Voll, W.A. Scheffers & J.P. van Dijken. 1990. Transient-state analysis of metabolite fluxes in Crabtree-positive and Crabtree-negative yeasts. *Appl. Environ. Microbiol.* 56:281-287.

In bakers' yeast, an immediate alcoholic fermentation begins when a glucose pulse is added to glucose-limited, aerobically grown cells. The mechanism of this short-term Crabtree effect was investigated via a comparative enzymic analysis of eight yeast species. It was established that the fermentation rate of the organisms upon transition from glucose limitation to glucose excess is positively correlated with the level of pyruvate decarboxylase (EC4.1.1.1). In the Crabtree-negative yeasts, the pyruvate decarboxylase activity was low and did not increase when excess glucose was added. In contrast, in the Crabtree-positive yeasts, the activity of this enzyme was on the average sixfold higher and increased after exposure to glucose excess. In Crabtree-negative species, relatively high activities of acetaldehyde dehydrogenases (EC 1.2.1.4 and EC 1.2.1.5) and acetyl coenzyme A synthetase (EC 6.2.1.1), in addition to low pyruvate decarboxylase activities were present. Thus, in these yeasts, acetaldehyde can be effectively oxidized via a bypass that circumvents the reduction of acetaldehyde to ethanol. Growth rates of most Crabtree-positive yeasts did not increase upon transition from glucose limitation to glucose excess. In contrast, the Crabtree-negative yeasts exhibited enhanced rates of biomass production which in most cases could be ascribed to the intracellular accumulation of reserve carbohydrates. Generally, the glucose consumption rate after a glucose pulse was higher in the Crabtree-positive yeasts than in the Crabtree-negative yeasts. However, the respiratory capacities of steady-state cultures of Crabtree-positive yeasts were not significantly different from those of Crabtree-negative yeasts. Thus, a limited respiratory capacity is not the primary cause of the Crabtree effect in yeasts. Instead, the difference between Crabtree-positive and Crabtree-negative yeasts is attributed to differences in the kinetics of glucose uptake, synthesis of reserve carbohydrates, and pyruvate metabolism.

6. Verduyn, C., E. Postma, W.A. Scheffers & J.P. van Dijken. 1990. Physiology of *Saccharomyces cerevisiae* in anaerobic glucose-limited chemostat cultures. *J. Gen. Microbiol.* 136:395-403.

The physiology of *Saccharomyces cerevisiae* CBS 8066 was studied in anaerobic glucose-limited chemostat cultures in a mineral medium supplemented with ergosterol and Tween 80. The organisms has a μ_{max} of 0.31 h^{-1} and a K_s for glucose of 0.55 mM . At a dilution rate of 0.10 h^{-1} , a maximal yield of $0.10 \text{ g biomass (g glucose)}^{-1}$ was observed. The yield steadily declined with increasing dilution rates, so a maintenance coefficient for anaerobic growth could not be estimated. At a dilution rate of 0.10 h^{-1} , the yield of the *S. cerevisiae* strain H1022 was considerably higher than for CBS 8066, despite a similar cell composition. The major difference between the two yeast strains was that *S. cerevisiae* H1022 did not produce acetate, suggesting that the observed difference in cell yield may be ascribed to an uncoupling effect of acetic acid. The absence of acetate formation in H1022 correlated with a relatively high level of acetyl-CoA synthetase. The uncoupling effect of weak acids on anaerobic growth was confirmed in experiments in which a weak acid (acetate or propionate) was added to the medium feed. This resulted in a reduction in yield and an increase in specific ethanol production. Both yeasts required approximately $35 \text{ mg oleic acid (g biomass)}^{-1}$ for optimal growth. Lower or higher concentrations of this fatty acid, supplied as Tween 80, resulted in uncoupling of dissimilatory and assimilatory processes.

7. Verduyn, C., E. Postma, W.A. Scheffers & J.P. van Dijken. 1990. Energetics of Saccharomyces cerevisiae in anaerobic glucose-limited chemostat cultures. J. Gen. Microbiol. 136:405-412.

