ISSN 0073-8751

NUMBER 14

INSTITUTE FOR FERMENTATION
OSAKA

RESEARCH COMMUNICATIONS

(ANNUAL REPORT 1987-1988)

1989

財団法人 発酵研究所

RESEARCH COMMUNICATIONS

No. 14

(*Annual Report 1987-1988*)

1989

INSTITUTE FOR FERMENTATION, OSAKA

Published by the Director,
INSTITUTE FOR FERMENTATION, OSAKA
17-85, JUSO-HONMACHI 2-CHOME
YODOGAWA-KU, OSAKA 532, JAPAN

INSTITUTE FOR FERMENTATION, OSAKA

BOARD OF TRUSTEES

Chairman: Shinbei KONISHI

Saburo FUKUI
Osamu HAYAISHI
Teiji IIJIMA
Yoshio OKADA
Takezi HASEGAWA
Motoyoshi HONGO
Katsura MORITA
Hideaki YAMADA

AUDITORS

Ryohei KISAKI
Toshio MIWATANI

COUNCILORS

Teruhiko BEPPU
Tsuguo HONGO
Masao ISONO
Ken-ichi MATSUBARA
Yoshio OKAMI
Shoichi TAKAO
Gakuzo TAMURA
Kiyoshi YORA
Tokuya HARADA
Hiroshi IIZUKA
Kazuo KOMAGATA
Einosuke OHMURA
Yasuji OSHIMA
Rokuro TAKEDA
Keisuke TUBAKI

HONORARY MEMBERS

Shigeyasu AKAI
Hideo KIKKAWA
Kin-ichiro SAKAGUCHI
Tsunesaburo FUJINO
Yoshio KOBAYASI
Yuji SASAKI

CONTENTS

****

The Institute for Fermentation, Osaka publishes the IFO Research communications on a biennial basis. Purchase orders of the Research Communications should be addressed to The Institute for Fermentation, Osaka, 17-85 Juso-honmachi 2-chome, Yodogawa-ku, Osaka 532, Japan.

Prices of back numbers are as follows: Nos. 1-5, 500 yen each; No. 6, 800 yen; No. 7, 1,000 yen; Nos. 8-9, 800 yen; No. 10, 1,000; and Nos. 11-13, 1,300 each, and plus postage.

REPORT OF THE DIRECTOR

Application of chemical and physical techniques to elucidate the chemical composition of whole cells or part of cells has produced information of great value in the classification and identification of microorganisms. The analysis of the chemical composition of cell components, hybridization of DNA from related microorganisms and analysis of rRNA are familiar techniques in culture collections. It is not possible to identify or classify microorganisms without sophisticated understanding of the recent developments in cell biology, microbiology and genetics. The renewal of facilities and the recruitment of young manpower are indispensable in establishing a solid basis for culture collections. During the period 1987 - 1988, new staff have joined and new facilities have been installed in the institute. A laboratory of physical containment P2 level to handle recombinant DNA, new model microscopes, HPLC and electrophoresis equipment have been installed and have begun to operate.

The chairman of the Board of Trustees, Mr. Shinbei Konishi, received a new fund amounting 150 million yen from Takeda Chemical Industries Ltd., in March 1987. Addition of the fund together with 45 million yen from the balance of the fiscal year 1986, to the foundation of the institute was approved at the 83rd annual meeting of the Board of Trustees in March 1987. As the result, the total amount of foundation reached one billion yen. This growth in the foundation has helped to increase the total activities of IFO by allowing recruitment of manpower and installation of new equipment. At the 84th Annual Meeting of the Councilors of IFO in June 1987, Dr. Saburo Fukui, Emeritus Professor of Kyoto University, was nominated as a member of the Board of Trustees. Prof. Kazuo Komagata, University of Tokyo and Prof. Yasuji Oshima, Osaka University, were nominated as councilors of the Board of Trustees in June 1988. The treasurer, Yasuyuki Yamada, moved to Takeda Service Ltd. in July 1988, and Mr. Shunji Ietsuka was appointed as treasurer and started his service from 1 July, 1988.

In April 1987 Dr. Toru Hasegawa, taxonomist of Actinomycetes, moved from the Central Research Division, Takeda Chemical Industries

Ltd., and joined IFO as a Senior Researcher on Actinomycetes and Fungi. Dr. Taiki Kusaka moved to the Corporate Technical Planning Department, Takeda Chemical Industries Ltd. His seven years of dedication in the Actinomycetes laboratory and qualification of ISP strains in IFO was much appreciated.

Dr. Akira Yokota, returned back from the Max-Planc Institute for Immunology, Freiburg, in September 1987 and resumed to his research project at IFO. His achievements in the Max-Planc Institute have helped us to carry out chemical taxonomy at IFO. A new member of staff, Dr. Akira Nakagiri, joined IFO from the University of Tsukuba in April 1988. His main research field at IFO is taxonomic studies on marine fungi. His four years experience as a researcher at the University of Tsukuba have allowed us to add another researching field to IFO. Dr. Sato joined the animal cell line laboratory from 1 November, 1988. Assistant curator Mr. Isamu Asano retired from the institute on 6 December, 1988 after 30 years of service in preserving fungus strains at IFO. Mr. Nishii succeeded to his position.

The total number of cultures stored in the IFO culture collection reached 12,943 at the end of 1987 and 13,177 at the end of 1988. The newly accepted strains during these two years are listed in the present issue of IFO Research Communications No. 13. The total number of cultures distributed from the IFO culture collection reached to 9,265 in 1987 and 9,000 in 1988. The distribution of phytopathogenic strains with permission from the phytoquarantine office reached 80 strains during this period.

IFO Research Communications No. 13 was published in March 1987, and IFO List of Cultures 8th edition vol.1 (Microorganisms) and vol.2 (Animal Cell Lines) were published in March 1988. These listed about 9000 strains: 5300 fungi, 2030 bacteria, 1600 yeasts, 50 bacteriophages and 50 animal cell lines. The manuscript of the publications was edited and arranged from the data base of the IFO culture collection in an IBM/23 computer by transferring to an NEC9800 and printer. We are now preparing IFO Research Communications No.14 (1989), which will be issued in March 1989.

Dr. I. Banno, attended the 7th International Symposium on Yeasts, from 1 to 5 August, 1988 in Perugia, Italy, and presented a paper on the taxonomical relation between *Saccharomyces cerevisiae* and *S.*

bayanus. He also visited MSDN and NCYC in U.K. and DSM in West Germany, before and after the Symposium.

The 6th International Congress for Culture Collections was held from 30 October to 4 November 1988, at the Center of Adult Education, University of Maryland, U.S.A. Director T. Iijima and Dr. Toru Hasegawa attended the congress and presented a paper on the ISP Checking Committee and introduced the activities of IFO and JFCC (Japan Federation for Culture Collections). They also visited NCTC and NCIMB in the U.K., to see the activities of these famous culture collections in the U.K.

IFO has received a number of guests in the past two years. Prof. M. Goodfellow, University of Newcastle upon Tyne, visited us on 21 July 1987, Dr. R. Stevenson, Director of American Type Culture Collection, visited us on 12 April 1988, and Dr. S. C. Jong, Department head, American type culture Collection, visited us on 19 August, 1988. The staff of IFO discussed matters of mutual interest with them. Lectures and Seminars were given by the following guest speakers.

Dr. S. Harashima, Department of Fermentation Technology, Faculty of Technology, Osaka University: Genetic control on biosynthesis of amino acids in yeasts.

Dr. H. Mizusawa, National Institute of Hygienic Science: Recent development of JCRB and its researching activities.

Dr. H. Oyaizu, Toyama University: Database of 16S-ribosomal RNA and possible approaches to identification in microorganisms.

Dr. H. Mayer, Max Planck Institute für Immunologie, Freiburg, FRD: Toxic and non-toxic lipopolysaccharides from non-enterobacterial species -- A contribution to phylogeny and endotoxin research.

Dr. Ji-sheng Ruan, Institute of Microbiology, Academia Sinica, Beijing, China: The study of rare actinomycetes in China.

Dr. R. B. Lacey, Plant Pathology Department, Rothamsted Experimental Station, Harpenden, U.K.: Thermophilic actinomycetes as causes of respiratory disease.

Dr. R. B. Batra, Mycology laboratory, U.S. Department of Agriculture, Beltsvill, U.S.A.: Floral Mimicry induced by mummy-berry fungus exploits host's pollinators as vectors.

As a cooperative activity, IFO received guest researchers during this period: Miss Celia R. Kamakura from Adolfo Lutz Institute, Brazil; Miss H. C. Yang from Taiwan Agricultural Chemicals and Toxic Substances Research Institute; Dr. Nakagawa from Shizuoka University; and Dr. Kikuko Takeuchi from Ehime College of Health Science. We accepted members of the JICA training course for lectures and a demonstration of the institute's activities. Dr. Banno, Dr. Yokoyama and Dr. Imai gave a lecture to the trainees.

(T. Iijima)

Heartfelt condolences are extended to the bereaved of:

Professor emeritus Hideo Katagiri, who passed away on 17 September, 1987

Professor emeritus Kei Arima, who passed away on 23 August, 1988

They made great contributions to the establishment and the development of the Institute for Fermentation, Osaka.

IFO Res. Comm. 14,
5-12, 1989 (March)

CHARACTERIZATION OF TWO BACTERIOPHAGES FOR BACILLUS PUMILUS, THE VIRULENT PHAGE 31 AND THE TEMPERATE PHAGE NP-5

KO IMAI

Summary

Two bacteriophages for Bacillus pumilus, the virulent phage 31 isolated from a soil sample and the temperate phage NP-5 carried by IFO 12088, were characterized. The latent period and the apparent minimal burst size of phage 31 were about 45 min and 30, respectively, and those of NP-5 were about 60 min and 100, respectively, when propagated in B. pumilus IFO 12093 in PY broth at 37 C. Electron micrographs of 31 and NP-5 revealed that both phages possess a hexagonal head measuring about 60 x 70 nm and a tail about 130 to 175 nm long with a base plate. Teichoic acid is a possible receptor for these phages.

Bacillus subtilis has been well characterized genetically by transformation and transduction (4), but Bacillus pumilus, which is a closely related species, has not. The generalized transducing phages of B. pumilus have been found to be infectious for only motile host strains (5), while the temperate phage Ø75 is productively infectious and lysogenizes certain asporogenic mutants of the host strain, but does not sporogenic strains (3). Bacteriophages can be useful tools for genetic analysis of bacteria. We attempted to isolate more bacteriophages for B. pumilus and found the virulent phage 31 from a soil sample and the temperate phage NP-5 from the B. pumilus collection in IFO. The present report deals with the preliminary characterization of 31 and NP-5.

Materials and Methods

Bacterial strains and growth conditions. The bacterial strains used here are shown in Table 1. The liquid bacterial cultures were incubated aerobically on a reciprocal shaker. The incubation temperature for liquid cultures and plates was 37 C.

Media. The PY broth used for liquid bacterial cultures and for dilution of phage suspensions for assay contained 10 g of Polypepton (Nippon Seiyaku, Co.), 2 g of yeast extract (Difco), 2 g of NaCl, and 1,000 ml of distilled water and was adjusted to pH 7.0 with NaOH. Solid and soft agar media consisted of PY plus 1.5% and 0.8% agar, respectively.

Isolation of phage from soil samples. Approximately 0.1 g of a soil sample and 0.1 ml of an overnight culture of B. pumilus IFO 12092, which is the type strain of the species, were inoculated into 10 ml of PY. After incubation for 16 hr, 1 ml of the culture was centrifuged at 15,000 rpm for 10 min. One drop of chloroform was added to the supernatant, which was allowed to stand at 37 C for 15 min. Suitable dilutions were made with PY, then 0.1 ml-portions of diluted samples and 0.5 ml of an overnight culture of IFO 12092 were mixed on solid plates with 5 ml of soft agar premelted and kept at 50 C. Plates were incubated for 20 hr, and some of plaques produced were repeatedly purified, then examined for their host range and plaque morphology.

Isolation of temperate phage from the B. pumilus collection in IFO. Overnight cultures of B. pumilus strains shown in Table 1 were centrifuged at 15,000 rpm for 10 min. After the treatment with chloroform, the supernatants were assayed for the plaque formation on the B. pumilus strains.

Phage assay. Infective phage particles (plaque-forming units, PFU) were determined by use of the agar layer technique (1).

One-step growth experiment. One ml of an overnight culture of B. pumilus IFO 12093 and 1 ml of a phage suspension containing 10^8 PFU were mixed, and immediately 0.2 ml of the mixture was filtered through a Millipore membrane (0.45 μm pore size). The filter was washed twice with 10 ml of ice-cold PY, transferred into a 200-ml flask containing 50 ml of prewarmed PY, then incubated at 37 C. The phage titer in the broth culture was determined at intervals of 15 min. Free phage particles which were not adsorbed to host cells were determined from the PFU in 0.1 ml of the

broth culture, in which host cells were killed with chloroform.

Electron microscopy. A diluted phage suspension was mixed with a saturated solution of uranyl acetate, and the mixture was placed on a standard electron microscope grid that was coated with a polyvinyl formvar film. Excess mixture was removed with the edge of a piece of filter paper, and the preparation was air-dried before examination in a JEM-1200EX electron microscope (JEOL, Ltd.).

Purification of the cell wall. To identify the host components required for attachment of 31 and NP-5, the cell wall of IFO 12093 was purified, and fractions obtained at each step of the purification were assayed for their ability to inactivate phage particles. About 10 g of wet cells of IFO 12093 was suspended in 50 ml of distilled water and disrupted with a Kubota sonic oscillator at 160 W for 60 min. Undisrupted cells were removed by centrifugation at 3,000 rpm for 10 min, and cell wall in the supernatant was collected and washed twice with distilled water by centrifugation at 35,000 rpm for 30 min (fraction I). The cell wall fraction I was resuspended in 2% sodium dodecyl sulfate (SDS) and boiled for 30 min. After cooling to room temperature, the cell wall was collected by centrifugation and washed three times with distilled water to remove SDS (fraction II). The final pellet was resuspended in 0.02 M HCl containing pepsin (1 mg/ml) and incubated at 37 C for 2 hr. The cell wall treated with pepsin was washed three times each with 0.02 M HCl and distilled water (fraction III). Fraction III was extracted with 5% trichloroacetic acid at 90 C for 6 min, then centrifuged at 35,000 rpm for 30 min. The pellet was washed three times with distilled water (Fraction IV), and the supernatant was extracted three times with ether to remove trichloroacetic acid (fraction V). All fractions were lyophilized and assayed for the inactivation of phages 31 and NP-5.

Inactivation of phage by the cell wall fraction. One ml of 0.1 M phosphate buffer (pH 8.0) containing ca. 10^5 PFU of phage particles, and 1 mg of a cell wall fraction were mixed with one drop of chloroform. After incubation at 37 C for 2 hr, the phage titer of the mixture was determined by the agar layer technique.

Analysis of fraction V. Five mg of fraction V was hydrolyzed with 2 M HCl at 100 C for 4 hr, and the hydrolysate was dried under vacuum. Amino acids and amino sugars in the dried sample were determined by use of an amino acid analyzer, and neutral sugars were determined by use of a high

performance liquid chromatograph equipped with a Shimadzu SCR-101H column (7.9 x 300 mm), with dilute sulfuric acid (pH 2.2) as the mobile phase. Phosphorus was determined by the method of Ames (2).

Table 1. Bacillus pumilus strains used and the sensitivity to bacteriophages 31 and NP-5.

IFO No.	No. of other collection	Sensitivity to	
		31	NP-5
3813	NCTC 8241, ATCC 14884	R	R
12086	NCIB 2595, ATCC 4520	S	R
12087	NCIB 7576	S	R
12088	NCIB 8081, ATCC 6632	S	R
12089	NCIB 8600	S	R
12090	NCIB 8738	R	R
12092	NCIB 9369, ATCC 7061	S	R
12093	CCM 77	S	S
12094	CCM 340	S	R
12097	CCM 386	S	R
12100	CCM 1697	S	R
12101	CCM 1725	S	R
12110	NRRL B-1489	R	R
12111	NRRL B-1875	S	R
12103	CCM 1995, BUCSAV 167, NCIB 8081, ATCC 6632	S	S

Abbreviations of culture collections are as follows: ATCC, American Type Culture Collection, Rockville, USA; BUCSAV, Institute of Biology, Czechoslovak Academy of Sciences, Prague, CSSR; CCM, Czechoslovak Collection of Microorganisms, J.E. Purkyne University, Brno, CSSR; NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; and NRRL, ARS Culture Collection, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, U.S.A.

R and S indicate resistance and sensitivity to phage, respectively.

Results and Discussion

Isolation of B. pumilus virulent phage 31 from soil

Phages were isolated from 2 of the 24 soil samples examined; and the 2 phages appeared, from the morphology of their plaques and their host range, to be of the same type. One of them was chosen for further study and was called 31. Of 15 strains of B. pumilus, 12 were sensitive to phage 31 (Table 1). On infection of these 12 strains with phage 31, 30 mutants resistant to phage 31 were isolated, none of which lysogenic for 31.

Isolation of temperate phage NP-5

Of the 15 strains of B. pumilus in the IFO collection, IFO 12088 was found to produce active phage particles on IFO 12093 and to be lysogenic for an inducible, nondefective temperate phage NP-5. IFO 12093 was infected but not lysogenized with NP-5. IFO 12088 is a subculture of NCIB 8081. IFO 12103, which also originated from NCIB 8081 but transferred through two institutions before arriving at IFO, was infected and lysogenized with NP-5. Ø75 is the only known inducible, nondefective temperate phage for B. pumilus. However, Ø75 infects some asporogenic host variants, but not sporogenic variants (3). IFO 12088 and 12103 are sporogenic, and NP-5 is a novel temperate phage infectious for the sporogenic host.

Latent period and minimal burst size of 31 and NP-5

Results obtained from the one-step growth experiments using IFO 12093 as the host indicated that the latent period and the apparent minimal burst size were respectively about 45 min and 30 for phage 31 and about 60 min and 100 for NP-5 (Fig. 1).

Morphology of 31 and NP-5

Electron micrographs of 31 and NP-5 negatively stained with uranyl acetate are shown in Fig. 2. Both bacteriophages possess a regular hexagonal head with a size of about 60 x 70 nm and a straight tail (about 130 to 175 nm long and about 10 nm in diameter) with a base plate.

Identification of receptor for phages 31 and NP-5

To identify the host components required for attachment of 31 and NP-5, the cell wall fractions obtained at each step of the purification were assayed for their ability to inactivate the phages. As shown in Table 2, the cell wall fractions treated with SDS and pepsin (fraction II and III) inactivated both phages. This result suggests that the cell membrane and

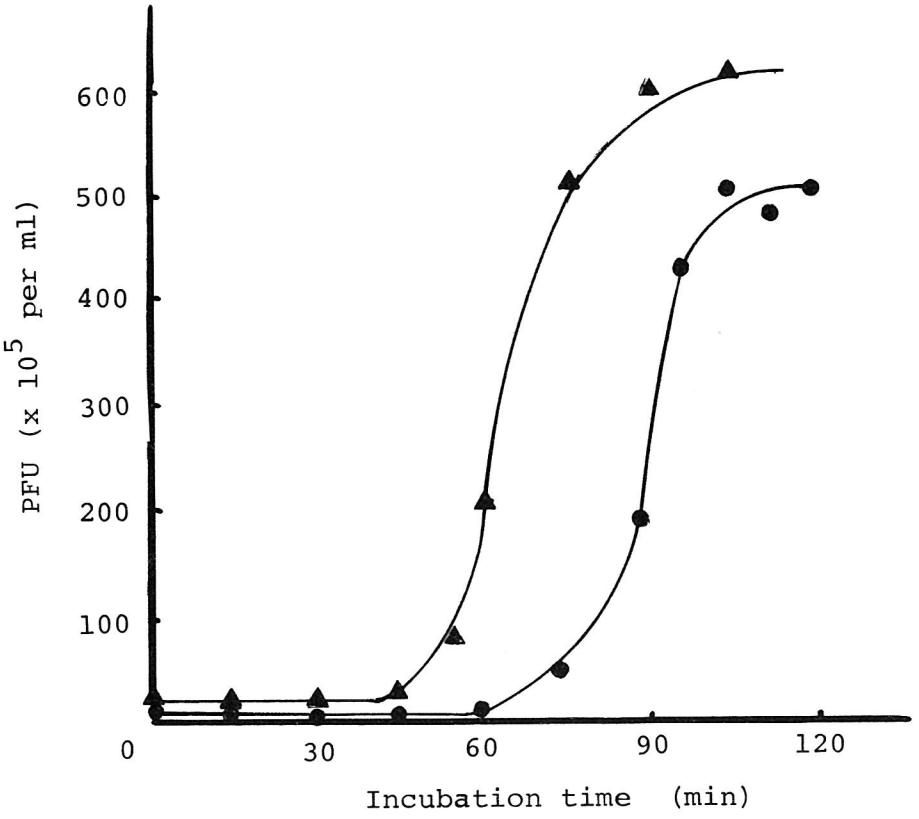


Fig. 1. One-step growth curves of 31 (▲) and NP-5 (●) on IFO 12093 in PY broth at 37 C. The PFUs of 31 and NP-5 at 0 min were 2.01 x 10^6 and 5.08 x 10^5 per ml, respectively.

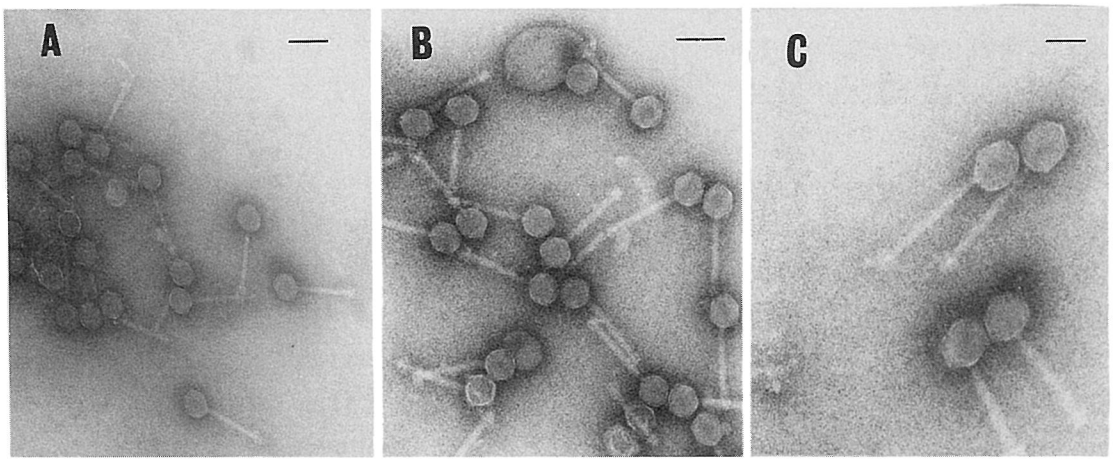


Fig. 2. Electron micrographs of 31 (A) and NP-5 (B and C) stained with uranyl acetate. Bars indicate 200 nm in A, 100 nm in B, and 50 nm in C.

Table 2. Inactivation of phage 31 and NP-5 by the cell wall fractions.

Cell wall fraction	PFU/ml	
	31	NP-5
None	8.93×10^4	4.58×10^4
I	0	0
II	0	0
III	0	0
IV	9.20×10^4	4.30×10^4
V	0	0

The cell wall fractions I to V were prepared as described in materials and methods. One ml of the reaction mixtures containing ca. 10^5 PFU of phage particles was incubated for 2 hr, then the phage titer of the mixtures was determined by the agar layer technique.

proteins are not the receptor for these phages. The cell wall treated with trichloroacetic acid (fraction IV) lost its ability to inactivate the phages, while the extract of the cell wall with trichloroacetic acid (fraction V) inactivated the phages. The hydrolysate of the extract with HCl contained glucosamine, galactosamine, alanine, glycerol, and phosphorus in a molar ratio of 1.00:0.11:4.73:4.21:9.36. These results suggest that fraction V contains mainly teichoic acid, and that teichoic acid is a possible receptor for phages 31 and NP-5.

The investigation reported here has dealt with the isolation of the virulent phage 31 and the temperate phage NP-5, and the determination of some of their characteristics. This investigation was a necessary preliminary to a more detailed study, now in progress, of the transducing ability and nucleic acid of these phages. NP-5 and 31 have been deposited in the Institute for Fermentation, Osaka, under the accession numbers of IFO 20060 and 20062, respectively.

We thank Ihomi Nishiura for excellent technical assistance.

References

1) Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2) Ames, B. N. 1966. Assay of inorganic phosphate, total phosphate, and phosphatases. In E. F. Neufeld and V. Ginsbrug (ed.), Methods in Enzymology, vol. 8, p. 115-118. Academic Press Inc., New York.
3) Bramucci, M. G., and P. S. Lovett. 1974. Temperate bacteriophage infectious for asporogenic variants of Bacillus pumilus. J. Virol. 14: 1281-1287.
4) Henner, D. J., and J. A. Hoch. 1980. The Bacillus subtilis chromosome. Microbiol. Rev. 44: 57-82.
5) Lovett, P. S. 1972. PBP1: a flagella-specific bacteriophage mediating transduction in Bacillus pumilus. Virology 47: 743-752.

IFO Res. Comm. 14,
13-19, 1989 (March)

IDENTIFICATION OF MYCOPLASMAS CONTAMINATING ANIMAL CELL LINES

TOUHO YOSHIDA, NOBUAKI YANAI*, MASAKO KAWASE**,
HIROSHI MIZUSAWA**, KOSHI YAMAMOTO***, AND MASAO TAKEUCHI

Summary

We identified species of mycoplasmas contaminating cell lines by an immunoblot assay using eight anti-mycoplasma antibodies. The immunoblot assay detected eight species of mycoplasma specifically and its sensitivity ranged from 1 x 10^5 to 4 x 10^6 colony-forming units (CFU)/ml of organisms depending on mycoplasma species. In 57 cell lines examined, the mycoplasmas detected were Mycoplasma hyorhinis (41%), Mycoplasma fermentans (24%), Mycoplasma orale (14%), Mycoplasma hominis (10%), Acholeplasma laidlawii (2%), Mycoplasma salivarium (2%), and other mycoplasma species (7%).

Mycoplasmas are common contaminants of cell lines and have been shown to affect the normal functions of cell lines in many ways (7). As one means of quality control of cell lines, we routinely tested for mycoplasmal contamination using both DNA staining and microbiological culture methods (9, 10). The rate of contamination in our survey at the Institute for Fermentation, Osaka (IFO) was 26% (10).

---

*) Present address: Research Institute for Tuberculosis and Cancer, Tohoku University.

**) National Institute of Hygienic Sciences.

***) National Institute of Animal Health.

To determine the sources of contamination, it is helpful to identify the contaminating mycoplasmas. Barile et al. (2) reported that 19 different mycoplasma species were isolated from cell lines. However, four species, Mycoplasma orale, Mycoplasma arginini, Mycoplasma hyorhinis, and Acholeplasma laidlawii, accounted for the vast majority of isolates (2). These isolates are human, bovine, or swine species of mycoplasmas. In the present study, we identified mycoplasmas in cell lines in the IFO collection using the immunoblot assay (5), in order to determine the major cause of mycoplasmal contamination.

Materials and Methods

Cell culture specimens. A total of 57 cell lines that had been submitted by 18 laboratories and preserved at the IFO were tested. Test specimens containing cells and their cultured media were taken 3 to 6 days after the last subcultivation. All cell cultures were incubated in antibiotic-free media.

Mycoplasmas. Mycoplasma arginini G230 (IFO 14476), Mycoplasma orale CH19299 (IFO 14477), Mycoplasma salivarium PG20 (IFO 14478), Mycoplasma hyorhinis BTS7, Acholeplasma laidlawii PG8 (IFO 14400), Mycoplasma buccale CH20247, Mycoplasma fermentans PG18, and Mycoplasma hominis PG21 were grown in a broth medium for mycoplasmas as described previously (9). As Mycoplasma hyorhinis DBS1050 is a fastidious strain in cell-free media (4), it was grown by co-cultivation with LoVo cells (IFO 50067) and the supernatant of LoVo cell cultures was used as the specimen for this strain. The number of viable mycoplasmas was determined by inoculation onto agar and recorded as the number of colony-forming units (CFU/ml).

Antibodies. Polyclonal antibodies to M. buccale, M. fermentans, or M. hominis were produced in rabbits by immunization with each organism. Mouse monoclonal antibodies to M. arginini, M. hyorhinis, M. orale, M. salivarium, and A. laidlawii were purchased from GIBCO Laboratories (Grand Island, NY).

Immunoblot. The immunoblot assay described by Kotani and McGarrity (5) was used to identify mycoplasmas. Ten μl of test specimens spotted onto a nitrocellulose paper (BIO-RAD Laboratories, Richmond, CA) and air dried. The nitrocellulose paper was pretreated with 0.3% H_2O_2 in Tris-

buffered saline (TBS, 50 mM Tris-HCl, 200 mM NaCl, pH7.4) for 10 min to inactivate endogenous peroxidase, then washed with TBS for 5 min. Blocking solution (10% horse serum, 0.02% Tween 20 in TBS) was applied for 30 min to block nonspecific protein on the paper. The three polyclonal and five monoclonal antibodies were diluted 1:1,000 and 1:100 with the blocking solution, respectively, and applied as first antibodies. The paper was incubated for 30 min at room temperature and washed 3 times with TBS. As secondary antibodies, biotinylated goat anti-rabbit IgG antibodies (Cappel Laboratories, Inc., Cochranville, PA) or biotinylated goat anti-mouse IgG antibodies (Cappel Laboratories, Inc., Cochranville, PA) were applied for 30 min at room temperature. These antibodies were employed at a 1:1,000 dilution in the blocking solution. The paper was washed 3 times with TBS. Finally, avidin conjugated peroxidase (Zymed Laboratories, Inc., San Francisco, CA) diluted 1:50,000 in the blocking solution was added and incubated for 30 min at room temperature. The paper was washed 3 times with TBS, then developed with 1 ml of substrate solution (0.05% 4-chloro-1-naphtol, 0.01% H_2O_2 in TBS). After a positive reaction had developed, the paper was washed with distilled water and air dried.

Results and Discussion

Specificity and sensitivity of the immunoblot assay

We certified the specificity of immunoblot assay using eight species of mycoplasma: M. arginini, M. orale, M. salivarium, M. hyorhinis, A. laidlawii, M. buccale, M. fermentans, and M. hominis. These eight species were reported to be the major isolates from contaminated cell lines in surveys performed in the United States (2, 8). A 10-μl suspension of each mycoplasma (1×10^5 CFU) was dotted on a nitrocellulose paper. When monoclonal antibodies to M. hyorhinis were used as first antibodies, a clear homologous reaction was obtained without non-specific reaction. Typical reactions are shown in Figure 1. When lower than 1:1,000 dilutions of the polyclonal antibodies were used as first antibodies, non-specific reactions appeared for some mycoplasmas. However, when the optimal concentration (1:1,000) was used, such artifacts were eliminated. As shown in Table 1, the eight antibodies employed in our studies reacted specifically for the eight respective species. The immunoblot assay also

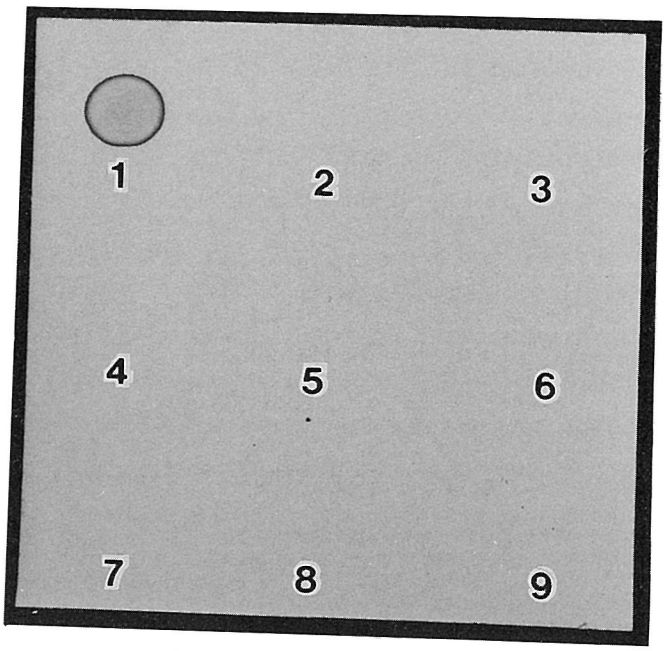


Fig. 1. Immunoblot assay using monoclonal antibody to M. hyorhinis.
Test samples were as follows: 1, M. hyorhinis BTS7; 2, M. orale CH19299; 3, M. arginini G230; 4, M. salivarium PG20; 5, A. laidlawii PG8; 6, M. fermentans PG18; 7, M. buccale CH20247; 8, M. hominis PG21; 9, medium for mycoplasmas.

detected M. hyorhinis DBS1050, a fastidious strain in cell-free media for mycoplasmas.

To determine the sensitivity of this method, 10-fold serial dilutions of broth cultures of each species were applied to nitrocellulose paper. The end-points of positive detection ranged from 1 x 10^5 to 4 x 10^6 CFU/ml depending on mycoplasma species (Table 2). In most cases the number of viable mycoplasmas in contaminated cell lines ranged from 10^5 to 10^8 CFU/ml (1). Hence, this procedure should be sensitive enough to detect even low levels of mycoplasmal contamination as it is possible to increase the number of mycoplasmas by cultivation in a medium for mycoplasmas or co-cultivation with LoVo cells.

Identification of mycoplasmas contaminating cell lines

We identified species of mycoplasmas in 57 cell lines. Mycoplasmal contamination was detected in these cell lines by the DNA staining method and microbiological culture method (10). Among them, one cell line (CTLL-2) contained both M. hyorhinis and M. salivarium. Table 3 lists the

Table 1. Specificity of immunoblot assay.

Mycoplasmas ($1x10^5$CFU/spot)	Antibodies							
	Anti-M. hyorhinis	Anti-M. arginini	Anti-M. salivarium	Anti-M. orale	Anti-A. laidlawii	Anti-M. fermentans	Anti-M. hominis	Anti-M. buccale
M. hyorhinis BTS7	+	-	-	-	-	-	-	-
M. hyorhinis DBS1050	+	-	-	-	-	-	-	-
M. arginini G230	-	+	-	-	-	-	-	-
M. salivarium PG20	-	-	+	-	-	-	-	-
M. orale CH19299	-	-	-	+	-	-	-	-
A. laidlawii PG8	-	-	-	-	+	-	-	-
M. fermentans PG18	-	-	-	-	-	+	-	-
M. hominis PG21	-	-	-	-	-	-	+	-
M. buccale CH20247	-	-	-	-	-	-	-	+

Table 2. Sensitivity of immunoblot assay.

Mycoplasma	Sensitivity (CFU/ml)
M. hyorhinis BTS7	1×10^5
M. arginini G230	4×10^6
M. salivarium PG20	2×10^5
M. orale CH19299	2×10^5
A. laidlawii PG8	1×10^5
M. fermentans PG18	4×10^5
M. hominis PG21	3×10^5
M. buccale CH20247	4×10^6

mycoplasmas isolated from 57 cell lines. For four cell lines, the contaminating mycoplasmas could not be identified. Since these cell lines contained more than 10^7 CFU/ml of viable mycoplasmas, the contaminants are considered to be mycoplasmas other than the eight species.

M. hyorhinis was the most frequent isolate, accounting for 41% of the contaminated cell lines examined. Among 21 cell lines that were contaminated by M. hyorhinis, both F111 and LoVo cells were contaminated by fastidious strains of M. hyorhinis that did not grow in cell-free

Table 3. Mycoplasma species in 57 contaminated cell lines.

Species	No. Cell Lines (%)
M. hyorhinis	24 (41)
M. fermentans	14 (24)
M. orale	8 (14)
M. hominis	6 (10)
A. laidlawii	1 (2)
M. salivarium	1 (2)
M. arginini	0 (0)
M. buccale	0 (0)
Others	4 (7)

media for mycoplasmas. M. hyorhinis is a very common inhabitant of the nasal cavity of swine, but none of the 24 cell lines contaminated by M. hyorhinis was isolated from swine. Researchers have considered that the contamination was originally introduced via bovine sera as well as A. laidlawii (1, 3); and attempts to isolate mycoplasmas from commercial trypsin that was derived from swine pancreas have failed (1). Furthermore, Barile and Kern (3) isolated M. hyorhinis from commercial sera. Since swine and cattle are frequently processed through the same slaughter houses, swine mycoplasmas may contaminate bovine sera during manufacture (1).

The next largest group of contaminants, including M. fermentans, M. orale, and M. hominis, consists of human oral and genital species of mycoplasmas. Contamination by these human mycoplasmas is probably caused by faulty or inadequate sterile procedures.

While bovine sera and laboratory personnel are considered to be the original sources of contamination, contaminated cell lines themselves are also significant sources of further contamination. McGarrity et al. showed that mycoplasmal droplets were generated easily during the handling of cell cultures and the mycoplasmas were resistant to drying (6).

Our results for species isolated from cell lines parallel on the whole those of Barile et al. (2) and McGarrity et al. (8). Barile et al. (2) reported four species, M. orale, M. hyorhinis, M. arginini, and A. laidlawii, as major isolates. In addition, M. fermentans was among the major isolates reported by McGarrity et al. (8). In our study, M.

hyorhinis, M. fermentans, and M. orale were the major isolates, whereas M. arginini was not among them. However, M. arginini, the rate of contamination by M. hominis was significant. Further investigation using a large number of cell lines from many laboratories must be made to determine the characteristics of mycoplasmal contamination. On the basis of these studies, effective methods for preventing and eliminating mycoplasmas must be developed.

References

1) Barile, M. F. 1973. Mycoplasmal contamination of cell cultures: mycoplasma-virus-cell culture interaction. In J. Fogh (ed.) Contamination in tissue culture, p. 131-156. Academic Press Inc. New York.
2) Barile, M. F., H. E. Hopps, and M. W. Grabowski. 1978. Incidence and sources of mycoplasma contamination: a brief review. In G. J. Murphy, and W. W. Nichols (ed.) Mycoplasma infection of cell cultures, p. 35-45. Plenum Press Inc. New York.
3) Barile, M. F., and J. Kern. 1971. Isolation of Mycoplasma arginini from commercial bovine sera and its implication in contaminated cell cultures. Proc. Soc. Exp. Biol. Med. 138: 432-437.
4) Del Giudice, R. A., R. S. Gardella, and H. E. Hopps. 1980. Cultivation of formerly noncultivable strains of Mycoplasma hyorhinis. Curr. Micro. 4: 75-80.
5) Kotani, H., and G. J. McGarrity. 1985. Rapid and simple identification of mycoplasmas by immunobinding. J. Immunol. Methods. 85: 257-267.
6) McGarrity, G. J. 1976. Spread and control of mycoplasmal infection of cell cultures. In Vitro. 12: 643-648.
7) McGarrity, G. J., and H. Kotani. 1985. Cell culture mycoplasmas. In S. Razin, and M. F. Barile (ed.) The mycoplasmas, vol. 4 p. 353-390. Academic Press Inc. New York.
8) McGarrity, G. J., H. Kotani, and D. Carson. 1986. Comparative studies to determine the efficiency of 6-methylpurine deoxyriboside to detect cell culture mycoplasmas. In Vitro. 22: 301-304.
9) Yoshida, T., M. Kawase, K. Sasaki, H. Mizusawa, M. Ishidate, and M. Takeuchi. 1988. Comparative studies on the sensitivity of three methods for detecting mycoplasmal contamination in cell cultures. Bull. JFCC. 4: 9-15.
10) Yoshida, T., and M. Takeuchi. 1987. Tests for mycoplasmal contamination in cell lines collected in IFO. IFO Res. Comm. 13: 52-58.

IFO Res. Comm. 14,
20-24, 1989 (March)

CRYOPRESERVATION OF ANIMAL CELL LINES GROWING ON MICROCARRIERS

NOBUAKI YANAI* AND MASAO TAKEUCHI

Summary

We tried to grow and cryopreserve three animal cell lines on two types of microcarrier. The number of cells adhering to a polystyrene microcarrier (Biosilon) was lower than that to a Sephadex microcarrier (Cytodex 1). The growth rate of cell on Biosilon was similar to that on Cytodex 1. When cells were frozen and thawed on Cytodex 1, the viability and the recovery ratio were low, because cells became detached from the microcarrier. With Biosilon, cell viability was in the range of 20-50%, and cell culture could be recovered from an ampule stored in liquid nitrogen. These results indicate that the polystyrene microcarrier was applicable for cryopreservation of these adhered cells.

Cells adhering to plastic or glass substrates generally need to be detached from the substrates to be cryopreserved. If the cells growing on substrates can be cryopreserved in situ, the use of protease to prepare samples can be avoided, and hence the risk of cell damage be protease.

Sephadex microcarrier (1, 3, 5) and polystyrene microcarrier (2) have been used for mass culture of animal cells. However, the cryopreservation

* Present address: Research Institute for Tuberculosis and Cancer, Tohoku University.

of cultured cells on microcarriers has not been reported. We tried to use microcarriers to grow cells and to cryopreserve growing cells without protease treatment.

Materials and Methods

Cells. L929 (ATCC CCL 1), KT-5 (IFO 50161) and HeLa S3 (IFO 50011), were used. Culture medium used was Dulbecco's modified Eagle's MEM supplemented with 10% fetal bovine serum and 10 mM HEPES. These cell lines were subcultured by incubation at 37 C for 5 min in 0.25% trypsin after washing with 0.02% EDTA in phosphate-buffered saline.

Microcarrier cultivation. Two microcarriers, Cytodex 1 (Pharmacia Fine Chemicals) and Biosilon (Nippon InterMed), were used at concentrations of 5 mg/ml and 20 mg/ml, respectively. Cells were cultivated in a siliconized glass tube of 50 ml capacity with a roller drum apparatus at 37 C. The roller drum was operated at 7.5 rpm for adhesion of cells, then at 20 rpm for cultivation.

Cell number and viability. Cell numbers were estimated from numbers of nuclei released by treatment of cells with 0.1% crystal violet in 0.1 M Na-citrate at 37 C for 30 min. Viabilities of cells were estimated by a dye-exclusion method. To assay adhesion efficiency to microcarriers, trypsiniazed cells were incubated in a plastic dish (Corning Science Products). After 4 hr, adhesion efficiency was calculated from cell numbers adhering to the dishes.

Cryopreservation. The cells growing on microcarriers were washed two times with the culture medium supplemented with 10% dimethylsulfoxide. Ampules containing the cells were cooled to -40 C at 1 C/min using a programmable controlled-rate freezing unit (Taiyo Sanso Co. Ltd.) and stored in liquid nitrogen (5). To thaw the cells stored in liquid nitrogen, the ampules were vigorously shaken at 37 C for 1 min.

Results and Discussion

The cultivation of animal cells on microcarriers has been described (1, 2, 3, 5). We also examined the growth of three cell lines, L929, HeLa S3

and KT-5, on the two microcarriers. Figure 1 shows that L929 cells grew uniformly on the surface of these microcarriers. Figure 2 shows the growth curves of the three cell lines on Cytodex 1 and Biosilon microcarriers, and plastic bottles (T-25). The number of adhering cells was lower for the microcarriers than the plastic bottles (T-25). Furthermore, more cells of

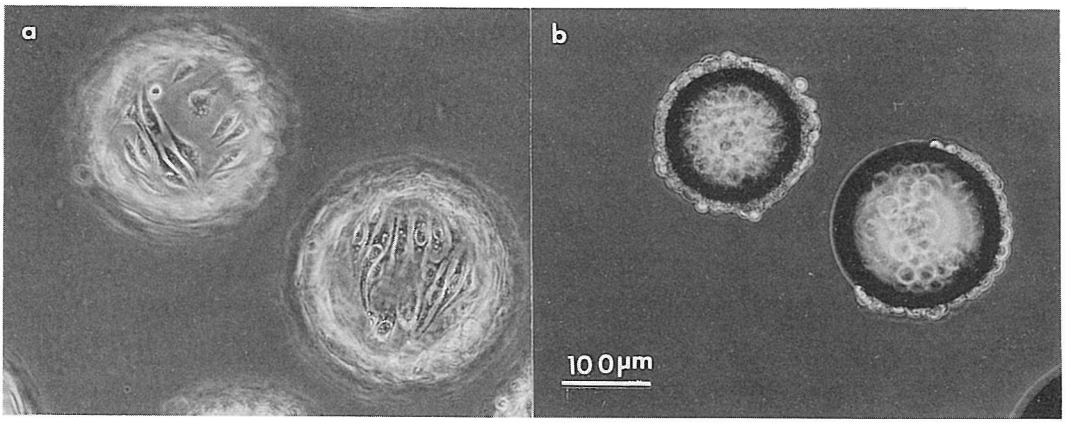


Fig. 1. L929 cells growing on microcarriers.
a: Cytodex 1, b: Biosilon.