The energetics of Saccharomyces cerevisiae were studied in anaerobic glucose-limited chemostat cultures via an analysis of biomass and metabolite production. The observed Y_{ATP} was dependent on the composition of the biomass, the production of acetate, the extracellular pH, and the provision of an adequate amount of fatty acid in the medium. Under optimal growth conditions, the Y_{ATP} was approximately 16 g biomass (mol ATP formed⁻¹). This is much higher than previously reported for batch cultures. Addition of acetic acid or propionic acid lowered the Y_{ATP} . A linear correlation was found between the energy required to compensate for import of protons and the amount of acid added. This energy requirement may be regarded as a maintenance energy, since it was independent of the dilution rate at a given acid concentration.

XXVI. Department of Genetics and Microbiology and Institute of Biotechnology, Charles University, Vinicna 5, Prague 2, CS 128 44 Czechoslovakia. Communicated by B. Janderová and O. Bendová.

The following paper has been recently published.

1. Vondrejs, V., I. Pavlicek, M. Kothera & Z. Palková. 1990. Electrofusion of oriented Schizosaccharomyces pombe cells through apical protoplast-protuberances. Biochem. Biophys. Res. Commun. 166:113-118.

The electrofusion of oriented Schizosaccharomyces pombe cells through apical protoplast-protuberances was demonstrated. The protuberances arose after an exposure of early-exponential phase cells to digestive enzymes from hepatopancreas of Helix pomatia. The orientation of cylindric cells within pearl chains was produced by the application of inhomogenous alternating electric fields.

The following is the abstract of an article that will appear in J. Basic Microbiol. 30(6) or (7), 1990.

2. Janderová, B., F. Cvrcková & O. Bendová. Construction of the dextrin degrading pof brewing yeast by protoplast fusion.

A haploid, glucoamylase producing strain of Saccharomyces diastaticus lacking the POF gene and thus unable to produce phenolic off-flavour in beer was constructed by classical genetic techniques. Protoplast fusion was performed between this strain and a brewing polyploid Saccharomyces uvarum strain. Some clones derived from fusion products can produce low-carbohydrate beer of acceptable flavour. Currently a selected clone is being tested for production of low-carbohydrate beer from wort pretreated by commercial α -amylase in production scale.

The following papers have been submitted for publication.

3. Cvrcková, F. & B. Janderová. Rapid methods for characterization of hybrid industrial yeasts. Submitted to Biotechnology Letters.

Rapid, small-scale techniques for preliminary screening of some technologically important features (melibiase activity, viability, growth and fermentation rate) of large numbers of brewer's or distiller's yeast clones are described.

4. Puta, F. & R. Wambutt. The construction of the new Escherichia coli - Saccharomyces cerevisiae shuttle plasmid cloning vectors allowing positive selection for cloned fragments. Submitted to Yeast.

The new shuttle plasmid cloning vectors with positive type of selection system for isolation of bacterial transformants harbouring recombinant plasmids have been constructed. The segment carrying the selection system from cloning plasmid pUN121 which is based on the principle of regulatory origin of (λ) phage controlling expression of tetracycline resistance has been used. The cloning sites EcoRI, HindIII and SmaI/XmaI have been supplemented by BglII created by oligonucleotide mediated mutagenesis. Relatively small size of the vector (7.8 kb) has been achieved using the URA3 yeast marker.

The following papers will be presented on 14th ISSY, Yeast Taxonomy, Theoretical and Practical Aspects, September 3-7, 1990, Smolenice, Czechoslovakia. The articles are in preparation.

5. Kothera, M., Z. Palková, B. Janderová, O. Bendová & V. Vondrejs. Killer systems in yeasts: application in taxonomy.

A rapid method for estimating the capability of various factors to cause permeabilization of cytoplasmic membranes in yeast cells based on the orientation of their asymmetric cells in high frequency electric fields (0.5 and 5 MHz) was evolved. The technique was applied to distinguish various killer producing and killer sensitive species respectively on the basis of mechanism of action of killer toxins. The method can be exploited for the rapid classification of killer strains, sensitive strains and killer toxins from the point of view of their ability to permeabilize cytoplasmic membrane of sensitive cells.