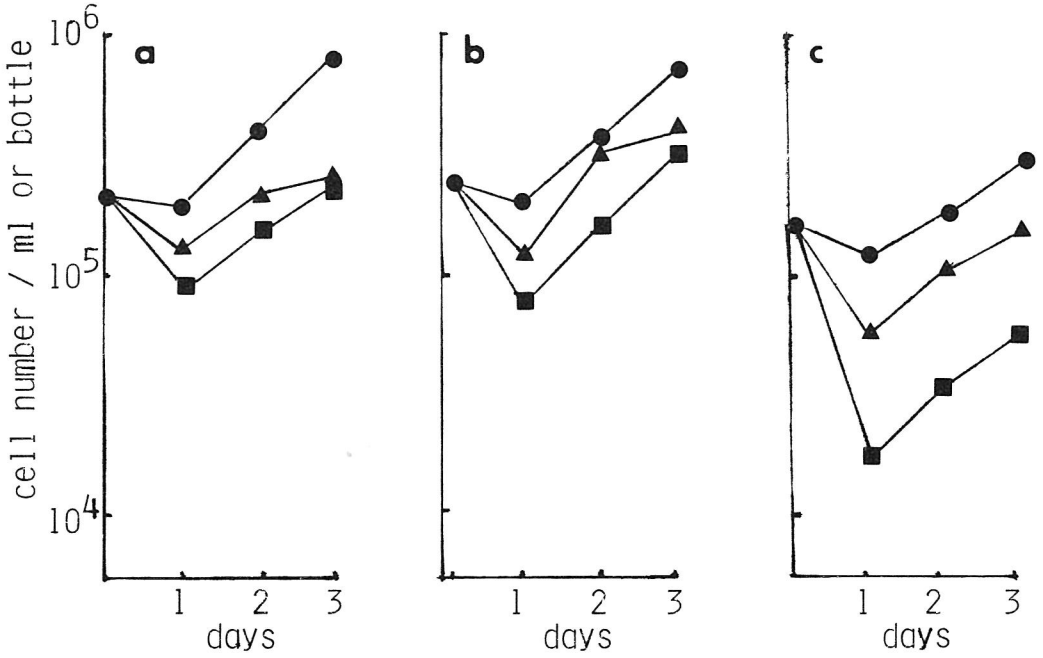


Fig. 2. Growth curves of three cell lines on microcarriers.
a: L929 cells, b: HeLa S3 cells, c: KT-5 cells.
●: plastic culture bottles, ■: Biosilon, ▲: Cytodex 1.

all three cell lines adhered to Cytodex 1 than to Biosilon. This evidence shows that the adhesiveness of cells to substrates depends on the electric charges of the cell surface and microcarriers surface. The growth rates of cells were similar on all three substrates.

It will be advantageous if cells growing on microcarriers can be cryopreserved without any treatment, as this will avoid the risk of damage by protease treatment, which is generally used to disperse cells. L929, HeLa S3 and KT-5 cells growing on the microcarriers were frozen with a programmable controlled-rate freezing unit (6). After thawing cells, their viabilities were determined by the dye-exclusion method and by the adhesion test (Table 1).

Table 1. Viability of cells growing on microcarriers before freezing and after freezing and thawing.

Cell lines	Micro-carriers	Viability (%) before freezing dye-exclusion	before freezing adhesion	after freezing and thawing dye-exclusion	after freezing and thawing adhesion
L929	Biosilon	84	98	89	35
	Cytodex 1	87	80	36	12
HeLa S3	Biosilon	94	82	89	24
	Cytodex 1	96	100	58	11
KT-5	Biosilon	98	63	84	50
	Cytodex 1	97	NT	43	18

Concentrations of cells before freezing were as follows: L929 (2×10^5/ml), HeLa S3 (2×10^5/ml).
NT, not tested.

The dye-exclusion test showed higher viabilities of cells cultivated on Biosilon than on Cytodex 1. The viabilities of the three cell lines on Biosilon were higher than 80%. After thawing, as shown in Figure 3 many cells remained attached to the Biosilon (24-50% in Table 1). It was also found that the adhesion efficiencies of cells on Cytodex 1 were 11-18%, lower than those on Biosilon.

These findings are due to the detachment cells from the Cytodex 1 swells, and ice crystals grow during the process of freezing. Therefore, the volume of the microcarrier is changed by freezing and thawing. On the

other hand, Biosilon is not swollen by water and its physicochemical characteristics hardly change (4).

From the data on viability and adhesiveness (Figure 2 and Table 1), Biosilon microcarrier was judged to be suitable for cryopreservation. This method of cryopreservation may be useful for protease-sensitive cells, such as certain primary cultures.

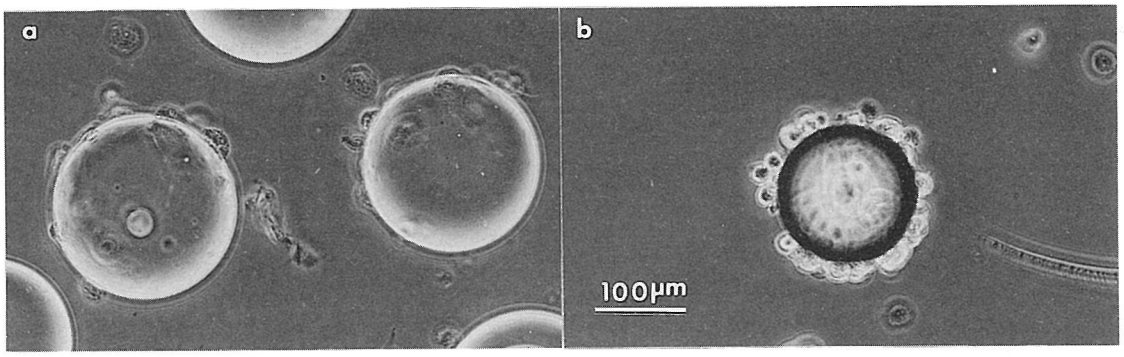


Fig. 3. L929 cells adhering to microcarriers after freezing and thawing.
a: Cytodex 1, b: Biosilon.

The authors gratefully acknowledge the helpful discussions of Mr. Touho Yoshida.

References

1) Crespi, C. L., and W. G. Thilly. 1981. Continuous cell propagation using low-charge microcarriers. Biotechnol. Bioeng. 23: 983-993.
2) Davis, P. F. 1981. Microcarrier culture of vascular endothelial cells on solid plastic beads. Exp. Cell Res. 134: 367-376.
3) Horst, J., M. Kern, and E. Ulmer. 1980. Subcultivation of mammalian cell with a Sephadex derivative instead of proteinases. Eur. J. Cell Biol. 22: 599.
4) Personal communication with the Nippon InterMed Co.
5) Ryan, U. S., M. Montara, and C, Whitaker. 1980. Methods for microcarrier culture of bovine pulmonary arterry endothelial cells avoiding the use of enzymes. Tissue and Cell, 12: 619-635.
6) Yoshida, T., N. Yanai, and M. Takeuchi. 1988. Cryopreservation of animal cell lines: Cooling velocity and survival. Bull. JFCC. 4: 16-20.

IFO Res. Comm. 14,
25-39, 1989 (March)

TAXONOMIC SIGNIFICANCE OF CELLULAR FATTY ACID COMPOSITION IN RHIZOBIUM, BRADYRHIZOBIUM AND AGROBACTERIUM SPECIES

AKIRA YOKOTA

Summary

The cellular fatty acid composition of 19 strains of the genera Rhizobium, Bradyrhizobium and Agrobacterium was investigated. Straight-chain unsaturated 18:1 and/or cyclopropane acid 19cyc were commonly found as major non-hydroxy fatty acids in all the strains tested. Hydroxy fatty acid profiles showed characteristics for each genus of Rhizobiaceae. The genus Bradyrhizobium is distinguished from the genus Rhizobium on the basis of 2- and 3-hydroxy fatty acid composition. The genus Agrobacterium is also distinguished from the genera Rhizobium and Bradyrhizobium on the basis of 2- and 3-hydroxy fatty acid composition. Strains of biovar 1 and biovar 2 of Agrobacterium species have different hydroxy fatty acid profiles, and strains belonging to different biovar clusters could be distinguished from each other on this basis. The significance of hydroxy fatty acids in the classification and identification of the strains of Rhizobiaceae is discussed.

Although conventional taxonomic techniques have been used in an attempt to distinguish Rhizobium, Bradyrhizobium and Agrobacterium species, the taxonomic grouping still depends largely on plant-affinity tests (7,9). Attempts to differentiate strains of Rhizobiaceae by chemotaxonomic approaches employing cellular fatty acid (10,12), polyacrylamide gel

electrophoresis of cellular protein (8,19), and the composition of extracellular gum (20) have been reported.

Hydroxy fatty acid profiles in whole-cell fatty acids have proven useful for differentiating certain Gram-negative bacteria (4,10,12-15,23, 24,26,27). Both 2- and 3-hydroxy fatty acids were found to be constituents of cells whose distribution varies with on the type of microorganism. Therefore, their profile has been shown also to be a tool for grouping of bacteria. For the strains of Rhizobiaceae, cellular fatty acids have already been described (1,2,10,12,15,22); but data are limited to a few species, and the descriptions of hydroxy fatty acid composition are insufficient. More precise knowledge of the cellular fatty acid profile may provide useful criteria in the taxonomy of Rhizobiaceae. For this reason a study on the cellular fatty acid composition of Rhizobiaceae was undertaken in relation to the chemotaxonomy of these bacteria.

This communication deals with the cellular fatty acid composition of three genera of Rhizobiaceae, Rhizobium, Bradyrhizobium, and Agrobacterium, and the significance of their hydroxy fatty acid profiles is discussed with regard to the classification and identification of these bacteria.

Materials and Methods

Microorganisms. The microorganisms used are listed in Table 1. Type strains are indicated by the superscript "T" above the strain number.

Cultivation of microorganisms. The medium (YEM) used contained 0.5 g of K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of NaCl, 0.4 g of yeast extract, and 10 g of mannitol per liter (pH 7.2). The bacteria were cultivated at 28 C for 3-5 days with shaking in 300 ml of YEM in 1-liter flasks. Cells were separated by centrifugation, then washed twice with distilled water and lyophilized.

Analysis of fatty acids. Lyophilized cells (50 mg) were methanolyzed with 2 ml of 5% HCl-methanol at 100 C for 3 hr in a screw-capped test tube. After methanolysis, the reaction mixture was extracted with n-hexane. The solvent fraction was washed with water, dried with Na_2SO_4, and concentrated under a nitrogen stream. Hydroxy fatty acids were separated from non-polar fatty acids by thin-layer chromatography using a solvent system of n-hexane and ethyl ether (1:1). Fatty acids on the chromatogram, visualized with

Table 1. Bacterial strains studied.

Taxon	Strain	Other strain designations[a]			Comments
		ATCC	IAM	Others	
Genus Bradyrhizobium					
B. japonicum	IFO 14783^T	10324	12608	NCIB 11477	Group I[b]
B. japonicum	IFO 14792			USDA 110	Group Ia
B. japonicum	IFO 14791			USDA 76	Group II
B. sp. (Lupinus)	IFO 14781	10319	12610		Group I
Genus Rhizobium					
R. leguminosarum	IFO 14778^T	10004	12609	NCIB 11478	
R. leguminosarum	IFO 14168				
R. leguminosarum biovar phaseoli	IFO 14785^T	14482	12612		
R. leguminosarum biovar trifolii	IFO 14784^T	14480	12613		
R. leguminosarum biovar trifolii	IFO 13337$_T$				
R. meliloti	IFO 14782^T	9930	12611		
R. loti	IFO 14779^T	33669			
R. fredii	IFO 14780^T	35423			
Genus Agrobacterium					
A. radiobacter	IFO 13532^T	19358		NCIB 9042	Biovar 1
A. tumefaciens	IFO 12667	4452			Biovar 1
A. tumefaciens	IFO 14793$_T$			AtR11[c]	Biovar 2
A. rhizogenes	IFO 13257^T	11325			Biovar 2
A. rhizogenes	IFO 14554				Biovar 1
A. rhizogenes	IFO 14555$_T$				Biovar 1
A. rubi	IFO 13261^T				

[a] Abbreviations for culture collections: ATCC, American Type Culture Collection, Rockville, Md., USA; IAM, Institute of Applied Microbiology, University of Tokyo, Japan; NCIB, National Collection of Industrial Bacteria, Aberdeen, U.K.; USDA, US Department of Agriculture, Beltsville, Md., U.S.A.

[b] DNA homology group by Hollis et al.(3).

[c] Isolated by Ohta and Nishiyama (16,17).

iodine vapor or by spraying dichlorofluorescein (0.02%, in ethanol), were extracted with ethyl ether, and the extract was concentrated under nitrogen. Fatty acids dissolved in acetonitrile were analyzed by gas-liquid chromatography.

Gas-liquid chromatography (GLC) and gas-liquid chromatography-mass spectrometry (GLC-MS). Fatty acid methyl esters were analyzed with a Shimadzu GC-9A gas chromatogram (Shimadzu, Kyoto, Japan) fitted with a flame ionization detector. The columns employed were: (A) a glass column (2 m x 0.28 cm) packed with 5% OV-1 on Chromosorb W at 165 C; (B) a glass column

(2 m x 0.28 cm and 5 m x 0.28 cm) packed with 10% diethyleneglycol succinate (DEGS) on Chromosorb W at 165 C or at 180 C. Helium was used as a carrier gas at a flow rate of 50 ml/min.

Fatty acids were primarily identified by comparison of the retention times of their methyl esters with those of the same esters of standard fatty acids. 2-Hydroxy and/or 3-hydroxy fatty acids of Pseudomonas aeruginosa PAO1, Pseudomonas marginata IFO 13700^{T}, Flavobaterium meningosepticum IFO 12535^{T}, Xanthobacter autotrophicus IFO 14758, Thiobacillus novellus IFO 12443^{T}, Thiobacillus versutus IFO 14567^{T}, and Pimelobacter simplex IFO 12069^{T} were used as fatty acid references. Further, the equivalent chain length (ECL) was determined from the logarithm of the retention time of methyl esters of saturated fatty acids and 2- and 3-hydroxy fatty acids, plotted against their carbon number, and some of the fatty acids were presumed on the basis of ECL. The percentage of each acid was estimated from the ratio of the peak area to the total area. Branched 3-hydroxy-pentadecanoic acid methyl esters were identified by GLC-MS using Shimadzu QP-1000 mass spectrometer.

Abbreviations for fatty acids are as follows: In the shorthand numbering system used to identify fatty acids, the figures preceding the colon indicate the number of carbon atoms in the fatty acids, while those following the colon represent the number of double bonds present. Cyc indicates cyclopropane acid, and i, ai and i(a) indicate iso-branched, anteiso-branched, and iso- or anteiso-branched acid, respectively. The prefix OH indicates a hydroxy group at the position indicated.

Results

The cellular fatty acid composition of 19 stains of Rhizobiaceae is summarized in Table 2. The major cellular fatty acids were 18:1 and/or 19cyc. 3-Hydroxy acids were found in all the strains tested. On the other hand, 2-hydroxy acid was detected only in the strains of Bradyrhizobium species and the strains of biovar 2 of Agrobacterium tumefaciens and Agrobacterium rhizogenes (Fig. 1).

Fatty acid composition in Bradyrhizobium species

Fatty acids of B. japonicum and Bradyrhizobium sp. (Lupinus) mainly consisted of straight-chain acids of 16:0 and 18:1, 3-hydroxy fatty acids of

Table 2. Cellular fatty acid composition of Rhizobium, Bradyrhizobium and Agrobacterium strains.

Strain	Non-hydroxylated fatty acid (%)[a]						3-Hydroxy fatty acid (%)[b]							2-Hydroxy fatty acid[c]
	16:0	16:1	18:0	18:1	19cyc	21:1	12:0	14:0	i-15:0	ai-15:0	16:0	18:0	Unknown	
Bradyrhizobium														
B. japonicum IFO 14783^T	17	7	2	74	-	-	36	64	-	-	-	-	-	+
B. japonicum IFO 14792	14	1	1	75	6	3	45	55	-	-	-	-	-	+
B. japonicum IFO 14791	13	1	1	71	10	5	38	62	-	-	-	-	-	+
B. sp. (Lupinus) IFO 14781	18	5	2	74	-	-	24	76	-	-	-	-	-	+
Rhizobium														
R. leguminosarum IFO 14778^T	10	-	16	42	17	15	-	39	-	17	14	30	-	-
R. leguminosarum IFO 14168	7	-	13	69	6	5	-	48	-	10	10	32	-	-
R. leguminosarum biovar phaseoli IFO 14785^T	9	-	22	36	23	11	-	58	-	3	8	32	-	-
R. leguminosarum biovar trifolii IFO 14784^T	8	-	15	55	14	9	-	50	-	7	11	32	-	-
R. leguminosarum biovar trifolii IFO 13337	7	1	16	71	2	4	-	43	-	11	11	35	-	-
R. meliloti IFO 14782^T	14	-	7	51	15	12	-	62	-	2	6	31	-	-
R. loti IFO 14779^T	17	5	5	44	15	14	-	59	-	-	12	29	-	-
R. fredii IFO 14780^T	8	1	10	76	-	4	-	66	-	-	3	31	-	-
Agrobacterium														
A. radiobacter IFO 13532^T	20	-	-	39	26	15	-	62	-	-	31	-	7	-
A. tumefaciens IFO 12667	17	-	-	51	23	9	-	64	-	-	30	-	6	-
A. tumefaciens IFO 14793	15	3	4	19	35	25	-	22	32	-	28	18	-	+
A. rhizogenes IFO 13257^T	21	-	7	19	35	18	-	20	34	-	27	19	-	+
A. rhizogenes IFO 14554	20	-	-	25	40	15	-	65	-	-	28	2	5	-
A. rhizogenes IFO 14555	20	-	-	29	38	14	-	65	-	-	29	2	4	-
A. rubi IFO 13261^T	18	12	-	60	7	4	-	72	-	-	23	2	3	-

[a] The numbers refer to the percentage of an acid relative to the total non-hydroxylated acids.
[b] The numbers refer to the percentage of an acid relative to the total 3-hydroxy acids.
[c] +, present; -, absent.

3-OH-12:0 and 3-OH-14:0. Small amount of 2-hydroxy fatty acids, which were tentatively identified from their retention times on GLC as i(ai)-2-OH-15:0, 2-OH-16:0, and i(ai)-2-OH-18:0, were present in these strains. Non-polar, 3-hydroxy- and 2-hydroxy fatty acid compositions of B. japonicum IFO 14792 and IFO 14791, which belong to DNA homology groups different from B. japonicum IFO 14783^T and Bradyrhizobium sp. IFO 14781, were the same as those of strains IFO 14783^T and IFO 14781 (Table 2). The fatty acid profile of B. japonicum IFO 14783^T is shown in Fig. 2.

Fatty acid composition in Rhizobium species

Fatty acids of Rhizobium strains were composed mainly of straight-chain acids of 16:0, 18:0, 18:1, 21:1, and cyclopropane acid of 19cyc. 3-OH-14:0, 3-OH-16:0 and 3-OH-18:0 were the major 3-hydroxy fatty acid in

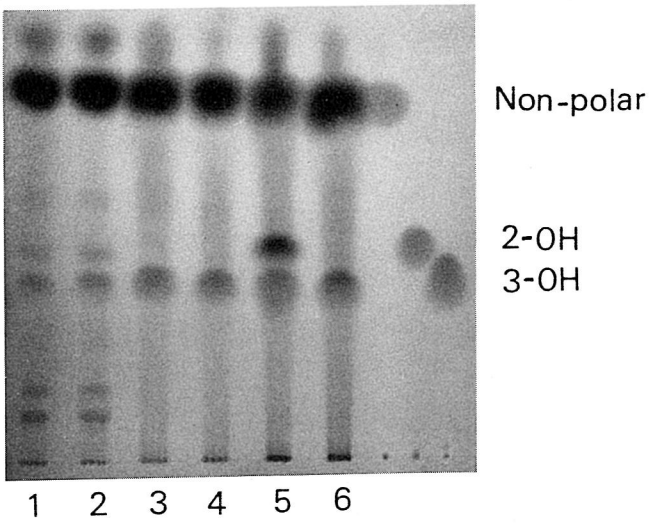


Fig. 1. Thin-layer chromatographic patterns of the fatty acid methyl esters of Rhizobiaceae strains.
Non-polar, non-polar fatty acid methyl esters; 2-OH, 2-hydroxy acid methyl esters; 3-OH, 3-hydroxy acid methyl esters.
Fatty acid methyl esters from: 1, B. japonicum IFO 14783^T; 2, Bradyrhizobium. sp. (Lupinus) IFO 14781; 3, R. leguminosarum biovar trifolii IFO 14784 ; 4, A. radiobacter IFO 13532 ; 5, A. rhizogenes IFO 13257 ; 6, A. rhizogenes IFO 14555.

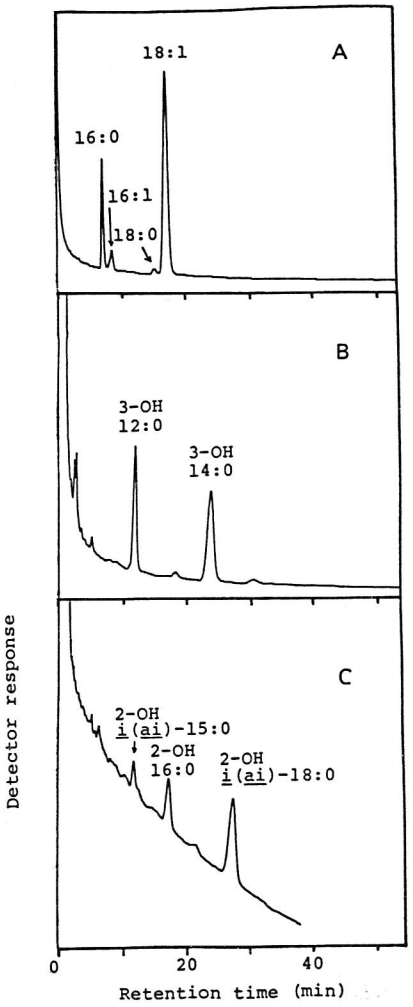


Fig. 2. Fatty acid profile of Bradyrhizobium japonicum IFO 14783.
A, non-polar acid fraction; B, 3-hydroxy acid fraction; C, 2-hydroxy acid fraction.
Column, DEGS (2 m); column temperature, 165 C (A and B) and 180 C (C).

these species. R. leguminosarum and R. meliloti strains were different from other Rhizobium species in the presence of branched 3-OH-15:0, which was assigned as ai-3-OH-15:0 from its mass spectrum (data not shown) and ECL value on GLC, following the method described by Zevenhuizen et al.

(28). As the mass spectra of iso and anteiso isomers are known to be the same (28), ECL values were used to distinguish between them; i-3-OH-15:0 prepared from Flavobacterium meningosepticum (27) showed an ECL value of 14.50, and the branched 3-OH-15:0 from R. leguminosarum are of 14.65. Based on these data, the 3-hydroxy fatty acid was identified as ai-15:0. 2-Hydroxy fatty acid was absent in the strains of Rhizobium species (Fig. 1 and Table 2). The fatty acid profile of R. leguminosarum and R. meliloti are shown in Figs. 3 and 4.

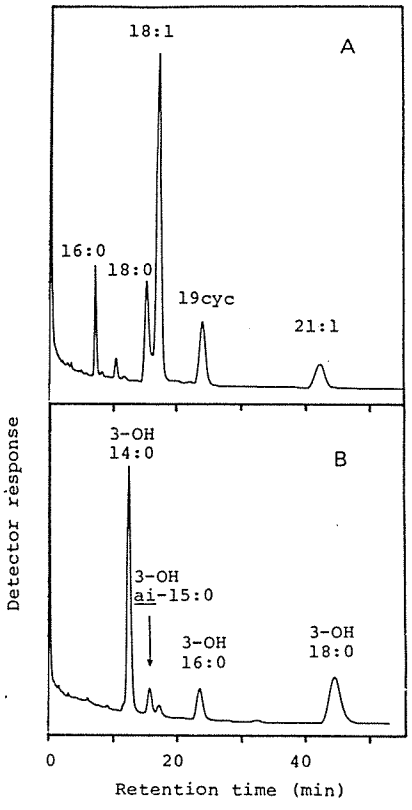


Fig. 3. Fatty acid profile of Rhizobium leguminosarum IFO 14778 .
A, non-polar acid fraction; B, 3-hydroxy acid fraction. Column, DEGS (2 m); column temperature, 165 C (A) and 180 C (B),

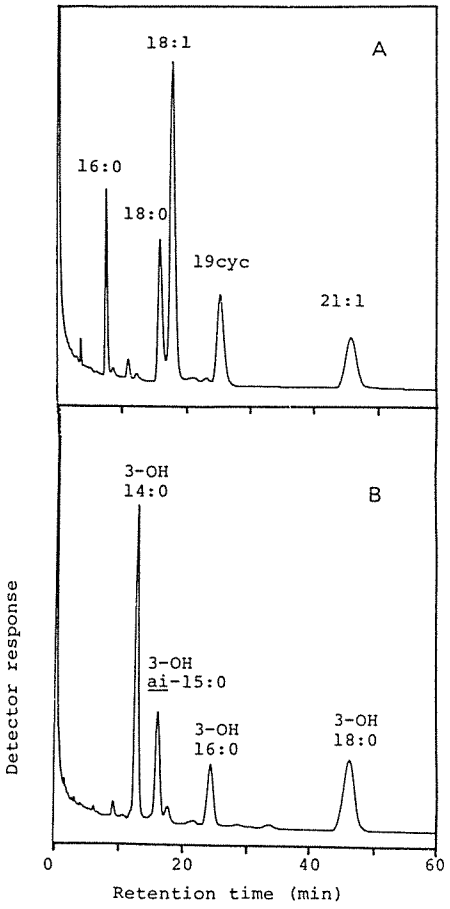


Fig. 4. Fatty acid profile of Rhizobium meliloti IFO 14782.
A, non-polar acid fraction; B, 3-hydroxy acid fraction. Column, DEGS (2 m); column temeprature, 165 C (A) and 180 C (B).

Fatty acid composition in Agrobacterium species

Fatty acids of Agrobacterium strains mainly consisted of straight-chain acids of 16:0, 18:1, 21:1 and cyclopropane acid of 19cyc. The main 3-hydroxy fatty acid were 3-OH-14:0 and 3-OH-16:0. However, A. rhizogenes IFO 13257^{T} (biovar 2) (Fig. 5) and A. tumefaciens IFO 14793 (biovar 2) had quite a different hydroxy fatty acid profile. These strains contained relatively high concentrations of 2-hydroxy fatty acids together with 3-hydroxy fatty acids (Fig. 1 and Table 2). 3-Hydroxy fatty acids were identified as 3-OH-14:0, branched 3-OH-15:0, 3-OH-16:0 and 3-OH-18:0. The branched 3-OH-15:0 was assigned as i-3-OH-15:0 from its mass spectrum and ECL value on GLC, by a similar method to that used in the identification of

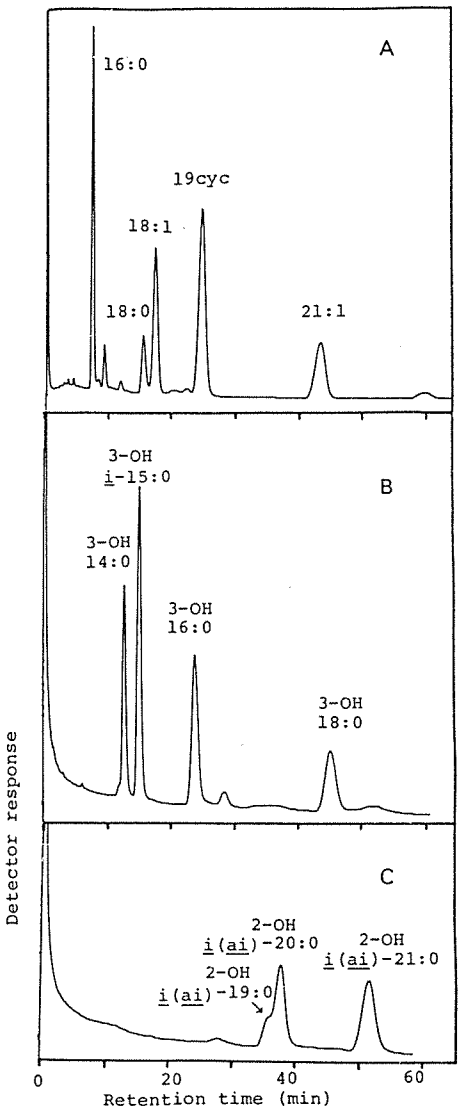


Fig. 5. Fatty acid profile of Agrobacterium rhizogenes IFO 13257 (biovar 2). A, non-polar acid fraction; B, 3-hydroxy acid fraction; C, 2-hydroxy acid fraction. Column, DEGS (2 m); column temperature, 165 C (A) and 180 C (B and C).

ai-3-OH-15:0. Electron impact mass spectrum (Fig. 6) of the branched 3-hydroxy fatty acid methyl ester from A. rhizogenes IFO 13257^{T} showed an intensive fragment m/z 103, but did not show a molecular ion peak. The molecular weight was deduced by the (M-18) and (M-50) peaks (Fig. 6) and by chemical ionization mass spectrum (Fig. 7). Both the spectrum and ECL value of the branched 3-OH-15:0 were identical to those of i-3-OH-15:0 from F. meningosepticum. 2-Hydroxy fatty acids were tentatively identified from their retention times on GLC as 2-OH-18:1, i(ai)-2-OH-19:0, i(ai)-2-OH-

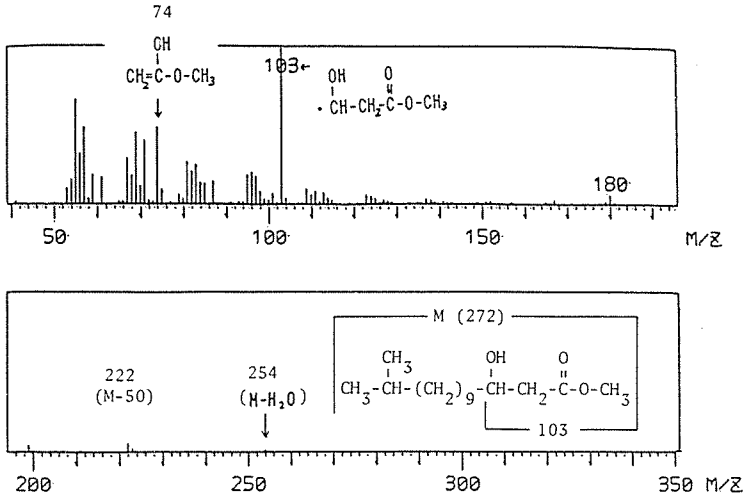


Fig. 6. Electron impact mass spectrum of i-3-OH-15:0 methyl ester from Agrobacterium rhizogenes IFO 13257^{T}.

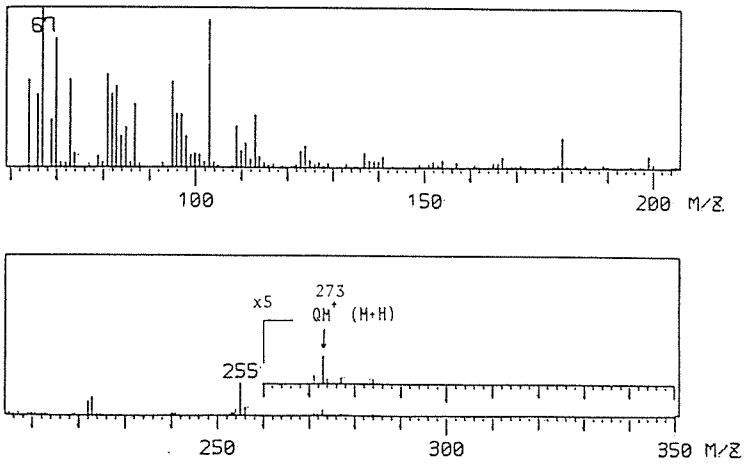


Fig. 7. Chemical ionization mass spectrum of i-3-OH-15:0 methyl ester from Agrobacterium rhizogenes IFO 13257^{T}.

20:0, and i(ai)-2-OH-21:0 (Fig. 5). The fatty acid profile of the strains of A. radiobacter (biovar 1) and A. rhizogenes (biovar 1 and 2) are shown in Figs. 5, 8 and 9. Thus, the biovar 2 strains were differentiated from biovar 1 strains by the presence of 2-hydroxy fatty acid and also by the 3-hydroxy fatty acid composition. The fatty acid profile of A. rubi was similar to that of biovar 1 strains (Table 2).

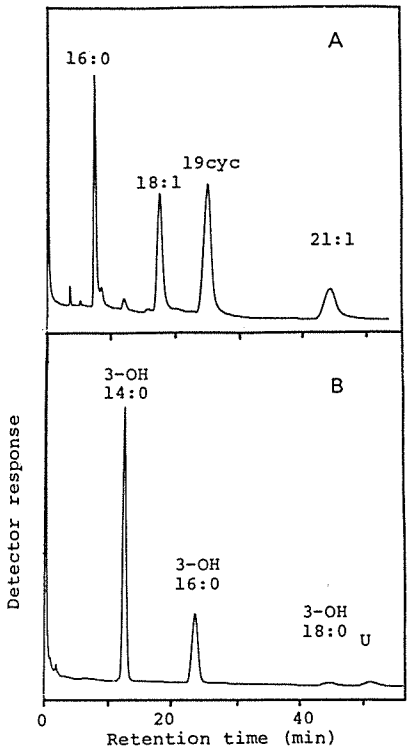


Fig. 8. Fatty acid profile of Agrobacterium rhizogenes IFO 14554 (biovar 1). A, non-polar acid fraction; B, 3-hydroxy acid fraction. Column, DEGS (2 m); column temperature, 165 C (A) and 180 C (B).

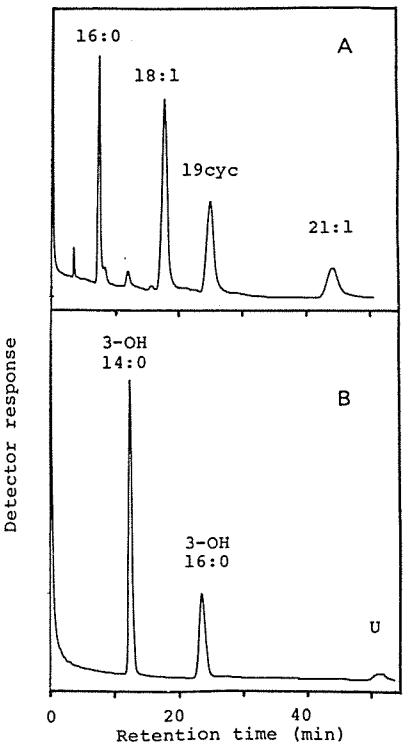


Fig. 9. Fatty acid profile of Agrobacterium radiobacter IFO 13532 (biovar 1). A, non-polar acid fraction; B, 3-hydroxy acid fraction. Column, DEGS (2 m); column temperature, 165 C (A) and 180 C (B).

Discussion

All the strains of Rhizobiaceae studied in this paper had in common unsaturated 18:1 and/or cyclopropane acid 19cyc as the major non-polar

fatty acid, and therefore the Rhizobiaceae strains could not be distinguished from each other on the basis of their non-polar fatty acid composition. On the other hand, these bacteria were found to be heterogeneous in hydroxy fatty acid composition, and the genera of Rhizobiaceae could be distinguished from each other based on their hydroxy fatty acid profiles. The 3-hydroxy fatty acids found were: 3-OH-12:0 and 3-OH-14:0 in Bradyrhizobium, 3-OH-14:0, ai-3-OH-15:0 and 3-OH-18:0 in Rhizobium, and 3-OH-14:0 and 3-OH-16:0 (biovar 1 cluster) or 3-OH-14:0, i-3-OH-15:0, 3-OH-16:0 and 3-18:0 (biovar 2 cluster) in Agrobacterium. Therefore, the genera Bradyrhizobium, Rhizobium and Agrobacterium could be differentiated from each other on the basis of their 3-hydroxy fatty acid profiles, and also by the presence or absence of 2-hydroxy fatty acid (Table 3). Thus, hydroxy fatty acids are useful to distinguish these bacteria at the genus level.

Rhizobium and Bradyrhizobium are bacteria capable of nitrogen-fixing symbiosis with leguminous plants. The taxonomic status of Rhizobium and

Table 3. Major fatty acids and hydroxy acids of Rhizobiaceae strains.

Genera and species	Major fatty acid	2-Hydroxy fatty acid	3-Hydroxy fatty acid					
			12:0	14:0	i-15:0	ai-15:0	16:0	18:0
Bradyrhizobium	18:1	+	+	+	-	-	-	-
R. leguminosarum	18:1 19cyc	-	-	+	-	+	+	+
R. meliloti R. loti R. fredii	18:1 19cyc	-	-	+	-	±	+	+
[Biovar 1]								
A. radiobacter A. tumefaciens A. rhizogenes	18:1 19cyc	-	-	+	-	-	+	-
[Biovar 2]								
A. tumefaciens A. rhizogenes	18:1 19cyc	+	-	+	+	-	+	+
A. rubi	18:1	-	-	+	-	-	+	±

+, fatty acid detected; ±, fatty acid detected in a small amount;
-, fatty acid not detected.

Bradyrhizobium is controversial because it is based on host infectivity. Within designated species, the bacteria exhibit fairly uniform biochemical characteristics (7). Two important manifestations of biochemical differences, growth rate and alkaline reaction in sugar media, have been used in differentiating rhizobia with different host affinities. As described above, hydroxy fatty acid profiles were found to be useful for identification of these genera.

DNA-DNA hybridization studies by Hollis et al. (3) suggested that strains of B. japonicum can be separated into at least three DNA homology groups. Hydroxy fatty acid compositions of B. japonicum strains belonging to different DNA homology groups (group I, Ia and II) were the same, which indicate that the hydroxy fatty acid profile of Bradyrhizobium is homogeneous at the intra-generic level.

The results on the fatty acid composition of Rhizobium species indicated that the genus Rhizobium is uniform in hydroxy fatty acid composition. One small variation is the presence of ai-3-OH-15:0 in R. leguminosarum. The presence of ai-3-OH-15 in lipopolysaccharides of R. leguminosarum and R. meliloti has already been reported (28).

The results obtained in this study are in accordance with published data (1,2,10,15,18,21,22,28) except for the report that Bradyrhizobium strains had no hydroxy fatty acid (12). Miyazaki et al. (12) reported the absence of 3-hydroxy fatty acids in the cellular fatty acids of the strains of Bradyrhizobium. However, the presence of 3-OH-12:0 and 3-OH-14:0 in lipopolysaccharides of B. japonicum and Bradyrhizobium sp. (Lupinus) was shown by Puvanesarajah et al. (18) and Mayer et al. (11). In the present study, 3-hydroxy fatty acids together with small amounts of 2-hydroxy fatty acids were found in the cells of Bradyrhizobium strains (Fig. 1). The non-detection of hydroxy fatty acids in Bradyrhizobium species by Miyazaki et al. (12) seems to be caused by the small number of cells used, because the content of hydroxy acids in the cells of these bacteria was low.

In Gram-negative bacteria, 3-hydroxy fatty acids are known to be distributed mostly in lipid A's of lipopolysaccharides (25), and 2-hydroxy acids are in phospholipids (1,2,26,27). 3-Hydroxy fatty acid profiles in cellular fatty acids described in this paper and those in lipid A's (lipopolysaccharides) (11,21,22) were correlated well in many strains of Rhizobiaceae. Recently, Mayer et al. (11) have found differences in the backbone amino sugar of lipid A of lipopolysaccharide between Rhizobium and

Bradyrhizobium species. B. japonicum and Bradyrhizobium sp. (Lupinus) have 2,3-diamino-2,3-dideoxyglucose in their lipid A's instead of the usual amino sugar, glucosamine. These results indicate that these genera can be distinguished on the basis of both the hydroxy fatty acid and the backbone amino sugar compositions of lipid A, and therefore, the lipopolysaccharide molecule is a useful chemotaxonomic marker for the systematics of Rhizobiaceae.

Agrobacterium is known to be a rather heterogeneous genus, consisting of two or three genetic clusters, biovar 1 group, biovar 2 group (and possibly biovar 3 group) and A. rubi (9). In Bergey's Manual of Systematic Bacteriology (9), four species, A. tumefaciens, A. radiobacter, A. rhizogenes and A. rubi, are included in this genus. However, no morphological, physiological or genotypical differentiation is possible within biovar 1 or biovar 2 strains of A. tumefaciens, A. radiobacter and A. rhizogenes. It is now firmly established that phytopathogenicity in Agrobacterium depends on the presence or absence of plasmid(s) only (9), and these biovar clusters seem to be separated at the species level. The differences in hydroxy fatty acid composition described here support this consideration. Biovar 1 and biovar 2 strains of Agrobacterium species can be easily and clearly distinguished from each other on the basis of the 2- and 3-hydroxy fatty acid profiles (Table 3). The 3-hydroxy fatty acids found were: 3-OH-14:0 and 3-OH-16:0 in biovar 1 strains of Agrobacterium and in A. rubi, and 3-OH-14:0, i-3-OH-15:0, 3-OH-16:0, and 3-OH-18:0 in the strains of biovar 2 of Agrobacterium.

2-Hydroxy fatty acids found in B. japonicum, A. tumefaciens IFO 14793 and A. rhizogenes IFO 13257^{T} were tentatively identified from their retention times on GLC as shown in Figs. 2 and 5. However, mass spectrometric studies are necessary to identify these acids precisely, and are now in progress.

It is noted that relatively high concentrations of 2-hydroxy fatty acids are present in the cells of Agrobacterium biovar 2 strains. Their cellular lipid compositions have not been reported yet.

From this study, hydroxy fatty acid compositions appear to be useful for differentiation and identification of Rhizobiaceae, as has been shown for many other Gram-negative bacteria, such as Pseudomonas species (4,14), Flavobacterium-Cytophaga complex (13), methanol-, methane- and methylamine-

utilizing bacteria (23), Rhodospirillaceae species (24), and myxobacteria (26).

The author wishes to express his thanks to Dr. K. Komagata, Professor of the Institute of Applied Microbiology, University of Tokyo, for his criticism of the manuscript.

References

1) Bunn, C.R., J.J. McNeil, and G.H. Elkan. 1976. Effect of biotin on fatty acids and phospholipids of biotin-sensitive strains of Rhizobium japonicum. J. Bacteriol. 102: 24-29.
2) Gerson, T., and J.J. Patel. 1975. Neutral lipids and phospholipids of free-living and bacteroid forms of two strains of Rhizobium infective on Lotus pedunculatus. Appl. Microbiol. 30: 193-198.
3) Hollis, A.B., W.E. Kloos, and G.H. Elkan. 1981. DNA:DNA hybridization studies of Rhizobium japonicum and related Rhizobiaceae. J. Gen. Microbiol. 123: 215-222.
4) Ikemoto, S., H. Kuraishi, K. Komagata, R. Azuma, T. Suto, and H. Murooka. 1978. Cellular fatty acid composition in Pseudomonas species. J. Gen. Appl. Microbiol. 24: 199-213.
5) Jarvis, B.D., M. Gillis, and J. De Ley. 1986. Intra- and intergeneric similarities between the ribosomal ribonucleic acid cistrons of Rhizobium and Bradyrhizobium species and some related bacteria. Int. J. Syst. Bacteriol. 36: 129-138.
6) Jordan, D.C. 1982. Transfer of Rhizobium japonicum Buchanan 1980 to Bradyrhizobium gen. nov, a genus of slow-growing root nodulate bacteria from leguminous plants. Int. J. Syst. Bacteriol. 32: 136-139.
7) Jordan, D.C. 1984. Family III. Rhizobiaceae Conn 1938. Genus I. Rhizobium Frank 1889, and Genus II. Bradyrhizobium. In N.R. Krieg and J.G. Holt (ed.) Bergey's Manual of Systematic Bacteriology, Vol. 1, p. 235-244. The Williams and Wilkins Co., Baltimore.
8) Kersters, K., and J. De Ley. 1975. Identification and grouping of bacteria by numerical analysis of their electrophoretic protein pattern. J. Gen. Microbiol. 87: 333-342.
9) Kersters, K., and J. De Ley. 1984. Genus III. Agrobacterium. In N.R. Krieg and J.G. Holt (ed.) Bergey's Manual of Systematic Bacteriology, Vol. 1, p. 244-254. The Williams and Wilkins Co., Baltimore.
10) MacKenzie, S.L., M.S. Lapp, and J.J. Child. 1979. Fatty acid composition of Rhizobium spp. Can. J. Microbiol. 25: 68-74.
11) Mayer, H., J.H. Krauss, V. Puvanesarajah, G. Stacey, and G. Auling. 1988. Lipid A with 2,3-diamino-2,3-dideoxyglucose in lipopolysaccharide from slow-growing Rhizobiaceae and from Pseudomonas carboxydovorans. Arch. Microbiol. (in press).
12) Miyazaki, K., K. Kuraishi, S. Morita, and K. Yamasato. 1983. Taxonomic studies of the genus Alkaligenes and the related bacteria. In Abstract of papers, Annual Meeting of The Agricultural Chemical Society of Japan. Sendai. April. p. 95. (in Japanese)

13) Oyaizu, H., and K. Komagata. 1981. Chemotaxonomic and phenotypic characterization of the strains of species in the Flavobacterium-Cytophaga complex. J. Gen. Appl. Microbiol. 27: 57-107.
14) Oyaizu, H., and K. Komagata. 1983. Grouping of Pseudomonas species on the basis of cellular fatty acid composition and the quinone system with special reference to the existence of 3-hydroxy fatty acid. J. Gen. Appl. Microbiol. 29: 17-40.
15) Oyaizu-Masuchi, Y., and K. Komagata. 1988. Isolation of free-living nitrogen-fixing bacteria from the rhizosphere of rice. J. Gen. Appl. Microbiol. 34: 127-164.
16) Ohta, K. and K. Nishiyama. 1984. Studies on the crown gall diseases of flower crops I. Occurrence of the disease and the characterization of the causal bacterium. Ann. Phytopathol. Soc. Japan. 50: 197-204. (in Japanese with English smmary)
17) Ohta, K. 1984. Studies on the crown gall diseases of flower crops II. Pathogenicity and the inoculum potential of the causal bacterium. Ann. Phytopathol. Soc. Japan. 50: 205-210. (in Japanese with English summary)
18) Puvanesarajah, V., F.M. Schell, D. Gerhold, and G. Stacey. 1987. Cell surface polysaccharides from Bradyrhizobium japonicum and a non-nodulating mutant. J. Bacteriol. 169: 137-141.
19) Roberts, G.P., W.T. Leps, L.E. Silver, and W.J. Brill. 1980. Use of two dimensional polyacrylamide gel electrophoresis to identify and classify Rhizobium strains. Appl. Env. Microbiol. 29: 414-422.
20) Robertsen, B.K., P. Aman, A.G. Darvill, M. McNeil, and P. Albersheim. 1981. Host-symbiont interactions. V. The structure of the acidic extracellular polysaccharides secreted by R. leguminosarum and T. trifolii. Plant Physiol. 67: 389-400.
21) Russa, R., O. Lüderitz, and E.Th. Rietschel. 1985. Structural analyses of lipid A from lipopolysaccharides of nodulating and non-nodulating Rhizobium trifolii. Arch. Microbiol. 141: 284-289.
22) Salkinoja-Salonen, M., and R. Boeck. 1978. Characterization of lipolysaccharides isolated from Agrobacterium tumefaciens. J. Gen. Microbiol. 105: 119-125.
23) Urakami, T., and K. Komagata. 1987. Cellular fatty acid composition with special reference to the existence of hydroxy fatty acids in Gram-negative methanol-, methane-, and methylamine-utilizing bacteria. J. Gen. Appl. Microbiol. 33: 135-165.
24) Urakami, T., and K. Komagata. 1988. Cellular fatty acid composition with special reference to the existence of hydroxy fatty acids, and the occurrence of squalene and sterols in species of Rhodospirillaceae genera and Erythrobacter longus. J. Gen. Microbiol. 34: 67-84.
25) Wollenweber, H.-W., U. Seydel, B. Lindner, O. Lüderitz, and E.Th. Rietschel. 1984. Nature and location of amide-bound (R)-3-acyloxyacyl groups in lipid A of lipopolysaccharides from various Gram-negative bacteria. Eur. J. Biochem. 145: 265-272.
26) Yamanaka, S., R. Fudo, A. Kawaguchi, and K. Komagata. 1988. Taxonomic significance of hydroxy fatty acids in myxobacteria with special reference to 2-hydroxy fatty acids in phospholipids. J. Gen. Appl. Microbiol. 34: 57-66.
27) Yano, I., Y. Ohno, M. Masui, K. Kato, E. Yabuuchi and A. Ohyama. 1976. Occurrence of 2- and 3-hydroxy fatty acids in high concentration in the extractable and bound lipids of Flavobacterium meningosepticum and Flavobacterium IIb. Lipids. 11: 685-688.
28) Zevenhuizen, L.P.T.M., I. Scholten-Koerselman, and M.A. Posthumus. 1980. Lipopolysaccharides of Rhizobium. Arch. Microbiol. 125: 1-8.

IFO Res. Comm. 14,
40-51, 1989 (March)

THE ANALYSIS OF MADUROSE, AN ACTINOMYCETE WHOLE-CELL SUGAR, BY ENZYMATIC HPLC

AKIRA YOKOTA AND TORU HASEGAWA

Summary

The analysis of madurose, a taxonomically important whole-cell sugar in actinomycetes, was studied using HPLC. D-Mannose, which shows the same retention time as madurose on HPLC, and therefore disturbs the estimation of madurose, could be eliminated from the whole-cell hydrolysate by treatment with hexokinase. Madurose was easily identified by its retention time and by the disappearance of the corresponding peak on HPLC after treatment with D-galactose oxidase.

This method was applied to the analyses of strains of nine actinomycete genera which are known to contain madurose, and it gave satisfactory results in all the strains tested. This enzymatic HPLC procedure is especially effective for strains with low madurose levels.

Since the early work of Lechevalier and Lechevalier (12), whole-cell sugar analysis has become a widely used technique to classify and identify actinomycetes. By such sugar analysis, four types of whole-cell sugar patterns are recognized in aerobic actinomycetes, in which arabinose, xylose, galactose, and madurose (3-O-methyl-D-galactose) are diagnostic sugars. All the sugars can be identified by conventional chromatographic techniques such as paper chromatography (PC) (4) and thin-layer chromato-

graphy (TLC) (10,14,20,21); but madurose, a diagnostically important sugar, is often present in very small amounts (6,16,19), and is frequently difficult to identify. PC and TLC have been used for rapid, quantitative analysis of the whole-cell sugar compositions. Another established technique, applied only rarely to such sugar analysis, is the conversion of sugars into volatile derivatives, which are then analyzed by combined gas-liquid chromatography / mass spectrometry (GC/MS) (1,3,8,9,17). However, this system is expensive.

Recently, high-performance liquid chromatography (HPLC) has been applied to the analysis of sugars (7). Among the methods reported, a system using an anion exchange column and fluorescence detector (15) is the most suitable to analyze a mixture of small amounts of sugars. However, a suitable HPLC method which permits the resolution of all of the diagnostic sugars has not yet been found (11), as it has not been possible to separate madurose and mannose on the chromatogram.

Here, we present an improved method involving the use of enzymes and HPLC to simply and reliably identify madurose, a taxonomically important whole-cell sugar.

Materials and Methods

Bacterial strains and culture conditions. The strains of actinomycetes used in this study are listed in Table 1. Type strains are indicated by the superscript "T". Strains were cultured in medium containing 1% yeast extract and 1% D-glucose (pH 7.0) at 28 C for 4 days with shaking. The biomass was washed twice with distilled water and lyophilized.

Preparation of whole-cell hydrolysate. The dried cells (100 mg) were hydrolyzed with 4N HCl at 100 C for 4 hr in a screw-capped test tube. After filtration, the hydrolysate was concentrated in vacuo. The residue was dissolved in distilled water and neutralized with 5N NaOH; water was added to make 1 ml.

Treatment of whole-cell hydrolysate with enzymes. D-Mannose and D-glucose in the hydrolysate were converted into their phosphate esters with hexokinase (grade II, Oriental Yeast Co. Ltd., Japan, EC 2.7.1.1.)

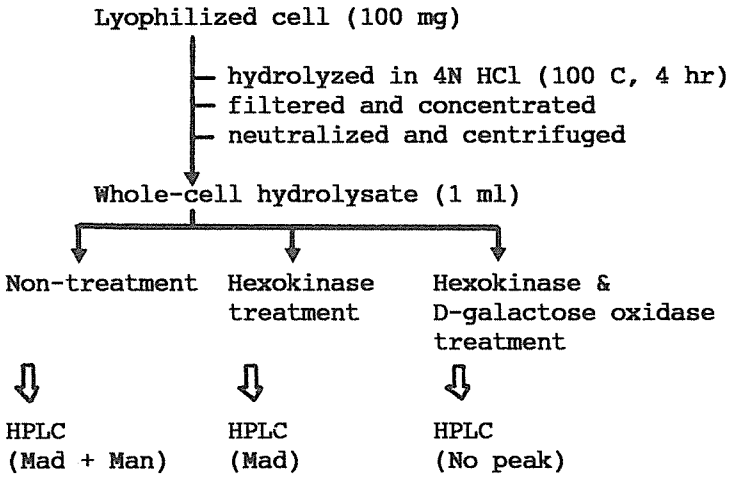


Fig. 1. Procedure of enzymatic HPLC.

(5) in the reaction mixture A, which was composed of 50 µl of the hydrolysate, 50 µl of 50 mM triethanolamine buffer (pH 7.6) containing 7 mM mercaptoethanol and 1.4 mM $MgCl_2$, 50 µl of 50 mM ATP, and 100 µg (12.8 units) of hexokinase dissolved in 20 µl of water (total 200 µl). To confirm the presence of madurose, the hydrolysate was treated with D-galactose oxidase (Sigma Co., St. Louis, USA, EC 1.1.3.9.) (2) and catalase (Sigma Co., EC 1.11.1.6.) in the reaction mixture B, which contained 170 µl of reaction mixture A, 200 µg (32 units) of D-galactose oxidase dissolved in 30 µl of water, and 70 µg (140 units) of catalase dissolved in 2 µl of water (total 202 µl). Both reaction mixtures were incubated at 37 C for 20 hr in the presence of 1 drop of chloroform. The scheme for analysis of madurose by the enzymatic-HPLC is shown in Fig. 1.

High-performance liquid chromatography (HPLC). HPLC was performed on a Shimadzu Model LC-5A pumping system, equipped with a manual 20 µl loop injector. An anion exchange column, Shim-pack ISA-07/S2504 (4.0 x 250 mm, Shimadzu), was heated to 65 C, and stepwise elution was performed with borate buffer at concentrations of 0.2 M (pH 8.5) for 10 min, 0.3 M (pH 9.0) for 10 min, and 0.4 M (pH 9.0) for 20 min. The column effluent (flow rate of 0.6 ml/min) and detection reagent, a mixture of 1% arginine and 3% borate (15) delivered by another Model LC-5A pump at a flow rate of 0.5 ml/min, were led to a Shimadzu Model CRB-3A chemical reaction bath and heated to 140 C. Fluorescence intensities of the effluent were measured

with a Shimadzu Model RF-530 spectrofluorometer and a Shimadzu Chromatopac integrator.

Chemicals. The madurose used in the experiment was prepared by Dr. T. Kusaka from a hydrolysate of Actinomadura kijaniata IFO 14229^T according to the method of Lechevalier and Gerber (13). Based on the data of optical rotation, ^{1}H-NMR, ^{13}C-NMR and GC/MS, the purified sugar was confirmed to be 3-O-methyl-D-galactose (madurose) (T. Kusaka, unpublished results). Hexokinase (baker's yeast) was purchased from Oriental Yeast Co., Ltd. (Osaka, Japan), and D-galactose oxidase (Dactylium dendroides), catalase (bovine liver) and ATP were from Sigma Chemical Co. (St. Louis, USA).

Results

Separation of sugar mixture by standard HPLC condition

As shown in Fig. 2, the HPLC system used in this study worked well for the identification of diagnostic sugars in whole-cell hydrolysate except for madurose and D-mannose. As the retention times of madurose and

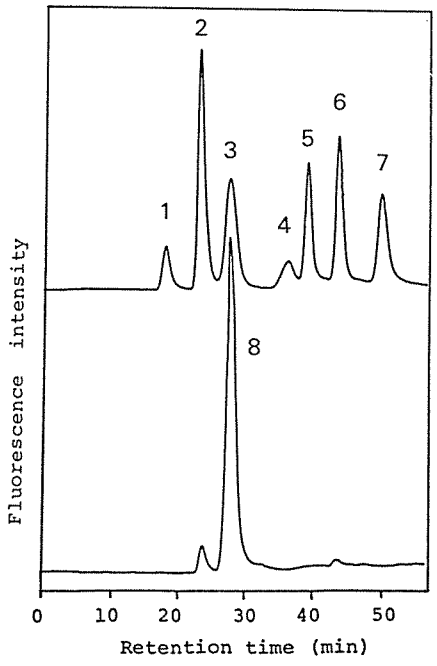


Fig. 2. HPLC separation of mixture of authentic sugars.
Peak designations: 1, L-rhamnose; 2, D-ribose; 3, D-mannose 4, L-arabinose; 5, D-galactose; 6, D-xylose; 7, D-glucose; 8, madurose.

D-mannose were very close (26.5 min and 27 min, respectively) in this HPLC system, it was necessary to eliminate D-mannose from the hydrolysate in order to estimate the madurose content; and we applied hexokinase to achieve this. To verify the reliability and accuracy of the enzymatic elimination procedure, mixtures of authentic sugars were used as a model system. Typical elution patterns of the mixtures of D-mannose, D-galactose and D-glucose (Fig.3), and L-rhamnose and madurose (Fig. 4) before and after enzymatic treatment are shown. D-Mannose and D-glucose completely disappeared after the hexokinase treatment (Fig. 3B); but D-galactose and madurose were not affected by this treatment (Fig. 3B and 4B). Thus, D-mannose in the sugar mixture was confirmed to be smoothly phosphorylated by hexokinase. The presence of relatively high concentration of ATP (12.5 mM) was necessary to completely phosphorylate hexoses in the reaction mixture.

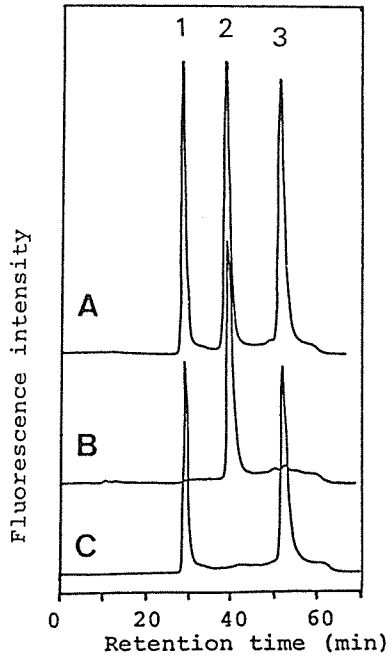


Fig. 3. HPLC separation of mixture of authentic sugars. A, non-treatment; B, hexokinase treatment; C, D-galactose oxidase treatment. Peak designations:1, D-mannose; 2, D-galactose;3, D-glucose.

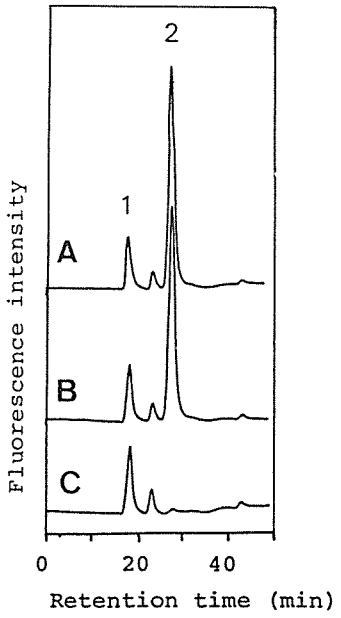


Fig. 4. HPLC separation of mixture of authentic sugars. A, non-treatment; B, hexokinase treatment; C, D-galactose oxidase treatment. Peak designations: 1, L-rhamnose; 2, madurose.

Identification of madurose by D-galactose oxidase treatment

We next used D-galactose oxidase to identify madurose. This enzyme is known to oxidize madurose as well as D-galactose (13). As shown in Fig. 3C and 4C, D-galactose and madurose were completely oxidized by D-galactose oxidase and disappeared from the chromatogram on HPLC. Thus, it was confirmed that the oxidation of madurose was smoothly catalyzed by D-galactose oxidase. Disappearance of the peak with a retention time of 26.5 min after oxidation of D-galactose confirms this to be the peak of madurose.

Analysis of whole-cell sugar of actinomycetes with type IIIB cell-walls

Figure 5-1 shows the HPLC chromatograms of the whole-cell hydrolysate of Microtetraspora fusca IFO 13915^{T}. The hydrolysate contained D-glucose, D-galactose, and, possibly, equal amount of D-mannose and madurose (Fig. 5-1A). The chromatogram of the sample treated with hexokinase clearly shows the presence of madurose in the hydrolysate (Fig. 5-1B). The peak

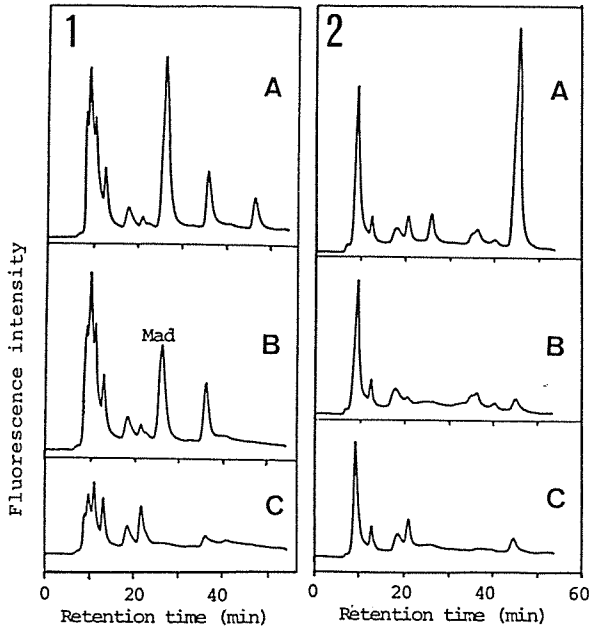


Fig. 5. HPLC chromatograms of whole-cell hydrolysates of Microtetraspora fusca IFO 13915 (1) and Streptomyces lavendulae subsp. lavendulae IFO 12340 (2).
A, non-treatment; B, hexokinase treatment; C, D-galactose oxidase treatment. Peak identification: Mad, madurose.

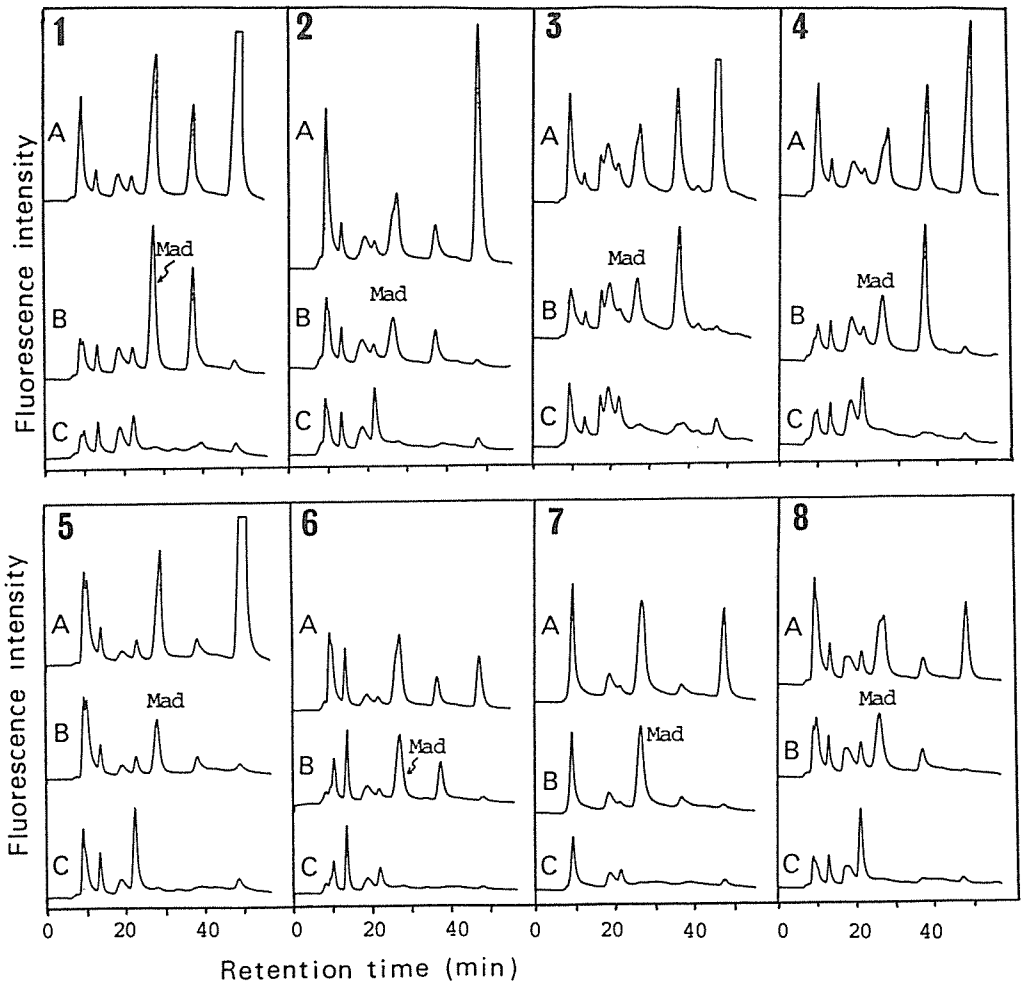


Fig. 6. HPLC chromatograms of whole-cell hydrolysates of actinomycete strains with type IIIB cell walls.
Whole-cell hydrolysate from: 1, Actinomadura madurae IFO 14263^{T}; 2, Planobispora longispora IFO 13879^{T}; 3, Spirillospora albida IFO 12248^{T}; 4, Excellospora viridilutea IFO 14480^{T}; 5, Microbispora rosea IFO 14044^{T}; 6, Planomonospora parontospora subsp. parontospora IFO 13880^{T}; 7, Dermatophilus congolensis IFO 13913^{T}; 8, Streptosporangium roseum IFO 3776^{T}. A, non-treatment; B, hexokinase treatment; C, D-galactose oxidase treatment.
Peak identification: Mad, madurose.

Mad was confirmed to be the peak of madurose by its retention time and its disappearance after incubation with D-galactose oxidase (Fig. 5-1C). As a negative control, the chromatograms of Streptomyces lavendulae subsp. lavendulae IFO 12340 (cell-wall type I, no diagnostic sugar) are also shown in Fig. 5-2.

Nine genera of aerobic actinomycetes are so far known to have madurose-containing whole-cell sugar patterns (cell-wall type IIIB); they are Actinomadura, Microbispora, Microtetraspora, Dermatophilus, Planomonospora, Planobispora, Spirillospora, Streptosporangium, and Excellospora. Nine representative strains were selected from these genera, and madurose content in their whole-cell hydrolysates was analyzed by the enzymatic HPLC method (Fig. 6-1~8). Quantitative data on the madurose in these actinomycete strains are summarized in Table 1; D-galactose was used as a standard for the estimation of madurose. Madurose could be detected clearly in all the strains except S. lavendulae subsp. lavendulae, which has no madurose. The madurose content in actinomycetes seems to vary significantly depending on the strain. Sugars identified by this HPLC method in the hydrolysates of the above strains are listed in Table 2.

Table 1. Madurose content in the whole-cell hydrolysate of various actinomycete strains.

Strain	Madurose content[a] (μg/mg of dried cells)
Actinomadura madurae IFO 14623^T	16.7
Microbispora rosea IFO 14044^T	5.8
Microtetraspora fusca IFO 13915^T	10.5
Dermatophilus congolensis IFO 13913^T	42.9
Planomonospora parontospora subsp. parontospora IFO 13880^T	9.8
Planobispora longispora IFO 13879^T	11.3
Spirillospora albida IFO 12248^T	12.0
Streptosporangium roseum IFO 3776^T	8.9
Excellospora viridilutea IFO 14480^T	3.0
Streptomyces lavendulae subsp. lavendulae IFO 12340	0.0

[a] Calculated from fluorescence intensity using D-galactose as a standard.

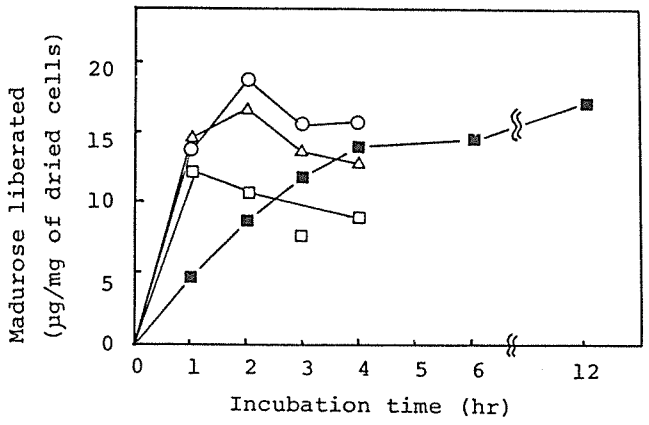


Fig. 7. Effects of acid concentration on the liberation of madurose from whole-cells.
Dried cells (100 mg) of Actinomadura madurae IFO 14263^T were heated at 100 C with 4 ml each of acid in a screw-capped test tube. Symbols: □, 1N HCl; △, 2N HCl; ○, 4N HCl; ■, 1N H_2SO_4.

Effects of acid concentration on the liberation of madurose from whole-cells

Sulfuric acid is usually used for the hydrolysis of cells in determining whole-cell sugar patterns (4). We examined the effect of acid concentration on the liberation of madurose from whole-cells. As shown in Fig. 7, the amount of madurose liberated was the highest under conditions of heating for 2 hr at 100 C in 4N HCl. Therefore, the usual hydrolysis conditions, heating for 2 hr at 100 C with 1N H_2SO_4, seem to be too mild for the liberation of madurose. Based on these results, we employed hydrolysis conditions of heating with 4N HCl for 2-4 hr at 100 C in this study.

Discussion

Staneck and Roberts (20) and Hasegawa et al. (10), and very recently Meyertons et al. (14), have reported a rapid analytical method for whole-cell sugars using TLC. In their methods, madurose was distinguished from other sugars by the differences in Rf value and color reaction on the chromatogram. A time advantage is apparent with their TLC methods. However, in the strains with trace amounts of madurose, it is difficult to

distinguish differences in color. Furthermore, the TLC methods have difficulty in determining amounts quantitatively.

The procedure described here involves two steps of enzyme reaction. D-Mannose, which shows the same retention time as madurose on HPLC, and therefore disturbs the estimation of madurose by HPLC, was phosphorylated with commercially available hexokinase. Madurose was identified from its retention time and by the disappearance of the corresponding peak on HPLC after treating the reaction mixture of the first step with commercially available D-galactose oxidase.

Thus, the enzymatic-HPLC procedure appears to be effective to distinguish madurose from D-mannose in the whole-cell hydrolysates, and also quantitatively to estimate madurose. Our method can be easily used to analyze strains with low madurose levels. Sugars in the hydrolysates of many actinomycete strains that were resolved well by this HPLC method were glucose, galactose, mannose, arabinose, xylose, madurose, ribose, and rhamnose (Table 2). Therefore, it is well suited to the needs of laboratories for clinical, ecological and taxonomic studies of actinomycetes.

Table 2. Whole-cell sugar analysis of actinomycete strains with type IIIB cell walls using enzymatic HPLC.

Strain	HexN	Rha	Rib	Mad	Man	Gal	Glc
Actinomadura madurae IFO 14623^T	+	tr	tr	+	-	+	+
Microbispora rosea IFO 14044^T	tr	tr	tr	+	+	tr	+
Microtetraspora fusca IFO 13915^T	+	tr	tr	+	+	+	tr
Dermatophilus congolensis IFO 13913^T	+	tr	tr	+	+	tr	+
Planomonospora parontospora subsp. *parontospora* IFO 13880^T	+	tr	tr	+	+	+	+
Planobispora longispora IFO 13879^T	+	tr	tr	+	+	+	+
Spirillospora albida IFO 12248^T	+	tr	tr	+	+	+	+
Streptosporangium roseum IFO 3776^T	+	+	tr	+	+	tr	+
Excellospora viridilutea IFO 14480^T	+	+	tr	+	+	+	+
Streptomyces lavendulae subsp. *lavendulae* IFO 12340	+	tr	tr	-	+	-	+

HexN = hexosamine Rha = rhamnose Rib = ribose Mad = madurose
Man = mannose Gal = galactose Glc = glucose
+ = positive - = negative tr = trace amount

Naumova et al. (18) have found that madurose is contained within the teichoic acid of the cell walls of Actinomadura carminata. Studies on the location of madurose in other strains with cell-wall type IIIB are of special interest in view of their taxonomic value among actinomycetes. Furthermore, madurose-containing actinomycete strains with cell-wall type IID (Micromonospora carbonacea, M. chalcea subsp. izumiensis, and M. rosaria) (14), and a strain with cell-wall type IVA (Kibdelosporangium aridum) (19) have recently been reported. These reports suggest that further studies on the taxonomic value of madurose are necessary, and hence the enzymatic HPLC method for the analysis of madurose might be useful for such biochemical and chemotaxonomic studies in actinomycetes.

We are indebted to Dr. T. Kusaka, Integrated Technology Laboratories of Takeda Chemical Industries Ltd., for supplying madurose.

References

1) Aamlid, K.H., and S. Morgenlie. 1983. Analysis of mixtures of some mono-O-methylaldoses with the common aldoses by G.L.C.-M.S. after isopropylidenation. Carbohydr. Res. 124: 1-10.
2) Avigad, G., D. Amaral, C. Asensio, and B.L. Horecker. 1962. The D-galactose oxidase of Polyporus circinatus. J. Biol. Chem. 237: 2736-2743.
3) Bacon, J.S.D., and M.V. Cheshire. 1971. Apiose and mono-O-methyl sugars as minor constituents of the leaves of deciduous trees and various other species. Biochem. J. 124: 555-562.
4) Becker, B., M.P. Lechevalier, R.E. Gordon, and H.A. Lechevalier. 1964. Rapid differentiation between Nocardia and Streptomyces by paper chromatography of whole-cell hydrolysates. Appl. Microbiol. 12: 421-423.
5) Darrow, R.A., and S.P. Colowick. 1962. Hexokinase from baker's yeast. Methods Enzymol. 5: 226-235.
6) Fischer, A., R.M. Kroppenstadt, and E. Stackebrandt. 1983. Molecular-genetic and chemotaxonomic studies on Actinomadura and Nocardiopsis. J. Gen. Microbiol. 129: 3433-3446.
7) Frei, R.W. 1981. In R.W. Frei and J.F. Lawrence (ed.) Chemical derivatization in analytical chemistry, Vol. 1. Chromatography, p. 233-234, p. 241-243, p. 259-260. Plenum Press, New York.
8) Fukuda, K., and A. Hamada. 1978. 3-O-Methyl-galactose found in polysaccharides of Lampteromyces japonicus. Biochim. Biophys. Acta. 544: 445-447.
9) Gerwig, G.J., J.P. Kamerling, and J.F.G. Vliegenthart. 1984. Characteristic differences in monosaccharide composition of glyco-conjugates from opposite mating types of Chlamydomonas eugametos.

Carbohydr. Res. 127: 245-251.
10) Hasegawa, T., M. Takizawa, and S. Tanida. 1983. A rapid analysis for chemical grouping of aerobic actinomycetes. J. Gen. Appl. Microbiol. 29: 319-322.
11) Labeda, D.P. 1987. Actinomycete taxonomy: generic characterization. J. Ind. Microbiol., Suppl. No. 2. 28: 115-121.
12) Lechevalier, H.A., and M.P. Lechevalier. 1965. Classification des actinomycètes aérobies basée sur leur morphologie et leur composition chimique. Ann. Inst. Pasteur. 108: 662-673.
13) Lechevalier, M.P., and N.N. Gerber. 1970. The identity of madurose with 3-O-methyl-D-galactose. Carbohydr. Res. 13: 451-454.
14) Meyertons, J.L., D.P. Labeda, G.L. Cote, and M.P. Lechevalier. 1988, A new thin-layer chromatographic method for whole-cell sugar analysis of Micromonospora species. Actinomycetes. 20: 182-192.
15) Mikami, H., and Y. Ishida. 1983. Post-column fluorometric detection of reducing sugars in high-performance liquid chromatography using arginine. Bunseki Kagaku. 32: E207-E210.
16) Miyadoh, S., S. Amano, H. Tohyama, and T. Shomura. 1987. Actinomadura atramentaria, a new species of the Actinomadura. Int. J. Syst. Bacteriol. 37: 342-346.
17) Morgenlie, S. 1979. Determination of the substituent position in mono-O-methylaldoses by oxidation with silver carbonate on Celite. Carbohydr. Res. 73: 315-319.
18) Naumova, I.B., N.V. Potekhina, C.Duigimbaye, A.S. Shashkov, L.P. Terekhova, and T.P. Preobrazhenskaya. 1986. Cell wall polymers of Actinomadura carminata INA 4281. Arch. Microbiol. 146: 256-262.
19) Shearer, M.C., P.M. Colman, R.M. Ferring, L.J. Nisbet, and C.H. Nash III. 1986. New genus of the Actinomycetales: Kibdelosporangium aridum gen. nov., sp. nov. Int. J. Syst. Bacteriol. 36: 47-54.
20) Staneck, J.L., and G.D. Roberts. 1974. Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. Appl. Microbiol. 28: 226-231.
21) Terekhova, L.P., T.P. Preobrazhenskoya, and O.A. Galtenko. 1986. Occurrence of galactose in the whole cell hydrolysates of Actinomadura strains. Actinomycetes. 19: 73-81.

IFO Res. Comm. 14,
52-79, 1989 (March)

MARINE FUNGI IN SEA FOAM FROM JAPANESE COAST

AKIRA NAKAGIRI

Summary

From sea foam collected on beaches around the Japanese coast, spores of marine fungi have been observed and isolated. Thirty-six species of marine fungi comprising 27 species of ascomycetes, one species of basidiomycetes, and 8 species of deuteromycetes, were recorded.

In foam and scum formed in rivers or the sea, there are many propagules of aquatic or semi-aquatic organisms as well as debris trapped and accumulated during transportation in water. Spores of aquatic hyphomycetes and other aquatic fungi were found to accumulate in foam in streams (2, 10). Foam has been used for research to learn the mycoflora of the stream system or to isolate fungal spores for culture.

On the sea shore, foam accumulates spores of marine fungi as well as other small marine organisms, e.g., protozoa, phytoplanktons, and bacteria, as well as debris (Fig. 2). Sea foam may be a useful sample for examining the flora of marine fungi and also for isolating fungal spores. In particular, sea foam on sand beaches has been found to contain many spores of marine fungi inhabiting sand (6, 15), whereas the sea foam on a rocky shore rarely accumulates spores of marine fungi (unpublished data).

In the course of studying the higher marine fungi since 1980, 27 species of ascomycetes, one species of basidiomycetes, and 8 species of deuteromycetes have been recorded from sea foam samples from the Japanese coast.

Materials and Methods

Collection of sea foam Sea foam was collected at a sand beach when the window blew onshore and rough waves produced foam at the shoreline. A heavy sea just after a storm produced "good" sea foam abundant in fungal spores; but even with a calm sea, foam or spume at the shore would be sampled if it was gathered repeatedly. Foam collected in bottles was kept cool during transportation to the laboratory to prevent spores from germination.

Collection sites Localities and dates of collection are shown in Figure 1 and Table 1. Collection site numbers on the map (Fig. 1) are referred to in the text to indicate ranges of fungal distribution. Marine fungi whose spores were found and isolated from the foam samples were recorded with reference to the collection sites.

Isolation Bottles of sea foam samples were left to settle in a cool place. Sediment of sea foam was pipetted onto agar plates of SWS medium (1% soluble starch, 0.1% soytone, 1.5% agar in 20‰ salinity artificial seawater [Jamarin S; Jamarin Lab., Japan], pH 8.2), and single spores of marine fungi contained in the foam were isolated under the microscope with Skerman's micromanipulator (14). Germinated hyphae from the isolated spores were transferred to a new medium to obtain isolates.

Culture The isolates were cultured mainly on SWS. In some cases other media, e.g., SWS with soluble starch replaced by cellulose powder or other carbohydrates, were used to induce ascocarp formation of ascomycete strains (7). Besides the agar media, sterilised quartz sand with balsa wood which was soaked in seawater containing 0.1% soytone was also used for incubation. Incubation was carried out at 20-28 C.

Results

Marine fungi recorded in sea foam samples are described briefly in terms of spore morphology and cultural properties. Localities where the species were recorded are also listed as range according to the numbers on the map (Fig. 1, Table 1).

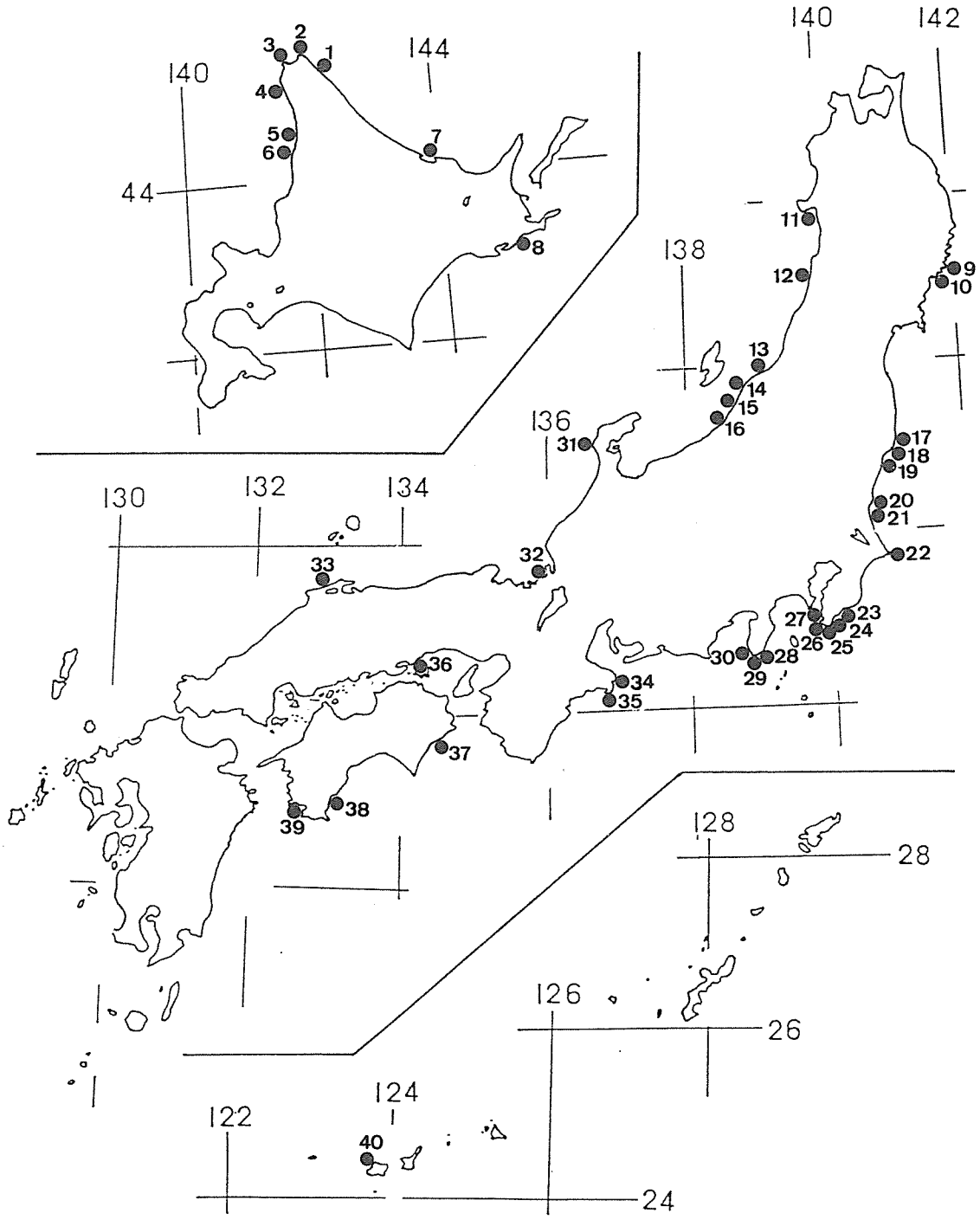


Fig. 1. Collection sites (Refer to Table 1).

Table 1. Collection sites and dates.

*1.	Higashiura, Soya mura, Wakkanai C., Hokkaido; Aug. 22, 1981
2.	Cape Soya, Soya mura, Wakkanai C., Hokkaido; Aug. 22, 1981
3.	Cape Noshyappu, Noshyappu, Wakkanai C., Hokkaido; Aug. 22, 1981
4.	Wakkasanai, Toyotomi cho, Teshio gun, Hokkaido; Aug. 27, 1984
5.	Kitasarakishi, Teshio cho, Teshio gun, Hokkaido; Sept. 24, 1981; Mar. 17, June 12, Nov. 17, 1982
6.	Cape Konpira, Shyosanbetsu mura, Tomamae gun, Hokkaido; Aug. 21, 1981; Mar. 17, June 13, 1982
7.	Wakka, Tokoro cho, Tokoro gun, Hokkaido; Aug. 29, 1984
8.	Hamanaka, Hamanaka cho, Akkeshi gun, Hokkaido; Aug. 30, 1984
9.	Ofunato Bay, Ofunato C., Iwate Pref.; Oct. 5, 1980
10.	Oya, Motoyoshi cho, Motoyoshi gun, Miyagi Pref.; Oct. 5, 1980; June 17, Oct. 29, 1981
11.	Funakoshi, Oga C., Akita Pref.; June 20, 1981
12.	Fukura, Uza machi, Akumi gun, Yamagata Pref.; June 20, 1981
13.	Fujitsukahama, Shiunji machi, Kitakanbara gun, Niigata Pref.; June 17, 1981
14.	Gokahama, Maki machi, Nishikanbara gun, Niigata Pref.; May 29; 1981
15.	Teradomari machi, Santo gun, Niigata Pref.; May 29, 1981
16.	Kujiranami, Kashiwazaki C., Niigata Pref.; Feb. 1, 1982
17.	Cape Shioya, Iwaki C., Fukushima Pref.; June 8, 1982; Mar. 18, 1983
18.	Izura, Kitaibaraki C., Ibaraki Pref.; Mar. 23, 1982
19.	Isohara, Isohara cho, Kitaibaraki C., Ibaraki Pref.; Mar. 23, 1982
20.	Ajigaura, Ajigaura cho, Nakaminato C., Ibaraki Pref.; Apr. 18, Dec. 17, 1980; Dec. 11, 1981; May 27, 1982
21.	Oarai, Oarai machi, Higashiibaraki gun, Ibaraki Pref.; Dec. 17, 1980; Dec. 11, 1981
22.	Kimigahama, Inubozaki; Choshi C., Chiba Pref.; Sept. 15, 1981; Apr. 27, July 10, Dec. 1, 1982; Mar. 24, 1983
23.	Maehara, Kamogawa C., Chiba Pref.; Dec. 21, 1982
24.	Shirahama, Shirahama machi, Awa gun, Chiba Pref.; Feb. 27, 1981
25.	Heisaura, Tateyama C., Chiba Pref.; Mar. 6, 1985
26.	Cape Suno, Sunosaki, Tateyama C., Chiba Pref.; July 10, 1982
27.	Kenbutsu, Tateyama C., Chiba Pref.; Feb. 27, 1981
28.	Shirahama, Shimoda C., Shizuoka Pref.; Apr. 4, 1980; Mar. 24, Apr. 6, May 13, June 15, July 11, Aug. 13, Sept. 16, 1981; Nov. 1, 1982; Feb. 6, Apr. 3, 1983
29.	Nabetahama, Shimoda C., Shizuoka Pref.; July 11, Aug. 14, 1981
30.	Iwachi, Matsuzaki cho, Kamo gun, Shizuoka Pref.; Apr. 11, Nov. 1, 1982
31.	Notokongo, Togi machi, Hakui gun, Ishikawa Pref.; Feb. 2, 1982
32.	Hayase, Mihama cho, Mikata gun, Fukui Pref.; Oct. 1, 1980
33.	Konami, Shimane cho, Yatsuka gun, Shimane Pref.; Sept. 27, 1981
34.	Chidorigahama, Osatsu, Toba C., Mie Pref.; Oct. 9, 1988
35.	Hirohama, Fuseda, Shima cho, Shima gun, Mie Pref.; Oct. 9, 1988
36.	Kashino, Ushimado cho, Oku gun, Okayama Pref.; Sept. 24, 1981
37.	Uchizuma, Mugi cho, Kaifu gun, Tokushima Pref.; July 21, 1982
38.	Ukibuchi, Ogata cho, Hata gun, Kochi Pref.; July 24, 1982
39.	Mukuzu, Sukumo C., Kochi Pref.; July 25, 1982
40.	Hoshizunanohama, Iriomote Is., Taketomi cho, Yaeyama gun, Okinawa Pref.; Apr. 22, June 20, 1982

* The figures refer to the locality numbers in Fig. 1.

Ascomycotina
Pyrenomycetes
Sphaeriales
Halosphaeriaceae

Arenariomyces trifurcatus Höhnk Fig. 3

Veroeff. Inst. Meeresforsch. Bremerhaven 3: 30, 1954

= Halosphaeria trifurcata (Höhnk) Cribb & Cribb, Univ. Queensl. Pap. Dep. Bot. 3: 99, 1956

≡ Peritrichospora trifurcata (Höhnk) Kohlm., Nova Hedwigia 3: 89, 1961

≡ Corollospora trifurcata (Höhnk) Kohlm., Ber. Dtsch. Bot. Ges. 75: 126, 1962

Ascospores: 22-35 x 7.5-10.5 μm (excluding appendages), elliptic-fusiform to ellipsoidal or oblong, one-septate, with or without slight constriction at the septum, hyaline.

Appendages: at both ends of the spore with (2-)3(-4) terminal appendages, 22.5-30 μm long, 1.5-2 μm in diam at base, attenuate, developed by outgrowth of spore wall, with a bulbous base, slender, rigid, round shaft, terminating in an apical hook which corresponds to the space between two bulbous bases of the opposite terminal appendages.

Culture: brown to dark brown colony on SWS. A few strains produced ascocarps in culture on the agar media. IFO 32095, 32096.

Range: 1, 4, 5, 6, 7, 8, 10, 11, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 33, 34, 35, 36, 37, 38, 39.

Carbosphaerella leptosphaerioides I. Schmidt Fig. 4

Nat. Naturschutz Mecklenburg 7: 9, 1969 (publ. 1971)

Ascospores: 31-46 x 17-23 μm (excluding sheath), ellipsoidal, triseptate, not or slightly constricted at the septa; central large cells, dark brown; apical small cells, hyaline or light brown; septa with central porus.

Appendages: reticulate or net-like sheath surrounding ascospore, becoming fibrillar by the loss of the cross-connections in the reticula,

flexuous, gelatinous, persistent, irregular shaped.

Culture: gray to black colony on SWS. Ascocarp was not produced in culture on the agar media. IFO 32097.

Range: 1, 5, 6, 10, 13, 14, 15, 16, 17, 20, 25, 28, 38.

Corollospora angusta Nakagiri & Tokura Fig. 5

Trans. mycol. Soc. Japan 28: 417, 1987

Ascospores: 35-57 x 3-7.5 μm (excluding polar appendages), fusiform, slender, 3(-5)-septate, hyaline.