6. Richter, V., B. Janderová, J. Hausler & O. Bendová. Fatty acid composition in strains of Saccharomyces cerevisiae.

As a new chemotaxonomic method for yeast identification fatty acid composition of yeast cells can be used. Our aim was to estimate differences of fatty acid composition between laboratory and various groups of industrially used strains of S. cerevisiae. We have tested 16 strains belonging to 4 groups of S. cerevisiae - laboratory, brewing, distillery and wine strains. It was shown that these yeasts can be distinguished by their fatty acid composition. All brewing yeasts tested can be characterized by considerably high concentration of unsaturated fatty acids (hexadecenoic, 16:1, and octadecenoic, 18:1) the relation of which is approximately 3:2. Wine and distillery strains can be distinguished from the brewing ones by lower relation of 16:1 and 18:1 (approximately 1:1) and by the presence of a component with ECN 24.3. Typical chromatographical pattern with a higher number of components can distinguish laboratory strains from the industrial ones.

XXVII. Institute of Chemistry of Slovak Academy of Sciences, Bratislava 842 38, Dubravská cesta 9, Czechoslovakia. Communicated by A. Kocková-Kratochvilová.

Recently published books.

1. Kocková-Kratochvilová, A. 1990. Yeasts and yeast-like organisms. 2nd ed. Verlag Chemie Weinheim (in English).

This is the 2nd edition of the book published by ALFA, Bratislava 1982 in Slovak, translated by Dr. K. Sigler into English. It offers basic information on biotechnology, fermentation industry, medicine, agriculture, science and education. Both first and second editions relate to the book "Yeasts" (ALFA, Bratislava, 1957) dedicated to brewer's and baker's yeasts. The book is divided into following chapters: Yeast populations; Cytology; Chemical composition; Regulating and control; Metabolism; Fodder yeasts; Pathogenic yeasts; Identification key.

2. Kocková-Kratochvilová, A. 1990. Taxonomy of yeasts and yeast-like organisms. ALFA, Bratislava (in Slovak).

The book is divided into two main parts: General and Systematics. The general part includes history of taxonomies, different kinds of taxonomies, basic informations in ecology, coding of Kocková-Kratochvilová and Slaviková, identification methods, nutritive media, etc. The systematic part is constructed on similarities among families, genera and species. The description of species is focussed on biotechnology (production and fermentation) and on biological models of eukaryotic cells.

3. Kocková-Kratochvilová, A. (editor) 1990. Thirty years of Czechoslovak activities in yeast research. VEDA, Bratislava (in English).

It is the survey of published papers in Czechoslovak persons permanently studying yeasts in the last decade. The first chapter in this book includes the biographies of fundators of both, Czechoslovak and International (ICY) Commissions for yeasts, who organized the first symposia in Smolenice (1964) and Bratislava (1966). The book will be distributed to participants on the 14th International Symposium on Yeasts, Smolenice, September 1990.

XXVIII. Laboratoire de Biologie et Cytophysiologie végétales, Faculté des Sciences, 2, rue de la Houssinière, Université de Nantes, F-44072 Nantes Cedex 03. Communicated by L. Simon.

The following is an abstract of a recently published paper.

1. Papon, P., L. Simon & C. Caye-Vaugien¹. 1989. Aureobasidium pullulans: Bilan morphologique, métabolique et énergétique. Cryptogamie Mycol. 10:227-242.

Five various composition culture media (with or without phosphorus and organic nitrogen) are described inducing different morphogenetic development in Aureobasidium pullulans (L.C.P. 87.43). Energetic phenomenons (as measured by intracellular ATP and polyphosphate contents), pH values correlated to organic or inorganic nitrogen uptake, and glucose consumption are factors that influence either cellular or filamentous development. Resting cellular forms (chlamydo-spores and swollen cells), induced by a rapid acidification of the culture medium and by an extracellular glucose excess, are ATP and polyphosphate energy consumers for the first days culture during growth and ammonium nitrogen consumption. Hyphal forms, induced by organic nitrogen source and alkalization of the culture medium, are high energy consumers for the 24h culture of the exponential growth but energy producers (ATP) the next days.

XXIX. Department of Microbiology and Immunology, The University of Western Ontario, London, Ontario, Canada N6A 5C1. Communicated by C.F. Robinow.

1. Robinow, C.F. & J.S. Hyams. 1989. General Cytology of Fission Yeasts. Molecular Biology of the Fission Yeast (see reference 7, communication XXI) Chapter 8, pp. 273-330.

Table of Contents:

- I. General Cytology of Fission Yeasts
 - A. Cytoplasmic Features
 - B. Nucleus
 - C. Cell Division
 - D. Conjugation
- II. Fluorescence Microscopy
 - A. Nucleus
 - B. Cell Wall
 - C. Cytoskeleton
 - D. Summary and Conclusions

XXX. Department of Food Science and Technology, University of California, Davis, CA 95616, U.S.A. Communicated by H.J. Phaff

The following is an abstract of a paper to be presented at the 14th ISSY in Smolenice, Czechoslovakia, in September 1990.

1. Phaff, H.J. & J. Blue. 1990. Convergent evolution among cactophilic yeast species that resemble Candida ingens.

Extensive investigations of the yeast flora in necrotic tissue of cactus species indigenous to the Americas have revealed a rather cactus-specific group of species (Starmer et al., 1982, 1990). One of the dominant yeast species in rotting tissue of columnar cacti in the subtribes Stenocereinae and the Pachycereinae was identified as Candida ingens in the above-cited surveys. Identification was based primarily on traditional carbon and nitrogen compound utilization and morphology (Lachance et al. 1988). A reinvestigation of many of the 63 strains in our collection by an expanded metabolic profile, vegetative reproduction, and by DNA-DNA hybridization has shown that apparently none of the isolates represents C. ingens. Instead, we found that they represent not a single species, but a complex of a least three new and undescribed species that resemble C. ingens in many respects. We have now established that there are several consistent differences between the cactus isolates and C. ingens. Physiologically, C. ingens is urease positive, lipase negative, vitamin independent, and has a maximum growth temperature of 36-38°C, whereas the cactus isolates are urease negative, strongly positive for lipase, vitamin dependent and their maximum temperature for growth ranges from 42-45°C. Although the nuclear DNA base composition of C. ingens and the cactus isolates is similar (approximately 38-39 mol % G-C) DNA complementarity is very low (10% or less). Morphologically, the cactus isolates tend to bud bipolarly on a very broad base and they may form septa in short mycelial fragments, while C. ingens also buds multi-laterally. We have conducted a number of DNA hybridization experiments among the isolates from cactus necroses collected in various geographic areas and from specimens belonging to the two major subdivisions of the columnar cacti, the Stenocereinae and the Pachycereinae with their fundamentally different chemical composition (Starmer et al., 1980). The results have given proof for the existence of at least three new species. Species A occurs in rots of Stenocereus hystrix on Jamaica, Haiti and the Dominican Republic. Species B is present in rots of Stenocereus gummosus and Stenocereus thurberi on the Baja California peninsula and in the State of Sonora, Mexico. Species C is associated with rots in Lophocereus schottii and Pachycereus pringlei (both members of the Pachycereinae) occurring in Baja California, Mexico. The only criteria we have found so far to differentiate these three species are ability to utilize L-lysine as sole nitrogen source and resistance of sensitivity to triterpene glycosides or digitonin. We postulate that a combination of host plant chemistry and geographic isolation was instrumental in producing evolutionary divergence and speciation for these organisms. Their similarities in most phenotypic characteristics are most likely caused by convergent evolution. The proposed taxonomic position of the new species will be discussed.

Starmer, W.T., H.J. Phaff, M. Miranda, M.W. Miller, and W.B. Heed. 1982. The yeast flora associated with the decaying stems of columnar cacti and Drosophila in North America. *Evol. Biol.* 14:269-295.

Starmer, W.T., M.A. Lachance, H.J. Phaff, and W.B. Heed. 1990. The biogeography of yeasts associated with decaying cactus tissue in North America, the Caribbean and Northern Venezuela. *Evol. Biol.* 24:253-296.