Appendages of two kinds: (i) a single terminal appendage at each end of the spore, 3-8 μm long, spine- or thorn-like, attenuate; (ii) fibrous and peritrichous appendages on the terminal appendages, 5.8-12.5 μm long, and around the central septum, 18-24.5 μm long, developed by fragmentation and peeling of the exospore.

Culture: white colony on SWS. Single-spore isolates produced ascocarps on the agar media and on the glass of slant tubes. IFO 32100, 32101, 32102.

Range: 1, 5, 6, 8, 10, 14, 15, 16, 17, 19, 20, 23, 25, 27, 28, 30, 31, 33, 34, 38.

Corollospora colossa Nakagiri & Tokura Fig. 6

Trans. mycol. Soc. Japan 28: 418, 1987

Ascospores: 60-108 x 13-26 μm, fusiform to ellipsoidal, (6-)7(-8)-septate, hyaline.

Appendages: fibrous, peritrichous, at both ends of the spore, 20-27 μm long and around the central septum, 20-28 μm long, developed by fragmentation and peeling of the exospore.

Culture: dark green to black colony on SWS. Single-spore isolates produced ascocarps on sand grains by the "quartz sand method", but not on the agar media. IFO 32103, 32104.

Range: 1, 5, 6, 7, 10, 13, 14, 15, 16, 20, 21, 22, 23, 27, 28, 34, 37, 38, 39.

Corollospora filiformis Nakagiri in Nakagiri and Tokura Fig. 7
Trans. mycol. Soc. Japan 28: 422, 1987

Ascospores: (73-)87-120 x 5-8(-10) μm, filiform, (9-)13(-17)-septate, hyaline.

Appendages: fibrous, peritrichous, at both ends of the spores, 18-25 μm long and around the central septum, 13-22 μm long, developed by fragmentation and peeling of the exospore.

Culture: brownish gray to black colony on SWS. Single-spore isolates produced ascocarps on sand grains by the "quartz sand method", but not on the agar media. IFO 32106.

Range: 39.

Corollospora fusca Nakagiri & Tokura Fig. 8
Trans. mycol. Soc. Japan 28: 424, 1987

Ascospores: 63-220 x 20-38 μm (excluding polar appendages), fusiform, muriform with transverse and longitudinal septa, (5-)12-21 transversally septate, dark brown, longitudinally finely striated on the spore surface. Ridges of striation run in parallel and sometimes dichotomize.

Appendages of two kinds: (i) a single terminal appendage at each end of the spore, 28.5-65 μm long, thorn-like, hyaline; (ii) fibrous and peritrichous appendages on the terminal appendages, 28-54 μm long, and around the central septum, 25-75 μm long, developed by fragmentation and peeling of the exospore.

Culture: grayish yellow to dark green colony on SWS. Single-spore isolates produced ascocarps on sand grains by the "quartz sand method", but not on the agar media. IFO 32107, 32108, 32109.

Range: 4, 5, 6, 10, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 25, 27, 28, 34, 38, 39, 40.

Corollospora gracilis Nakagiri & Tokura Fig. 9
Trans. mycol. Soc. Japan 28: 426, 1987

Ascospores: 26-45 x 3-5.5(-7) μm (excluding polar appendages), fusiform, slender, one-septate, hyaline.

Appendages of two kinds: (i) a single terminal appendage at each end of the spore, 6.5-12 μm long, spine- or thorn-like, attenuate; (ii) fibrous and peritrichous appendages on the terminal appendages, 4-8 um long, and around the central septum, 12-20 μm long, developed by fragmentation and peeling of the exospore.

Culture: white colony on SWS. Single-spore isolates produced ascocarps abundantly on the agar media as well as on the glass of slant tubes. IFO 32110, 32111.

Range: 4, 5, 8, 10, 13, 14, 15, 16, 18, 20, 22, 23, 28, 29, 30, 34, 35, 37, 38.

Corollospora intermedia I. Schmidt Fig. 10

Nat. Naturschutz Mecklenburg 7: 6, 1969 (publ. 1971)

Anamorph: Varicosporina prolifera Nakagiri, Trans. mycol. Soc. Japan 27: 198, 1986

Ascospores: 25-40 x 8-11 μm (excluding polar appendages), ellipsoidal, three-septate, constricted at the septa, hyaline.

Appendages of two kinds: (i) a single terminal appendage at each end of the spore, 5-9 μm long, spine- or thorn-like, attenuate; (ii) fibrous and peritrichous appendages on the terminal appendages, 5-10 μm long, and around the central septum, 10-18 μm long, developed by fragmentaion and peeling of the exospore.

Culture: white colony on SWS, turning to dark olive to black in age. Conidia were produced abundantly. Single-spore isolates produced ascocarps on SWS, which, however, did not mature inside. IFO 32119, 32120.

Range: 1, 28, 30, 35.

Corollospora lacera (Linder) Kohlm. Fig. 11

Ber. Dtsch. Bot. Ges. 75: 126, 1962

≡ Peritrichospora lacera Linder in Barghoorn and Linder, Farlowia 1: 415, 1944

Ascospores: 39-58 x 10-15 μm (excluding polar appendages), fusiform, straight or slightly curved, (4-)5-septate, constricted at the septa, hyaline.

Appendages of two kinds: (i) a single terminal appendages at each end of the spore, (11-)17-40 μm long, thorn-like, attenuate; (ii) fibrous and peritrichous appendages on the terminal appendages, 23-45 μm long and around the central septum, 12-20 μm long, developed by fragmentation and peeling of the exospore.

Culture: dark green to black colony on SWS. Single-spore isolates produced ascocarps on sand grains by the "quartz sand method", but not on the agar media. IFO 32121, 32122.

Range: 1, 4, 5, 6, 8, 10, 16, 18, 19, 20, 22, 27, 28.

Corollospora luteola Nakagiri & Tubaki Fig. 12

Trans. mycol. Soc. Japan 23: 102, 1982

Anamorph: Sigmoidea luteola Nakagiri & Tubaki, ibid. 23: 102, 1982

Ascospores: 50-85 x 4.8-7.5 μm, fusiform, straight or slightly curved, (4-)5(-6)-septate, hyaline.

Appendages: fibrous, peritrichous, at both ends of the spore, 12-17.5 μm long, and around the central septum, 16-28 μm long, developed by fragmentation and peeling of the exospore.

Culture: pale yellow to yellow colony on SWS. Single-spore isolates produced ascocarps and conidia on the agar media. IFO 31315, 31316.

Range: 1, 8, 10, 27, 28.

Corollospora maritima Werdermann Fig. 13

Notizbl. Bot. Gart. Berlin 8: 248, 1922

= Arenariomyces cinctus Höhnk, Veroeff. Inst. Meeresforsch Bremerhaven 3: 28, 1954

= Peritrichospora integra Linder in Barghoorn and Linder, Farlowia 1: 414, 1944

Ascospores: 27.5-37.5 x 7-11.5 μm (excluding polar appendages), elliptic-fusiform to ellispoidal, one-septate, hyaline.

Appendages of two kinds: (i) a single terminal appendage at each end of the spore, 10-20.5 μm long, spine- or thorn-like, slender attenuate; (ii) fibrous and peritrichous appendages on the terminal appendages, 3-12

μm long, and around the central septum, 8-15 μm long, developed by fragmentation and peeling of the exospore.

Culture: olive gray to black colony on SWS. Single-spore isolates produced ascocarps on the glass of slant tubes. Catenulate chlamydospores of globose to oval or oblong, brown cells were produced on the agar media. IFO 32117, 32118.

Range: 1, 2, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 27, 28, 29, 30, 33, 35, 36, 37, 38, 39.

Corollospora pseudopulchella Nakagiri & Tokura Fig. 14

Trans. mycol. Soc. Japan 28: 428, 1987

Ascospores: 65-97.5 x 7.5-11.5 μm, fusiform, slender, 7-11 septate, hyaline.

Appendages: fibrous, peritrichous, at both ends of the spore, 7.5-12.5 μm long, and around the central septum, 18-31 μm long, developed by fragmentation and peeling of the exospore.

Culture: olive to olive gray colony on SWS. Single-spore isolates produced ascocarps on sand grains by the "quartz sand method". Catenulate or bulbil-like chlamydospores of globose to subglobose, brown cells were produced on the agar media. IFO 32112, 32113.

Range: 1, 4, 6, 10, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 26, 28, 30, 33, 34.

Corollospora pulchella Kohlm., Schmidt & Nair Fig. 15

Ber. Dtsch. Bot. Ges. 80: 98, 1967

Anamorph: Clavatospora bulbosa (Anastasiou) Nakagiri & Tubaki, Bot. Mar. 28: 489, 1985

Ascospores: 60-97 x 8-12.5 um, fusiform, straight or slightly curved, (5-)7-9(-10)-septate, constricted at the septa, hyaline.

Appendages: fibrous, peritrichous at both ends of the spore, 7.5-13 um long and around the central septum, 13-28 μm long, developed by fragmentation and peeling of the exospore.

Culture: olive to olive gray colony on SWS. Single-spore islolates produced ascocarps on sand grains by the "quartz sand method", but not on

agar media. Conidia and catenulate chlamydospores of subglobose to oblong, olive brown cells were produced on the agar media. IFO 32123, 32124.

Range: 37, 38, 39.

Corollospora quinqueseptata Nakagiri in Nakagiri and Tokura Fig. 16
Trans. mycol. Soc. Japan 28: 430, 1987

Ascospores: (37.5-)41.3-58.8 x 7.5-9.8 μm (excluding polar appendages), fusiform, (3-)5(-8)-septate, hyaline.

Appendages of two kinds: (i) a single terminal appendage at each end of the spore, 5.3-12 μm long, spine- or thorn-like, attenuate; (ii) fibrous and peritrichous appendages on the terminal appendages, 7.5-11.3 μm long, and around the central septum, 16.8-24.5 μm long, developed by fragmentation and peeling of the exospore.

Culture: white to brownish gray colony on SWS. Single-spore isolates produced ascocarps on the agar media and on the glass of slant tubes. IFO 32115, 32116.

Range: 2, 10, 20, 23, 28, 34, 35.

Halosphaeria appendiculata Linder in Barghoorn and Linder Fig. 17
Farlowia 1: 412, 1944
= Remispora ornata Johnson & Cavaliere, Nova Hedwigia 6: 188, 1963

Ascospores: 18.8-25 x 7.5-10 μm, ellipsoid, one-septate, not or slightly constricted at the septum, hyaline.

Appendages: a single terminal appendage at each end of the spore, 7.5-12.5 μm long, membranous, obclavate, attenuate, curved, spoon-shaped at the base; (3-)4 similar radiating appendages around the septum; developed by outgrowth of the spore.

Culture: dark brown colony with white aerial hyphae on SWS. Single-spore isolates produced ascocarps on SWS. IFO 32147, 32148.

Range: 6, 10, 12, 22, 29.

Halosphaeria torquata Kohlm. Fig. 18

Nova Hedwigia 2: 311, 1960

Ascospores: 22-28 x 10-15 μm, broadly ellipsoidal, one-septate, not or slightly constricted at the septum, hyaline.

Appendages: a single terminal appendage at each end of the spore, 5-11 μm long, 2-3 μm in diam at the base, subcylindrical, attenuate; a tubular annulus around the septum, 2-4 μm thick.

Culture: brown to dark brown colony on SWS. Catenuate chlamydospores of globose to subglobose, brown cells were produced on SWS. AN-627, 628, 687.

Range: 1, 6, 15, 16, 18, 28, 31, 33.

Halosphaeriopsis mediosetigera (Cribb & Cribb) Johnson Fig. 19

J. Elisha Mitchell Sci. Soc. 74: 44, 1958

≡ Halosphaeria mediosetigera Cribb & Cribb, Univ. Queensl. Pap., Dep. Bot. 3: 100, 1956

= Halosphaeria mediosetigera var. grandispora Kohlm., Nova Hedwigia 2: 310, 1960

Anamorph: Trichocladium achrasporum (Meyers & Moore) Dixon in Sheare & Crane, Mycologia 63: 244, 1971

Ascospores: 24.5-36.3 x 6.5-10 μm, ellipsoid to fusiform, one-septate, not constricted at the septum, hyaline.

Appendages: a single terminal appendages at each end of the spore, inverted cap-shaped; 3(-4) crescent-shaped appendages around the septum, 12.5-17.5 μm long, rigid, attenuate, obliquely attatched to the septum; developed by spiral fragmentation and peeling of the exospore.

Culture: dark brown to dark gray colony on SWS. Single-spore isolates produced ascocarps on SWS. Japanese strains have never produced Trichocladium conidia in culture; but catenulate and brown colored chlamydospores, which were similar in shape to the conidia, were produced. IFO 32127, 32128.

Range: 1, 14, 15, 17, 18, 22, 27, 28, 30, 32, 38, 39.

Kohlmeyeriella tubulata (Kohlm.) Jones, R. G. Johnson & Moss Fig. 20
Bot. J. Linn. Soc. 87: 210, 1983
≡ Corollospora tubulata Kohlm., Ber. Dtsch. Bot. Ges. 81: 53, 1968

Ascospores: 130-165 x 16-25 μm (including polar appendages), fusiform, curved, frequently C-shaped, repand on outer side, smooth on inner side, one-celled, thick-walled, hyaline.

Appendages: polar, tube-like, (20-)32-47 X 3-6 μm, curved, rigid, slightly tapering, mucus-filled; mucus released from an apical pore, forming a persistent gelatinous globule at the mouth of the tube.

Culture: grayish brown colony on SWS. Single-spore isolates did not produce ascocarps on the agar media. Catenulate chlamydospores of globose to subglobose or oblong, brown to dark brown cells were formed in culture. IFO 32149, 32150.

Range: 1, 5, 19.

Lindra obtusa Nakagiri & Tubaki Fig. 21
Mycologia 75: 488, 1983
Anamorph: Anguillospora marina Nakagiri & Tubaki, ibid. 75: 488, 1983

Ascospores: 182.5-250(-313) x 2.3-3.2(-3.8) μm, filiform, rounded at the ends (not inflated or tapering), curved or crooked (S, U, α-shaped), 9-16(-21)-septate, not or barely constricted at the septa, hyaline.

Appendages: absent.

Culture: white to pale yellow colony on SWS. Single-spore isolates produced ascocarps and conidia on the agar media. IFO 31317, 31318.

Range: 6, 8, 9, 10, 30, 33, 34.

Lindra thalassiae Orpurt, Meyers, Boral & Simms Fig. 22
Bull. Mar. Sci. Gulf. Caribb. 14: 406, 1964

Ascospores: 275-438 x 5-6 μm, filiform, tapering toward both apices, curved (S, U, α-shaped), 18-24-septate, not or barely constricted at the septa, hyaline; tips slightly inflated.

Appendages: absent.

Culture: white colony on SWS. Single-spore isolates freely produced ascocarps in culture on the surface of the agar media, on the mycelia and on the glass wall. IFO 32131, 32132.

Range: 1, 4, 5, 10, 20, 33, 34, 35, 40.

Lulworthia crassa Nakagiri Fig. 23

Trans. mycol. Soc. Japan 25: 378, 1984

Ascospores: 140-205 x 5-8 μm (including appendages), allantoid, curved, non-septate, hyaline.

Appendages: mucus filling chamber at each end of spore, conical or tubular, 20-33 μm long; a drop of mucilage is released through an apical pore.

Culture: dark brown to dark gray or black colony on SWS. Single-spore isolates produced ascocarps on sand grains by the "quartz sand method", but not on the agar media. IFO 32133, 32134.

Range: 5, 6, 10, 16, 18, 19, 20, 21, 28, 39.

Lulworthia lignoarenaria Koch & Jones Fig. 24

Mycotaxon 20: 389, 1984

Ascospores: 350-450 x 4-5.5 μm (including appendages), filiform, curved, 25-31-septate, hyaline.

Appendages: mucus filling chamber at each end of spore, conical or tubular, 33-53 μm long, ; a drop of mucilage is released through an apical pore.

Culture: dark brown or dark green to black colony on SWS. Ascocarp was not produced in culture. IFO 32135, 32136.

Range: 4, 5, 6, 8, 16, 17, 19, 28.

Marinospora calyptrata (Kohlm.) Cavaliere Fig. 25

Nova Hedwigia 11: 548, 1966

≡ Ceriosporopsis calyptrata Kohlm., Nova Hedwigia 2: 301, 1960

≡ Ceriosporella calyptrata (Kohlm.) Cavaliere, ibid. 10: 394, 1966

Ascospores: 23-32 x 8-13 μm (excluding appendages), ellipsoid, one-septate, constricted at the septum, hyaline.

Appendages: a single terminal appendage at each end of spore, obclavate or subcylindrical, tapering, 5-17.5 x 2.5-5 μm; around the septum, 3-4 similar, radiating appendages; small, 1-2 μm high, cupuliform, thin caps, which may invert, cover the apices of appendages.

Culture: brown to dark brown colony on SWS. Ascocarp was not produced in culture. IFO 32151.

Range: 1, 5, 6, 18, 28.

Nereiospora cristata (Kohlm.) Jones, R. G. Johnson & Moss Fig. 26
Bot. J. Linn. Soc. 87:206, 1983
≡ Peritrichospora cristata Kohlm., Nova Hedwigia 2: 324, 1960
≡ Corollospora cristata (Kohlm.) Kohlm., Ber. Dtsch. Bot. Ges. 75: 126, 1972

Ascospores: 29-37 x 14-19 μm (excluding appendages), ellipsoid, 2-3-septate, constricted at the septa, central cells brown, apical cells hyaline.

Appendages: seta-like, flexible, attached in a tuft to each apex and in several tufts around the central septum; apical setae, 7-10 μm long; lateral setae, 9-13 μm long developed by outgrowth of the spore.

Culture: brownish gray to black colony on SWS. Catenulate and branched chlamydospores of globose to subglobose or ellipsoidal to oblong, light brown cells were formed in culture. AN-671, 672, 673, 891, 892, 893.

Range: 1.

Trailia ascophylli Sutherland Fig. 27
Trans. Br. mycol. Soc. 5: 149, 1915

Ascospores: 85-110 x 3-4 μm, filamentous, tapering, curved, 1-4-septated, not constricted at the septa, hyaline.

Appendages: absent.

Culture: white to cream colored colony on SWS. Ascocarp primordium-like structure was observed in culture. AN-509.

Range: 1, 2, 8, 9, 18, 20, 22, 27, 28, 29, 38.

Sphaeriaceae

Chaetosphaeria sp. Fig. 28

Ascospores: (33-)40-66 x 6-9 μm, ellipsoid or cylindrical, 3(-6)-septate, hyaline.

Appendages: peritrichous appendages around the central septum, 13-25 μm long, developed by fragmentation and peeling of the exospore.

Culture: white colony on SWS. Single-spore isolates produced ascocarps on the agar media.

Range: 1, 8.

Note: this fungus is similar to Ch. chaetosa Kohlm. except that the latter has smaller ascospores (24-36.5 x 6-11.5 μm) and produces coriaceous ascocarps. Further reseach on the taxonomic position of this fungus is necessary.

incertae sedis

Torpedospora radiata Meyers Fig. 29

Mycologia 49: 496, 1957

Ascospores: 20-47 x 3.5-5 μm, cylindrical or clavate, broader at the apex, (2-)3-septate, not or slightly constricted at the septa, hyaline.

Appendages: 3(-4) radiating appendages on the lower end, 10-26 x 1.0-2.5 μm, semirigid, straight or slightly curved, with a thick base, tapering toward the apex.

Culture: hyaline to light brown colony on SWS. Single-spore isolates produced ascocarps in culture. IFO 32145, 32146.

Range: 20, 27, 28, 29, 30, 32, 33, 34, 36, 38.

Basidiomycotina

Gasteromycetes

Melanogastrales
Melanogastraceae

Nia vibrissa Moore & Meyers Fig. 30
Mycologia 51: 874, 1959

Basidiospores: 10-16 x 4-8 μm (excluding appendages), ovoid to pyriform, one-celled, hyaline, at the point of attachment to the basidium with a short cylindrical projection.

Appendages: a single appendage at the apex, slender, flexible, attenuate, hyaline, 20-33 μm long, less than 1.5 μm in diam, terminally slightly inflated; 3-4 similar, subterminal radiating appendages around the base, 15-27 μm long.

Culture: hyaline to cream colored colony on SWS. Some strains of single-spore isolates produced basidiocarps on the agar media. IFO 32088, 32089, 32090.

Range: 1, 5, 6, 8, 10, 17, 18, 20, 22, 26, 27, 28, 30, 38.

Deuteromycotina
Hyphomycetes
Hyphomycetales
Moniliaceae

Anguillospora marina Nakagiri & Tubaki Fig. 31
Mycologia 75: 488, 1983
Teleomorph: Lindra obtusa Nakagiri & Tubaki, ibid. 75: 488, 1983

Conidia: 150-255(-312.5) x 2.5-4 μm, filiform, straight or curved, 9-13(-19)-septate, swollen at both ends, hyaline.

Culture: white to yellow colony on SWS. Single-spore isolates produced conidia and ascocarps on the agar media.

Conidiogenous cells: hyaline, holoblastic, terminal, percurrent, without a separating cell.

Range: 3, 6, 7, 8, 9, 10, 30, 33.

Sigmoidea luteola Nakagiri & Tubaki Fig. 32

Trans. mycol. Soc. Japan 23: 102, 1982

Teleomorph: Corollospora luteola Nakagiri & Tubaki, ibid. 23: 102, 1982

Conidia: 106-222.5 μm long, 1.3-2.5 μm in diam at the base, 4.5-7.5 μm in diam at the central cell, filiform, curved, 7-13(-18) septate, constricted at the septa, hyaline; terminal and basal cells of mature conidia are devoid of contents.

Culture: pale yellow to yellow colony on SWS. Single-spore isolates produced conidia and ascocarps on the agar media.

Conidiogenous cells: hyaline, holoblastic, terminal, irregularly sympodial and denticulate.

Range: 1, 4, 5, 6, 8, 14, 15, 16, 24, 27.

Sigmoidea marina Haythorn & Jones in Haythorn, Jones and Harrison Fig. 33

Trans. Br. mycol. Soc. 74: 620, 1980

Conidia: (88-)103-153 μm long, 1.5-2.5 μm in diam at the base, 2.8-5 μm in diam at the central cell, filiform, curved, 6-10-septate, constricted at the septa, hyaline; terminal and basal cells of mature conidia are devoid of contents.

Culture: hyaline to white colony on SWS. Isolates produced conidia on the agar media. IFO 32159, 32160.

Conidiogenous cells: hyaline, holoblastic, terminal, sympodial and denticulate.

Range: 1, 3, 7, 8, 10, 22, 28.

Varicosporina prolifera Nakagiri Fig. 34

Trans. mycol. Soc. Japan 27: 198, 1986

Teleomorph: Corollospora intermedia I. Schmidt, Nat. Naturschutz Mecklenburg 7: 6, 1969 (publ. 1971)

Conidia: three-dimensionally branched, septate, hyaline; main axis,

25-57 μm long, 2.5-5 μm in diam at the apex, 1-2(-4)-septate; first side branch, 18-38.8 μm long, 2.5-4(-5) μm in diam at the apex, 1-2(-3)-septate, arising perpendicularly from the apical or central cell of the main axis; second side branch, 15-32.5 μm long, 2,7-5 μm in diam at the apex, 1-2(-3)-septate, arising perpendicularly from the apical or central cell of the first side branch; third side branch, rare in some strains, 8-21.3 μm long, 3-4.5 μm in diam at the apex, 1-2-septate, arising perpendicularly from the apical or central cell of the second side branch. Very often, conidia are released before the third side branch grows out. The basal cell of the main axis, which is attached to the conidiogenous cell, is often devoid of content when conidia are released.

Culture: white colony on SWS, turning dark olive to black in age. Isolates produced conidia and immature ascocarps on the agar media.

Conidiogenous cells: hyaline, holoblastic, terminal, sympodial and flat-topped denticulated, sometimes producing conidia in a manner intermediate between the sympodial-type and percurrent proliferation.

Range: 28, 34.

Varicosporina ramulosa Meyers & Kohlmeyer Fig. 35

Can. J. Bot. 43: 916, 1965

Conidia: three-dimensionally branched, septate, hyaline; main axis, 27.5-62.5 μm long, 1.5-2.5 μm in diam at the apex, 1-3-septate; first side branch, 30-58 μm long, 2.7-5 μm in diam at the apex, 3-5-septate, arising perpendicularly from the central cell of the main axis; second side branch, 27.5-46.5 μm long, 3.3-5 μm in diam at the apex, 2-4-septate, arising perpendicularly from the central cell of the first side branch; third side branch, 22.5-37.5 μm long, 4-5 μm in diam at the apex, 2(-4)-septate, arising perpendicularly from the central cell of the second side branch. Very often, conidia are released before the third side branch grows out.

Culture: white colony on SWS and turned to dark olive to black in age. Isolates produced conidia and "sclerocarps", degenerate ascocarps, which was considered to have lost the ability to produce ascospores (3). IFO 32163.

Conidiogenous cells: hyaline, holoblastic, terminal, sympodial and denticulate or monoblastic on lateral or terminal determinate conidiophore.

Range: 7, 14, 15, 28, 29, 35, 38, 39.

Dematiaceae

Asteromyces cruciatus Moreau & Moreau ex Hennebert Fig. 36
Can. J. Bot. 40: 1213, 1962

Conidia: 7.5-13 x 4.8-6 μm, ovoid to pyriform, one-celled, thin-walled, brown, originating successively on denticles on the conidiogenous cell; the first conidium is apical, the following ones are in one or more lateral whorls of usually eight conidia. Conidia are usually released in aggregates attached to the conidiogenous cell.

Culture: brown to greenish gray colony on SWS. Greenish grey conidial masses concentrically scatter on the surface of the agar media. Reddish brown pigment diffuses into the media. IFO 32141, 32142.

Conidiogenous cells: hyaline to brown, holoblastic, 7.5-20 μm long (including stalk), 3-5.5 μm in diam, subglobose with stalk, hyaline or light brown, bearing up to 15 conidia on the denticles.

Range: 1, 4, 5, 8, 10, 14, 20, 24, 25, 27, 28, 33.

Clavatospora bulbosa (Anastasiou) Nakagiri & Tubaki Fig. 37
Bot. Mar. 28: 489, 1985
≡ Clavariopsis bulbosa Anastasiou, Mycologia 53: 11, 1961
Teleomorph: Corollospora pulchella Kohlm., Schmidt & Nair, Ber. Dtsch. Bot. Ges. 80: 98, 1967

Conidia: tetraradiate, with (1-)2-3 arms, septate, slightly constricted at the septa, light brown; main axis, one-septate; proximal cell, 9-17 μm high, 5.5-9.5 μm in diam, ellipsoidal to ovoid, truncate at the base; distal cell, 5.5-13 μm high, 7.5-12 μm in diam, arising simultaneously from the inflated distal cell; arms, 7-45 x 4.5-7 μm, cylindrical, 1-4-septate.

Culture: olive to olive gray colony on SWS. Isolates produced conidia on the aerial hyphae on the agar media.

Conidiogenous cells: olive, holoblastic, terminal, proliferating

simpodially or rarely percurrently at the apex.

Range: 28, 29, 34, 36, 38, 39.

Orbimyces spectabilis Linder in Barghoorn & Linder Fig. 38

Farlowia 1: 404, 1944

Conidia: consisting of a large dark cell with one or two crowns of septate, light colored appendages at the distal end; basal cell, 15.5-27.5 x 15-27.5 μm, globose to subglobose, thick-walled, shining black, smooth, with a scar of detachment from conidiophore at the base; radiating appendages, septate, consisting of a central cell and (1-)3-6(-7) arms; arms, 11-37 x 3-4 μm, cylindrical, (0-)1-4(-5)-septate, slightly constricted at the septa, light brown at the base, subhyaline at the apex.

Culture: dark green to black colony on SWS. Isolates produced conidia abundantly on the agar media. IFO 32157, 32158.

Conidiogenous cells: hyaline, holoblastic, terminal, determinate.

Range: 1, 5, 8, 16.

Of the marine fungi whose spores were accumulated in sea foam, the halosphaeriaceous ascomycetes are dominant as shown in the above list. Ascospores of the species of Arenariomyces, Carbosphaerella, Corollospora, Kohlmeyeriella and some species of Lindra and Lulworthia, which are so-called arenicolous fungi (4), were frequently found in sea foam. They live among or on grains of sand and produce hard, carbonaceous ascocarps on sand grains or shells of marine animals. Spores of the lignicolous fungi., e.g., Halolsphaeria appendiculata, H. torquata, Halosphaeriopsis mediosetigera, which live in wood and produce thin-walled ascocarps in the wood, were barely found in sea foam. Basidiospores of Nia vibrissa were also observed in sea foam around the Japanese coast. In hyphomycetes, conidia of Asteromycetes cruciatus, Sigmoidea spp. and Varicosporina ramulosa were common in sea foam.

Isolation of fungal spores from sea foam samples was useful for correct identification and life historical study (7, 8). Most of the arenicolous ascomycetes produced ascocarps on the glass wall of a slant tube or on sand grains by the "quartz sand method". Single spore isolates

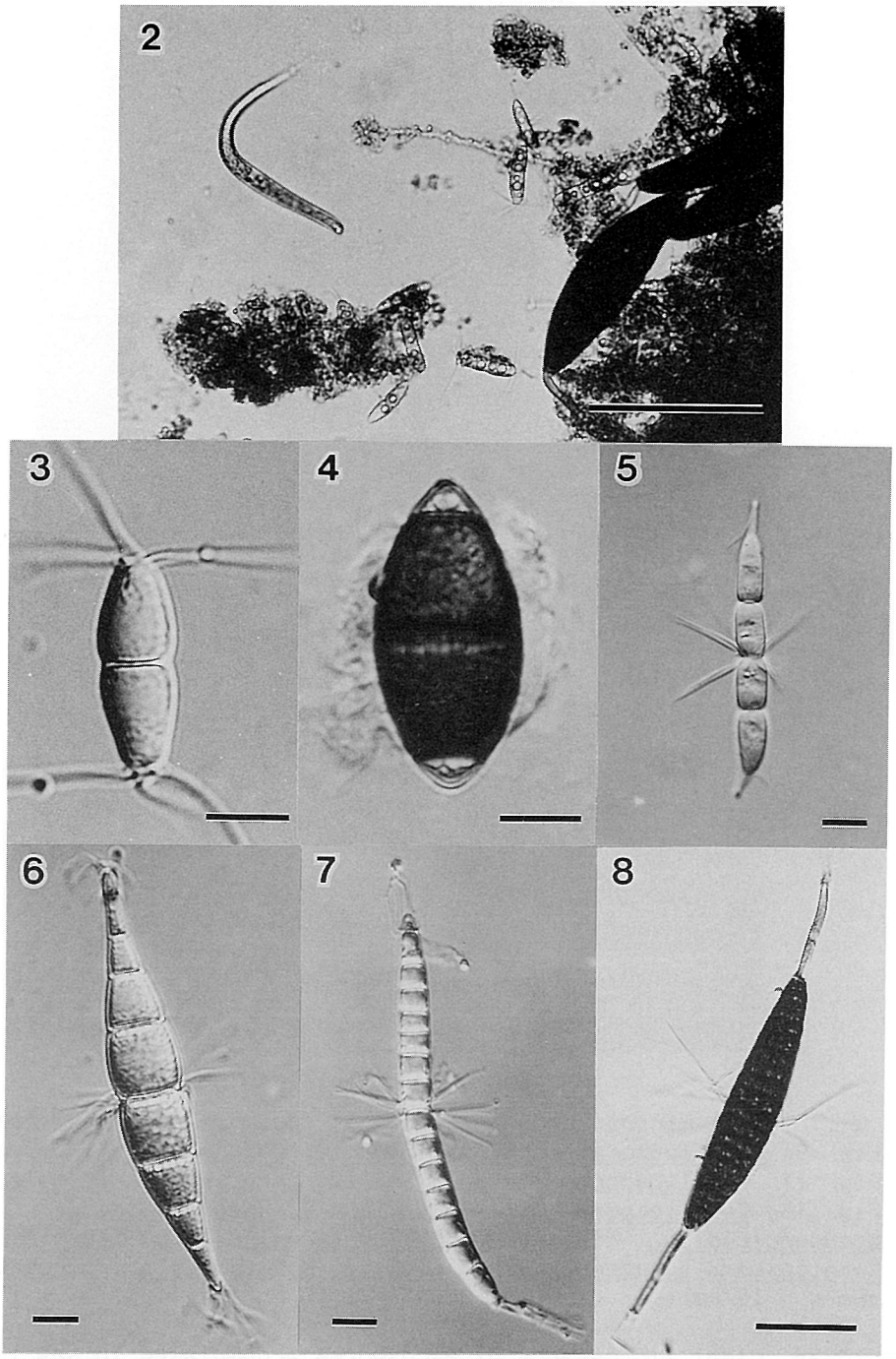
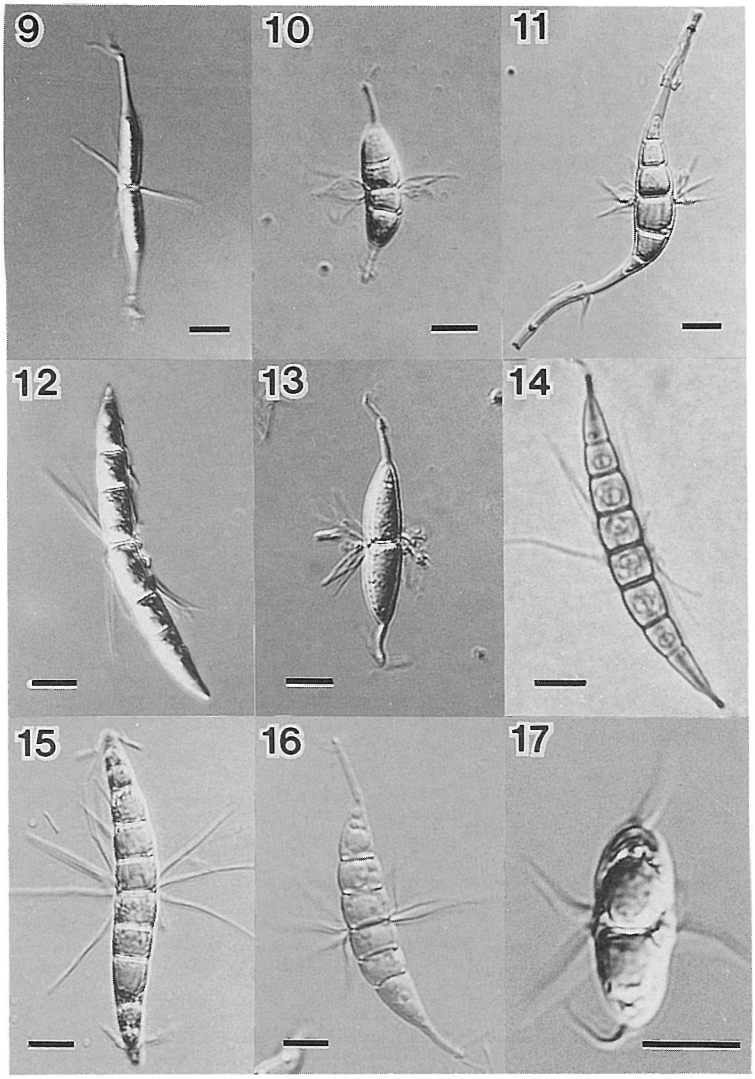


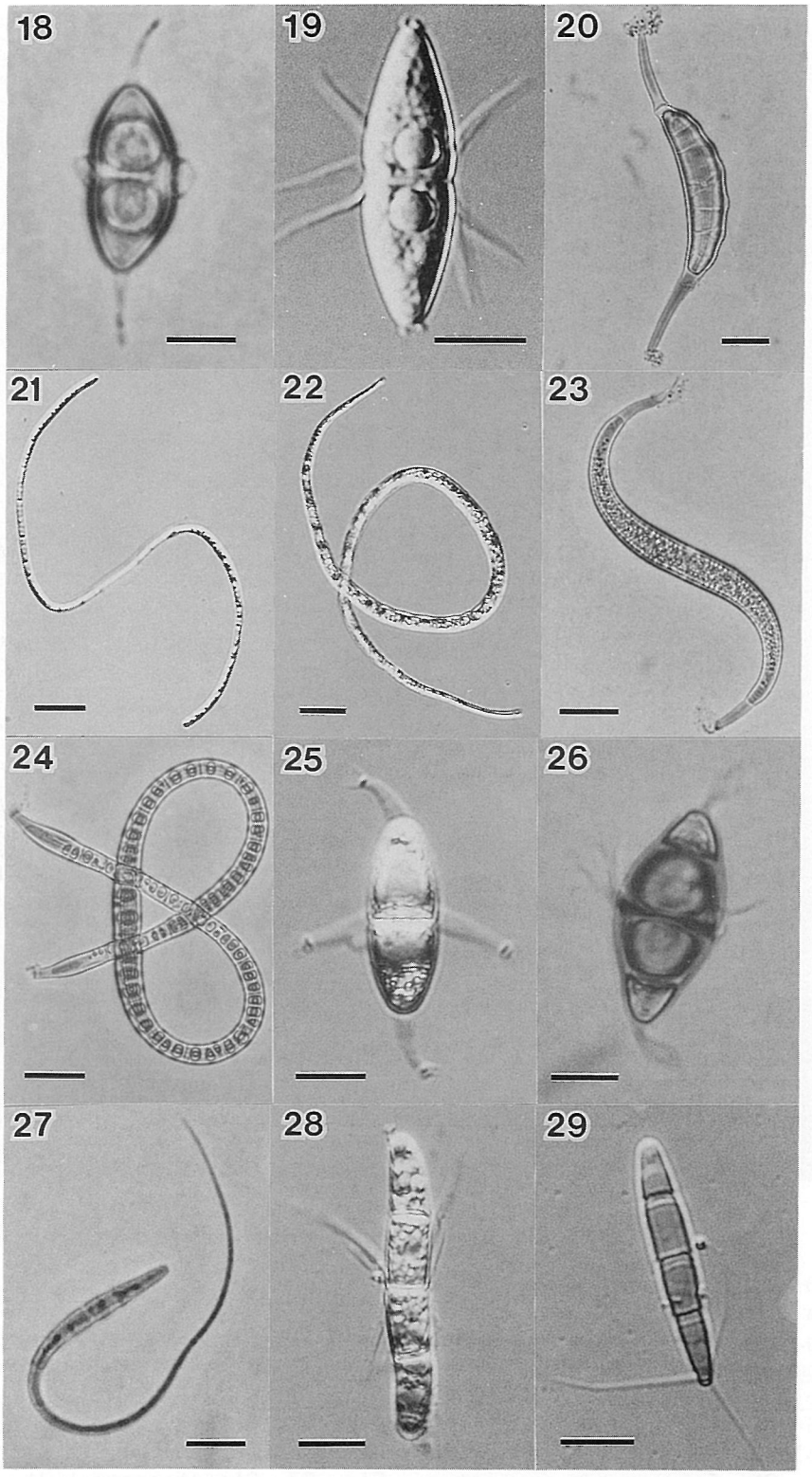
Fig. 2. Light micrograph of sea foam with accumulated marine fungal spores and debris. (Bar = 100 μm)

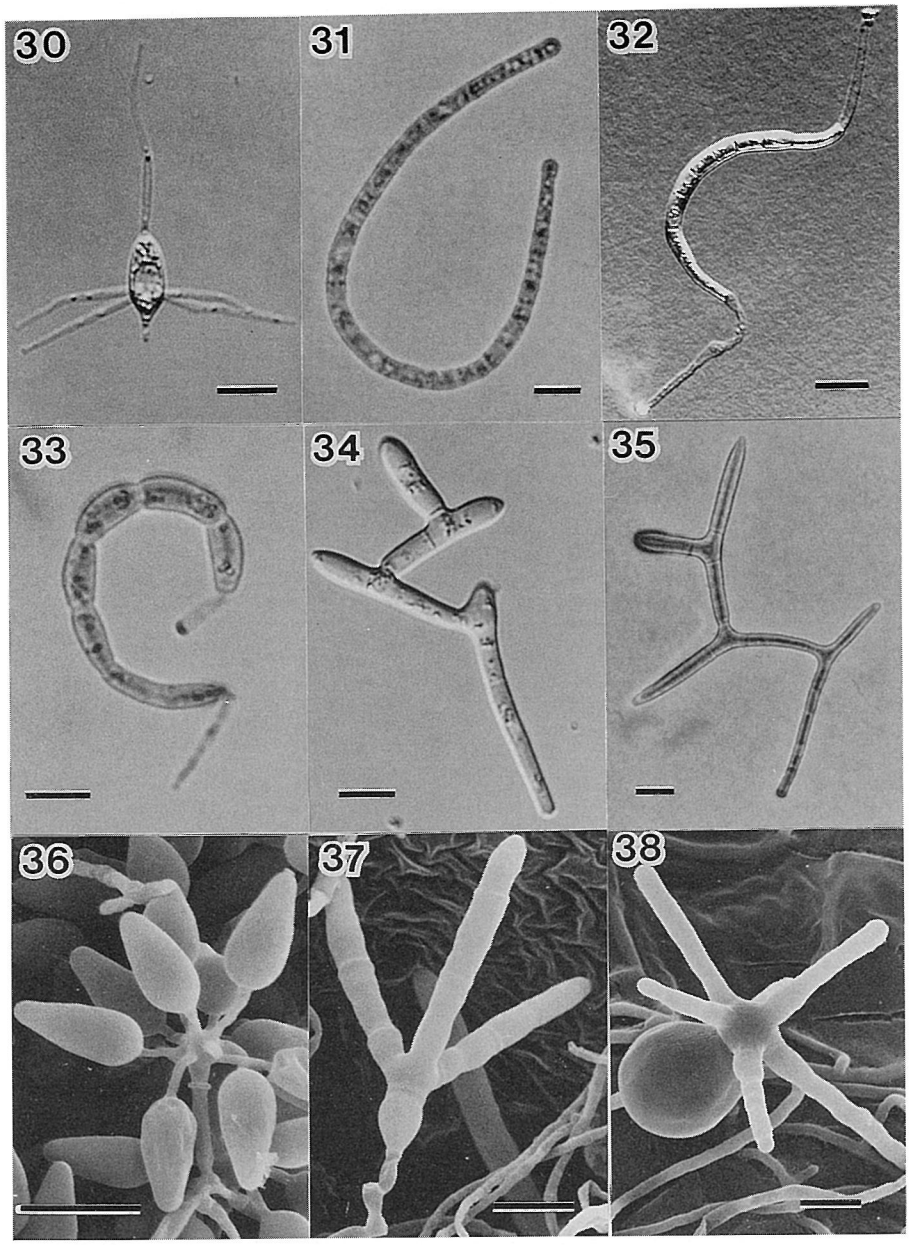
Figs. 3-8. Light micrographs of ascospores accumulated in sea foam. Fig. 3. Arenariomyces trifurcatus. Fig. 4. Carbosphaerella leptosphaerioides. Fig. 5. Corollospora angusta. Fig. 6. Corollospora colossa. Fig. 7. Corollospora filiformis. Fig. 8. Corollospora fusca. (Bars = 10 μm)



Figs. 9-17. Light micrographs of ascospores accumulated in sea foam.
Fig. 9. Corollospora gracilis. Fig. 10. Corollospora intermedia.
Fig. 11. Corollospora lacera. Fig. 12. Corollospora luteola.
Fig. 13. Corollospora maritima. Fig. 14. Corollospora pseudopulchella. Fig. 15. Corollospora pulchella. Fig. 16. Corollospora quinqueseptata. Fig. 17. Halosphaeria appendiculata.
(Bars = 10 μm)

Figs. 18-29. Light micrographs of ascospores accumulated in sea foam.
Fig. 18. Halosphaeria torquata. Fig. 19. Halosphaeriopsis mediosetigera. Fig. 20. Kohlmeyeriella tubulata. Fig. 21. Lindra obtusa. Fig. 22. Lindra thalassiae. Fig. 23. Lulworthia crassa.
Fig. 24. Lulworthia lignoarenaria. Fig. 25. Marinospora calyptrata.
Fig. 26. Nereiospora cristata. Fig. 27. Trailia ascophylli.
Fig. 28. Chaetosphaeria sp. Fig. 29. Torpedospora radiata.
(Bars: 18-19, 25-29 = 10 μm; 20-24 = 20 μm)


18
19
20
21
22
23
24
25
26
27
28
29



Figs. 30-35. Light micrographs of basidiospore and conidia accumulated in sea foam.
Fig. 30. Nia vibrissa. Fig. 31. Anguillospora marina. Fig. 32. Sigmoidea luteola. Fig. 33. Sigmoidea marina. Fig. 34. Varicosporina prolifera. Fig. 35. Varicosporina ramulosa. (Bars = 10 um)
Figs. 36-38. Scanning electron micrographs of conidia produced in culture. Isolates were obtained from sea foam.
Fig. 36. Asteromyces cruciatus. Fig. 37. Clavatospora bulbosa.
Fig. 38. Orbimyces spectabilis. (Bars = 10 um)

succeeded in producing ascocarps and ascospores, so they are homothalic in sexual reproduction. Some single-spore isolates of Nia vibrissa reproduced basidiospores in culture. All of the marine hyphomycetes easily produced conidia on the agar media.

Discussion

Spores of marine fungi were often contained abundantly in sea foam; and some samples yielded more than 20 species. Although sea foam contains phytoplanktons, protozoa, nematodes, blue-green algae, bacteria, other marine organisms and fungal spores with debris, spores of marine fungi are easily detectable under the microscope because of their peculiar morphology. Besides the marine fungi, plenty of spores of freshwater aquatic hyphomycetes, which are tetraradiate, sigmoid or helicoid in shape, and terrestrial fungi, e.g., Pestalotiopsis, Camposporium, Fusarium, were often accumulated in sea foam on shores close to the mouths of rivers. These fungal spores that had flowed from the river into the sea were considered unable to germinate and grow in a marine environment (1). Sea foam on beaches contained abundant spores of the arenicolous fungi. Arenicolous fungi attaching to sand grains were observed and 11 species were reported from the Japanese coast by Tokura (13). These species from sand samples were mostly included in the species found in foam samples. However, lignicolous or algicolous species were rarely contained in sea foam. So, other methods of collecting samples, e.g., drift wood and living or washed-up algal thallus, are necessary to investigate the total marine fungal flora of a location.

The spores of marine fungi accumulated in sea foam are mostly appendaged or sigmoid or branched and contain conspicuous oil globules in the cells. These characteristics were considered to be advantageous for arenicolous fungi in floating in seawater, becoming trapped between air bubbles and attaching to new substrates (4). These hyphotheses were investigated experimentally (5, 11, 12), and the roles of the spore appendages and sigmoid or branched shapes of marine fungal spores were found to be to reduce the rate sinking in seawater and to anchor the spore on the substrate.

The frequency of marine fungal species in sea foam was found to depend

on locality and region. The sea foam collected on rocky shores, even those which adjoined a sand beach, barely contained arenicolous marine fungal spores, whereas the foam on sand beaches accumulated many spores. So, the fungal spores may not be dispersible over a long distance. This, however, should be examined carefully in further research, with consideration of the spore survival through transportation. With respect to geographical distribution, three types of arenicolous fungi were observed, that is, (i) species distributed widely throughout Japan, e.g., A. trifurcatus, C. marintima, N. vibrissa; (ii) species distributed in the northern part of Japan, e.g., K. tubulata, N. cristata, Chaetosphaeria sp.; (iii) species distributed in the southern part of Japan, e.g., C. filiformis, C. pulchella, V. prolifera and Clav. bulbosa. In addition to the geographical distribution, a seasonal change in reproduction was observed in some species. In winter in the northern part of Japan, conidia of marine hyphomycetes were rarely contained in sea foam whereas a small number of ascospores were still observed. In warmer seasons, there were abundant conidia accumulated in sea foam. Alternation of morphs according to seasons were detected in a holomorphic species, C. intermedia - V. prolifera by collecting sea foam throughout a year (9).

Sea foam is a useful source for research on marine fungi, especially to know the fungal flora of a beach. Coastal sea foam is expected to be examined by marine mycologists world-wide to accumulate distribution data on marine fungi.

This work was carried out at the Institute of Biological Sciences, University of Tsukuba. The author is indebted to Professor Keisuke Tubaki (now of the College of Pharmacy, Nihon University) for his constant guidance in the course of this study.

References

1) Byrne, P. J., and E. B. G. Jones. 1975. Effect of salinity on the reproduction of terrestrial and marine fungi. Trans. Br. mycol. Soc. 65: 185-200.
2) Ingold, C. T. 1975. An illustrated guide to aquatic and water-borne Hyphomycetes (Fungi Imperfecti) with notes on their biology. Freshwater Biol. Assoc., Sci. Publ. 30: 1-96.

3) Kohlmeyer, J., and T.M. Charles. 1981. Sclerocarps: undescribed propagules in a sand-inhabiting marine fungus. Can. J. Bot. 59: 1787-1791.
4) Kohlmeyer, J., and E. Kohlmeyer. 1979. Marine mycology, The higher fungi. Academic Press, New York.
5) Nakagiri, A. 1988. Structure and function of conidia of Varicosporina species (marine Hyphomycetes). Trans. Br. mycol. Soc. 90: 265-271.
6) Nakagiri, A., and R. Tokura. 1987. Taxonomic studies of the genus Corollospora (Halosphaeriaceae, Ascomycotina) with descriptions of seven new species. Trans. mycol. Soc. Japan 28: 413-436.
7) Nakagiri, A., and K. Tubaki. 1982. A new marine ascomycete and its anamorph from Japan. Trans. mycol. Soc. Japan 23: 101-110.
8) Nakagiri, A., and K. Tubaki. 1983. Lindra obtusa, a new marine ascomycete and its Anguillospora anamorph. Mycologia 75: 487-497.
9) Nakagiri, A., and K. Tubaki. 1985. Teleomorph and anamorph relationships in marine Ascomycetes (Haloshpaeriaceae). Bot. Mar. 28: 485-500.
10) Nilsson, S. 1964. Freshwater Hyphomycetes. Taxonomy, morphology and ecology. Symb. Bot. Ups. 18: 1-130.
11) Rees, G. 1980. Factors affecting the sedimentation rate of marine fungal spores. Bot. Mar. 23: 375-385.
12) Rees, G., and E. B. G. Jones. 1984. Observations on the attachment of spores of marine fungi. Bot. Mar. 27: 145-160.
13) Tokura, R. 1982. Arenicolous marine fungi from Japanese beaches. Trans. mycol. Soc. Japan 23: 423-433 (in Japanese).
14) Tubaki, K. 1978. On the Skerman's micromanipulator and microforge. Trans. mycol. Soc. Japan 19: 237-239 (in Japanese).
15) Tubaki, K., and A. Nakagiri. 1982. Marine fungi in and around Teshio-cho. III. Human Cult. Environ. Stud. Northern Hokkaido 3: 35-46 (in Japanese).

IFO Res. Comm. 14,
80-103, 1989 (March)

PRESERVATION OF YEAST CULTURES BY L-DRYING: VIABILITY AFTER 5 YEARS OF STORAGE AT 5 C

KOZABURO MIKATA and ISAO BANNO

Summary

Viabilities of the L-dried cultures of 701 strains preserved for 5 years at 5 C were examined. The survival counts of 28 strains were less than 10^4 per ampule. It was estimated from the survival reduction rates that dried cultures preserved at 5 C would retain survival counts of more than 10^4 for over 25 years in 505 strains, over 15 years in 83 strains, and over 10 years in 85 strains.

The survival values after 5 years of storage at 5 C corresponded fairly well to the survival values in the 60-day accelerated storage test at 37 C in the case of yeast strains with viabilities higher than 1% in the accelerated test.

The method of L-drying has been successfully applied to the long-term preservation of yeasts (1, 2). All the yeasts maintained in the IFO yeast collection were L-dried and have been preserved in a cold room at 5 C. Viabilities of these dried cultures in an accelerated storage test at 37 C for 60 days were previously reported (2). In a preliminary investigation with 42 strains of various yeasts, the viability decreased exponentially at a constant rate during storage and their survival values after 5 years of storage at 5 C corresponded to those in the 60-day accelerated storage test (2). On the basis of this result, the decimal reduction time in the viability at 5 C was estimated for each of the dried cultures from their

viabilities in the accelerated storage test.

In this study, viabilities of the dried cultures of 701 strains which had been preserved for 5 years at 5 C were examined and compared with those in the accelerated storage test reported in the previous paper (2).

Materials and Methods

Seven hundred and one strains belonging to 271 species in 42 genera, shown in Tables 1-6, were L-dried by the standard method described previously (1) and have been stored in a cold room at 5 C. Two of 18 ampules of the dried cultures which had been preserved for 5 years were rehydrated.

Viability was determined as reported previously (1). Viable counts per ampule were determined and survival values were expressed as percentages of surviving cells relative to the viable counts before drying.

Reduction rate per year (R_y) is estimated by equation [1]: $R_y = (S_{5y} / S_0)^{\frac{1}{5}}$ (S_{5y}: survival value after 5-year storage, S_0: survival value immediately after drying.

On the assumption that the survival count will decrease exponentially at a constant reduction rate during preservation at 5 C, the time taken for the survival count of dried cultures to reduce to 10^4 per ampule (T_4) was calculated by equation [2]: $T_4 = (4 - \log V_0)/\log R_y$ (V_0: viable count per ampule immediately after drying, R_y: reduction rate per year). Below, T_4 will be called the limit survival time.

Results and Discussion

Strains examined are divided into two categories according to their viabilities: yeasts with survival values higher than 1% in the accelerated storage test for 60 days, and those with survival values lower than 1%.

1. Viabilities after 5-year storage at 5 C of dried cultures with viabilities higher than 1% in the accelerated storage test.

Results obtained for the 553 yeast strains with survival values higher than 1% in the accelerated test are presented in Tables 1-3. In addition

to the survival count and survival value after 5 years of storage at 5 C, viable counts before drying, survival immediately after drying and after the 60-day accelerated storage test are shown for comparison. The survival count is expressed as the logarithm of the number of colony-forming-units. Tables 1, 2, and 3 include respectively 323 ascomycetous yeasts representing to 180 species in 25 genera, 128 deuteromycetous yeasts representing 93 species in 10 genera, and 35 basidiomycetous yeasts representing 12 species in 4 genera. There is no significant difference in the distribution of survival values between ascomycetous, deuteromycetous and basidiomycetous yeasts.

The survival values of 366 of 553 strains were higher than 10% after 5 years at 5 C; those of 169 strains ranged from 1 to 10%; and 12 strains had survival values of 0.5 to 1.0%. Only the following 6 dried cultures had survival values below 0.5%: Debaryomyces pseudopolymorphus IFO 1358, Nadsonia commutata IFO 10029, Pichia humboldtii IFO 10060, Candida albicans IFO 1067, Cryptococcus hungaricus IFO 1379, and Rhodosporidium toruloides IFO 0413. Among these 6 strains, the survival counts of the 4 strains IFO 1358, 10029, 1067, 1379 were already less than 10^4 per ampule after 5 years of storage.

Experience suggests that a survival count of at least 10^4 per ampule is necessary for inexperienced workers to recover sufficient viable cells from a dried culture without failure. The dried cultures whose survival counts have decreased to less than 10^4 should be promptly renewed. The time taken for the survival count of dried cultures to decrease to 10^4 per ampule will be hereafter called limit survival time (LST).

The reduction rate was estimated by equation [1]. The rate ranges from 0.22 to 1.00 and averages 0.87. Dried cultures of 13 strains were reduced at rapid rates of 0.22 to 0.6: Debaryomyces pseudopolymorphus IFO 1358, Nadsonia commutata IFO 10029, Pichia farinosa IFO 0464, P. membranaefaciens IFO 1284, Zygosaccharomyces bailii IFO 1098, Z. rouxii IFO 0443 & 0526, Candida albicans IFO 1067 & 1398, C. halonitratophila IFO 1561, C. versatilis IFO 10038, Cryptococcus hungaricus IFO 1379 and Rhodosporidium toruloides IFO 0413. This implies that these strains are sensitive in the dried state and their dried cells tend to die rapidly.

Limit survival times (LST) of the dried cultures were estimated by equation [2] from their viable counts immediately after drying and their reduction rates, and these are presented in the last column of the tables.

Table 1. Viabilities at 5 C of dried cultures of ascomycetous yeasts with survival values higher than 1% in the 60-day accelerated test at 37 C.

Species	IFO No.	log CFU* per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C** (years)
Ambrosiozyma platypodis	1471	7.50	6.75 (17.2)	6.14 (3.7)	6.54 (10.9)	69.3
Arthroascus javanensis	1848	7.52	6.75 (17.3)	6.08 (3.7)	6.40 (7.6)	38.6
Arxiozyma telluris	1331	7.11	6.20 (11.9)	5.54 (2.6)	5.34 (1.6)	12.7
Botryoascus synnaedendrus	1604	8.51	8.36 (70.0)	8.32 (65.4)	8.38 (72.3)	NC***
Debaryomyces cantarellii	1363	7.11	6.17 (11.5)	5.65 (3.5)	5.74 (4.2)	24.9
D. castellii	1359	7.38	6.11 (5.4)	5.70 (2.1)	5.88 (3.2)	46.5
D. formicarius	10028	7.55	7.30 (55.6)	6.85 (20.0)	7.11 (36.5)	90.1
D. hansenii	1751	8.34	8.14 (65.7)	6.66 (2.1)	7.86 (32.6)	68.3
D. hansenii	1752	8.25	7.97 (52.2)	7.14 (7.8)	7.48 (16.8)	40.4
D. marama	1878	7.99	7.64 (44.6)	7.08 (11.9)	7.78 (60.8)	NC
D. marama	1879	8.18	8.11 (84.2)	7.71 (33.9)	8.08 (77.3)	552.3
D. melissophilus	1900	8.20	7.80 (40.0)	6.95 (5.6)	7.43 (17.3)	52.3
D. melissophilus	1901	7.93	7.58 (44.4)	6.00 (1.2)	7.40 (29.3)	99.2
D. polymorphus	1153	7.16	6.84 (47.6)	6.34 (15.2)	6.44 (19.3)	36.2
D. pseudopolymorphus	1026	7.80	6.72 (8.3)	6.34 (3.5)	6.23 (2.7)	27.9
D. pseudopolymorphus	1358	7.77	6.75 (9.5)	6.08 (2.1)	3.48 (0.005)	4.1
D. vanriji	0934	7.17	6.64 (29.3)	6.47 (20.0)	6.55 (23.7)	143.5
D. yarrowii	1818	7.54	7.34 (61.0)	6.81 (18.5)	6.81 (18.2)	31.7
Dekkera intermedia	1591	7.73	7.00 (19.4)	6.36 (4.3)	6.55 (6.7)	32.7
Dipodascus albidus	1984	5.70	4.50 (6.5)	3.78 (1.2)	4.00 (2.1)	5.2
Endomyces magnusii	0110	6.41	5.87 (29.0)	5.32 (8.2)	5.20 (6.5)	14.5
E. reessii	1112	7.20	6.41 (16.3)	5.92 (5.3)	6.25 (11.7)	83.7
Hanseniaspora guilliermondii	1411	7.59	7.00 (26.4)	6.58 (9.6)	6.82 (16.9)	77.9
H. occidentalis	1718	7.57	7.07 (31.9)	6.98 (25.7)	6.71 (13.9)	42.6
H. occidentalis	1819	8.14	7.82 (46.5)	7.08 (8.6)	7.30 (14.1)	36.9
H. osmophila	1753	8.11	7.77 (45.4)	7.60 (30.8)	7.57 (28.5)	93.2
H. osmophila	1754	8.26	7.91 (43.9)	7.81 (35.3)	7.72 (28.3)	102.5
H. uvarum	1341	7.64	7.05 (25.5)	6.65 (10.2)	6.86 (16.7)	83.0
H. uvarum	1413	7.63	7.25 (41.6)	7.17 (34.4)	7.23 (39.4)	689.2
H. uvarum	1755	8.10	8.00 (79.7)	7.91 (63.9)	7.71 (40.5)	68.1
H. uvarum	1756	8.25	8.04 (59.8)	7.98 (53.8)	7.85 (38.7)	106.7
H. uvarum	1757	7.90	7.75 (70.3)	7.48 (37.7)	7.34 (28.1)	47.1
H. valbyensis	1758	8.25	7.78 (34.1)	7.60 (23.1)	7.71 (29.2)	280.2
H. valbyensis	1759	8.15	7.84 (49.8)	7.76 (42.0)	7.75 (39.9)	199.5
Hansenula anomala	0118	7.11	6.41 (20.0)	6.22 (12.7)	6.04 (8.5)	32.5
H. anomala	1470	7.24	6.62 (22.9)	6.51 (18.9)	6.49 (17.7)	116.3
H. anomala	1760	8.18	7.88 (50.7)	7.70 (33.6)	7.73 (36.0)	130.4
H. anomala	1761	7.88	7.23 (21.6)	7.04 (14.5)	7.08 (16.1)	126.0
H. beckii	0803	7.42	7.08 (45.4)	6.83 (25.9)	6.74 (21.4)	46.9
H. beckii	0983	7.74	7.46 (52.4)	7.34 (40.3)	7.40 (46.2)	316.0
H. beckii	1216	7.75	7.52 (57.2)	7.30 (34.3)	7.43 (46.9)	203.8
H. beijerinckii	0992	7.86	6.76 (7.9)	6.36 (3.1)	6.04 (1.5)	19.2

* Viable counts are expressed as the logarithm of the number of colony forming units.

** Limit survival time (LST) is the time taken for the survival count to decrease to 10^4 per ampule. See text.

*** NC indicates the case in which the survival count after 5 years of storage was more than the viable count immediately after drying.

Table 1. (continued)

Species	IFO No.	log CFU[‡] per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C[‡‡] (years)
Hansenula beijerinckii	1191	7.71	6.64 (8.5)	6.40 (4.9)	6.20 (3.2)	31.1
H. beijerinckii	1762	7.93	7.52 (38.0)	7.28 (22.4)	7.20 (18.0)	54.2
H. beijerinckii	1763	7.81	7.26 (27.7)	6.93 (13.3)	6.90 (12.3)	46.1
H. californica	1764	8.23	7.91 (46.9)	7.72 (30.8)	7.81 (38.6)	230.9
H. californica	1765	8.30	7.88 (39.2)	7.71 (25.6)	7.70 (25.5)	104.1
H. californica	1766	8.30	7.97 (47.5)	7.94 (44.4)	7.85 (35.6)	158.5
H. californica	1767	7.95	7.49 (34.5)	7.23 (18.7)	7.25 (19.7)	71.6
H. capsulata	0801	6.98	6.00 (10.5)	5.17 (1.6)	5.65 (4.8)	29.4
H. capsulata	1768	8.34	8.08 (54.5)	7.62 (19.5)	7.70 (23.1)	54.6
H. capsulata	1769	8.25	7.97 (52.4)	7.53 (19.2)	7.68 (26.8)	68.2
H. capsulata	1770	8.25	8.04 (59.4)	7.52 (18.0)	7.85 (38.8)	109.1
H. ciferrii	0793	7.58	6.99 (25.3)	6.69 (12.7)	6.88 (19.7)	137.4
H. ciferrii	0904	7.76	7.14 (24.2)	6.73 (9.2)	6.80 (11.0)	46.0
H. dimennae	1880	8.04	7.56 (33.4)	7.25 (16.7)	7.32 (18.8)	71.3
H. dimennae	1881	8.11	7.53 (26.5)	7.25 (14.3)	7.34 (17.4)	96.6
H. dimennae	1882	8.00	7.50 (30.8)	7.28 (18.5)	7.20 (15.6)	59.3
H. dryadoides	1820	7.77	7.00 (17.6)	5.91 (1.4)	6.71 (8.9)	50.9
H. fabianii	1371	7.61	7.21 (40.0)	6.84 (17.0)	7.18 (37.0)	474.8
H. fabianii	1874	8.42	8.34 (83.8)	8.30 (75.7)	8.32 (80.0)	1077.1
H. fabianii	1875	8.11	7.70 (40.0)	7.34 (17.5)	7.62 (33.0)	221.6
H. henricii	1478	7.48	6.57 (12.1)	5.59 (1.3)	6.41 (8.6)	86.6
H. jadinii	0804	8.00	7.70 (47.9)	7.66 (44.6)	7.61 (39.8)	229.8
H. jadinii	0987	7.13	6.50 (23.7)	6.13 (10.0)	6.40 (18.7)	121.7
H. muscicola	1383	8.23	7.94 (50.6)	7.79 (36.4)	7.75 (33.3)	108.3
H. philodendra	1821	8.45	8.25 (65.5)	7.48 (10.9)	7.91 (29.7)	62.0
H. saturnus	0131	7.84	6.93 (12.5)	6.45 (4.1)	6.70 (7.4)	64.5
H. saturnus	0132	7.84	6.82 (9.6)	6.40 (3.7)	6.50 (4.7)	45.5
H. saturnus	0133	7.71	6.72 (10.2)	6.38 (4.6)	6.66 (8.8)	212.5
H. saturnus	0811	7.80	6.54 (5.6)	6.40 (4.0)	6.52 (5.3)	532.7
H. saturnus	1466	7.08	6.28 (15.8)	5.70 (4.2)	5.75 (4.7)	21.6
H. saturnus	1467	7.24	6.50 (18.3)	6.23 (9.7)	6.27 (10.6)	52.8
H. saturnus	1772	7.79	7.41 (41.5)	7.00 (16.5)	7.00 (16.7)	43.1
H. saturnus	1773	7.81	7.43 (41.5)	7.34 (33.1)	7.23 (26.2)	85.9
H. saturnus	1774	7.77	7.38 (40.6)	7.25 (30.8)	7.04 (18.1)	48.1
H. saturnus	1775	7.83	7.48 (44.9)	7.23 (24.6)	7.04 (15.8)	38.3
H. saturnus	1776	7.93	7.43 (32.0)	7.30 (23.2)	7.23 (20.1)	85.0
Issatchenkia occidentalis	1904	8.00	7.69 (47.3)	7.30 (19.1)	7.40 (24.2)	63.4
I. scutulata var. exigua	1896	7.71	6.94 (16.9)	6.60 (7.7)	6.54 (6.9)	37.8
I. scutulata var. exigua	10050	7.65	6.81 (14.3)	6.14 (3.2)	5.86 (1.6)	14.8
I. scutulata var. scutulata	1895	7.78	7.17 (23.9)	7.04 (18.0)	6.91 (13.1)	60.6
I. terricola	0933	7.87	7.50 (42.7)	6.20 (2.2)	6.55 (4.9)	18.6
I. terricola	1798	8.20	8.04 (68.4)	7.92 (52.4)	7.95 (56.0)	232.6
I. terricola	1799	7.61	7.23 (42.3)	6.54 (8.8)	6.53 (8.5)	23.2
I. terricola	1888	8.00	7.88 (74.6)	7.70 (48.5)	7.67 (45.7)	91.3
I. terricola	1907	7.72	7.43 (51.2)	7.23 (32.0)	7.30 (38.4)	137.3

Table 1. (continued)

Species	IFO No.	log CFU[‡] per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C[‡‡] (years)
Kluyveromyces fragilis	0541	7.83	6.76 (8.5)	6.11 (1.9)	6.41 (3.8)	39.5
K. fragilis	1735	7.45	6.64 (15.8)	6.48 (10.7)	6.55 (12.7)	139.2
K. fragilis	1777	8.25	7.81 (35.6)	7.43 (14.8)	7.52 (18.2)	65.3
K. fragilis	1963	7.71	7.15 (27.0)	7.00 (20.4)	7.04 (21.6)	162.0
K. lactis	1902	7.74	7.38 (42.7)	7.28 (34.3)	7.52 (59.2)	NC[‡‡‡]
K. lactis	1903	7.98	7.65 (47.5)	7.38 (25.2)	7.46 (30.3)	93.6
K. marxianus	0219	7.54	6.40 (7.1)	6.20 (4.5)	6.41 (7.3)	NC
K. marxianus	0273	7.71	6.50 (6.2)	6.32 (4.1)	6.54 (6.8)	NC
K. marxianus	0277	7.82	6.61 (6.2)	6.30 (3.0)	6.54 (5.3)	191.7
K. marxianus	0480	7.78	6.67 (7.6)	6.45 (4.6)	5.93 (1.4)	18.2
K. marxianus	0481	7.79	6.49 (5.0)	6.25 (2.9)	6.43 (4.4)	224.4
K. marxianus	0484	7.78	6.64 (7.3)	6.23 (2.9)	6.28 (3.2)	36.9
K. phaffii	1884	7.79	6.87 (12.0)	5.84 (1.1)	5.87 (1.2)	14.4
K. phaffii	1885	7.51	6.75 (17.1)	5.55 (1.1)	5.97 (2.9)	17.8
K. thermotolerans	1778	7.81	7.66 (70.2)	7.30 (30.6)	7.30 (30.0)	49.6
K. thermotolerans	1779	7.91	7.78 (75.0)	7.46 (36.5)	7.50 (39.3)	67.4
K. thermotolerans	1780	7.80	7.48 (48.3)	7.34 (34.7)	7.30 (31.6)	94.4
K. thermotolerans	1985	7.49	6.92 (26.5)	7.00 (32.7)	6.75 (18.0)	86.9
Nadsonia commutata	10029	6.84	5.28 (2.8)	5.43 (3.9)	<1.69 (<0.000)	3.7
N. elongata	0665	6.48	5.48 (10.0)	5.00 (3.5)	4.75 (2.7)	13.0
N. fulvescens	0666	6.54	5.60 (11.4)	5.54 (10.0)	5.54 (10.0)	140.7
Pachytichospora transvaalensis	1625	6.65	5.77 (13.1)	5.40 (5.6)	5.46 (6.4)	28.5
Pichia adadieae	1822	8.68	8.36 (47.9)	8.17 (32.4)	8.25 (38.1)	219.1
P. amethionina	10014	7.99	7.69 (49.1)	7.63 (44.1)	7.67 (47.3)	1136.0
P. amethionina var. pachycereana	10015	7.56	7.28 (51.9)	6.98 (26.5)	7.20 (45.1)	268.3
P. bovis	1886	8.14	7.88 (56.2)	7.72 (39.2)	7.72 (39.2)	124.0
P. burtonii	6130	7.85	6.80 (8.9)	6.49 (4.4)	6.30 (2.9)	28.8
P. cactophila	10017	8.43	8.23 (65.6)	8.08 (45.6)	8.06 (43.7)	120.2
P. carsonii	0946	8.04	7.78 (53.1)	6.25 (1.6)	7.11 (11.4)	28.4
P. carsonii	1989	8.25	8.11 (71.9)	7.61 (22.9)	7.88 (42.8)	91.2
P. castillae	1823	8.69	8.46 (58.8)	8.00 (21.4)	8.14 (28.9)	72.2
P. cellobiosa	1909	7.94	7.32 (24.1)	6.97 (10.6)	7.11 (14.6)	76.4
P. dispora	1781	7.95	7.70 (56.2)	7.40 (27.5)	7.41 (28.9)	64.1
P. dispora	1782	8.11	7.78 (48.4)	7.48 (23.7)	7.54 (27.5)	77.1
P. dispora	1783	7.71	7.53 (64.3)	7.38 (45.1)	7.41 (49.9)	160.1
P. etchellsii	1987	8.04	7.74 (50.5)	7.62 (38.4)	7.68 (43.6)	293.1
P. farinosa	0459	7.28	6.88 (40.5)	5.74 (2.9)	6.04 (5.8)	17.1
P. farinosa	0464	7.68	7.48 (63.9)	6.20 (3.4)	6.20 (3.4)	13.7
P. fermentans	0815	7.38	6.60 (16.7)	5.61 (1.7)	6.20 (6.7)	32.8
P. fluxuum	1784	7.96	7.59 (42.2)	7.30 (21.5)	7.25 (19.5)	53.6
P. fluxuum	1785	8.04	7.55 (32.5)	7.25 (16.5)	7.20 (14.3)	49.8
P. fluxuum	1786	7.57	7.28 (51.7)	6.60 (10.6)	6.70 (13.4)	28.0
P. fluxuum	1787	7.78	7.53 (55.8)	7.17 (24.2)	7.78 (26.4)	54.3
P. heedii	10018	8.20	7.80 (43.8)	7.68 (30.7)	7.71 (32.9)	154.3
P. heedii	10019	8.32	8.00 (48.4)	7.62 (20.1)	7.85 (33.8)	128.4
P. heedii	10020	8.36	8.04 (47.3)	7.70 (22.2)	7.85 (33.0)	128.9

Table 1. (continued)

Species	IFO No.	log CFU' per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C‡‡ (years)
Pichia humboldtii	10060	6.70	5.11 (2.7)	5.78 (1.2)	4.17 (0.3)	5.9
P. kluyveri	1165	7.50	7.26 (56.9)	7.12 (41.3)	7.24 (54.5)	871.0
P. kluyveri	1988	8.08	7.82 (56.5)	7.75 (47.5)	7.74 (46.8)	233.5
P. media	1824	7.45	7.08 (43.2)	7.08 (42.9)	6.92 (30.5)	101.7
P. membranaefaciens	0563	8.11	7.66 (36.0)	6.43 (2.1)	7.04 (8.8)	30.0
P. membranaefaciens	0815	7.38	6.60 (16.7)	5.61 (1.7)	5.91 (3.4)	18.8
P. membranaefaciens	1284	7.43	6.94 (32.4)	5.58 (1.4)	5.34 (0.8)	9.2
P. membranaefaciens	1788	7.62	7.30 (46.3)	7.11 (30.1)	7.08 (27.1)	70.8
P. membranaefaciens	1789	7.63	7.45 (64.7)	6.98 (22.0)	6.88 (17.4)	30.2
P. membranaefaciens	1790	7.73	7.48 (55.2)	7.28 (35.3)	7.28 (35.4)	90.1
P. mucosa	1825	8.11	7.71 (39.9)	7.45 (21.6)	7.17 (11.2)	33.6
P. ohmeri	0163	7.93	7.45 (33.4)	6.92 (9.9)	7.04 (13.1)	42.5
P. ohmeri	0202	7.73	6.88 (14.2)	6.68 (9.0)	6.59 (7.3)	49.9
P. opuntiae	10021	8.14	7.78 (42.3)	7.65 (31.8)	7.65 (31.5)	147.6
P. opuntiae	10022	8.08	7.66 (38.4)	7.41 (22.0)	7.46 (23.8)	88.2
P. opuntiae	10023	7.91	7.50 (38.7)	6.93 (10.3)	7.20 (18.8)	55.8
P. opuntiae var. thermotolerans	10024	8.40	8.04 (45.0)	7.88 (30.2)	7.98 (37.8)	267.7
P. opuntiae var. thermotolerans	10025	8.08	7.50 (27.5)	7.11 (11.3)	7.28 (16.3)	77.1
P. opuntiae var. thermotolerans	10026	8.17	7.74 (36.0)	7.54 (22.8)	7.63 (28.3)	179.0
P. pijperi	1290	7.16	6.68 (33.1)	6.58 (26.2)	6.57 (25.2)	113.2
P. pijperi	1791	7.76	7.58 (65.2)	7.48 (52.3)	7.23 (29.9)	52.9
P. pijperi	1792	8.14	7.94 (63.7)	7.67 (34.2)	7.52 (24.2)	46.8
P. pijperi	1887	7.69	7.46 (66.3)	7.41 (58.5)	7.25 (40.0)	80.1
P. pinus	1793	7.98	7.70 (52.9)	7.28 (20.2)	7.49 (32.1)	85.3
P. pinus	1794	7.80	7.71 (80.4)	7.28 (30.1)	7.27 (29.8)	43.1
P. pinus	1795	7.54	7.32 (59.5)	6.72 (15.1)	6.87 (21.2)	37.0
P. rhodanensis	1272	7.19	6.58 (24.5)	6.45 (18.4)	6.46 (18.5)	105.7
P. saitoi	1796	7.76	7.50 (55.9)	7.25 (31.7)	7.20 (26.8)	55.0
P. saitoi	1797	7.79	7.54 (56.3)	7.20 (26.0)	7.28 (30.5)	66.5
P. sargentensis	1826	8.38	7.65 (18.4)	7.41 (10.5)	7.25 (7.4)	46.1
P. scolyti	1114	8.30	8.20 (81.6)	7.78 (29.8)	8.04 (56.0)	129.0
P. scolyti	1115	8.57	8.48 (82.2)	8.38 (66.0)	8.40 (68.2)	276.1
P. spartinae	1827	7.80	7.11 (20.0)	6.64 (7.0)	6.77 (9.3)	46.7
P. spartinae	1905	8.38	8.11 (54.3)	7.93 (36.0)	8.04 (44.2)	230.1
P. stipitis	10063	8.01	6.78 (5.9)	6.32 (2.0)	6.61 (4.0)	82.5
P. toletana	1800	7.83	7.70 (74.5)	7.30 (29.9)	7.38 (36.0)	58.5
P. wickerhamii	1278	7.14	6.66 (32.9)	6.49 (21.8)	6.57 (26.5)	141.7
Saccharomyces bayanus	1802	7.17	6.95 (59.0)	6.90 (52.5)	6.82 (44.0)	115.7
S. bayanus	1803	7.63	6.99 (22.7)	6.87 (17.2)	6.71 (12.1)	54.7
S. bayanus	1943	7.45	6.70 (17.6)	6.66 (16.2)	6.40 (8.9)	45.5
S. beticus	1831	6.91	6.25 (21.4)	6.11 (15.6)	5.97 (11.4)	41.0
S. capensis	1991	7.70	7.08 (23.7)	7.00 (20.5)	6.76 (11.5)	49.0
S. cerevisiae	0636	7.22	6.67 (28.5)	5.73 (3.3)	6.20 (9.9)	29.1
S. cerevisiae	1804	7.82	7.64 (67.2)	7.54 (53.5)	7.54 (53.7)	187.2
S. cerevisiae	1805	7.75	7.63 (76.3)	7.49 (54.9)	7.41 (47.1)	86.7

Table 1. (continued)

Species	IFO No.	log CFU* per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C** (years)
Saccharomyces cerevisiae	1947	7.43	6.98 (34.6)	6.81 (23.7)	6.84 (25.0)	105.4
S. cerevisiae	1948	7.73	7.32 (38.3)	7.34 (40.1)	7.11 (24.9)	88.6
S. cerevisiae	1949	7.53	7.04 (32.1)	6.84 (20.7)	7.04 (32.6)	NC***
S. cerevisiae	1950	7.36	6.62 (18.1)	6.73 (23.6)	6.48 (12.8)	87.0
S. cerevisiae	1951	6.92	6.52 (39.0)	6.41 (30.3)	6.28 (22.4)	51.1
S. cerevisiae	1952	7.34	6.93 (39.6)	6.68 (22.3)	6.61 (19.1)	46.3
S. cerevisiae	1953	7.63	7.11 (31.0)	7.08 (26.5)	7.23 (39.5)	NC
S. cerevisiae	1954	7.73	6.86 (13.2)	6.65 (8.3)	6.61 (7.6)	59.6
S. chevalieri	1726	7.63	6.86 (16.8)	6.65 (10.5)	6.48 (6.9)	36.9
S. chevalieri	1727	7.67	7.17 (31.5)	6.94 (18.5)	6.85 (14.9)	48.8
S. chevalieri	1728	7.04	6.55 (32.1)	6.46 (26.4)	6.43 (24.4)	107.0
S. chevalieri	1729	7.71	7.34 (41.1)	7.32 (40.0)	7.33 (40.7)	3921.8
S. chevalieri	1955	7.97	7.81 (70.2)	7.80 (68.4)	7.65 (48.7)	120.1
S. cordubensis	1832	7.38	6.91 (34.6)	6.86 (30.6)	6.57 (15.7)	42.4
S. coreanus	0573	7.06	6.14 (12.2)	5.74 (4.8)	7.96 (7.9)	56.9
S. coreanus	1833	7.86	7.45 (37.6)	7.38 (33.2)	7.43 (36.2)	1044.0
S. dairensis	1992	7.82	7.23 (24.9)	6.96 (13.8)	7.04 (16.5)	90.0
S. diastaticus	1958	7.81	7.49 (47.9)	6.96 (14.1)	7.28 (28.7)	78.5
S. gaditensis	1834	7.52	7.30 (60.4)	7.28 (58.1)	7.17 (45.8)	137.0
S. globosus	0752	7.84	7.11 (19.7)	6.30 (2.9)	6.66 (6.7)	33.5
S. globosus	1889	7.49	6.85 (23.0)	6.57 (12.0)	6.72 (17.2)	112.9
S. globosus	1890	7.36	6.76 (24.9)	6.53 (14.3)	6.79 (26.6)	NC
S. globosus	1891	7.48	6.99 (32.2)	6.78 (19.8)	6.91 (27.0)	195.2
S. hienipiensis	1994	7.59	7.11 (33.5)	7.08 (29.9)	7.11 (33.4)	12004.6
S. hispanica	1995	7.36	6.93 (36.6)	6.97 (39.7)	6.78 (25.4)	92.4
S. inusitatus	1343	7.14	6.80 (8.6)	5.73 (3.9)	5.80 (4.5)	37.0
S. kluyveri	1811	7.41	7.17 (57.3)	6.91 (31.5)	6.97 (36.2)	79.4
S. kluyveri	1892	7.60	7.00 (25.0)	6.63 (10.8)	6.71 (13.1)	53.4
S. kluyveri	1893	7.87	7.49 (41.4)	7.11 (18.1)	7.28 (26.0)	86.3
S. mrakii	1835	7.98	7.48 (31.7)	7.27 (19.5)	7.28 (19.7)	84.3
S. norbensis	1836	7.60	7.11 (31.6)	7.00 (25.3)	6.79 (15.5)	50.1
S. oleaceus	1997	7.64	7.40 (56.3)	7.32 (47.2)	7.23 (37.2)	94.4
S. oleaginosus	1998	7.74	7.20 (28.3)	7.25 (32.1)	7.17 (26.9)	723.5
S. prostoserdovii	1837	7.84	7.40 (35.0)	7.20 (22.8)	6.98 (13.7)	41.6
S. servazzii	1838	7.80	7.66 (72.0)	7.40 (39.5)	7.28 (29.9)	48.0
S. uvarum	0615	6.90	6.00 (12.5)	5.60 (5.0)	5.87 (9.4)	80.8
S. uvarum	1815	7.61	7.08 (30.1)	6.98 (23.1)	6.93 (20.6)	93.9
S. uvarum	1816	7.60	7.15 (34.3)	7.08 (28.8)	7.00 (25.2)	79.7
S. uvarum	1961	7.72	7.04 (18.8)	6.92 (14.7)	6.87 (13.0)	94.4
S. uvarum	1962	7.36	6.64 (18.6)	6.48 (12.9)	6.28 (7.8)	34.9
S. uvarum	10010	7.38	6.78 (25.4)	6.20 (6.6)	6.23 (7.1)	25.2
S. uvarum	10011	7.52	6.79 (18.8)	6.76 (17.5)	6.69 (14.8)	134.4
S. uvarum	10012	7.32	6.53 (16.1)	6.36 (10.9)	6.23 (7.8)	40.1
Saccharomycodes ludwigii	0798	7.45	5.95 (3.2)	5.77 (2.1)	5.45 (1.0)	19.3
S. ludwigii	1714	6.97	6.52 (34.9)	6.30 (20.8)	6.41 (27.9)	129.2
S. ludwigii	1722	7.36	6.70 (21.6)	6.50 (13.9)	6.40 (10.9)	45.4
S. ludwigii	1723	7.30	6.78 (29.5)	6.60 (19.7)	6.64 (21.6)	102.5
S. ludwigii	1725	7.31	6.43 (13.4)	6.00 (5.2)	6.17 (7.3)	46.2

Table 1. (continued)

Species	IFO No.	log CFU‡ per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C‡‡ (years)
Saccharomycodes ludwigii	10036	6.98	6.50 (33.3)	6.32 (21.4)	6.17 (14.9)	35.9
Saccharomycopsis fibuligera	0103	7.40	6.82 (26.6)	6.38 (9.6)	6.64 (17.5)	77.7
S. fibuligera	0106	7.23	6.70 (30.1)	6.40 (14.6)	6.46 (17.1)	55.0
S. fibuligera	0107	7.74	7.53 (61.5)	7.36 (41.4)	7.30 (35.5)	74.0
S. fibuligera	0111	7.06	5.95 (7.8)	5.65 (3.9)	5.75 (4.9)	48.4
S. fibuligera	1744	7.78	7.38 (39.6)	7.40 (41.3)	7.40 (41.8)	NC‡‡‡
S. fibuligera	1745	7.98	7.77 (61.4)	7.64 (45.7)	7.61 (42.3)	116.5
S. lipolytica	0717	7.11	6.87 (59.3)	6.67 (37.0)	6.84 (54.4)	383.6
S. lipolytica	1209	7.32	7.23 (78.1)	7.11 (60.1)	7.04 (52.1)	91.5
S. lipolytica	1542	7.30	6.36 (11.5)	6.65 (22.5)	6.48 (15.1)	NC
S. lipolytica	1602	7.86	7.50 (44.8)	7.04 (22.8)	7.43 (37.8)	237.6
S. lipolytica	1659	7.34	7.34(100.0)	7.25 (84.0)	7.32 (98.8)	3178.0
S. vini	1748	7.46	7.14 (47.6)	7.00 (35.3)	7.15 (48.1)	NC
Schizosaccharomyces pombe	0346	7.00	6.60 (40.0)	6.50 (31.5)	6.51 (32.5)	144.3
S. pombe	0362	6.90	6.59 (48.8)	6.42 (33.1)	6.38 (30.0)	61.3
S. japonicus	1609	6.70	5.90 (16.0)	5.40 (5.0)	5.50 (6.4)	23.9
S. japonicus	1712	6.30	5.54 (17.3)	5.04 (5.8)	5.53 (17.0)	1012.9
Schwanniomyces alluvius	1839	7.84	7.20 (22.9)	6.78 (8.8)	6.91 (11.6)	54.3
S. castellii	1840	8.13	7.40 (18.6)	6.98 (7.1)	7.04 (8.2)	47.8
S. occidentaris	0371	7.79	6.17 (2.4)	5.90 (1.3)	6.11 (2.2)	287.0
S. persoonii	1842	7.84	7.04 (15.4)	6.82 (9.6)	6.80 (9.1)	66.4
Sporopachydermia cereana	10013	7.96	7.75 (60.9)	7.59 (42.6)	7.65 (48.3)	186.3
S. lactativora	1867	8.25	7.50 (18.5)	7.25 (10.0)	7.36 (13.2)	119.7
Torulaspora delbrueckii	1739	7.04	6.71 (47.4)	6.55 (33.9)	6.28 (17.1)	30.5
T. delbrueckii	1956	7.93	7.40 (28.3)	7.08 (13.9)	7.08 (13.8)	54.3
T. delbrueckii	1957	7.82	7.43 (40.0)	7.08 (18.5)	6.97 (14.0)	37.5
T. delbrueckii	1959	7.99	7.64 (44.2)	7.53 (34.7)	7.43 (27.6)	89.0
T. globosa	0016	7.36	6.43 (11.7)	5.97 (4.1)	6.09 (5.4)	36.2
T. globosa	0038	7.19	6.79 (40.0)	6.44 (17.7)	6.49 (20.1)	46.7
T. globosa	1160	7.19	6.63 (27.7)	6.37 (15.2)	6.03 (6.5)	20.9
Wickerhamiella domercqii	1857	8.75	8.62 (73.0)	8.11 (23.7)	8.30 (35.9)	74.9
Wingea robertsii	1277	7.65	7.22 (37.8)	6.96 (20.8)	7.15 (32.0)	223.0
Zygosaccharomyces bailii	0722	7.53	6.79 (18.2)	6.04 (3.2)	6.34 (6.6)	31.7
Z. bailii	1047	7.20	6.25 (11.3)	5.32 (1.3)	5.72 (3.3)	21.1
Z. bailii	1098	7.70	6.82 (13.4)	6.30 (4.0)	5.40 (0.5)	9.9
Z. bailii	1137	7.20	6.55 (23.2)	5.87 (4.8)	6.04 (7.2)	25.2
Z. bailii	1610	7.53	6.53 (10.0)	6.08 (3.7)	6.43 (8.0)	130.6
Z. bailii	1738	7.46	7.36 (80.0)	6.84 (24.1)	6.93 (29.9)	39.3
Z. bailii	1801	7.32	6.97 (45.4)	6.75 (27.0)	6.71 (24.8)	56.5
Z. bisporus	1734	7.63	7.28 (43.2)	6.77 (13.5)	6.87 (17.0)	40.4
Z. bisporus	1736	7.73	7.54 (65.4)	7.49 (58.6)	7.34 (40.9)	86.9
Z. bisporus	1737	7.78	7.49 (52.4)	7.25 (29.4)	7.26 (30.8)	75.7
Z. bisporus	1944	7.84	7.28 (26.9)	7.11 (18.3)	6.95 (13.0)	51.7
Z. cidri	1990	7.86	7.41 (35.3)	7.25 (24.9)	7.40 (34.5)	1712.6
Z. fermentati	1996	8.08	7.87 (61.3)	7.84 (58.3)	7.80 (52.4)	283.8
Z. florentinus	1806	7.99	7.87 (76.0)	7.71 (53.2)	7.78 (62.7)	231.5
Z. florentinus	1807	8.04	7.93 (78.5)	6.36 (2.1)	7.73 (49.5)	98.2
Z. florentinus	1808	8.04	7.93 (80.0)	7.11 (12.4)	7.73 (50.5)	98.4
Z. florentinus	1809	8.04	7.93 (82.2)	7.54 (33.3)	7.68 (45.4)	76.3

Table 1. (continued)

Species	IFO No.	log CFU[‡] per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C[‡‡] (years)
Zygosaccharomyces florentinus	1810	7.88	7.77 (77.4)	7.52 (43.9)	7.38 (31.7)	48.6
Z. florentinus	1993	8.04	7.71 (46.7)	7.58 (34.1)	7.64 (39.7)	263.4
Z. microellipsoides	1740	8.04	7.71 (49.6)	7.28 (17.6)	7.14 (13.0)	32.0
Z. rouxii	0321	7.25	6.30 (11.1)	5.58 (2.1)	5.73 (3.0)	20.2
Z. rouxii	0322	7.00	6.60 (40.0)	5.14 (1.4)	6.38 (24.2)	59.6
Z. rouxii	0323	7.20	6.67 (30.3)	5.54 (2.3)	6.25 (11.7)	32.3
Z. rouxii	0329	7.16	6.14 (9.7)	5.65 (3.1)	5.43 (1.9)	15.2
Z. rouxii	0330	7.45	6.87 (26.3)	5.85 (2.5)	6.50 (11.3)	39.2
Z. rouxii	0332	7.23	6.93 (50.6)	5.55 (2.1)	6.25 (10.5)	21.5
Z. rouxii	0439	6.17	5.49 (21.0)	4.60 (2.7)	5.11 (8.6)	19.3
Z. rouxii	0442	7.08	6.50 (27.8)	5.53 (3.0)	5.96 (8.0)	23.2
Z. rouxii	0443	7.08	6.57 (32.2)	5.48 (2.6)	5.00 (0.9)	8.3
Z. rouxii	0451	7.11	6.86 (56.2)	5.60 (3.1)	5.87 (5.8)	14.5
Z. rouxii	0489	6.87	6.04 (14.7)	5.32 (2.8)	5.52 (4.4)	19.5
Z. rouxii	0506	7.32	6.17 (7.1)	5.92 (4.0)	5.97 (4.5)	54.9
Z. rouxii	0507	7.49	7.20 (52.39	6.17 (4.8)	7.08 (39.9)	136.6
Z. rouxii	0510	7.56	6.59 (10.7)	6.14 (4.0)	5.92 (2.3)	19.4
Z. rouxii	0513	6.81	6.20 (24.6)	5.30 (3.1)	5.58 (5.8)	17.6
Z. rouxii	0514	7.42	6.46 (10.9)	6.00 (3.8)	6.25 (6.7)	58.2
Z. rouxii	0521	7.58	6.78 (15.6)	5.78 (1.6)	6.53 (8.8)	55.9
Z. rouxii	0523	7.58	6.89 (20.5)	6.08 (3.2)	6.48 (7.9)	34.9
Z. rouxii	0525	6.84	6.28 (27.1)	5.78 (8.6)	6.08 (17.1)	57.0
Z. rouxii	0526	7.87	7.04 (14.7)	6.11 (1.7)	5.57 (0.5)	10.4
Z. rouxii	0528	6.81	6.50 (49.2)	5.51 (7.7)	6.36 (35.1)	85.4
Z. rouxii	0529	7.11	6.43 (20.8)	5.84 (5.4)	6.46 (22.5)	NC[‡‡‡]
Z. rouxii	0531	7.09	6.66 (36.8)	5.78 (4.8)	5.82 (6.8)	18.2
Z. rouxii	0532	7.56	6.59 (10.7)	6.08 (3.4)	6.45 (7.7)	90.7
Z. rouxii	0533	7.64	6.95 (20.5)	6.17 (3.5)	6.49 (7.0)	31.7
Z. rouxii	0543	7.41	6.84 (26.5)	5.90 (3.1)	6.62 (16.1)	65.6
Z. rouxii	0845	7.08	6.72 (44.2)	5.50 (2.7)	5.84 (5.8)	15.5
Z. rouxii	1730	7.61	7.30 (49.5)	6.36 (5.6)	6.91 (20.2)	42.5
Z. rouxii	1732	7.16	7.11 (88.8)	5.23 (1.2)	6.52 (22.8)	26.4
Z. rouxii	1733	7.53	6.93 (24.9)	5.76 (1.7)	6.74 (16.2)	78.5
Z. rouxii	1812	7.27	7.14 (75.6)	5.93 (4.6)	6.34 (12.1)	19.8
Z. rouxii	1813	7.65	6.65 (10.0)	6.25 (4.0)	6.71 (11.3)	NC
Z. rouxii	1814	7.45	7.17 (53.3)	6.77 (20.6)	7.08 (40.6)	134.5
Z. rouxii	1876	7.69	7.20 (32.4)	6.04 (2.2)	6.52 (6.8)	23.6
Z. rouxii	1914	7.75	7.45 (50.1)	6.86 (12.8)	7.38 (42.2)	231.2
Z. rouxii	1945	8.25	8.04 (64.2)	7.97 (53.3)	7.82 (37.1)	85.1
Z. rouxii	1960	7.29	6.92 (42.1)	5.94 (4.5)	6.61 (20.9)	48.0
Baker's yeast	0555	7.74	6.61 (7.4)	6.36 (4.2)	6.32 (3.8)	45.1
Brewer's yeast	2031	7.78	7.11 (21.5)	6.43 (4.6)	6.56 (6.1)	28.4
Distillery yeast	2363	7.65	6.60 (9.0)	5.80 (1.4)	5.86 (1.6)	17.4
Wine yeast	2226	7.75	6.32 (3.6)	6.17 (2.6)	5.65 (0.8)	17.7
Lactose fermented yeast	2124	8.29	7.52 (16.9)	6.82 (3.4)	7.04 (5.5)	36.1
Lactose fermented yeast	2126	8.25	7.43 (15.6)	6.87 (4.3)	6.86 (4.2)	30.2
Lactose fermented yeast	2127	7.97	6.90 (8.5)	5.97 (1.0)	6.20 (1.7)	20.8
Lactose fermented yeast	2128	8.17	7.28 (12.7)	6.63 (2.9)	6.91 (5.5)	45.1

Table 2. Viabilities at 5 C of dried cultures of deuteromycetous yeasts with survival values higher than 1% in the 60-day accelerated test at 37 C.