Starmer, W.T., H.W. Kircher, and H.J. Phaff. 1980. Evolution and speciation of host plant specific yeasts. *Evolution* 34:137-146.

Lachance, M.A., W.T. Starmer, and H.J. Phaff. 1988. Identification of yeasts found in decaying cactus tissue. *Can. J. Microbiol.* 34:1025-1036.

XXXI. École Nationale Supérieure Agronomique de Montpellier, Chaire de Microbiologie Industrielle et de Génétique des Microorganismes. Communicated by P. Galzy.

1. Matte, O., C. Chabaliér, R. Ratomahenina, J.P. Bossy & P. Galzy. 1990. Isolation and characterization of a RNA virus like particle from Candida curvata. Biol. Cell. 68:159-162.
2. Gunata, Y.Z., C.L. Bayonove, R.E. Cordonnier, A. Arnaud & P. Galzy. 1990. Hydrolysis of grape monoterpenyl glycosides by Candida molischiana and Candida wikerhamii β -glucosidases. J. Sci. Food Agric. 50:499-506.
3. Vasserot, Y., P. Chemardin, A. Arnaud & P. Galzy. 1990. Evidence for the β -glucosidase activity and cellobiose fermentation by various Koecckera strains. Acta Biotechnologica (in press).
4. Mouillet-Loevenbruk, D., M. Nicolas, G. Moulin & P. Galzy. 1989. Purification and properties of an alcohol dehydrogenase (ADHc) of mutant strains of Schwanniomyces castellii SC-ADH5P. J. Ferment. Bioengin. 68:404-411.
5. Dubreucq, E., H. Boze, M. Fouilhes, G. Moulin & P. Galzy. 1990. Alternative respiration pathways in Schwanniomyces castellii. I. Isolation and characterization of cytochrome-deficient mutants. Antonie van Leeuwenhoek 57:123-130.
6. Dubreucq, E., H. Boze, G. Moulin & P. Galzy. 1990. Alternative respiration pathways in Schwanniomyces castellii. II. Characteristics of oxidation pathways. Antonie van Leeuwenhoek 57:131-137.
7. Antier, Ph., G. Moulin & P. Galzy. 1990. Influence of composition of the culture medium on the behaviour of K. fragilis in chemostat culture. Process Biochemistry 25:9-13.
8. Antier, Ph., H. Boze, G. Moulin & P. Galzy. 1990. Isolation and analysis of K. fragilis mutants displaying alcohol dehydrogenase deficiency. Biotechnology Letters 12: 45-50.

XXXII. Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7. Communicated by M.A. Lachance.

The following review paper has appeared recently.

1. Starmer, W.T., Lachance, M.A., Phaff, H.J., & W.B. Heed 1990. The biogeography of yeasts associated with decaying cactus tissue in North America, the Caribbean, and Northern Venezuela. Evol. Biol. 24:253-296.

The following is an abstract of a recently accepted paper.

2. Lachance, M.A., Starmer, W.T. & Phaff, H.J., Metschnikowia hawaiiensis sp. nov., a heterothallic haploid yeast from hawaiian morning glory and associated drosophilids. Int. J. Syst. Bacteriol.

A new haploid, heterothallic yeast species was isolated repeatedly from morning glory (Ipomoea acuminata) and two associated drosophilid species, Scaptomyza calliginosa and Drosophila floricola, in a Hawaiian kipuka. Haploid strains multiply asexually by budding and, under nutrient deprivation, by the formation of long germ tubes developing into branching true mycelium. Mating compatibility is controlled by two alleles of a single locus. Plasmogamy between compatible strains is followed by the development of very large elongate asci bearing vestiges of the zygote, and forming two unusually large aciculate ascospores similar to those formed by members of the genus Metschnikowia. Membership in that genus is supported by the yeast's physiological profile which is typical, but not identical to that of any known species. The epithet hawaiiensis is proposed to emphasize the geography of the new species, while expressing uncertainties about its precise habitat. The holotype of M. hawaiiensis is strain UWQ(PS)87-2167.2 (CBS 7432) and the designated isotype is strain UWQ(PS)87-2203.2 (CBS 7433).