Species	IFO No.	log CFU‡ per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C‡‡ (years)
Brettanomyces abstinens	1589	7.52	6.78 (18.5)	5.59 (1.2)	6.58 (11.6)	68.7
B. claussenii	0627	8.08	7.11 (11.2)	6.32 (1.8)	6.96 (7.7)	90.1
B. lambicus	0797	7.80	6.53 (5.3)	6.11 (2.0)	6.20 (2.5)	38.8
B. lambicus	1154	7.71	6.92 (16.3)	5.82 (1.3)	6.71 (10.3)	73.2
B. lambicus	1243	7.83	7.00 (15.1)	6.11 (2.0)	6.67 (6.9)	43.0
B. naardenensis	1588	8.25	7.68 (27.0)	6.87 (4.2)	7.00 (5.8)	27.6
Bullera alba	1192	6.74	6.11 (23.6)	5.40 (4.5)	4.78 (1.1)	7.9
B. alba	1244	6.54	5.60 (11.4)	5.11 (3.9)	5.20 (4.5)	19.8
B. alba	1245	7.04	6.23 (15.5)	6.00 (9.1)	6.72 (12.3)	111.1
Candida acutus	1912	7.96	7.89 (86.1)	7.80 (69.1)	7.91 (89.5)	NC‡‡‡
C. albicans	0692	7.72	7.43 (52.2)	6.25 (3.5)	7.17 (29.9)	71.0
C. albicans	1067	7.89	7.46 (37.7)	5.93 (1.1)	<4.74 (<0.07)	6.4
C. albicans	1262	8.05	7.62 (37.4)	6.43 (2.4)	7.04 (10.2)	11.1
C. albicans	1398	7.61	6.76 (14.1)	5.78 (1.5)	5.52 (0.8)	32.2
C. apicola	1093	8.07	7.58 (32.1)	6.28 (1.6)	7.00 (8.4)	30.7
C. apis var. galacta	10031	8.04	7.81 (58.1)	7.50 (28.9)	7.82 (59.1)	NC
C. atmospherica	1969	8.45	8.08 (42.6)	7.62 (15.0)	7.67 (16.7)	50.1
C. boidnii	10035	7.53	7.03 (31.3)	6.50 (9.2)	6.72 (15.5)	49.7
C. cariosilignicola	1910	8.32	8.18 (72.0)	8.14 (66.2)	8.16 (69.7)	1482.1
C. catenulata	1338	7.76	7.46 (49.0)	7.11 (22.6)	7.11 (22.6)	51.4
C. catenulata	1452	7.91	7.90 (99.2)	7.63 (52.6)	7.62 (51.4)	68.4
C. conglobata	0959	7.86	7.30 (27.4)	7.14 (19.2)	7.11 (17.5)	84.6
C. curvata	0732	7.71	6.71 (10.1)	5.71 (1.0)	5.91 (1.6)	17.0
C. curvata	1599	7.51	6.17 (4.6)	5.59 (1.2)	5.55 (1.1)	17.5
C. curvata	1858	7.00	6.25 (17.5)	6.17 (15.4)	6.00 (10.1)	47.0
C. diddensii	1970	8.47	8.17 (51.7)	7.90 (26.8)	8.11 (43.6)	282.6
C. diddensii	1971	8.52	8.23 (52.3)	8.01 (31.9)	8.08 (36.7)	137.6
C. diffluens	1524	7.37	5.95 (3.8)	6.29 (8.3)	6.16 (6.1)	NC
C. diffluens	1525	7.54	6.18 (4.3)	6.62 (11.9)	6.54 (10.0)	NC
C. diffluens	1526	7.59	6.20 (4.1)	6.84 (17.9)	6.62 (10.8)	NC
C. etchelisii	10037	8.03	7.77 (55.5)	6.46 (2.7)	7.08 (11.6)	27.8
C. guilliermondii	1454	7.66	7.46 (62.9)	7.14 (30.5)	7.25 (38.4)	80.7
C. guilliermondii	1913	8.38	8.20 (68.2)	8.05 (48.1)	8.23 (69.0)	NC
C. guilliermondii	1972	8.32	7.97 (44.1)	7.99 (45.4)	7.94 (41.2)	672.4
C. haemulonii	10001	8.17	7.88 (51.8)	7.74 (37.3)	7.70 (34.0)	106.2
C. halonitratophila	1561	8.40	8.14 (55.5)	6.68 (1.9)	6.76 (2.3)	15.0
C. halophilus	1941	7.90	7.60 (50.0)	7.11 (15.9)	7.30 (25.4)	61.2
C. humicola	0753	7.89	7.38 (30.3)	6.43 (3.5)	6.36 (3.0)	16.8
C. humicola	0760	7.85	7.14 (20.2)	6.28 (2.7)	6.36 (3.3)	20.0
C. humicola	1117	7.90	7.20 (20.7)	6.28 (2.4)	6.50 (4.0)	22.5
C. hydrocarbofumaria	1973	8.14	7.96 (66.3)	7.90 (57.2)	7.70 (36.4)	76.1
C. ingens	10057	7.00	5.99 (9.9)	5.70 (5.0)	6.23 (16.7)	NC
C. intermedia	0062	7.52	6.66 (13.7)	6.38 (7.2)	6.63 (12.7)	404.3

‡ Viable counts are expressed as the logarithm of the number of colony forming units.

‡‡ Limit survival time (LST) is the time taken for the survival count to decrease to 10^4 per ampule. See text.

‡‡‡ NC indicates the case in which the survival count after 5 years of storage was more than the viable count immediately after drying.

Table 2. (continued)

Species	IFO No.	log CFU[‡] per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C[‡‡] (years)
Candida intermedia	1118	8.10	7.67 (36.8)	6.59 (3.1)	7.49 (24.6)	105.0
C. kefyr	0008	7.85	6.93 (12.0)	6.36 (3.3)	6.65 (6.3)	52.4
C. kefyr	0432	7.69	6.40 (5.1)	6.17 (3.0)	6.23 (3.5)	73.3
C. lactis-condensi	1325	7.53	6.34 (6.5)	5.60 (1.2)	5.64 (1.3)	16.7
C. lactis-condensi	1326	7.03	6.04 (9.9)	5.23 (1.6)	5.03 (1.0)	10.2
C. langeronii	1974	8.11	7.61 (31.6)	7.30 (15.8)	7.50 (25.1)	180.5
C. maltosa	1975	7.80	7.25 (27.1)	7.04 (17.3)	7.05 (17.6)	86.5
C. maltosa	1976	7.89	7.17 (19.6)	7.14 (17.4)	7.11 (17.2)	280.5
C. maltosa	1977	7.88	7.44 (36.6)	7.38 (30.6)	7.23 (21.7)	76.0
C. maltosa	1978	7.45	7.12 (47.8)	7.11 (46.5)	7.10 (45.7)	799.6
C. mannitofaciens	1908	7.95	7.25 (20.0)	6.97 (10.6)	7.36 (26.1)	NC[‡‡‡]
C. marina	1979	8.04	7.48 (27.7)	7.23 (15.7)	7.14 (13.2)	53.9
C. maris	10003	8.36	7.46 (12.4)	7.65 (19.1)	7.68 (20.3)	NC
C. melinii	1431	7.50	7.20 (51.5)	6.08 (3.9)	7.00 (32.9)	82.5
C. mesenterica	1211	8.05	6.72 (4.8)	6.73 (4.9)	6.52 (3.0)	66.8
C. mesenterica	1301	7.86	7.41 (35.2)	5.91 (1.1)	6.71 (7.0)	24.3
C. musae	1582	8.22	7.77 (35.4)	6.71 (3.1)	6.82 (4.0)	19.9
C. nitratophila	10004	8.38	7.84 (29.4)	7.63 (18.1)	7.75 (23.9)	213.4
C. nodaensis	1942	8.07	7.80 (52.8)	6.98 (8.0)	6.78 (5.1)	18.7
C. oregonensis	1980	8.11	7.90 (59.6)	7.73 (40.0)	7.84 (52.5)	354.2
C. rugosa	0591	7.61	7.30 (48.9)	7.25 (44.9)	7.04 (27.4)	65.6
C. sake	1149	8.14	7.78 (44.0)	7.52 (24.3)	7.57 (27.2)	90.5
C. sake	1981	7.11	6.84 (55.2)	6.60 (31.9)	6.62 (33.0)	63.5
C. santamariae	1982	7.61	6.83 (16.6)	6.58 (9.3)	6.52 (8.0)	44.7
C. shehatae	1983	7.82	6.75 (8.6)	6.17 (2.3)	6.70 (7.7)	286.6
C. sonorensis	10027	7.88	7.63 (56.5)	7.41 (34.4)	7.71 (67.2)	NC
C. sorboxylosa	1578	7.93	7.65 (52.1)	7.57 (42.9)	7.56 (42.6)	208.8
C. succiphila	1911	8.44	8.08 (43.0)	7.72 (19.2)	8.04 (40.5)	782.8
C. tenuis	1303	8.20	7.43 (16.2)	6.53 (2.1)	6.92 (5.1)	34.1
C. tropicalis	1187	7.06	6.11 (11.3)	5.84 (6.1)	6.17 (13.0)	NC
C. tropicalis	1556	7.55	7.32 (58.4)	7.07 (32.1)	7.08 (33.5)	68.9
C. tsukubaensis	1940	7.79	7.65 (72.1)	6.46 (4.7)	6.80 (10.3)	21.6
C. utilis	0396	7.67	6.87 (15.8)	6.84 (14.7)	6.65 (9.4)	63.7
C. utilis	0639	7.77	7.00 (17.4)	6.98 (16.1)	6.78 (10.2)	64.8
C. valida	0166	7.57	7.23 (44.7)	7.04 (30.1)	6.82 (17.9)	40.5
C. valida	0842	7.31	7.04 (52.7)	5.90 (3.9)	6.48 (14.5)	27.1
C. versatilis	10038	7.34	6.61 (18.3)	5.49 (1.4)	5.34 (1.0)	10.3
C. vinaria	1092	8.17	7.71 (33.8)	6.73 (3.5)	7.38 (15.7)	55.8
Cryptococcus albidus	0612	6.98	6.74 (57.9)	6.65 (47.4)	6.19 (16.4)	25.0
C. albidus	0763	6.74	5.78 (10.9)	5.84 (12.7)	5.65 (8.2)	71.9
C. albidus	0953	6.90	6.70 (62.5)	6.69 (61.9)	6.63 (53.1)	190.6
C. albidus	0937	6.84	6.64 (62.9)	6.41 (36.4)	6.41 (36.4)	55.6
C. albidus	1420	7.28	6.79 (32.3)	6.30 (10.3)	6.40 (13.1)	35.6
C. albidus	1859	7.54	5.85 (2.0)	5.54 (1.0)	5.50 (0.9)	26.7
C. albidus	1860	6.95	6.15 (15.6)	5.60 (4.4)	5.33 (2.4)	13.2
C. albidus var. diffluens	1861	7.11	6.65 (35.4)	6.49 (24.1)	6.48 (23.6)	75.1
C. ater	1862	7.08	6.70 (41.6)	6.60 (32.6)	6.43 (22.1)	49.1
C. dimennae	1863	7.24	6.78 (34.3)	6.70 (28.9)	6.72 (29.7)	222.1

Table 2. (continued)

Species	IFO No.	log CFU[‡] per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C[‡‡] (years)
Cryptococcus flavus	0407	7.30	5.70 (2.5)	5.55 (1.8)	5.20 (0.8)	17.2
C. flavus	0710	7.11	5.90 (6.2)	5.54 (2.7)	5.71 (4.0)	50.1
C. flavus	1193	7.11	6.36 (17.7)	6.00 (7.7)	6.17 (11.5)	63.1
C. flavus	1222	7.25	6.34 (12.3)	5.81 (3.6)	5.61 (2.3)	16.1
C. hungaricus	1379	6.54	5.11 (3.9)	4.58 (1.1)	4.00 (0.3)	5.1
C. hungaricus	1864	6.58	5.92 (21.9)	5.83 (17.9)	5.76 (15.2)	60.5
C. kuelzingii	1866	8.04	7.40 (23.9)	7.17 (14.3)	7.14 (13.5)	63.6
C. laurentii	0372	6.81	6.32 (32.3)	6.39 (37.7)	6.02 (16.2)	38.7
C. laurentii	0384	6.84	6.70 (71.4)	6.41 (37.1)	6.53 (48.6)	80.8
C. laurentii	0698	6.65	6.36 (51.1)	6.36 (51.1)	6.29 (43.3)	164.2
C. laurentii	0757	6.54	6.32 (60.0)	6.09 (35.7)	6.07 (34.1)	47.3
C. laurentii	0765	7.02	6.71 (49.5)	6.64 (41.9)	6.59 (37.4)	111.5
C. laurentii	0766	7.09	6.86 (58.4)	6.84 (56.0)	6.80 (50.4)	223.8
C. laurentii	0930	7.70	7.23 (33.5)	6.80 (12.6)	7.15 (28.2)	215.8
C. laurentii	1011	6.84	6.50 (45.7)	6.38 (34.3)	6.14 (20.0)	34.9
C. laurentii	1321	6.65	6.32 (46.7)	5.81 (14.4)	5.59 (8.7)	15.9
C. laurentii	1376	7.02	6.66 (43.8)	6.39 (23.3)	6.56 (34.8)	133.3
C. laurentii	1487	6.90	6.45 (35.0)	6.24 (21.9)	6.40 (31.3)	252.2
C. laurentii	1898	5.30	4.41 (13.4)	3.58 (4.4)	4.53 (17.4)	NC[‡‡‡]
C. laurentii var. flavescen	1868	7.11	6.32 (15.9)	5.80 (4.8)	5.75 (4.4)	20.8
C. laurentii var. magnus	1869	6.70	6.23 (33.3)	6.11 (24.5)	5.60 (7.8)	17.6
C. luteolus	0411	6.74	6.11 (23.6)	5.95 (16.4)	5.70 (9.1)	25.5
C. luteolus	0611	7.04	6.28 (17.3)	6.20 (14.5)	6.25 (16.4)	491.2
C. macerans	0943	7.13	6.62 (20.8)	6.00 (7.4)	5.65 (3.3)	15.3
C. melibiosum	1871	7.93	7.60 (47.0)	7.55 (41.9)	7.54 (41.5)	332.8
C. skinneri	1872	6.04	5.77 (53.4)	5.55 (32.2)	4.93 (7.8)	10.6
C. terreus	1873	6.89	5.92 (10.8)	5.71 (6.5)	5.77 (7.6)	63.1
Kloeckera africana	0177	7.59	6.90 (20.0)	6.58 (9.6)	6.75 (14.2)	97.4
K. africana	1155	7.16	6.86 (50.0)	6.16 (10.0)	6.57 (25.5)	48.1
K. apiculata	0150	7.29	7.20 (81.5)	6.48 (15.4)	6.75 (29.2)	36.1
K. apiculata	0865	7.76	7.33 (37.4)	7.21 (28.4)	7.37 (40.6)	NC
K. apiculata	0866	7.42	7.05 (42.6)	6.78 (22.8)	6.82 (25.1)	66.4
K. jansenii	1157	7.50	7.02 (33.1)	6.43 (8.4)	7.00 (31.4)	660.5
K. javanica	0669	7.63	7.07 (27.2)	6.70 (11.6)	6.96 (21.2)	141.7
K. javanica	1094	7.19	6.91 (52.9)	6.25 (11.6)	6.63 (27.7)	50.4
K. javanica	1095	6.87	6.23 (22.7)	5.34 (2.9)	5.45 (3.8)	14.4
K. javanica	1096	7.49	6.91 (26.5)	6.84 (22.1)	6.83 (21.8)	171.9
K. javanica	1156	7.42	6.88 (28.7)	6.73 (20.2)	6.73 (20.2)	74.4
K. javanica	1158	7.73	6.86 (13.4)	6.85 (13.2)	7.03 (19.6)	NC
K. javanica	1248	7.55	7.25 (49.7)	7.06 (31.7)	6.80 (17.8)	36.5
K. javanica	1328	7.50	7.04 (34.7)	6.59 (12.3)	6.85 (22.3)	79.3
Malassezia pachydermatis	1041	8.62	8.20 (39.3)	8.08 (27.5)	8.11 (30.8)	199.2
Rhodotorula aurantiaca	0754	7.47	7.23 (58.3)	7.08 (40.5)	7.15 (48.1)	193.7
R. aurantiaca	0756	7.44	7.12 (47.9)	7.13 (48.2)	7.17 (53.0)	NC
R. aurantiaca	0951	8.01	7.57 (36.2)	7.03 (10.5)	6.70 (4.9)	20.5
R. aurantiaca	1221	7.64	7.34 (50.5)	7.29 (44.2)	7.20 (36.1)	114.3
R. glutinis	0386	7.39	6.84 (28.6)	6.26 (7.5)	6.04 (4.5)	17.7

Table 2. (continued)

Species	IFO No.	log CFU[‡] per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C[‡‡] (years)
Rhodotorula glutinis	0667	7.41	6.11 (4.9)	5.87 (2.8)	5.11 (0.5)	10.7
R. glutinis	0695	6.95	6.25 (20.0)	6.05 (12.4)	5.95 (10.0)	37.5
R. glutinis	1099	7.53	7.23 (50.0)	7.16 (43.2)	7.02 (31.2)	78.9
R. glutinis	1125	7.02	6.34 (21.0)	6.31 (19.5)	6.11 (12.4)	51.2
R. glutinis	1240	7.43	6.86 (27.1)	6.47 (10.9)	6.45 (10.4)	34.4
R. glutinis	1241	7.35	6.78 (27.2)	6.23 (7.6)	6.57 (16.7)	65.8
R. glutinis	1438	7.73	7.46 (53.7)	7.23 (31.7)	7.15 (26.7)	57.0
R. glutinis	1503	7.60	7.23 (42.8)	6.49 (7.8)	6.89 (19.4)	47.1
R. glutinis	1535	7.92	7.40 (30.3)	7.20 (19.3)	7.15 (17.0)	67.7
R. graminis	0190	6.60	6.11 (32.5)	5.54 (8.8)	5.18 (3.8)	11.3
R. graminis	1422	7.46	6.80 (21.7)	6.62 (14.3)	6.62 (14.3)	77.3
R. lactosa	1058	7.29	7.00 (51.8)	6.98 (49.7)	6.95 (45.4)	262.3
R. lactosa	1423	7.31	6.69 (23.9)	6.88 (36.8)	6.55 (17.3)	95.8
R. lactosa	1424	7.52	7.04 (33.1)	6.96 (27.6)	6.77 (17.9)	56.9
R. marina	0928	7.71	7.15 (27.6)	6.97 (18.0)	6.99 (18.9)	95.9
R. marina	1421	7.94	7.63 (48.3)	7.34 (28.2)	7.40 (28.4)	78.7
R. minuta	0932	7.52	7.16 (44.3)	7.11 (38.8)	7.05 (34.1)	139.2
R. minuta	1006	7.78	7.40 (41.7)	7.24 (28.8)	7.28 (31.9)	146.0
R. minuta	1435	7.74	7.58 (68.5)	7.43 (48.3)	7.47 (53.1)	161.8
R. psychophila	1122	6.58	6.19 (40.8)	6.37 (61.8)	5.83 (17.1)	29.0
R. rubra	0915	7.24	6.59 (22.3)	6.33 (12.3)	6.16 (8.3)	30.2
R. rubra	0931	7.53	7.03 (31.5)	6.96 (27.2)	7.09 (36.3)	NC[‡‡‡]
Sporobolomyces gracilis	1033	7.13	6.36 (17.0)	6.15 (10.4)	6.19 (11.5)	69.5
S. holsaticus	0923	6.81	4.71 (0.8)	4.99 (1.5)	5.07 (1.8)	NC
S. holsaticus	1109	5.48	4.17 (5.2)	4.00 (3.4)	3.71 (1.7)	2.0
S. pararoseus	0376	6.74	6.25 (32.7)	6.08 (21.8)	5.74 (10.0)	21.9
S. pararoseus	0471	7.23	6.32 (12.5)	5.67 (2.8)	5.81 (3.9)	23.0
S. pararoseus	1036	7.13	5.77 (4.4)	5.47 (2.2)	5.17 (1.1)	14.7
S. pararoseus	1103	7.00	6.11 (13.0)	5.74 (5.5)	5.70 (5.0)	25.4
S. pararoseus	1104	7.30	6.73 (27.0)	6.31 (10.3)	6.33 (10.8)	34.3
S. pararoseus	1105	6.60	6.04 (27.5)	5.17 (8.8)	5.00 (5.0)	13.8
S. pararoseus	1107	6.95	6.46 (32.2)	6.23 (18.9)	6.17 (16.7)	43.2
S. roseus	0373	6.74	6.34 (40.0)	6.13 (24.5)	6.00 (18.2)	34.2
S. roseus	0925	6.60	5.70 (12.5)	5.28 (4.8)	5.30 (5.0)	21.4
S. roseus	0927	6.74	5.60 (7.3)	5.40 (4.5)	5.48 (5.5)	65.2
S. roseus	1037	6.70	5.90 (16.0)	5.30 (4.0)	5.48 (6.0)	22.3
S. roseus	1040	6.65	5.17 (3.4)	5.00 (2.3)	5.17 (3.3)	456.8
S. roseus	1106	6.65	5.60 (8.9)	4.97 (2.1)	5.17 (3.3)	18.6
S. odorus	1597	6.00	4.34 (2.2)	4.34 (2.2)	4.04 (1.1)	5.7
Sterigmatomyces elviae	1999	7.84	7.64 (63.4)	7.50 (45.6)	7.67 (66.9)	NC
S. halophilus	1488	7.73	7.48 (56.5)	7.28 (35.5)	7.26 (34.2)	79.9
S. indicus	1844	7.52	6.91 (25.0)	5.99 (3.0)	6.48 (9.2)	33.6
Trichosporon brassicae	1584	7.74	7.34 (39.0)	7.00 (18.1)	6.86 (13.1)	35.2
T. capitatum	0743	6.79	6.75 (91.9)	6.46 (46.9)	6.79 (100.0)	NC
T. cutaneum	0009	7.85	6.74 (7.7)	6.17 (2.2)	6.06 (1.6)	20.1
T. cutaneum	0173	7.65	6.60 (8.9)	6.41 (5.9)	6.41 (5.9)	72.8
T. cutaneum	0174	7.71	7.53 (65.7)	7.43 (51.2)	7.34 (42.9)	95.5
T. cutaneum	1198	7.43	6.70 (18.5)	6.20 (5.8)	6.60 (14.8)	139.5
T. cutaneum	1200	7.49	6.94 (28.3)	6.45 (9.1)	6.78 (19.4)	89.8

Table 2. (continued)

Species	IFO No.	log CFU‡ per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C‡‡ (years)
Trichosporon cutaneum	1202	7.50	7.42 (82.9)	7.49 (95.7)	7.43 (84.0)	NC‡‡‡
T. cutaneum	1203	7.57	7.57(100.0)	7.57(100.0)	7.49 (84.2)	238.5
T. cutaneum	1204	7.99	7.82 (67.8)	7.75 (58.8)	7.68 (49.6)	140.6
T. cutaneum	1205	8.17	7.99 (64.8)	7.88 (51.0)	7.86 (48.3)	156.0
T. cutaneum	1206	7.53	7.20 (47.0)	7.04 (32.4)	7.08 (35.5)	131.2
T. cutaneum	1500	7.62	6.49 (7.3)	6.00 (2.5)	6.48 (7.2)	2078.0
T. cutaneum	1534	7.69	6.11 (2.7)	6.00 (2.1)	6.11 (2.7)	NC
T. pullulans	0116	7.54	7.08 (32.6)	6.92 (23.9)	6.78 (17.3)	55.6

Table 3. Viabilities at 5 C of dried cultures of basidiomycetous yeasts with survival values higher than 1% in the 60-day accelerated test at 37 C.

Species	IFO No.	log CFU‡ per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C‡‡ (years)
Filobasidium floriforme	1915	7.46	7.20 (54.5)	6.97 (32.5)	6.87 (25.6)	48.7
F. floriforme	1916	7.54	7.34 (63.5)	7.30 (56.5)	7.08 (34.5)	63.1
Leucosporidium scottii	0736	7.28	6.36 (12.1)	5.74 (2.9)	5.64 (2.3)	16.4
L. scottii	1306	7.78	7.36 (37.4)	6.40 (4.2)	6.59 (6.5)	22.1
L. scottii	1923	7.58	6.80 (16.9)	6.59 (10.2)	6.65 (11.7)	87.8
L. scottii	1924	7.58	6.60 (10.6)	6.38 (6.3)	6.08 (3.2)	25.0
L. scottii	1925	7.57	6.65 (12.1)	6.43 (7.2)	6.45 (7.6)	65.7
Rhodosporidium bisporidiis	1927	7.92	7.04 (13.7)	6.62 (5.0)	6.71 (6.1)	43.6
R. bisporidiis	1928	7.96	7.00 (11.1)	6.40 (2.8)	6.62 (4.6)	41.3
R. capitatum	1929	6.84	6.05 (16.2)	5.84 (10.1)	6.04 (15.9)	1261.0
R. dacryoidum	1930	7.80	7.57 (57.9)	7.41 (41.0)	7.54 (54.6)	699.7
R, dacryoidum	1931	7.73	7.50 (58.4)	7.48 (56.0)	7.53 (63.3)	NC‡‡‡
R. diobovatum	1932	7.58	7.28 (50.3)	7.17 (39.2)	6.93 (22.4)	46.8
R. infirmo-miniatum	1005	7.16	6.20 (11.1)	5.88 (5.2)	6.04 (7.6)	67.1
R. infirmo-miniatum	1057	7.28	6.58 (20.0)	6.52 (17.4)	6.48 (16.1)	136.9
R. infirmo-miniatum	1223	7.41	6.25 (7.0)	6.11 (5.0)	5.67 (1.8)	19.2
R. infirmo-miniatum	1378	7.45	6.54 (12.3)	6.57 (13.2)	6.30 (7.0)	52.0 NC
R. infirmo-miniatum	1865	7.75	6.98 (17.0)	6.61 (7.4)	6.11 (2.3)	17.1
R. infirmo-miniatum	1933	7.89	7.38 (31.1)	7.17 (19.7)	7.14 (17.9)	69.7

‡ Viable counts are expressed as the logarithm of the number of colony forming units.

‡‡ Limit survival time (LST) is the time taken for the survival count to decrease to 10^4 per ampule. See text.

‡‡‡ NC indicates the case in which the survival count after 5 years of storage was more than the viable count immediately after drying.

Table 3. (continued)

Species	IFO No.	log CFU[‡] per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C[‡‡] (years)
Rhodosporidium infermo-miniatum	1934	7.92	7.52 (40.0)	7.17 (17.4)	7.38 (29.0)	126.1
R. malvinellum	1935	7.80	6.94 (13.8)	6.04 (1.7)	6.61 (6.4)	44.1
R. malvinellum	1936	7.92	7.54 (41.5)	7.00 (12.0)	7.47 (35.5)	261.1
R. toruloides	0388	7.58	6.41 (6.8)	6.14 (3.6)	6.00 (2.6)	29.0
R. toruloides	0413	7.59	6.58 (9.7)	6.52 (8.5)	5.07 (0.3)	8.5
R. toruloides	0871	7.84	7.31 (29.4)	7.19 (22.2)	7.31 (29.6)	NC[‡‡‡]
R. toruloides	0880	7.77	7.25 (29.9)	6.98 (16.1)	7.04 (18.7)	79.7
R. toruloides	1236	7.67	7.02 (22.5)	6.92 (17.7)	6.93 (18.0)	156.0
R. toruloides	10032	7.97	7.11 (14.2)	6.82 (7.0)	6.83 (7.1)	51.9
R. toruloides	10033	8.23	7.63 (26.3)	7.53 (20.7)	7.20 (9.4)	40.7
R. toruloides	10034	8.08	7.25 (14.2)	6.64 (3.5)	6.86 (5.7)	41.0
R. sphaerocarpum	1937	7.73	7.48 (55.9)	7.31 (37.8)	7.29 (36.4)	93.3
R. sphaerocarpum	1938	6.73	6.00 (18.6)	5.95 (16.5)	5.78 (11.5)	47.9
R. sphaerocarpum	1939	7.17	6.66 (30.3)	6.32 (13.4)	6.28 (12.2)	33.6
Sporidiobolus ruinenii	1689	7.34	6.08 (5.2)	5.75 (2.5)	6.20 (7.2)	NC
S. salmonicolor	1845	7.31	6.40 (12.0)	5.75 (2.8)	5.69 (2.4)	17.1

Of the 553 strains examined, it is estimated that the survival count of the following 9 strains will reach 10^4 between 5 and 10 years of storage: Dipodascus albidus IFO 1984, Pichia humboldtii IFO 10060, P. membranaefaciens IFO 1284, Zygosaccharomyces bailii IFO 1098, Z. rouxii IFO 0443, Bullera alba IFO 1192, Candida albicans IFO 1067, Sporobolomyces odorus IFO 1597, Rhodosporidium toruloides IFO 0413.

Dried cultures whose survival values are predicted to decrease to 10^4 between 10 and 15 years of storage are the following 19 strains: Arxiozyma telluris IFO 1331, Endomyces magnusii IFO 0110, Issatchenkia scutulata var. exigua IFO 10050, Klyveromyces phaffii IFO 1884, Nadsonia elongata IFO 0665, Pichia farinosa IFO 0464, Zygosaccharomyces rouxii IFO 0451 & 0526, Candida albicans IFO 1262, C. halonitratophila IFO 1561, C. lactis-condensi IFO 1326, C. versatilis IFO 10038, Cryptococcus albidus IFO 1860, Cryp. skinneri IFO 1872, Kloeckera javanica IFO 1095, Rhodotorula glutinis IFO 0667, R. graminis IFO 0190, Sporobolomyces pararoseus IFO 1036 & 1105.

Of the 553 strains, the limit survival time of dried cultures of 61 strains are over 15 years and those of other majority, 460 strains, are over 25 years.

The survival counts after 5 years at 5 C are plotted against the survival counts in the 60-day accelerated storage test on a log scale in

Fig. 1. The correlation coefficient is as high as 0.939. The relation is expressed by a regressive equation [3]:

$$V_{5y} = 1.07 \times V_{60d} + 1.45 \times 10^3$$

(V_{5y}: survival count after 5 years of storage at 5 C, V_{60d}: survival count in 60-day accelerated test).

This high correlation coefficient supports the idea that the viability after preservation at 5 C can be reliably estimated for dried cultures from the data of a short-term accelerated test and that its limit survival time at 5 C can also be predicted. If a dried culture shows a higher survival value than 1% in the accelerated test, it is unnecessary to check its viability during preservation at 5 C.

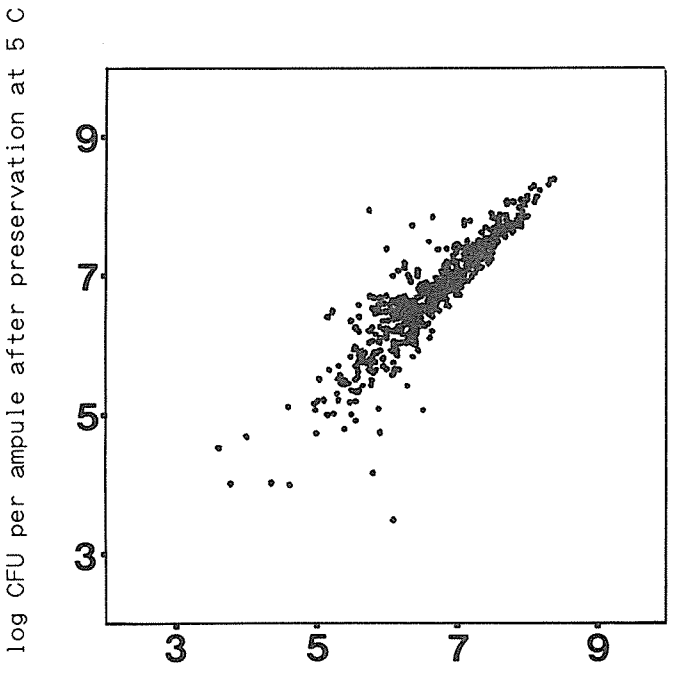


Fig. 1. Correlation between the survival counts (log CFU) of L-dried cells after 5 years of preservation at 5 C and after 60 days of accelerated storage at 37 C in strains with survival values higher than 1%.

2. Viabilities after 5-year storage at 5 C of dried cultures with viabilities lower than 1% in the accelerated test.

The results for 148 yeast strains with survival values below 1% in the 60-day accelerated test are presented in Tables 4-6. Tables 4, 5, and 6 list ascomycetous yeasts (19 genera, 38 species, 73 strains),

deuteromycetous yeasts (7 genera, 40 species, 61 strains), and basidiomycetous yeasts (2 genera, 7 species, 14 strains) respectively. In these dried cultures, survival values ranged from 0.0001 to 15%. The survival values of 53 of the 148 strains are higher than 2%. It seems likely that the dried cells of these strains are very sensitive in the dried state at a high temperature but not so at a lower temperature.

In the dried cultures of only 2 strains, Eremothecium ashbyi IFO 1425, and Saccharomyces exiguus IFO 1616, were the survival counts less than 10^4 per ampule immediately after drying. But the reduction rate at 5 C of IFO 1425 is greater than 0.8. This strain is sensitive to the drying process but stable in the dried state. If a dried culture with a higher viable count could be prepared by an improved drying method, this strain would be safely preserved for a long term.

Dried cultures of the following 22 strains gave viable counts lower than 10^4 after 5 years at 5 C: Ambrosiozyma monospora IFO 4841, Arxiozyma telluris IFO 1017, 1329 & 1330, Lipomyces starkeyi IFO 0678 & 1289, Nadsonia commutata IFO 10030, Nematospora coryli IFO 0658, Saccharomyces exiguus IFO 0956 & 1128, Saccharomycopsis fibuligera IFO 0104, Candida bovina IFO 1069, C. famata IFO 0405, Cryptococcus hungaricus IFO 1052, Rhodotorula glutinis IFO 1224, R. rubra IFO 0712, Trichosporon pullulans IFO 1232, Leucosporidium antarcticum IFO 1917 & 1918, L. nivalis IFO 1852, and L. scottii IFO 1529.

The reduction rates and LSTs of the 148 strains were estimated by equations [1] and [2] respectively. The reduction rates range from 0.3 to 0.99 and average 0.65. The LSTs are presented in the last column of the tables.

Dried cultures whose survival counts are estimated to decrease to 10^4 between 5 to 10 years of storage are the following 37 strains: Ambrosiozyma cicatricosa IFO 1846, Ambrosiozyma monospora IFO 1965, A. philentoma IFO 1847, Arthroascus javanensis IFO 1579, Debaryomyces polymorphus IFO 1166 & 1357, Dekkera bruxellensis IFO 1590, Issatchenkia scutulata var. exigua IFO 10051, Kluyveromyces phaffii IFO 1883, K. polysporus IFO 0996, Lipomyces lipofer IFO 0673 and 1288, Nematospora coryri IFO 1220, Pichia chambardii IFO 1029, P. pinus IFO 1342, Saccharomyces dairensis IFO 10009, S. exiguus IFO 1141 & 1169, Zygosaccharomyces rouxii IFO 1615, Brettanomyces bruxellensis IFO 0628, Brettanomyces custersianus IFO 1585, Candida bovina IFO 0873 & 1018, C. curvata IFO 1159, C. holmii IFO 0660 & 1629, C.

Table 4. Viabilities at 5 C of dried cultures of ascomycetous yeasts with survival values lower than 1% in 60-day accelerated test at 37 C.

Species	IFO No.	log CFU‡ per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C‡‡ (years)
Ambrosiozyma cicatricosa	1846	6.99	5.34 (2.3)	3.70 (0.05)	4.30 (0.2)	6.4
A. monospora	1965	7.77	6.71 (8.8)	4.08 (0.02)	5.08 (0.2)	8.3
A. monospora	4841	6.61	4.38 (0.6)	<2.91 (<0.02)	3.52 (0.08)	2.2
A. philentoma	1847	7.49	5.57 (1.2)	4.17 (0.05)	4.38 (0.08)	6.7
Arthroascus javanensis	1579	7.78	5.86 (1.2)	3.63 (0.007)	4.38 (0.04)	6.3
Arxiozyma telluris	1017	6.78	4.68 (0.8)	3.68 (0.08)	2.68 (0.008)	1.7
A. telluris	1329	6.78	4.89 (1.3)	4.08 (0.2)	3.48 (0.05)	3.2
A. telluris	1330	6.65	5.48 (6.7)	3.65 (0.1)	3.95 (0.2)	4.9
A. telluris	1897	7.30	5.97 (4.7)	4.00 (0.05)	5.66 (2.3)	31.8
Debaryomyces coudertii	1817	8.04	7.71 (46.3)	4.04 (0.01)	7.04 (10.3)	28.4
D. hansenii	1084	8.11	5.98 (0.7)	5.83 (0.5)	7.38 (12.5)	NC‡‡‡
D. polymorphus	1166	7.20	6.38 (15.0)	4.91 (0.5)	4.04 (0.07)	5.1
D. polymorphus	1357	7.04	6.08 (11.3)	4.36 (0.2)	4.53 (0.3)	6.7
D. tamarii	0854	7.28	6.45 (14.7)	<4.75 (<0.3)	5.48 (1.6)	12.7
Dekkera bruxellensis	1590	7.32	5.95 (4.2)	<4.63 (<0.2)	4.63 (0.2)	7.4
Eremothecium ashbyi	1425	6.54	4.02 (0.3)	4.14 (0.4)	3.54 (0.1)	0.2
Hansenula ciferrii	0905	6.98	6.04 (11.6)	4.88 (0.8)	5.60 (4.2)	23.1
H. dimennae	1771	7.92	7.38 (28.9)	5.87 (0.9)	6.94 (10.5)	38.5
H. subpelliculosa	0808	7.89	7.14 (18.0)	5.59 (0.5)	6.57 (4.8)	27.4
Issatchenkia scutulata var. exigua	10051	7.69	6.68 (9.8)	5.53 (0.7)	5.28 (0.4)	9.7
Kluyveromyces phaffii	1883	7.83	6.40 (3.8)	4.80 (0.1)	4.82 (0.1)	7.6
K. polysporus	0996	8.08	6.20 (1.3)	5.08 (0.1)	4.99 (0.08)	9.1
Lipomyces lipofer	0673	7.02	4.71 (0.5)	4.32 (0.2)	4.32 (0.2)	9.0
L. lipofer	1288	6.70	5.20 (3.2)	<4.44 (<0.5)	4.30 (0.4)	6.7
L. starkeyi	0678	6.70	5.60 (8.0)	3.70 (0.1)	3.70 (0.1)	4.2
L. starkeyi	1289	7.28	5.78 (3.2)	4.98 (0.5)	3.98 (0.05)	4.9
Nadsonia commutata	10030	6.65	4.55 (0.8)	4.34 (0.5)	<3.82 (<0.01)	1.4
Nematospora coryli	0658	6.65	4.11 (0.3)	<3.82 (<0.06)	2.00 (0.0004)	0.2
N. coryli	1220	6.65	4.91 (1.8)	3.95 (0.2)	4.25 (0.4)	7.0
Pichia angophorae	10016	7.43	5.90 (3.0)	4.90 (0.3)	5.57 (1.4)	28.8
P. chambardii	1029	7.30	6.59 (19.1)	5.14 (0.7)	5.27 (0.9)	9.8
P. fermentans	0815	7.83	6.82 (10.9)	5.80 (0.2)	6.00 (1.7)	17.5
P. fluxuum	0773	7.75	6.91 (14.2)	5.34 (0.4)	6.38 (4.2)	27.5
P. membranaefaciens	0457	7.80	7.17 (24.3)	5.40 (0.4)	6.11 (2.1)	15.0
P. membranaefaciens	0460	7.72	7.00 (19.7)	5.49 (0.6)	5.95 (1.7)	14.2
P. membranaefaciens	0814	7.77	7.17 (25.6)	5.66 (0.8)	6.20 (2.7)	16.3
P. membranaefaciens	0916	7.40	6.32 (8.4)	<4.70 (<0.2)	6.57 (15.0)	NC
P. pinus	1342	7.66	6.14 (3.1)	3.28 (0.004)	4.14 (0.03)	5.4
P. stipitis	1720	8.20	6.61 (2.6)	6.04 (0.7)	6.55 (2.3)	245.4
P. stipitis	1968	8.20	6.23 (1.1)	4.67 (0.03)	5.67 (0.3)	19.9
Saccharomyces dairensis	10008	7.85	6.57 (5.1)	4.14 (0.02)	5.46 (0.4)	11.6
S. dairensis	10009	7.75	6.48 (5.3)	5.36 (0.4)	5.23 (0.3)	9.6

‡ Viable counts are expressed as the logarithm of the number of colony forming units.

‡‡ Limit survival time (LST) is the time taken for the survival count to decrease to 10^4 per ampule. See text.

‡‡‡ NC indicates the case in which the survival count after 5 years of storage was more than the viable count immediately after drying.