The following is an abstract of a paper to be presented at the 14th ISSY in Smolenice, Czechoslovakia, in September 1990.

3. Lachance, M.A. 1990. Ribosomal DNA restriction analysis and the microevolution of the cactophilic yeast Clavispora opuntiae.

Many yeasts are capable of various forms of sexual interaction in addition to their omnipresent ability to multiply asexually, but the respective roles of sexual and asexual reproduction on gene frequency changes through time and space (microevolution) are poorly understood. Restriction site polymorphisms in the intergenic spacer region of ribosomal DNA of Clavispora opuntiae have served as genetic markers used to document microevolutionary events (Lachance 1990 Mol. Biol. Evol. 7:178-193). When analyzed with 12 hexanucleotide-

specific restriction endonucleases, the spacer regions of 125 isolates collected from cacti worldwide each possessed one of 30 possible repetypes. Particularly interesting was the observation that mating types and rDNA spacer repetypes are jointly distributed randomly at the global level, but in disequilibrium at the local level. This was not completely unexpected since it was known that the mating types themselves are not distributed as expected (1:1) under the assumption that meiosis is commonplace (Phaff et al. 1986 Int. J. Syst. Bacteriol. 36:372-379). This was studied further on yeasts isolated from two cactus populations, each from a different Hawaiian island. One cactus stand (Hawaii) is presumed to have acquired its yeast flora through several introductions of exogenous microbial communities, whereas the other (Maui) is known to have been infected relatively recently with a sample of decaying cactus material from the Island of Hawaii. In the older population, rDNA spacer repetype distributions have changed rapidly through time but reassortments have been rare and slow to occur, suggesting that changes in the frequencies of haploid genotypes make important contributions to the evolution of *Clavispora opuntiae* as compared to the generation of new genotypic combinations through sexual exchange. In Maui the founder effect expected from a single infection was clearly corroborated by the identification of a single repetype in all strains collected.

The following papers, whose abstracts appeared in the last issue of the Yeast Newsletter, have now been published.

4. Eisikowitch, D., P.G. Kevan and M.A. Lachance. 1990. The nectar inhabiting yeast, *Metschnikowia reukaufii* and the control of pollen germination in common milkweed, *Asclepias syriaca*. Israel J. Bot. 39:217-225.
5. Lachance, M.A. 1990. Ribosomal DNA spacer variation in the cactophilic yeast *Clavispora opuntiae*. Mol. Biol. Evol. 7:178-193.

RECENT MEETINGS

1. 22nd Annual Meeting of the Yeast Genetics and Molecular Biology Society of Japan, July 1989, Nara Women's University, Nara, Japan. Communicated by O. Niwa.

The 22nd Annual Meeting of the Yeast Genetics and Molecular Biology Society of Japan was held from July 25th through 27th, 1989 at Nara Women's University in Nara. Eighty-eight papers were presented in the following 14 sessions: Session I, Organella; II, Nucleus, replication, segregation; III, Chromosome; IV, Repair, Recombination; V, Biosynthesis, metabolism, physiology; VI, Biotechnology; VII, Regulation, transcription; VIII, Translation, post-translational modification; IX, Cell cycle; X, Conjugation; XI, Meiosis, sporulation; XII, Calcium, calmodulin; XIII, Protein kinase; XIV, cAMP, inositol phosphate, RAS; XV, Transport, secretion; XVI, Plasmid. The abstracts of these presentations were published in Japanese in "Yeast Genetics and Molecular Biology News, Japan" issued on November 28, 1989. Communicated by Osami Niwa, Department of Biophysics, Faculty of Science, Kyoto University, Sakyo-Ku, Kyoto 606, Japan.

FORTHCOMING MEETINGS AND COURSES

1. Fifteenth International Conference on Yeast Genetics and Molecular Biology, The Hague, The Netherlands, July 21-26, 1990.

Contact: Holland Organizing Centre
Attn: Lidy Groot or Marijanka Schoor
16, Lange Voorhout 2514 EE
The Hague The Netherlands

Tel.: (+31-70)365.78.50

2. Society for Industrial Microbiology, 1990 Meetings Schedule.

WORKSHOP (July 28-29 1990)

"Biotechnology: GMPs, GLPs and Other Regulatory Issues." Co-sponsored with the Parenteral Drug Association. July 28-29, 1990, Marriott Orlando World Center, Orlando, Florida.

1990 ANNUAL MEETING (July 29 - August 3 1990)

47th Annual Meeting of the Society for Industrial Microbiology, July 29 - August 3, 1990, Marriott Orlando World Center, Orlando, Florida.

For further information on the workshop or the meeting, contact:

Mrs. Ann Kulback, SIM,
P.O. Box 12534,
Arlington VA 22209-8534
USA

Telephone (703)941-5373 or FAX: (703)941-8790.

CONFERENCE (October 14-17 1990)

Second International Conference on the Biotechnology of Microbial Products: Novel Pharmacological and Agrobiological Activities. October 14-17, 1990, Hyatt Sarasota Hotel, Sarasota, Florida. For information contact: Mrs. Ann Kulback (address above) or:

Dr. Jenny Hunter-Cevera,
Cetus Corporation,
1400 53rd St.,
Emeryville, CA 94608
USA

Telephone: (415)420-3211.

3. 6th International Symposium on Genetics of Industrial Microorganisms (GIM 90), Strasbourg, France, 12-18 August 1990. Communicated by H. Heslot.

The 6th International Symposium of Genetics of Industrial Microorganisms (GIM 90) will be held at the Palais des Congrès, Strasbourg (France), 12-18 August, 1990 under the auspices of the GIM-International Committee. The GIM 90 is organized by the SOCIÉTÉ FRANÇAISE DE MICROBIOLOGIE (SFM) with the support of the French Ministries of Agriculture, Education, Industry, Research, the Institut National de la Recherche Agronomique, the Centre National de la Recherche Scientifique. The scientific programme, of international scope, will include lectures by renowned scientists and industrialists. Poster sessions will play a key role in the scientific programme. Lectures and posters will be arranged in sessions covering the following themes: protein engineering, genetic instability heterologous expression, secretion, extrachromosomal elements - transposons, metabolic pathways - manipulation, corynebacteria - production of aminoacids, streptomycetes - antibiotics - resistance mechanisms, lactic and bacteria, industrial yeasts, filamentous fungi, methylotrophs - methanogens, genetics of bacillus, clostridia, plant-bacterial interactions, alcoholic and malolactic fermentations - aroma, biomass degradation, toxins of microorganisms, bioconversions.

Contact:

Symposium Secretariat, GIM 90
Société Française de Microbiologie
28, rue du Docteur Roux
75724 Paris Cedex 15
France

Phone: (1) 45.68.81.79 - Telecopy: (1) 45.67.46.98 - Telex 214 403 F

4. Fourth International Mycological Congress, Regensburg, Fed. Rep. of Germany, August 28 - September 3 1990).

Contact:

Prof. Dr. Andreas Bresinsky,
Botanisches Institut der Universität,
D-8400 Regensburg,
Federal Republic of Germany

Tel: 941 9433108

5. 14th International Specialized Symposium on Yeasts, Yeast Taxonomy, Theoretical and Practical Aspects, September 3-7 1990, Smolenice, Czechoslovakia.

Organized by the Commission for Yeasts of the Czechoslovak Microbiological Society; Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, Bratislava, in accordance with resolution of the meeting of the International Yeast Commission held in Perugia in 1988, the 14th International Specialized Symposium on Yeasts, YEAST TAXONOMY, THEORETICAL AND PRACTICAL ASPECTS will be held in the Smolenice Castle near Bratislava on September 3-7, 1990. The Symposium will cover the following topics:

1. Traditional and modern approaches in yeast taxonomy
2. New properties of yeasts
3. Evolution of yeasts
4. Taxonomy in relation to biotechnology
5. Environment-induced changes in yeasts
6. Morphological properties and surface structures
7. Preservation of yeasts

Contact:

Secretariat of the 14th ISSY
Institute of Chemistry
Dubravska cesta 9
842 38 Bratislava, Czechoslovakia