Table 4. (continued)

Species	IFO No.	log CFU[‡] per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C[‡‡] (years)
Saccharomyces exiguus	0956	7.55	5.78 (1.7)	<4.55 (<0.1)	3.55 (0.01)	4.0
S. exiguus	1128	7.57	5.53 (0.9)	<4.57 (<0.1)	3.87 (0.02)	4.6
S. exiguus	1141	7.63	6.11 (3.1)	<4.63 (<0.1)	4.62 (0.1)	7.1
S. exiguus	1142	7.94	6.65 (5.1)	5.72 (0.6)	5.54 (0.4)	12.0
S. exiguus	1169	7.25	5.50 (1.8)	<3.73 (<0.03)	4.55 (0.2)	7.9
S. exiguus	1170	7.58	6.32 (5.5)	5.36 (0.6)	5.36 (0.6)	12.1
S. exiguus	1616	7.36	<3.66 (<0.02)	<3.66 (<0.02)	1.48 (0.0001)	NC[‡‡‡]
Saccharomycodes ludwigii	1724	6.96	6.11 (14.0)	3.80 (0.07)	5.48 (3.3)	16.8
Saccharomycopsis capsularis	0672	6.38	5.32 (8.9)	4.23 (0.8)	4.84 (2.9)	13.7
S. fibuligera	0104	6.78	4.25 (0.3)	<4.48 (<0.5)	3.78 (0.1)	2.7
S. vini	1749	6.00	4.54 (3.5)	<3.70 (<0.5)	4.45 (2.8)	28.1
S. vini	1750	6.65	5.78 (13.3)	4.11 (0.3)	5.03 (2.4)	12.0
Schizosaccharomyces octosporus	0353	7.62	6.63 (10.2)	5.52 (0.8)	5.32 (0.5)	10.1
S. octosporus	0360	7.95	6.04 (1.3)	4.95 (0.1)	5.90 (0.9)	64.7
S. japonicus	1713	6.36	5.04 (4.9)	4.25 (0.8)	4.63 (1.9)	12.8
Schwanniomyces occidentalis	1841	8.25	6.49 (1.6)	5.23 (0.1)	6.00 (0.6)	28.6
Zygosaccharomyces rouxii	0320	6.98	6.46 (30.5)	4.88 (0.8)	6.04 (11.7)	29.6
Z. rouxii	0325	7.36	6.58 (16.5)	4.96 (0.4)	5.84 (3.0)	17.4
Z. rouxii	0326	7.17	6.59 (26.0)	4.78 (0.4)	5.75 (3.8)	15.5
Z. rouxii	0328	7.17	6.53 (22.7)	4.65 (0.3)	5.87 (5.0)	19.3
Z. rouxii	0331	7.17	6.96 (27.3)	5.11 (0.9)	6.28 (12.5)	38.5
Z. rouxii	0542	7.63	6.82 (15.8)	5.53 (0.8)	6.43 (6.4)	36.0
Z. rouxii	0570	7.48	6.38 (7.9)	5.38 (0.8)	6.23 (5.5)	75.7
Z. rouxii	0595	7.38	6.41 (10.8)	5.32 (0.9)	6.17 (6.3)	51.6
Z. rouxii	0596	7.16	6.32 (14.5)	5.04 (0.8)	5.87 (5.5)	27.6
Z. rouxii	0597	7.13	6.23 (12.6)	4.60 (0.3)	5.38 (6.3)	37.1
Z. rouxii	0687	6.98	5.95 (9.5)	4.93 (0.9)	5.84 (7.4)	90.1
Z. rouxii	1615	7.45	7.30 (70.2)	5.23 (0.6)	5.30 (0.7)	8.3
Z. rouxii	1731	7.07	6.57 (31.3)	3.54 (0.03)	5.81 (5.5)	17.0
Z. rouxii	1877	7.61	6.62 (10.5)	4.45 (0.07)	6.78 (14.8)	NC
Z. rouxii	1946	7.46	7.23 (58.2)	4.46 (0.1)	6.50 (11.1)	22.4

Table 5. Viabilities at 5 C of dried cultures of deuteromycetous yeasts with survival values lower than 1% in the 60-day accelerated test at 37 C.

Species	IFO No.	log CFU[‡] per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C[‡‡] (years)
Brettanomyces anomalus	0642	7.48	6.70 (16.4)	<4.48 (<0,1)	6.37 (7.7)	41.1
B. anomalus	0796	7.36	6.74 (23.9)	<4.36 (<0.1)	6.47 (13.0)	51.8
B. bruxellensis	0628	7.90	7.14 (18.4)	5.67 (0.6)	5.49 (0.4)	9.5
B. bruxellensis	0629	7.73	6.63 (7.9)	4.73 (0.1)	6.40 (4.6)	56.1
B. bruxellensis	0677	7.85	5.96 (1.3)	<4.27 (<0.1)	5.14 (0.2)	12.1
B. custersianus	1585	7.70	6.32 (4.1)	5.00 (0.2)	4.70 (0.1)	7.2
B. custersii	1586	7.27	6.68 (25.9)	<4.27 (<0.1)	6.15 (7.6)	25.2
B. intermedius	1587	7.33	5.85 (3.3)	<4.63 (<0.2)	5.41 (1.2)	21.1

Table 5. (continued)

Species	IFO No.	log CFU[‡] per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C[‡‡] (years)
Candida auriculariae	1580	7.47	6.66 (15.6)	<4.77 (<0.2)	5.54 (1.2)	12.0
C. boidnii	1967	7.97	6.99 (10.5)	5.66 (0.5)	6.75 (6.1)	63.4
C. bovina	0873	7.34	5.54 (1.6)	3.64 (0.02)	4.64 (0.2)	8.6
C. bovina	1018	7.19	4.97 (0.6)	<3.67 (<0.03)	4.08 (0.08)	5.5
C. bovina	1069	7.03	4.50 (0.3)	2.64 (0.004)	3.50 (0.03)	2.6
C. bovina	1087	7.44	5.78 (2.2)	4.74 (0.2)	4.92 (0.3)	10.3
C. bovina	1312	7.31	6.11 (6.3)	4.30 (0.1)	5.93 (4.2)	59.9
C. bovina	1313	7.35	5.95 (4.0)	<4.65 (<0.2)	5.20 (0.7)	12.9
C. buinensis	1642	8.33	7.43 (13.6)	6.08 (0.6)	6.34 (1.1)	15.7
C. catenulata	0731	7.87	7.48 (39.7)	<4.72 (<0.07)	6.69 (6.6)	22.3
C. colliculosa	1557	7.73	7.36 (42.4)	<4.73 (<0.1)	6.87 (13.9)	34.7
C. curvata	1159	7.38	6.04 (4.6)	5.14 (0.6)	4.68 (0.2)	7.5
C. curvata	1858	7.36	5.76 (2.5)	5.14 (0.6)	5.74 (2.4)	497.3
C. diffluens	1522	7.40	6.75 (22.4)	5.00 (0.4)	6.25 (7.3)	28.2
C. diversa	1091	8.51	7.75 (18.6)	6.32 (0.7)	7.14 (4.8)	31.9
C. etchelisii	1229	7.67	7.17 (31.6)	4.98 (0.2)	6.11 (2.8)	15.1
C. famata	0405	7.71	5.49 (0.6)	5.41 (0.5)	3.41 (0.005)	3.6
C. fructus	1581	8.14	7.67 (33.9)	5.14 (0.1)	6.87 (5.4)	23.0
C. glabrata	0861	7.93	6.99 (11.4)	5.83 (0.8)	5.62 (0.5)	11.0
C. glabrata	1085	7.91	6.99 (12.3)	5.75 (0.7)	5.81 (0.8)	12.6
C. halonitratophila	1595	7.97	6.63 (4.6)	<4.67 (<0.05)	5.57 (0.4)	12.4
C. halonitratophila	1906	8.17	7.30 (13.4)	5.46 (0.2)	6.69 (3.3)	27.1
C. holmii	0660	7.54	6.30 (5.8)	4.53 (0.1)	4.30 (0.06)	5.8
C. holmii	1629	7.50	5.78 (1.9)	4.80 (0.2)	4.80 (0.2)	9.1
C. humicola	1527	8.31	7.28 (9.3)	<4.61 (<0.02)	6.58 (1.9)	23.8
C. ingeniosa	10002	7.46	6.11 (4.6)	5.30 (0.7)	5.04 (0.4)	10.0
C. kefyr	0888	7.61	6.98 (23.4)	5.08 (0.3)	6.38 (6.0)	25.2
C. lactis-condensi	1324	7.03	5.99 (9.1)	4.88 (0.7)	5.34 (2.0)	15.2
C. mesenterica	0969	7.17	6.50 (21.3)	4.48 (0.2)	5.45 (1.9)	11.9
C. mesenterica	1210	7.85	5.92 (1.1)	5.34 (0.3)	5.58 (0.5)	28.1
C. mesenterica	1292	6.65	5.23 (3.8)	4.25 (0.4)	4.49 (0.7)	8.4
C. pintolopesii	0729	7.07	5.96 (7.8)	3.67 (0.04)	4.67 (0.4)	7.6
C. pinus	0741	8.27	7.11 (7.1)	5.25 (0.1)	6.70 (2.7)	37.2
C. pinus	1327	7.83	6.95 (13.1)	5.68 (0.7)	6.69 (7.3)	58.1
C. psychrophila	1532	7.29	5.69 (<2.5)	<4.59 (<0.2)	6.08 (6.4)	NC[‡‡‡]
C. psychrophila	1533	7.55	5.69 (<1.4)	<4.55 (<0.1)	6.08 (3.5)	NC
C. vinaria	1259	8.14	7.40 (17.4)	<5.64 (<0.3)	6.41 (1.8)	17.3
Cryptococcus albidus	1322	6.87	4.95 (1.2)	4.78 (0.8)	4.78 (0.8)	27.1
C. albidus var. aerius	1860	6.49	5.45 (9.2)	4.25 (0.6)	4.32 (0.7)	6.5
C. dimennae	1863	6.86	5.11 (1.8)	4.34 (0.3)	4.14 (0.2)	5.9
C. hungaricus	1052	7.49	5.49 (1.0)	<4.49 (<0.1)	2.97 (0.003)	3.0
C. hungaricus	1380	6.93	5.04 (1.3)	4.57 (0.4)	4.23 (0.2)	6.4
Kloeckera apiculata	0151	7.81	6.84 (10.6)	5.59 (0.6)	6.33 (3.3)	28.0
Rhodotorula glutinis	0391	6.00	5.54 (35.0)	3.60 (0.4)	4.32 (2.1)	6.3
R. glutinis	0667	6.78	4.85 (1.2)	4.48 (0.5)	5.01 (1.7)	NC
R. glutinis	1224	6.08	5.65 (37.9)	3.55 (0.3)	3.08 (0.1)	3.2
R. rubra	0712	6.32	4.80 (3.0)	2.17 (0.007)	2.32 (0.01)	1.6
Sporobolomyces holsaticus	1032	6.78	4.08 (0.2)	3.78 (0.1)	4.08 (0.2)	NC

Table 5. (continued)

Species	IFO No.	log CFU* per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C** (years)
Sporobolomyces holsaticus	1034	6.78	4.25 (0.3)	4.25 (0.3)	4.38 (0.4)	NC***
S. pararoseus	0471	6.98	5.84 (7.4)	3.98 (0.1)	4.67 (0.5)	7.9
Trichosporon cutaneum	0113	7.41	5.61 (1.6)	4.41 (0.1)	4.41 (0.1)	6.7
T. cutaneum	0598	6.60	5.90 (20.0)	<3.60 (<0.1)	5.30 (5.0)	15.8
T. pullulans	1232	6.74	5.28 (3.6)	<3.74 (<0.1)	3.74 (0.1)	4.2

Table 6. Viabilities at 5 C of dried cultures of basidiomycetous yeasts with survival values lower than 1% in the 60-day accelerated test at 37 C.

Species	IFO No.	log CFU* per ampule (survival value %) before drying	preservation at 37 C 0	preservation at 37 C 60 days	at 5 C 5 years	predicted LST at 5 C** (years)
Leucosporidium antarcticum	1917	6.82	4.30 (0.3)	1.00 (0.0001)	2.30 (0.003)	0.7
L. antarcticum	1918	7.73	4.20 (0.03)	1.78 (0.0001)	3.20 (0.003)	1.0
L. antarcticum	1919	7.48	5.08 (0.4)	4.95 (0.3)	4.08 (0.04)	5.4
L. frigidum	1851	7.49	6.11 (4.4)	4.49 (0.1)	5.70 (1.6)	24.3
L. frigidum	1920	7.20	5.08 (0.8)	4.49 (0.2)	4.49 (0.2)	9.1
L. gelidum	1921	7.04	4.34 (0.2)	3.83 (0.06)	4.53 (0.3)	NC***
L. nivalis	1852	6.17	4.36 (4.8)	3.48 (0.2)	3.48 (0.2)	3.1
L. nivalis	1922	7.34	5.49 (1.4)	5.30 (0.9)	4.82 (0.3)	11.2
L. scottii	1287	7.00	6.41 (26.0)	<4.70 (<0.5)	5.85 (7.1)	21.4
L. scottii	1304	8.25	7.82 (36.6)	5.25 (0.1)	7.40 (13.6)	44.5
L. scottii	1528	7.75	<5.71 (<0.9)	<3.76 (<0.01)	3.23 (0.003)	3.5
L. scottii	1529	7.92	5.23 (0.2)	<4.70 (<0.06)	3.92 (0.01)	4.7
L. stokesii	1926	7.76	5.80 (1.1)	5.36 (0.4)	5.36 (0.4)	20.6
Rhodosporidium toruloides	0559	8.03	7.74 (52.7)	5.32 (0.2)	5.81 (0.6)	9.6

* Viable counts are expressed as the logarithm of the number of colony forming units.
** Limit survival time (LST) is the time taken for the survival count to decrease to 10^4 per ampule. See text.
*** NC indicates the case in which the survival count after 5 years of storage was more than the viable count immediately after drying.

mesenterica IFO 1292, C. pintolopesii IFO 0729, Cryptococcus albidus var. aerius IFO 1860, Crypt. dimennae IFO 1863, Crypt. hungaricus IFO 1380, Rhodotorula glutinis IFO 0391, Sporobolomyces pararoseus IFO 0471, Trichosporon cutaneum IFO 0113, Leucosporidium antarcticum IFO 1919, L. frigidum IFO 1920, and Rhodosporidium toruloides IFO 0559.

Dried cultures whose viable counts are predicted to decrease to 10^4 between 10 to 15 years of preservation are the following 20 strains: Debaryomyces tamari IFO 0854, Pichia membranaefaciens IFO 0457 & 0460, Saccharomyces dairensis IFO 10008, S. exiguus IFO 1142 & 1170,

Saccharomycopsis capsularis IFO 0672, S'psis vini IFO 1750, Schizosaccharomyces octosporus IFO 0353, Sch. japonicus IFO 1713, Brettanomyces bruxellensis IFO 0677, Candida auriculariae IFO 1580, C. bovina IFO 1087 & 1313, C. glabrata IFO 0861 & 1085, C. halonitratophila IFO 1595, C. ingeniosa IFO 10002, C. mesenterica IFO 0969, and Leucosporidium nivalis IFO 1922.

The 67 strains with LSTs shorter than 15 years are considered to be rather labile during the preservation in the dried state. Most of the yeasts strains belonging to genera Ambrosiozyma, Arthroascus, Arxiozyma, Dipodascus, Eremothecium, Nadsonia, Nematospora, and Leucosporidium are sensitive in the dried state.

Of the 148 strains, the survival counts of dried cultures of 22 strains are expected to fall to 10^4 with 15 to 25 years of storage and those of 45 strains will probably remain higher than 10^4 for over 25 years. The living cells of these 67 strains are sensitive to the drying process, but their dried cells are very stable during preservation.

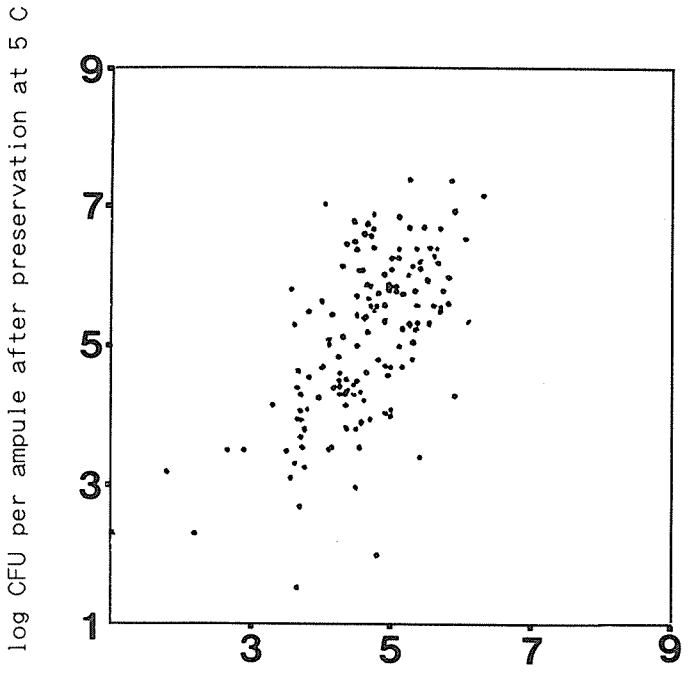


Fig. 2. Correlation between the survival count (log CFU) of L-dried cell after 5 years of preservation at 5 C and after 60 days of accelerated storage at 37C in strains with survival values lower than 1%.

The relation between the survival counts after 5 years of storage at 5 C and the survival counts in the 60-day accelerated test are plotted on a log scale for the 142 cultures in Fig. 2. The correlation coefficient is as low as 0.43. This low coefficient means that for dried cultures with low viabilities of less than 1% in the accelerated test, it is risky to estimate the viability on preservation at 5 C from the results of the accelerated test. Consequently, it is necessary to check their viabilities 5 years after preparation.

In conclusion, of a total of the 701 strains preserved in L-dried state at 5 C, it was estimated that 505 strains would retain a survival count of more than 10^4 for over 25 years, and further 83 strains for over 15 years. With the remaining 113 strains, the limit survival time of the dried cultures of 39 strains will expire between 10 and 15 years of storage, and that of 46 strains between 5 and 10 years. The survival counts of 28 strains had already fallen below 10^4 in 5 years of storage.

The results suggest that, for cultures with viabilities higher than 1% in the accelerated test, it is possible to predict the limit survival time of the L-dried cultures preserved at 5 C from their survival values in the accelerated test at 37 C.

References

1) Banno, I., K. Mikata, & T. Sakane. 1979. Viability of various yeasts after L-drying. IFO Res. Comm. 9: 27-34.
2) Mikata, K., S. Yamauchi, & I. Banno. 1983. Preservation of yeast cultures by L-drying: Viabilities 1710 yeasts after drying and storage. IFO Res. Comm. 11: 25-46.

IFO Res. Comm. 14,
104-110, 1989 (March)

ISOLATION AND GENETIC CHARACTERIZATION OF AUXOTROPHIC MUTANTS IN SACCHAROMYCES BAYANUS

YOSHINOBU KANEKO and ISAO BANNO

Summary

Auxotrophic mutants of S. bayanus were isolated from a spore progenitor of type strain IFO 1127 by ethylmethanesulfonate mutagenesis. Genetic analyses of four auxotrophic mutations (ade, his, trp, and ura) showed that each defect is a recessive mutation of a single nuclear gene. Tetrad analysis indicated that these four genes are unlinked to each other but that those other than the ade gene are linked to a centromere. By complementation tests, the his, trp, and ura mutant loci were found to correspond to the his6, trp5, and ura3 loci of S. cerevisiae, respectively. The his6, trp5, and ura3 genes were mapped from their respective centromeres at 2.1 centimorgans (cM), 10 cM, and 1.8 cM. Comparison with the genetic map of S. cerevisiae suggests that the chromosome structure of the S. bayanus type strain is slightly different from that of S. cerevisiae or that meiotic recombination frequencies are reduced in these regions.

Saccharomyces bayanus was originally classified as a different species from S. cerevisiae because of a difference in their fermentation ability of galactose (11). At present, however, S. bayanus is included in S. cerevisiae species (13). Recently, Martini and Kurtzman (6) reported that

the DNA relatedness between their type strains is very low. Their electrophoretic karyotypes are also slightly different (4). Furthermore, although diploid hybrids between S. bayanus and S. cerevisiae were obtained normally and produced normal four-spored asci, no ascospores were viable (1). Therefore, it is unclear in point of phylogenetic taxonomy whether S. bayanus is conspecific to S. cerevisiae.

To facilitate the genetic analysis of S. bayanus, we have attempted to isolate auxotrophic mutants of S. bayanus. Three centromere-linked genes were obtained. The map distances of these genes to their respective centromeres were calculated and compared with those of the corresponding genes of S. cerevisiae.

Materials and Methods

Strains and media. Strains used in this study are shown in Table 1. Nutrient medium (YPD) contained 1% yeast extract (Daigo-Eiyo), 2% polypeptone (Daigo-Eiyo), and 2% glucose. Minimal medium (SD) contained 0.67% Yeast Nitrogen Base w/o amino acids (Difco) and 2% glucose. To score auxotrophic markers, omission media were prepared by omitting an appropriate nutrient from the synthetic complete medium (10). Sporulation medium contained 0.5% potassium acetate. Agar plates were prepared by adding 2% agar. Cultivation was performed at 28 C.

Genetic analysis. Intraspecific mating of S. bayanus was carried out by the spore-to-spore method (3). To cross S. bayanus with S. cerevisiae, vegetative cell(s) of S. cerevisiae was placed next to a spore of S. bayanus by micromanipulation, and hybrid cells were selected by complementation of auxotrophic markers after growth on the YPD plate. Asci were dissected by the standard method (10).

Isolation of mutants. Spores of S. bayanus were treated with ethylmethanesulfonate (EMS) as described by Oshima and Takano (8). After mutagenesis, spore suspension was diluted adequately and spread on YPD plates. To select auxotrophic mutants, colonies appearing on YPD plates were replicated onto SD plates, and clones failing to grow on the SD plates were isolated from the original YPD plates. They were purified and subjected to genetic analysis.

Table 1. Strains used in this study.

Strain	Genotype	Source
S. cerevisiae		
AX55-2B	MAT**a** pho3-1 his6 trp1	our stock
AX66-10D	MAT**a** pho3-1 leu2 lys1 ura3	our stock
KYC77	MAT**a** gal1 ade1 ade2 ura1 trp5	our stock
N248-1C	MATα gal1 ade1 his2 leu1 met14 ura3	YGSC[a]
3654-1D	MATα ade6 arg4 his4 leu2 lys2 thr4 trp1 tyr1 ura4 gal7 MAL2	YGSC[a]
S. bayanus[b]		
Sb3A-1C	gal4	progeny of IFO 1127
SbAX5	gal4 ade	this study
SbAX10	gal4 trp5	this study
SbAX13	gal4 ura3	this study
SbAX14	gal4 his6	this study
SbAX10-1B	gal4 trp5	spore clone of SbAX10
B1-5A	gal4 ade ura3	spore clone of SbAX5 x SbAX13[c]
B18-2A	gal4 his6 trp5	spore clone of SbAX10-1B x SbAX14[c]
B19-3C	gal4 his6 ura3	spore clone of B1-5A x B18-2A[c]
B19-3D	gal4 ade trp5	spore clone of B1-5A x B18-2A[c]

a) Yeast Genetic Stock Center.
b) *S. bayanus* strains used in this study are homothallic and homozygous diploids except for the MAT gene. Mating type gene is omitted. Locus numbers correspond to the genes of *S. cerevisiae*.
c) Crossing was carried out by spore-to-spore mating.

Results and Discussion

Isolation of auxotrophic mutants

Since *S. bayanus* Sb3A-1C is a homothallic strain, spores were subjected to mutagenesis. The vegetative cells of Sb3A-1C were incubated on sporulation agar medium at 24 C for 5 days to sporulate. The sporulation frequency was 27%. Spore suspension was treated with EMS for 40 min, and the survival ratio after the treatment was about 4%.

Out of approximately 8×10^3 colonies, eight auxotrophic mutants were obtained. One mutant, SbAX5, requires adenine; 3 mutants, SbAX2, SbAX10, and SbAX16, tryptophan; one mutant, SbAX13, uracil; one mutant, SbAX14, histidine. The nutritional requirement of other two mutants was not determined. Four mutants (SbAX5, SbAX10, SbAX13, and SbAX14) were analyzed

further.

The four mutants could sporulate themselves. The pattern of spore germination and segregation of auxotrophic markers in tetrads is shown in Table 2. All viable spores of each mutant required the same nutrient as the parent strain. This result indicates that these mutants are homozygous for their respective auxotrophic markers.

Table 2. Tetrad analysis of auxotrophic mutants of S. bayanus.

	No. of viable spores in ascus					Segregation of marker		
Strain	4	3	2	1	0	4-:0+	3-:1+	2-:2+
SbAX5	7	2	5	0	13	7	0	0
SbAX10	0	0	5	4	19	nt*	nt	nt
SbAX13	6	0	0	1	5	6	0	0
SbAX14	4	1	4	4	1	4	0	0

* not tested

Auxotrophic mutations are recessive single nuclear mutations

To examine whether mutation is recessive or dominant, spore-to-spore mating was carried out between SbAX5 and SbAX13, and between SbAX10 and SbAX14. If the mutation is recessive, prototrophic diploids should be obtained. If the mutation is dominant, the resultant diploids should fail to grow on SD.

Forty-eight spore pairs were constructed by contacting a spore of SbAX5 with a spore of SbAX13. Out of 43 colonies grown on YPD, 8 colonies (B1-B8) were prototrophs. This result indicates that both adenine and uracil auxotrophic mutations are recessive. B1 and B3 were sporulated and 13 asci and 5 asci were dissected, respectively. Both auxotrophic markers segregated with a pattern of 2+:2-, indicating that each auxotrophy is, probably, caused by a single nuclear mutation.

The same analysis was carried out with SbAX10 and SbAX14. From 40 spore pairs, 3 prototrophic clones (B9-B11) were obtained. This result indicates that tryptophan and histidine auxotrophies are recessive mutations. Since no complete tetrads were obtained from B9, B10, and B11, another prototrophic diploid (B18) was constructed by spore-to-spore mating between SbAX10-1B and SbAX14. Eighteen tetrads of B18 showed a segregation of 2+:2- in tryptophan and histidine auxotrophy. Therefore, it was concluded that both mutations are single nuclear mutations.

Linkage analysis between auxotrophic mutations

Linkage between the four genes (ade, his, trp, and ura) was analyzed. Tetrad distributions of each pair are shown in Table 3. The ratio of parental ditype : nonparental ditype for each pair was nearly equal to 1:1, indicating that four genes were not linked to each other. However, tetrad distributions in the combinations of his, trp, and ura showed a smaller proportion of tetratype asci than would be expected if the genes segregated randomly, indicating that three genes are centromere-linked. The distances between three genes and their respective centromeres were calculated by using the equation of Whitehouse (12) with data shown in Table 3. The his, trp, and ura genes are located 2.1 cM, 10 cM, and 1.8 cM from their respective centromeres.

Correspondence of the three centromere-linked genes of S. bayanus with S. cerevisiae genes

In S. cerevisiae, three genes (his2, his4, and his6) of the histidine biosynthesis pathway are known to be centromere-linked (7). Two tryptophan biosynthetic enzyme genes (trp1 and trp5) and a uracil biosynthetic enzyme gene ura3 are also centromere-linked (7). To determine the correspondence of the S. bayanus his, trp, and ura genes with S. cerevisiae genes, complementation tests were carried out by crossing S. bayanus B18-2A or B19-3C and S. cerevisiae strains listed in Table 1. S. bayanus his, trp, and ura mutations did not complement the his6, trp5, and ura3 mutations of S. cerevisiae. From this result, the three genes of S. bayanus were designated his6, trp5, and ura3, according to the nomenclature of S. cerevisiae.

In S. cerevisiae, the genes his6, trp5, and ura3 have been mapped at 19.7 cM, 18.5 cM, and 8.0 cM from their centromeres, respectively (7). The distances of the three genes of S. bayanus were shorter than those of S. cerevisiae. These differences in map distance between the two yeasts suggest that the lengths of chromosome between the three genes and their centromeres is physically shorter in S. bayanus than S. cerevisiae, or that meiotic recombination between these genes and their centromeres is reduced in S. bayanus in contrast with S. cerevisiae.

Auxotrophic mutants obtained in this study are useful for constructing and analyzing genetically intra- and inter-specific hybrids of S. bayanus. We have already used these mutants to analyze hybrids between S. bayanus and S. cerevisiae (1). Furthermore, S. bayanus ura3 mutants are also

useful for genetic manipulation of S. bayanus because vector plasmids such as YEp24 carrying the URA3 gene of S. cerevisiae as a selectable marker can be used for transformation of S. bayanus.

Table 3. Linkage relationship of auxotrophic markers in S. bayanus.

Gene pair	Strain[a]	Tetrad distribution[b] PD	NPD	T
his - trp	B18	5	7	6
	B19	10	12	8
	B22	27	44	19
	Total	42	63	33
ade - ura	B1	2	2	15
	B3	2	0	3
	B19	5	5	23
	Total	9	7	41
trp - ura	B19	10	15	9
	B22	36	33	20
	Total	46	48	29
ura - his	B19	10	14	6
	B22	41	45	3
	Total	51	59	9
his - ade	B19	5	5	19
ade - trp	B19	2	8	23

a) All diploid strains were constructed by spore-to-spore mating. B1= SbAX5 x SbAX13, B3= SbAX5 x SbAX13, B18= SbAX10-1B x SbAX14, B19= B1-5A x B18-2A, B22= B19-3C x B19-3D.
b) Abbreviation of ascus type is as follows: PD, parental ditype; NPD, nonparental ditype; T, tetratype.

Electrophoretic karyotyping of S. bayanus showed that the type strain has 17 chromosome bands and its chromosome DNA banding pattern is slightly different from that of S. cerevisiae X2180-1A (4, 9). Although S. cerevisiae is known to have 17 linkage groups, only 16 chromosomes has been identified physically (2, 5). The analysis of chromosome structure of S. bayanus will give more information about the phylogenetic relationship between two yeasts.

References

1) Banno, I. and Y. Kaneko. A genetic analysis of taxonomic relation between Saccharomyces cerevisiae and Saccharomyces bayanus. The proceedings of 7th International Symposium on Yeasts. (submitted).
2) Carle, G. F., M. Frank, and M. V. Olson. 1986. Electrophoretic separations of large DNA molecules by periodic inversion of the electric field. Science 232: 65-68.
3) Hicks, J. B., and I. Herskowitz. 1976. Interconversion of yeast mating type I. Direct observations of the action of the homothallism (HO) gene. Genetics 83: 245-258.
4) Johnston, J. R., and R. K. Mortimer. 1986. Electrophoretic karyotyping of laboratory and commercial strains of Saccharomyces and other yeasts. Int. J. Syst. Bacteriol. 36: 569-572.
5) Kuroiwa, T., H. Kojima, I. Miyakawa, and N. Sando. 1984. Meiotic karyotype of yeast Saccharomyces cerevisiae. Exp. Cell. Res. 153: 259-265.
6) Martini, A. V., and C. P. Kurtzman. 1985. Deoxyribonucleic acid relatedness among species of the genus Saccharomyces sensu stricto. Int. J. Syst. Bacteriol. 35: 508-511.
7) Mortimer, R. K., and D. Schild. 1980. Genetic map of Saccharomyces cerevisiae. Microbiol. Rev. 44: 519-571.
8) Oshima, T., and I. Takano. 1980. Mutants showing heterothallism from a homothallic strain of Saccharomyces cerevisiae. Genetics 94: 841-857.
9) Pedersen, M. B. 1986. DNA sequence polymorphisms in the genus Saccharomyces IV. Homoeologous chromosome III of Saccharomyces bayanus, S. carlsbergensis, and S. uvarum. Carlsberg Res. Commun. 51: 185-202.
10) Sherman, F., G. R. Fink, and J. B. Hicks. 1983. Methods in yeast genetics, laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
11) Walt, J. P. van der. 1970. Genus 16. Saccharomyces Meyen emend. Reess, In J. Lodder (ed.) The yeasts: a taxonomic study, 2nd ed., p. 555-718. North-Holland Publishing Company, Amsterdam.
12) Whitehouse, H. L. K. 1950. Mapping chromosome centromeres by the analysis of unordered tetrads. Nature (London) 165: 893.
13) Yarrow, D. 1984. Genus 22. Saccharomyces Meyen ex Reess, In N. J. W. Kreger-van Rij (ed.) The yeasts: a taxonomic study, 3rd ed., p. 382-386. Elsevier Science Publishers B. V., Amsterdam.

IFO Res. Comm. 14,
111-117, 1989(March)

KARYOTYPING OF SACCHAROMYCES EXIGUUS BY PULSED-FIELD GEL ELECTROPHORESIS

YOSHINOBU KANEKO, KOZABURO MIKATA, and ISAO BANNO

Summary

The electrophoretic karyotypes of six strains of Saccharomyces exiguus have been determined using pulsed-field gel electrophoresis (PFGE). Each strain showed 8-12 DNA bands in range from ca. 360 kilobase pairs (kb) to 2,000 kb. In their electrophoretic karyotypes, the six strains fell into 3 groups. Southern hybridization of chromosomal DNA bands was carried out using total DNA of the type strain (IFO 1128) as a probe. In the group containing the type strain, all DNA bands hybridized strongly with the probe. The other groups, however, had weakly hybridized bands. These results indicate that S. exiguus contains at least two closely related groups.

Recently, a novel technique, pulsed-field gel electrophoresis (PFGE), has been developed by Schwartz and Cantor (13) for fractionating huge DNA fragments. Several improved methods have also been reported (1, 3, 4, 15). By the orthogonal field alternation gel electrophoresis (OFAGE) technique, Carle and Olson (2) assigned DNA bands to 16 chromosomes of S. cerevisiae and showed the possibility of karyotyping in yeasts. Several researchers have already applied electrophoretic karyotyping to characterization and identification of various yeasts (5, 6, 9, 10, 12).

In this study, we examined the electrophoretic karyotypes of six strains of S. exiguus, and found that S. exiguus contains three groups of different electrophoretic karyotype. Southern blot analysis using total

DNA of the type strain as probe showed that the two of the karyotype groups had slightly low DNA homology with the type strain.

Materials and Methods

Strains and Media. S. exiguus strains were IFO 0271, IFO 0956 (= CBS 1084), IFO 1128 (= CBS 379; type strain), IFO 1141 (= NRRL Y-2308), IFO 1142 (= NRRL Y-1349), and IFO 1169 (= CBS 2141). S. cerevisiae SH964 kindly provided by S. Harashima (Osaka University) was used as size marker strain in PFGE. As SH964 has the chromosome VII split into 2 fragments at RAD2 locus, we can separate chromosome VII from chromosome XV in PFGE. Lambda DNA size marker (Clontech Laboratories) was purchased for estimating the size of chromosomal DNA in PFGE. Nutrient medium (YPD) contained 1% yeast extract, 2% polypeptone, and 2% glucose. Cultivation was carried out at 28 C.

Preparation of chromosomal DNA for PFGE. The sample DNAs applied to PFGE were prepared essentially according to the method of Carle and Olson (2). Cells were grown to late logarithmic phase in 5 ml of YPD, then harvested and washed twice with ice-cold 50 mM EDTA (pH 7.5). A 0.3-ml portion of cell suspension (ca. 3×10^8 cells) was mixed at 37 C with 0.5 ml of 1% low melting point agarose (Bethesda Research Laboratories) and 0.1 ml of solution I (2) containing 1 mg/ml of Zymolyase 60000 (Seikagaku Kogyo). The agarose mixtures were solidified in LKB insert moulds instead of a small Petri plate. Ten pieces of agarose plug were transferred into a capped-test tube, immersed in 1 ml of solution II (2), then incubated overnight at 37 C. The solution was changed to 1 ml of solution III (2) containing 1 mg/ml of proteinase K (Merck), and incubated for 1 day at 50 C. The sample DNAs were stable for over one year when stored in 0.5 M EDTA (pH 9.0) at 4 C.

Gel electrophoresis. PFGE was performed by using the Pulsarphor system with hexagonal or point electrode array (Pharmacia-LKB). Electrophoretic buffer (0.5 x TBE; 45 mM Tris base, 45 mM boric acid, 1.25 mM EDTA) was cooled to 8-10 C. When the hexagonal electrode array was used, 1% agarose gel (15 x 15 x 0.5 cm; Agarose 1600, Wako), 240 V, a running time of 20 hr, and a pulse time of 60 sec were employed. When the point electrode array was used, 1% agarose gel (10 x 10 x 0.5 cm), 450 V, a

running time of 16 hr, and pulse time of 55 sec were employed. The agarose plugs were cut 0.5-2 mm in thickness and inserted into a gel well.

Southern blot analysis. The gel was treated with 0.25 M HCl for 20 min at room temperature and DNA bands were transferred to Biodyne A nylon membrane (1.2 μm; Pall Ultrafine Filtration Corporation) according to the protocol of the supplier based on the method of Southern (14). Hybridization and washing were performed according to the protocol (method A) of the supplier. The stringency of hybridization and washing is high. Autoradiography was performed at -80 C using Kodak X-Omat RP film with an intensifying screen (Cronex Lightning Plus, DuPont).

Preparation of radiolabeled probe. Total DNA of type strain IFO 1128 was prepared by the method of Holm et al. (8). Fragmentation of chromosomal DNA by sonication is effective for obtaining a highly specific radioactive probe (11). DNA was sonicated and fragments of approximately 500-3,000 base pairs (bp) in size were collected using a Geneclean kit (BIO 101). The DNA fragments were radiolabeled with [α-^{32}P] dCTP by using the Multiprime DNA labelling system (Amersham) based on the method of Feinberg and Vogelstein (7). The radioactivity was measured in the ^{3}H channel of the Cherenkov radiation.

Results and Discussion

Electrophoretic karyotypes in S. exiguus

Six strains of S. exiguus maintained in IFO were analyzed for their karyotype by PFGE with the hexagonal electrode array. As shown in Fig. 1, six strains had 8 to 12 DNA bands ranging in size from ca. 360 kb to 2000 kb. The DNA bands in the range above 1,200 kb were compressed and were not well resolved. Although the 7th DNA band from the bottom of the type strain, IFO 1128 (lane 5), was not observed in IFO 1141 (lane 6), the two strains showed very similar patterns. The DNA banding pattern of IFO 0271 (lane 3) was also similar to that of the type strain. On the other hand, IFO 0956 and IFO 1169 (lanes 4 & 8) showed an identical pattern characterized by the presence of only two bands in the region below 800 kb, in contrast to the type strain. In the range of 360 to 500 kb, IFO 1142 (lane 7) showed only one band, as did IFO 0956 and IFO 1169. However, the middle range (600-900 kb) was similar to the type strain.

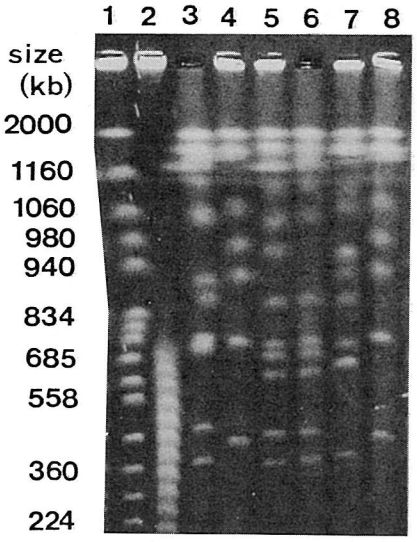


Fig. 1. Electrophoretic karyotypes of S. exiguus by PFGE. PFGE was carried out with the hexagonal array at 240 V and 60 sec of pulse time for 20 hr. Buffer (0.5 x TBE) was kept at 8-10 C. After PFGE, the gels were stained with 0.5 μg/ml of ethidium bromide for 10 min, destained with distilled water over 30 min, then photographed. The DNA band of chromosome XII of S. cerevisiae SH964 was not obsereved under this condition. Lane 1, S. cerevisiae SH964; lane 2, lambda DNA oligomers; lane 3, S. exiguus IFO 0271; lane 4, S. exiguus IFO 0956; lane 5, S. exiguus IFO 1128 (type strain); lane 6, S. exiguus IFO 1141; lane 7, S. exiguus IFO 1142; lane 8, S. exiguus IFO 1169.

We classified the electrophoretic karyotypes of the six strains examined into three groups. Group 1 contains IFO 0271, IFO 1128, and IFO 1141. Group 2, containing IFO 0956 and IFO 1169, differs from group 1 in the number and size of chromosomal DNA bands in the range below 800 kb. Group 3, represented by IFO 1142, has a chromosomal DNA banding pattern that is intermediate between those of groups 1 and 2. Chromosome length polymorphism (CLP) in the electrophoretic karyotype has been observed in S. cerevisiae (2, 5, 6, 9). We think empirically that the minor differences in DNA banding pattern within group 1 can be considered as CLP. However, it is unclear whether the differences in DNA banding pattern among three groups are explainable by CLP in the same species, because their meiotic segregants and hybridization between them have not been examined due to their lacking sporulation and mating.

DNA homology with the type strain

To clarify the relationship among these three groups of S. exiguus, DNA similarities were examined by Southern blot analysis. The chromosomal

DNA bands were transferred to the filter after PFGE and hybridized with total DNA of the type strain, IFO 1128, as probe. As shown in Fig. 2B, all chromosomal DNA bands of IFO 0271 and IFO 1141 were strongly hybridized, while other three strains (IFO 0956, IFO 1142, and IFO 1169) showed weak signals of DNA bands of sizes less than ca. 1,000 kb. Negative control of S. cerevisiae SH964 showed no hybridized bands in the region below 1,000 kb (Fig. 2B, lane 1). Although this analysis is not exactly quantitative, the result suggests that the three strains of groups 2 and 3 may be separable from S. exiguus on the basis of DNA similarity. Further quantitative analysis of DNA relatedness is required to confirm this idea.

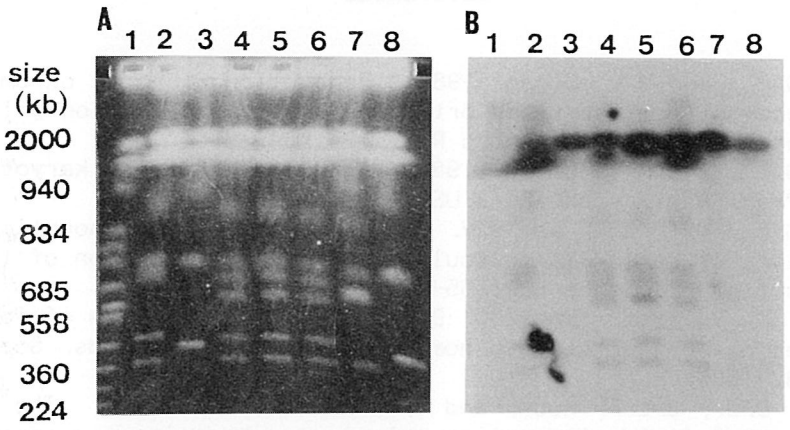


Fig. 2. Southern blot analysis of chromosome DNA separated by PFGE in S. exiguus. **A.** PFGE was carried out with the point electrode array at 450 V and 55 sec of pulse time for 16 hr. Buffer (0.5 x TBE) was kept at 8-10 C. After PFGE, the gels were stained with 0.5μg/ml of ethidium bromide for 10 min, then photographed. Lane 1, S. cerevisiae SH964; lane 2, S. exiguus IFO 0271; lane 3, S. exiguus IFO 0956; lane 4 & 5, S. exiguus IFO 1128 (type strain); lane 6, S. exiguus IFO 1141; lane 7, S. exiguus IFO 1142; lane 8, S. exiguus IFO 1169. **B.** Southern blot of the gel of panel **A** with ^{32}P-labeled total DNA of IFO 1128. Lanes are same as panel **A**. Hybridization was carried out overnight at 65 C in 1 ml of hybridization cocktail (5 x SSPE, 5 x Denhardt's buffer, 0.2% SDS, 0.5 mg/ml of denatured calf thymus DNA, and 1.4 x 10^7 cpm of probe DNA). The filter was washed 3 times with 5 mM sodium phosphate buffer, pH 7.0 containing 1 mM EDTA and 0.2% SDS at room temperature.

Candida holmii is known to be the imperfect state of S. exiguus (16). Candida yeasts have been classified mainly by differences in physiological characters. The combination of electrophoretic karyotyping and DNA-DNA hybridization should be useful to confirm the relationship between S. exiguus and C. holmii. Classification based on the similarity of genetic components is biologically more significant than that based on the

physiological and morphological characters, because the physiological and morphological characters appear as a result of gene expression and are easily variable by a single mutation.

We are grateful to Satoshi Harashima and Hiroaki Matsuzaki for provision of a yeast strain and information about PFGE, and to Shun'ichi Kuroda for information about high specific radiolabeling of DNA.

References

1) Carle, G. F., and M. V. Olson. 1984. Separation of yeast chromosomal DNA molecules from yeasts by orthogonal-field-alternation gel electrophoresis. Nucleic Acids Res. 12: 5647-5664.
2) Carle, G. F., and M. V. Olson. 1985. An electrophoretic karyotype for yeast. Proc. Natl. Acad. Sci. USA. 82: 3756-3760.
3) Carle, G. F., M. Frank, and M. V. Olson. 1986. Electrophoretic separations of large DNA molecules by periodic inversion of the electric field. Science 232: 65-68.
4) Chu, G., D. Vollrath, and R. W. Davis. 1986. Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science 234: 1582-1585.
5) Coetzee, D. J., J. L. F. Kock, and G. H. J. Pretorius. 1987. The value of orthogonal-field-alternation gel electrophoresis as a rapid identification process for some species representing the genus Saccharomyces. J. Microbiol. Methods 7: 219-225.
6) De Jonge, P., F. C. M. De Jongh, R. Meijers, H. Y. Steensma, and W. A. Scheffers. 1986. Orthogonal-field-alternation gel electrophoresis banding patterns of DNA from yeasts. Yeast 2: 193-204.
7) Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132: 6-13.
8) Holm, C., D. W. Meeks-Wagner, W. L. Fangman, and D. Botstein. 1986. A rapid, efficient method for isolating DNA from yeast. Gene 42: 169-173.
9) Johnston, J. R., and R. K. Mortimer. 1986. Electrophoretic karyotyping of laboratory and commercial strains of Saccharomyces and other yeasts. Int. J. Syst. Bacteriol. 36: 569-572.
10) Johnston, J. R., C. R. Contopoulou, and R. K. Mortimer. 1988. Karyotyping of yeast strains of several genera by field inversion gel electrophoresis. Yeast 4: 191-198.
11) Kuroda, S., Y. Itoh, T. Miyazaki, and Y. Fujisawa. 1988. A supersensitive dot-hybridization method: Rapid and quantitative detection of host-derived DNA in recombinant products at the one picogram level. Biochem. Biophys. Res. Commun. 152: 9-14.
12) Magee, B. B., and P. T. Magee. 1987. Electrophoretic karyotypes and chromosome numbers in Candida species. J. Gen. Microbiol. 133: 425-430.