6. **Third International Symposium on Gas, Oil, Coal, and Environmental Biotechnology, December 3-6, 1990, New Orleans, Louisiana, USA. Communicated by C. Akin.**

Contact:

Dr. Cavit Akin
Associate Director
Biotechnology Research
Institute of Gas Technology
3424 South State Street
Chicago, IL 60616-3896
USA

Phone: 312/567-3724. Fax: 312/567-5209. Telex: 25-6189

7. **XIth Congress of the International Society for Human and Animal Mycology, Montréal, Québec, Canada, June 24-28, 1991.**

To receive the second announcement, contact:

XIth Congress of the International Society for Human and Animal Mycology
c/o JPdl Multi-Management Inc.
1410 Stanley, Suite 609
Montréal, Québec, Canada
H3A 1P8

Tel: (514) 287-1070

BRIEF NEWS ITEMS

1. ***In Memoriam* - D. Minoru Yoneyama**

My father passed away of illness March 23 1989, after a long happy life of 77 years. I thank wholeheartedly those who gave my father so much kindness.

Kiichiro Yoneyama, Hiroshima, Japan

2. **Change of address: Dr. Gerald Reed**

I have retired from Universal Foods but will continue to write, edit, and consult. My new address is:

Dr. Gerald Reed
1016 Monmouth
Durham, NC 27701
U.S.A.

3. **Change of address: Dr. Gode B. Calleja**

Dr. Gode B. Calleja
Room 112, Institute of Biology
College of Science
University of the Philippines
Dilliman, Quezon City
The Philippines

4. **Change of address: Dr. Byron F. Johnson**

Effective in the autumn, 1990:

Dr. Byron F. Johnson
Department of Biology
Carleton University
Ottawa, Ontario
Canada K1S 5B6

5. Brew Info

Offered through collaboration between the European Brewery Convention (EBC) and the Brewing Research Foundation (BRF) the BREW-INFO system is specially designed to serve the interests of the international brewing industry.

The Brew-Info System

The BREW-INFO system consists of the computer database "BREW-INFO DATABASE" and the monthly publication "CURRENT AWARENESS MONTHLY" which contains the most recent published references on brewing and related topics.

About 35% of the references have supporting abstracts to give full information on new developments and matters of current interest. These abstracts can be retrieved by subscribers from the BREW-INFO DATABASE, available on-line 24 hours a day, all year round. Abstracts to selected publication are now included in "CURRENT AWARENESS MONTHLY" as well. In order to use the DATABASE on-line subscribers will need a personal computer (PC) and printer or a word processor (in many cases this equipment will be available already). In addition an appropriate telephone link (modem and software) will be required. Subscriber's terminals may be connected to the on-line system through the telephone network data handling system. Costs for this are less than for normal telephone calls over the same distance and are less subject to interference. A subscriber's communication with the DATABASE is confidential and at the end of any search session, all enquiries and answers obtained by the user are destroyed. In order to learn how to operate the system, subscribers are supplied with a Manual and receive a starting period of 3 hours free of charge for connection to the DATABASE, beginning at the date the password is used for the first time.

Current Price List

CURRENT AWARENESS MONTHLY: subscription for one year	(12 issues) NLG 375
ditto, for each supplementary copy	(MAX 15) NLG 200
BREW-INFO SYSTEM: same as above, plus access to DATABASE	(per hour) NLG 150

Contact:

Secretariat General,
European Brewery Convention,
P.O. Box 510,
NL-2380 BB Zoeterwoude,
The Netherlands.

Telephone: 31-71-456047/456614

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is essential for ensuring transparency and accountability in the organization's operations.

2. The second part of the document outlines the various methods and tools used to collect and analyze data. It highlights the need for consistent data collection procedures and the use of advanced analytical techniques to derive meaningful insights from the data.

3. The third part of the document focuses on the implementation of data-driven decision-making processes. It describes how the organization uses the insights gained from data analysis to inform strategic planning and operational decisions, leading to improved performance and efficiency.

4. The final part of the document discusses the challenges and opportunities associated with data management and analysis. It notes that while data provides valuable insights, it also presents significant challenges in terms of data quality, security, and privacy.