13) Schwartz, D. C., and C. R. Cantor. 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell 37: 67-75.
14) Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503-517.
15) Southern, E. M., R. Anand, W. R. A. Brown, and D. S. Fletcher. 1987. A model for the separation of large DNA molecules by crossed field gel electrophoresis. Nucleic Acids Res. 15: 5925-5943.
16) Yarrow, D. 1984. Genus 22. Saccharomyces Meyen ex Reess, In N. J. W. Kreger-van Rij (ed.) The yeasts: a taxonomic study. p. 382-386. Elsevier Science Publishers B. V., Amsterdam.

IFO Res. Comm. 14,
118-142, 1989(March)

FILAMENTOUS FUNGI ISOLATED FROM SOILS IN THE XINJIANG UIGHUR AUTONOMOUS REGION, CHINA

TATSUO YOKOYAMA, TADAYOSHI ITO AND YU-QI YIN*

Summary

One hundred and twelve species of filamentous fungi isolated mainly from soil samples collected in Xinjiang Uighur, China, are reported. Among these, 6 belong to the Zygomycotina, 36 to the Ascomycotina, and 70 to the Deuteromycotina. The most prevalent species detected in this region were Geomyces pannorum var. pannorum and Mortierella alpina followed by Aspergillus terreus, Talaromyces ucrainicus, T. flavus var. flavus, A. fumigatus, Pseudeurotium zonatum, A. niger var. niger, and Gliomastix cerealis. In the natural desert sites in the Junggar Pendi, Geomyces pannorum var. pannorum, A. terreus and A. fumigatus were found to some extent, but no fungus was detected in about half of the soil samples collected. In agricultural and horticultural crop fields in the same region, Geomyces pannorum var. pannorum was the most predominant, followed by Aspergillus terreus, Mortierella alpina, Talaromyces ucrainicus, T. flavus var. flavus, A. fumigatus, Pseudeurotium zonatum, Gliomastix cerealis, and Metarhizium anisopliae. Whereas, in the mountains sites with coniferous vegetation, Mortierella alpina and Geomyces pannorum var. pannorum were the predominant fungi,

---

*XINJIANG SHIHEZI AGRICULTURAL COLLEGE

followed by Pseudogymnoascus roseus. Overall, it is concluded that this region is very poor in fungus populations, in the numbers of both species and isolates.

In September 1986, Yokoyama visited the Xinjiang Shihezi Agricultural College, Shihezi, Xinjiang Uighur Autonomous Region where he worked with Prof. Yin to survey the fungus flora in the desert and agricultural soils. The work was conducted as a cooperative research project between the Institute for Fermentation, Osaka and the Xinjiang Shihezi Agricultural College.

Mycological flora in China has been recorded in "Sylloge Fungorum Sinicorum" (20), a comprehensive monographic treatise published by F.-L. Tai in 1979. However, most of the fungi recorded there and in other publications are either plant pathogenic and wood rotting fungi or wild mushrooms (1, 2, 3, 7, 8, 15, 16, 17, 20, 23, 26, 27, 28, 29); and only a few papers dealing with the soil fungi have been published in China (5, 11, 13, 21, 24, 25).

This paper is to contribute to the information on the mycological flora of China and the distribution mainly of soil microfungi in the Xinjiang Uighur Autonomous Region.

Materials and Methods

One hundred and sixty-four samples, mostly of soil, were collected in Xinjiang Uighur Zizhiqu (Sinkiang Uighur Autonomous Region) in 8 September through 5 October 1986. The collection sites were mostly located in three areas with different edaphic conditions: desert fields with or without xerophytes; agricultural and horticultural crop fields; and mountain areas with coniferous vegetation. These three were located in southern Junggar Pendi (Junggar basin) and the former two in Turpan Pendi (Fig. 1). The samples collected for our mycological surveys were composed mostly of soil, but some were feces, seed, fruits, fungi, and others. In addition, nine soil samples were also collected in crop fields and paddy fields in Beijing. The total of 173 samples thus obtained are listed in Table 1.

Fig. 1. Map of the People's Republic of China showing where samples were collected.

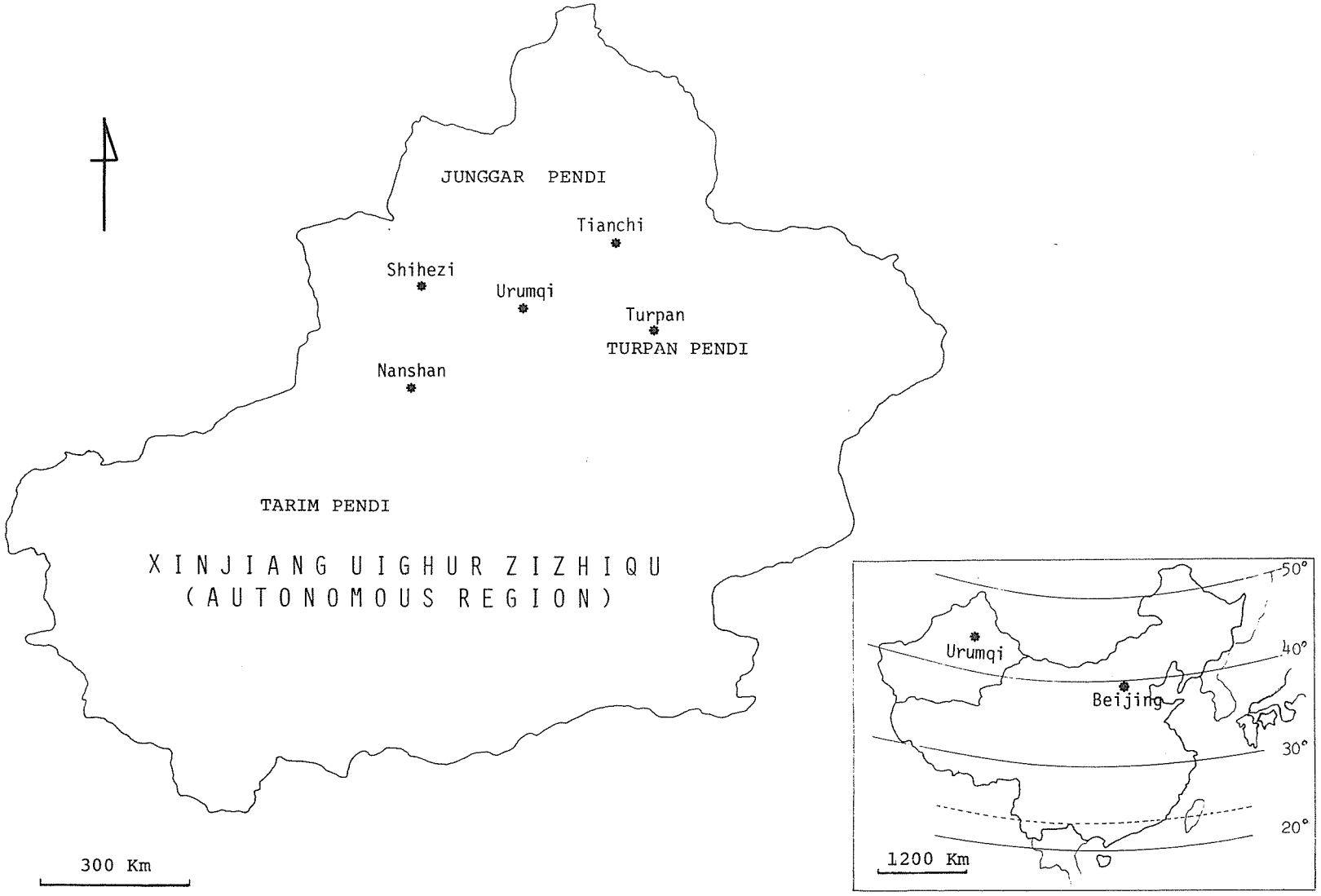


Table 1. List of samples collected in China.

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1701	Sep. 8, '86	soil	Beijing	Paeonia sp.	Summer palace	17	7, 8, 11, 30, 31, 35, 38, 56, 57, 67, 70, 74, 81, 89, 94, 95, 98
1702	Sep. 9, '86	〃	Shihezi		lake mud	1	3
1703	〃	〃	〃		〃	1	75
1704	〃	〃	〃	weeds	river bank	5	17, 48, 67, 93, 94
1705	Sep. 10, '86	〃	〃	cotton		9	3, 17, 30, 56, 58, 63 67, 75, 84
1706	〃	〃	〃	〃		6	3, 17, 25, 30, 64, 67
1707	〃	〃	〃	peanut		10	3, 17, 30, 39, 46, 58, 67, 72, 88, 93
1708	〃	〃	〃	〃		12	3, 16, 17, 30, 35, 46, 56, 67, 71, 72, 73, 75
1709	〃	〃	〃	cabbage		0	
1710	〃	〃	〃	〃		9	25, 30, 31, 48, 57, 58, 67, 72, 75
1711	〃	〃	〃	Allium fistulosum		13	3, 6, 31, 37, 39, 48, 56, 58, 67, 69, 79, 81, 86

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1712	Sep. 10, '86	soil	Shihezi	carrot		7	3, 17, 24, 31, 37, 39, 58
1713	〃	〃	〃	dent corn		11	17, 24, 31, 34, 36, 37, 39, 57, 67, 68, 79
1714	〃	fungus	〃		Ustilago maydis on dent corn	0	
1715	〃	soil	〃	grape		7	17, 39, 56, 67, 72, 75, 87
1716	〃	〃	〃	water melon		7	5, 31, 39, 43, 48, 58, 67
1717	〃	〃	〃	domestic apple		13	3, 16, 24, 31, 36, 39, 48, 67, 68, 72, 75, 91, 110
1718	〃	〃	〃	suger beet		9	16, 17, 24, 31, 39, 41, 46, 58, 67
1719	〃	〃	〃	〃		7	3, 31, 39, 46, 56, 57, 58
1720	〃	〃	〃	〃		10	3, 12, 24, 31, 39, 56, 58, 72, 74, 81
1721	〃	〃	〃	spring wheat		2	58, 67

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1722	Sep. 10, '86	soil	Shihezi	spring wheat		9	16, 31, 56, 58, 67, 72, 75, 84, 93
1723	〃	〃	〃	Chinese cabbage & radish		7	3, 35, 38, 43, 54, 67, 70
1724	〃	〃	〃	carrot		13	1, 3, 27, 28, 29, 35, 38, 43, 57, 58, 60, 67, 87
1725	〃	〃	〃	grape & grass		6	20, 31, 39, 45, 58, 67
1726	〃	〃	〃		cotton waste	5	1, 27, 52, 103, 106
1727	Sep. 11 '86	〃	Nanshan	Sorbus sp.		2	3, 32
1728	〃	〃	〃	Abies sp.		2	3, 32
1729	〃	〃	〃	〃		2	3, 32
1730	〃	〃	〃	〃		3	3, 32, 101
1731	〃	〃	〃	〃		5	3, 32, 93, 98, 101
1732	〃	〃	〃		rodent tunnel	3	67, 76, 104
1733	〃	〃	〃		〃	5	4, 31, 32, 67, 106
1734	〃	〃	〃		rocky	2	16, 67
1735	〃	〃	〃		〃	1	67
1800	〃	dung	〃		sheep	0	
1801	〃	〃	〃		〃	4	11, 53, 60, 93
1802	〃	〃	〃		horse	0	

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1849	Sep. 13, '86	soil	Shihezi		under *Peziza* sp.	2	58, 60
1850	〃	〃	〃		under *Coprinus* sp.	4	11, 56, 58, 67
1736	Sep. 16, '86	〃	〃 *2	winter wheat		12	6, 10, 31, 35, 43, 57, 67, 72, 79, 86, 92, 97
1737	〃	〃	〃	〃		5	1, 38, 58, 67, 98
1738	〃	〃	〃	suger beet		5	31, 38, 48, 72, 98
1739	〃	〃	〃	〃		7	6, 10, 35, 43, 73, 95, 107
1740	〃	〃	〃	clover		6	35, 38, 43, 54, 66, 67
1741	〃	〃	〃	〃		6	11, 38, 39, 48, 66, 67
1742	〃	〃	〃	flint corn		6	1, 3, 31, 38, 58, 67
1743	〃	〃	〃	〃		8	3, 26, 31, 35, 39, 48, 84, 98
1744	〃	〃	〃	soy bean		8	10, 39, 46, 56, 58, 60, 67, 91
1745	〃	〃	〃	〃		7	3, 35, 36, 39, 48, 74, 75
1746	〃	〃	〃	〃		5	3, 48, 58, 60, 74

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1747	Sep. 16, '86	soil	Shihezi	Hibiscus sp.		7	39, 48, 58, 60, 67, 75, 102
1748	〃	〃	〃	〃		6	1, 46, 48, 58, 60, 67
1749	〃	〃	〃	sunflower		8	1, 3, 39, 46, 48, 58, 76, 89
1750	〃	〃	〃	〃		8	3, 10, 35, 39, 56, 58, 84, 98
1751	〃	fungus	〃		Ustilago maydis on corn	0	
1752	〃	seed	〃		Cuscuta sp. seeds	0	
1753	Sep. 18, '86	soil	〃 *3	cowpea		3	58, 67, 98
1754	〃	〃	〃	〃		8	10, 35, 39, 43, 58, 67, 83, 98
1755	〃	〃	〃	snake gourd		13	3, 16, 19, 32, 35, 37, 39, 54, 58, 67, 72, 75, 98
1756	〃	〃	〃	〃		14	1, 3, 31, 35, 38, 39, 43, 49, 56, 64, 67, 72, 80, 98
1757	〃	〃	〃	Allium sp.		13	1, 3, 16, 34, 35, 37, 38, 57, 60, 67, 72, 75, 85

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1758	Sep. 18, '86	soil	Shihezi	Allium sp.		11	15, 18, 24, 34, 35, 37, 39, 43, 65, 66, 67
1759	〃	〃	〃	tomato		10	20, 35, 39, 43, 54, 56, 58, 63, 67, 72
1760	〃	〃	〃	〃		11	3, 34, 39, 40, 57, 58, 60, 67, 72, 81, 110
1761	〃	〃	〃	〃		10	1, 3, 12, 31, 35, 39, 40, 43, 56, 64
1762	〃	〃	〃	〃		14	1, 3, 16, 35, 38, 39, 43, 50, 55, 60, 62, 67, 72, 84
1763	〃	〃	〃	Chinese cabbage		12	3, 9, 31, 35, 37, 39, 43, 67, 75, 80, 91, 98
1764	〃	〃	〃	〃		8	14, 34, 35, 39, 58, 67, 75, 80
1765	〃	〃	〃	carrot		14	3, 24, 31, 35, 37, 39, 40, 43, 60, 67, 72, 75, 91, 112

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1766	Sep. 18, '86	soil	Shihezi	carrot		11	31, 34, 35, 38, 39, 43, 67, 72, 75, 81, 98
1767	〃	〃	〃	green pepper		7	12, 39, 43, 48, 58, 67, 75
1768	〃	〃	〃	〃		15	1, 3, 11, 16, 17, 31, 35, 39, 43, 57, 67, 72, 75, 88, 112
1769	〃	〃	〃	pepper		8	15, 16, 34, 35, 58, 60, 67, 72
1770	〃	〃	〃	cabbage		8	3, 34, 35, 39, 58, 67, 75, 112
1771	〃	〃	〃	cauliflower		10	3, 35, 37, 39, 40, 43, 48, 67, 72, 75
1772	〃	〃	〃	kaoliang		10	3, 10, 35, 39, 43, 54, 58, 67, 72, 98
1773	〃	〃	〃	〃		13	31, 35, 39, 40, 42, 43, 54, 56, 67, 75, 84, 98, 110
1774	〃	〃	〃	Allium tuberosum		11	15, 16, 21, 31, 39, 54, 56, 57, 58, 67, 75

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1775	Sep. 18, '86	soil	Shihezi	Allium tuberosum		2	13, 69
1776	〃	〃	〃		lime	0	
1777	〃	〃	〃 *4	hop		8	1, 3, 17, 39, 43, 54, 56, 81
1778	〃	〃	〃	〃		9	3, 10, 11, 16, 35, 56, 60, 81, 98
1779	Sep. 21, '86	〃	near Urumqi			0	
1780	〃	〃	〃			0	
1781	〃	〃	〃			2	72, 87
1782	〃	dung	〃		horse	2	48, 56
1783	〃	soil	〃		dry river mud	5	12, 31, 57, 58, 67
1784	〃	〃	〃	xerophyte		4	28, 45, 57, 67
1785	〃	〃	〃			4	51, 56, 67, 88
1786	〃	dung	〃		horse	1	51
1787	〃	soil	〃		rodent tunnel	3	53, 67, 78
1788	〃	〃	〃			0	
1789	〃	〃	〃			0	
1790	〃	〃	〃			0	
1791	〃	〃	〃			0	
1792	〃	〃	Tianchi	Abies sp.	lake side	2	67, 88
1793	〃	〃	〃	〃	〃	2	67, 108
1794	〃	〃	〃	Picea sp.		2	16, 44

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1795	Sep. 21, '86	mycorrhiza	Tianchi	*Abies* sp.		1	98
1796	〃	〃	〃	〃		0	
1797	〃	soil	〃	〃		5	2, 18, 33, 41, 109
1798	〃	〃	〃	〃		1	3
1799	〃	dung	near Urumqi		sheep	2	60, 61
1803	〃	〃	〃		camel	4	58, 63, 88, 93
1804	〃	〃	〃		〃	4	1, 11, 48, 58
1805	〃	〃	〃		〃	1	58
1806	〃	soil	〃			6	47, 48, 56, 57, 76, 96
1807	〃	〃	〃			3	47, 48, 56
1808	〃	〃	〃	wheat		7	3, 17, 28, 56, 58, 67, 88
1809	〃	〃	〃	sunflower		7	3, 32, 57, 58, 67, 72, 75
1810	〃	〃	〃	corn		4	57, 58, 67, 84
1811	〃	〃	〃	*Allium* sp.		4	11, 28, 43, 48
1812	〃	〃	〃	*Populus* sp. seedlings		11	3, 16, 17, 45, 48, 55, 56, 57, 67, 75, 94
1813	〃	〃	〃		rocky	3	11, 56, 89

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1814	Sep. 21, '86	manure	near Urumqi		wheet straw	8	1, 11, 17, 36, 45, 56, 67, 93
1815	Sep. 23, '86	soil	near Shihezi	apple		6	3, 17, 57, 75, 84, 110
1816	〃	〃	〃	〃		9	17, 31, 47, 58, 66, 67, 70, 72, 106
1817	〃	〃	〃	grape		4	3, 57, 60, 67
1818	〃	〃	〃	〃		8	3, 46, 47, 56, 57, 67, 72, 75
1819	〃	〃	〃	tomato		7	56, 57, 67, 75, 81, 98, 107
1820	〃	〃	〃	〃		10	3, 35, 48, 56, 57, 58, 61, 67, 72, 81
1860	〃	〃	〃	xerophytes	hill side	1	67
1861	〃	〃	〃		〃	2	21, 67
1862	〃	〃	〃		〃	3	28, 56, 65
1863	〃	〃	〃		〃	0	
1864	〃	〃	〃		〃	0	
1865	〃	gravel	〃		dry river base	2	56, 65
1866	〃	〃	〃		〃	3	11, 50, 94
1867	〃	〃	〃		dry river side	0	
1868	〃	〃	〃		〃	0	

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1869	Sep. 23, '86	soil	near Shihezi		clay for brick	2	66, 106
1870	〃	〃	〃		〃	1	32
1871	〃	〃	〃		〃	1	16
1821	Sep. 25, '86	〃	Shihezi		under *Peziza* sp.	8	3, 46, 48, 51, 56, 57, 58, 60
1822	〃	〃	〃		under *Cyathus* sp.	6	31, 48, 58, 61, 77, 98
1823	〃	〃	〃	*Zinnia elegans*		4	10, 35, 49, 57, 67
1824	〃	〃	〃	〃		10	3, 17, 32, 48, 56, 60, 61, 67, 69, 88,
1825	〃	〃	〃	*Pinus* sp.		4	32, 67, 69, 73
1826	〃	〃	〃	〃		6	3, 32, 48, 56, 58, 88
1847	〃	raisins	〃			1	57
1848	〃	seeds	〃		star anise	3	1, 17, 85
1852	〃	red rice	〃			1	22
1853	〃	seeds	〃		Ziren	1	6
1854	〃	dry apricot	〃			0	
1855	〃	Jew's ear	〃			2	82, 100
1856	〃	soil	〃			0	
1857	〃	〃	〃			0	
1858	〃	〃	〃			0	
1859	〃	〃	〃			0	

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1827	Sep. 27, '86	soil	Turpan			0	
1828	〃	〃	〃			3	11, 48, 57
1829	〃	〃	〃			1	60
1830	〃	〃	〃		hill	0	
1831	〃	〃	〃		〃	0	
1832	〃	〃	〃		〃	0	
1833	〃	〃	〃		〃	0	
1834	〃	〃	〃		〃	0	
1835	〃	〃	〃	Vitis sp.	spring	6	3, 48, 57, 59, 60, 62
1836	〃	humus	〃	〃		10	1, 35, 36, 56, 58, 67, 74, 81, 91, 93
1837	〃	soil	〃	〃		5	17, 57, 67, 94, 105
1838	〃	〃	〃	〃		1	57
1851	〃	〃	〃	〃	under Peziza sp.	6	11, 57, 62, 91, 105, 111
1872	〃	〃	〃		castle wall	0	
1873	〃	〃	〃			0	
1874	〃	〃	〃	xerophyte		3	11, 58, 105
1875	〃	〃	〃			0	
1876	〃	〃	〃			0	
1877	〃	〃	〃			0	
1878	〃	〃	〃			0	

Table 1. (continued)

Sample No.	Date Sampled	Samples	Locality	Predominant vegetation	Remarks	Number of species isolated	Fungus species number*1
1879	Sep. 27, '86	soil	Turpan			0	
1880	〃	〃	〃			0	
1881	〃	〃	〃			0	
1839	Oct. 3, '86	〃	Beijing		botanical garden	9	1, 31, 32, 56, 60, 66, 67, 75, 112
1840	〃	〃	〃		〃	10	1, 3, 11, 15, 16, 32, 57, 67, 106, 112
1841	Oct. 5, '86	〃	〃	Oryza sativa	paddy field	7	29, 31, 35, 56, 64, 67, 74
1842	〃	〃	〃	〃	〃	4	10, 31, 32, 67
1843	〃	〃	〃	〃	〃	7	23, 31, 60, 64, 67, 74, 112
1844	〃	〃	〃	Koendoro	crop field	8	3, 19, 23, 35, 56, 72, 74, 91
1845	〃	〃	〃	Chinese cabbage	〃	10	11, 38, 39, 56, 67, 72, 74, 81, 88, 112
1846	〃	〃	〃	turnip	〃	2	67, 74

*1 These numbers are the ordinal numbers of identified species shown in Table 2.
*2 Xinjiang Agricultural Experiment Station.
*3 Shihezi Vegetable Research Institute.
*4 Xinjiang Agricultural College Experiment Station.

Soil samples were stored in a refrigerator at 4 C, and fungi were isolated three to four weeks after collection. The following four isolation methods were applied as reported previously (10): incubation at 45 C, treatment with 50% ethanol, heat treatment at 70 C, and the standard dilution plate method. The isolation medium used was malt extract-yeast extract-agar (MYA) at pH 5.6. This was rich enough to detect as many kinds of fungi as possible, so as to provide detailed information on the mycological flora and the population and distribution of soil microfungi in these regions. Isolates were identified by cultivating them on media and at temperatures appropriate for each species.

Results and Discussion

Table 2 lists all of the species of fungi isolated from the 173 samples. One hundred and twelve species in 56 genera were identified and classified into 6 species in 3 genera of Zygomycotina, 36 in 19 genera of Ascomycotina, and 70 in 34 genera of Deuteromycotina. Almost all of the species isolated were common, typical soil fungi which have been recorded worldwide (6, 9, 10, 12, 14).

The total number of fungus species (Table 2) and the number of species detected per sample were both low for the total number of samples from which the fungi were isolated. For instance, many soil samples collected at Turpan, Urmuqi, and Shihezi seemed not to contain any viable fungal propagules, even though we tried to isolate them by the four isolation methods described above. This was also confirmed by the direct soil plate method.

The Xinjiang district has a typical continental climate, with average recorded temperatures of below -20 C in January and above 33 C in July, and annual rainfall of about 150 mm. These severe and extremely dry climatic conditions must be the most important limiting factor for fungi to survive. In particular, we failed to detect any fungi in 25 of the total of 51 soil samples collected at the desert sites. Although we did isolate fungi from the other 26 soil samples, the numbers of both species and isolates were small. In total, only 28 fungus species were detected. The most prevalent one was Geomyces pannorum (Link) Sigler & Carmichael var. pannorum (8

Table 2. List of fungus species with number of strains isolated from sample collected in China and representative strain number.

	Fungus species*	Number of strains isolated**	Representative strain number
ZYGOMYCOTINA			
1	Absidia corymbifera (Cohn) Saccardo & Trotter	18	1726-451
2	A. cylindrospora Hagem	1	1797-1
3	Mortierella alpina Peyronel	55	1707-703
4	M. globalpina W. Gams & Veenbas-Rijks	1	1733-1
5	M. minutissima van Tieghem	1	1716-2
6	Rhizopus oryzae Went & Prinsen-Geerligs	4	1853-451
ASCOMYCOTINA			
7	Chaetomium bostrychodes Zopf	1	1701-9
8	C. brasiliense Batista & Pontual	1	1701-E5
9	C. globosum Kunze:Fries	1	1763-452
10	Dichotomomyces cejpii (Milko) Scott var. cejpii	9	1736-E1
11	Emericella nidulans (Eidam) Vuillemin var. nidulans	21	1701-453
12	E. quadrilineata (Thom & Raper) C. R. Benjamin	4	1761-453
13	E. rugulosa (Thom & Raper) C. R. Benjamin	1	1775-451
14	Emericellopsis glabra (van Beyma) Backus & Orpurt	1	1764-10
15	Eupenicillium brefeldianum (Dodge) Stolk & Scott	4	1758-E2
16	E. javanicum (van Beyma) Stolk & Scott	17	1722-E1
17	Eurotium amstelodami Mangin	21	1704-E1
18	E. chevalieri Mangin	2	1758-704
19	Gymnoascus reessii Baranetzky	2	1755-11
20	Microascus cinereus (Emile-Weil & Gaudin) Curzi	2	1725-6
21	M. trigonosporus Emmons & Dodge	2	1774-9
22	Monascus anka Nakazawa & Sato	3	1852-451
23	Neosartorya fischeri (Wehmer) Malloch & Cain var. fischeri	2	1843-701
24	N. fischeri (Wehmer) Malloch & Cain var. glabra (Fennell & Raper) Malloch & Cain	8	1758-451
25	N. fischeri (Wehmer) Malloch & Cain var. spinosa (Fennell & Raper) Malloch & Cain	2	1706-451
26	N. quadricincta (Yuill) Malloch & Cain	1	1743-702
27	Petriellidium boydii (Shear) Malloch	5	1724-E2
28	Petromyces alliaceus Malloch & Cain	5	1811-1
29	Pithoascus intermedius (Emmons & Dodge) von Arx	2	1793-13
30	Pseudeurotium ovale Stolk	6	1701-E1
31	P. zonatum van Beyma	40	1701-E3
32	Pseudogymnoascus roseus Raillo	21	1727-E1
33	Sordaria humana (Fuckel) Winter	1	1797-451
34	Talaromyces byssochlamydoides Stolk & Samson	8	1713-453
35	T. flavus (Klöcker) Stolk & Samson var. flavus	45	1701-E11
36	T. helicus C. R. Benjamin apud Stolk & Samson var. helicus	6	1713-E2

Table 2. (continued)

	Fungus species*	Number of strains isolated**	Representative strain number
37	Talaromyces stipitatus C. R. Benjamin apud Stolk & Samson	9	1711-E2
38	T. trachyspermus (Shear) Stolk & Samson	16	1740-453
39	T. ucrainicus Udagawa apud Stolk & Samson	54	1711-E4
40	Thermoascus crustaceus (Apinis & Chesters) Stolk	5	1760-453
41	Thielavia arenaria Mouchacca	2	1718-452
42	T. terrestris (Apinis) Malloch & Cain	1	1773-453
DEUTEROMYCOTINA			
43	Acremonium alabamense Morgan-Jones	24	1716-451
44	A. butyri (van Beyma) W. Gams	1	1794-708
45	A. curvulum W. Gams	5	1725-2
46	A. fusidioides (Nicot) W. Gams	9	1719-E3
47	A. potronii Vuillemin	3	1806-9
48	A. strictum W. Gams	29	1704-702
49	Acrodontium crateriforme (van Beyma) de Hoog	1	1823-2
50	Acrophialophora levis Samson & Tariq Mahmood	3	1756-453
51	Alternaria alternata (Fries) Keissler	3	1821-701
52	Arthrobotrys oligospora Fresenius	2	1726-702
53	Aspergillus candidus Link	2	1801-E1
54	A. carneus (van Tieghem) Blochwitz	8	1723-451
55	A. flavus Link:Fries	2	1762-451
56	A. fumigatus Fresenius	45	1701-454
57	A. niger van Tieghem var. niger	35	1701-452
58	A. terreus Thom	54	1705-453
59	A. versicolor (Vuillemin) Tiraboschi	1	1835-702
60	Cladosporium cladosporioides (Fresenius) de Vries	20	1746-6
61	C. herbarum (Persoon) Link: S. F. Gray	4	1820-8
62	C. sphaerospermum Penzig	4	1835-704
63	Doratomyces microsporus (Saccardo) Morton & Smith	3	1705-702
64	D. nanus (Ehrenberg:Link) Morton & Smith	3	1761-3
65	Fusarium oxysporum Schlechtendahl emend. Snyder & Hansen	3	1758-2
66	F. solani (Martius) Appel & Wollenweber emend. Snyder & Hansen	6	1741-704
67	Geomyces pannorum (Link) Sigler & Carmichael var. pannorum	100	1705-705
68	Geotrichum candidum Link:Persoon emend. Carmichael	2	1713-4
69	Gliocladium catenulatum Gilman & Abbott	4	1775-702
70	G. roseum (Link) Bainier	5	1701-4
71	G. virens Miller et al.	1	1708-1
72	Gliomastix cerealis (Karsten) Dickinson	33	1708-704
73	G. murorum (Corda) Hughes var. felina (Marchal) Hughes	2	1708-2
74	Malbranchea pulchella Saccardo & Penzig var. sulfurea (Miehe) Cooney & Emerson	10	1701-451
75	Metarhizium anisopliae (Metschnikoff) Sorokin	27	1705-703
76	Monocillium indicum S. B. Saksena	3	1749-8

Table 2. (continued)

	Fungus species*	Number of strains isolated**	Representative strain number
77	Monocillium mucidum W. Gams	1	1822-8
78	M. tenue W. Gams	1	1787-7
79	Myrothecium cinctum (Corda) Saccardo	3	1711-1
80	M. roridum Tode:Fries	3	1756-7
81	M. verrucaria (Albertini & Schweinitz) Ditmar:Fries	11	1711-703
82	Oedocephalum fimetarium (Riess) Lindau	1	1855-E1
83	Oidiodendron griseum Robak	1	1754-8
84	Paecilomyces marquandii (Massee) Hughes	8	1705-706
85	P. variotii Bainier	3	1848-451
86	Penicillium funiculosum Thom	2	1736-454
87	P. janthinellum Biourge	3	1715-702
88	Penicillium lilacinum Thom	9	1792-E1
89	P. piceum Raper & Fennell	3	1701-455
90	Phialocephala humicola Jong & Davis	1	1778-4
91	Scolecobasidium variabile Barron & Busch	7	1717-704
92	Scopulariopsis brevicaulis (Saccardo) Bainier	1	1736-5
93	S. brumptii Salvanet-Duval	6	1707-7
94	Stachybotrys chartarum (Ehrenberg) Hughes	5	1704-705
95	S. microspora (Mathur & Sankhla) Jong & Davis	1	1701-1
96	Torulomyces lagena Delitsch	1	1806-8
97	Trichoderma aureoviride Rifai	1	1736-1
98	T. harzianum Rifai	22	1701-10
99	T. koningii Oudemans	1	1731-701
100	T. pseudokoningii Rifai	1	1855-1
101	T. viride Persoon:Fries	3	1731-702
102	Trichothecium roseum (Persoon) Link: S. F. Gray	1	1747-7
103	Trichurus spiralis Hasselbring	1	1726-7
104	Truncatella angustata (Persoon:Link) Hughes	1	1732-4
105	Ulocladium botrytis Preuss	3	1837-4
106	Verticillium fungicola (Preuss) Hassebrauk	5	1726-6
107	V. leptobactrum W. Gams	2	1739-3
108	V. psalliotae Treschow	1	1793-9
109	V. suchlasporium W. Gams & Dackman apud W. Gams var. catenatum W. Gams & Dackman apud W. Gams	1	1797-4
110	V. tenerum (Nees:Persoon) Link	4	1717-703
111	Wallemia sebi (Fries) von Arx	1	1851-17
112	Wardomyces inflatus (Marchal) Hennebert	8	1845-704

* Since only a single colony of each species was isolated from an isolation medium, the number of strains isolated is equal to the number of samples from which a given fungus was detected.

** Ordinal numbers preceding fungus names are the numbers ascribed to the species isolated in this work.

isolates)*, followed by Aspergillus fumigatus Fresenius (6 isolates), A. terreus Thom (5 isolates), and Acremonium strictum W. Gams (5 isolates). Aspergillus niger van Tieghem var. niger (4 isolates), Emericella nidulans (Eidam) Vuillemin var. nidulans (4 isolates), Eupenicillium javanicum (van Beyma) Stolk & Scott (2 isolates), Petromyces alliaceus Malloch & Cain (2 isolates), Pseudogymnoascus roseus Raillo (2 isolates), and Acremonium potronii Vuillemin (2 isolates) were also detected. Sixteen of the 28 species were found as a single isolate.

In the mountain areas where subalpine coniferous vegetations were predominant, Mortierella alpina Peyronel (6 isolates), Pseudogymnoascus roseus (6 isolates), and Geomyces pannorum var. pannorum (6 isolates) were the most dominant fungi. Eupenicillium javanicum (2 isolates), Trichoderma harzianum Rifai (2 isolates), and T. viride Persoon: Fries (2 isolates) were also found. The total number of species detected in 15 samples was 20. One exceptional sample was of soil within the mycorrhiza of Abies sp., from which no soil fungus was detected. Of these 20 species, only one isolate each was detected for 10 species.

In contrast, we found a richer fungus population in the crop field soils. Ninety species in total were detected in a total of 97 samples collected in agricultural and horticultural crop fields. This means that approximately 80.5% of the total of 112 fungus species listed in Table 2 were found in crop field soils. The most predominant fungus in these sites was again Geomyces pannorum var. pannorum (59 isolates), followed by Aspergillus terreus (42 isolates), Mortierella alpina (41 isolates), Talaromyces ucrainicus Udagawa apud Stolk & Samson (38 isolates), T. flavus (Klöcker) Stolk & Samson var. flavus (27 isolates), Aspergillus fumigatus (26 isolates), Pseudeurotium zonatum van Beyma (25 isolates), Gliomastix cerealis (Karsten) Dickinson (25 isolates), Metarhizium anisopliae (Metschnikoff) Sorokin (25 isolates), Acremonium alabamense Morgan-Jones (22 isolates), and A. strictum (21 isolates). Thus, we isolated more than 10 isolates each for around 20 species, indicating a richer population in both fungus species and isolates in crop field soils as compared with the desert and mountain soils.

From these results it is concluded that Geomyces pannorum var. pannorum is the most predominant fungus in Xinjiang, irrespective of the

*Since we isolated only one colony of each fungus species from any sample, "8 isolates" means that we detected this fungus species in 8 soil samples.

geographical location or vegetation. This fungus was detected in about 60% of all the soil samples collected in Xinjiang. Aspergillus terreus is predominant in both crop field soils and desert soils, and Mortierella alpina in both the crop field and mountain soils. These two fungi were each detected in about 30% of soil samples.

Geomyces pannorum var. pannorum (7 isolates) was also isolated in both the vegetable and paddy field soils in Beijing: 7 isolates were obtained from 9 soil samples collected. However, Malbranchea pulchella Saccardo & Penzig var. sulfurea (Miehe) Cooney & Emerson (5 isolates), Aspergillus fumigatus (4 isolates), Wardomyces inflatus (Marchal) Hennebert (4 isolates), Pseudeurotium zonatum (4 isolates), Pseudogymnoascus roseus (3 isolates) were also isolated, indicating the different microfungus flora in the Beijing district. Also, a total of 28 fungus species was detected in 9 soil samples, suggesting very rich soil fungus flora and population in agricultural field soils in the Beijing district as compared with the Xinjiang district.

Autoecological considerations of several noteworthy fungi, together with their distributional characteristics, are as follows. Two species of Mortierella were isolated, M. alpina was the most predominant, being detected in many soil samples, while M. globalpina W. Gams & Veenbas-Rijks was found in very few. Although some species of the subgenus Micromucor in Mortierella and the section Hiemalis in Mucor are considered to be very dominant in soil, especially in coniferous forest soils and in cold areas (6, 10, 12, 14), these two groups of fungi were not detected in this investigation. The reason is not clear, but it can probably be ascribed to the unsuitability for these fungi of the cold, but very dry climate in Xinjiang.

Nineteen genera of Ascomycotina were encountered in this survey. Of these, Emericella nidulans var. nidulans, Eupenicillium javanicum, Eurotium amstelodami Mangin, Pseudeurotium zonatum, Pseudogymnoascus roseus, Talaromyces flavus var. flavus, T. trachyspermus (Shear) Stolk & Samson, T. ucrainicus were common. These species have often been detected in soil, cereals, and other organic materials in many parts of the world (4, 6, 9, 10, 12, 18).

Twenty-one strains each of Emericella nidulans var. nidulans and Eurotium amstelodami were isolated from dry soil samples in Shihezi and Nanshan. These two species are typical soil fungi with a worldwide

distribution, being recorded most frequently in warm regions and in various dried and aged plant materials. Four strains of Emericella quadrilineata (Thom & Raper) C. R. Benjamin and one of E. rugulosa (Thom & Raper) C. R. Benjamin were also isolated in Shihezi and Nanshan. These two species have been reported in soils in tropical and temperate countries, groundnuts, and hay in a compost heap.

Pseudeurotium ovale Stolk and P. zonatum are the commonest species of the genus and they have been isolated predominantly from soils (6, 10), especially paddy field soils (unpublished data). However, the former was isolated only rarely in this study.

Talaromyces flavus var. flavus and T. ucrainicus, which were among the dominant isolates from soil samples in Xinjiang, are regarded as typical soil fungi (6, 10, 12).

Two strains of Microascus cinereus (Emile-Weil & Gaudin) Curzi were found in soil samples collected in Shihezi. This species is known from soil, dog's dung, and decaying wood (18, 22). Two strains of Microascus trigonosporus Emmons & Dodge were also detected in soils in Shihezi. According to Domsch et al. (6), this species has frequently been isolated from various soils (e. g., cultivated, desert, salt-marsh, costal sediments, alkaline soils, and loess) in the USSR, USA, Kuwait, Norway, France, and Israel. Other substrates are dead wood, seeds of various cereals, mouse, chicken manure, rat dung, and a skin lesion on a human foot. It is known that this species can grow at 30 C and in sea water containing 4% salt. Udagawa (22) reported that this species was found in milled rice imported from Taiwan and Burma. It has not yet been found in Japan.

Five strains of Petromyces alliaceus were detected in dry soil samples from Shihezi and Nanshan. Raper and Fennell (19) reported that this species is not abundantly distributed in nature. It has been isolated from a dead blister beetle, onion and garlic bulbs, cactus plant, and from soils collected in Texas, Arizona, Mexico, Calcutta, and Australia. Since this species produces many stroma on various media, it is considered to be able to endure the dry and severe climatic conditions of Xinjiang.

Strains of Pithoascus intermedius (Emmons & Dodge) von Arx were also isolated from soils in Shihezi and paddy field soil in Beijing. This species, like Microascus trigonosporus, is considered to favour dry conditions.

Five strains of Thermoascus crustaceus (Apinis & Chesters) Stolk were

detected in soils from Shihezi. Ito et al. (9) emphasized that T. aurantiacus Miehe was the most common species in paddy field soils in Japan, whereas T. crustaceus was less common.

Three species of Aspergillus in the Deuteromycotina, i.e., A. fumigatus, A. niger var. niger, A. terreus, were dominant isolates from about 30% of all the samples collected. These species are commonly isolated from various materials in many parts of the world, particularly the warmer regions.

Geomyces pannorum var. pannorum was found to be the most common and predominant species in the Xinjiang region. This fungus is very common in most soils (10, 12) and has been isolated particularly from various forest soils outside of tropical and subtropical areas.

The other Deuteromycotina fungi were sparsely isolated (Table 2). These species are mostly typical soil fungi (6, 10, 12), and overall fungus flora in the Xinjiang district agreed well with those in the Far Eastern USSR (10) and in paddy field soils in Japan (unpublished data).

The fungus flora in soils in the Xinjiang Uighur District were thus characterized principally by these typical mesophilic soil fungi. However, some species, e.g., Emericella quadrilineata, E. rugulosa, Petromyces alliaceus, were xerophilic fungi which were not detected in the Far Eastern USSR (10) or in paddy field soils in Japan (unpublished data).

The authors wish to thank the authorities of the Xinjiang Shihezi Agricultural College and the Ministry of Agriculture, Animal Husbandry and Fisheries, China, for financial support. We thank Dr. W. Gams, Centraalbureau voor Schimmelcultures, Baarn, the Netherlands, for his kind help in identification of some of the isolates.

References

1) Bi, C.S., G.Y. Zheng, J.Q. Liang, C. Li, T.H. Li, W.I. Zheng, and M.Z. Lian. 1983. Taxonomic studies on Marasmiellus from Dinghu Mountain of China. Acta Mycol. Sin. 2: 26-33.
2) Bi, Z.S., T.H. Li, and G.Y. Zheng. 1987. Taxonomic studies on Mycena from Guangdong Province of China. Acta Mycol. Sin. 6: 8-14.
3) Bi, Z.S., T.H. Li, G.Y. Zheng, and C. Li. 1985. Four new species of Agaricales. Acta Mycol. Sin. 4: 155-161.
4) Carmichael, J.W. 1962. Chrysosporium and some other aleuriosporic Hyphomycetes. Can. J. Bot. 40: 1137-1173.

5) Chen, Q. T., S. R. Xiao, and Z. Y. Shi. 1984. Paecilomyces sinensis sp. nov. and its connection with Cordyceps sinensis. Acta Mycol. Sin. 3: 24-28.
6) Domsch, K. H., W. Gams, and T. H. Anderson. 1980. Compendium of soil fungi. Academic Press, London.
7) Guo, Y. L. 1984. Four new species of the genus Corynespora. Acta Mycol. Sin. 3: 161-169.
8) Guo, Y. L. 1987. Studies on Hyphomycetes of Shennongjia IV. Cercospora, Cercosporidium and Phaeoramularia. Acta Mycol. Sin. 6: 225-228.
9) Ito, T, M. Ueda, and T. Yokoyama. 1981. Thermophilic and thermotolerant fungi in paddy field soils. IFO Res. Comm. 10: 20-32.
10) Ito, T., and T. Yokoyama. 1985. Filamentous fungi collected in the Far Eastern USSR. IFO Res. Comm. 12: 34-62.
11) Jiang, C. L., L. H. Xu, and Q. T. Chen. 1985. A new species of Scopulariopsis Bainier. Acta Mycol. Sin. 4: 167-170.
12) Kobayasi, Y., N. Hiratsuka, R. P. Korf, K. Tubaki, K. Aoshima, M. Soneda, and J. Sugiyama. 1967. Mycological studies of the Alaskan Arctic. Ann. Rep. Inst. Fermentation, Osaka 3: 1-138.
13) Kong, H. Z., and Z. T. Qi. 1985. Some new records and rare taxa of Aspergillus of China. Bull. Bot. Res. (Harbin) 5: 145-150.
14) Linnemann, C. 1985. Untersuchungen zur Verbreitung und Systematik der Mortierellen. Arch. Mikrobiol. 30 : 256-267.
15) Liu, B., and Y. S. Bau. 1980. Fungi and mycological literature of the Szechwan Province in China. Mycologia 72: 1117-1126.
16) Liu, T. R., C. K. Pai. 1985. Some new species of Peronosporaceae in China. Acta Mycol. Sin. 4: 5-11.
17) Mao, X. L. 1988. Wild edible fungi and their habitat in China. Acta Mycol. Sin. 7: 36-43.
18) Morton, F. J., and G. Smith. 1963. The genera Scopulariopsis, Microascus and Doratomyces. Mycol. Pap. 86: 1-96.
19) Raper, K. B., and D. I. Fennell. 1965. The genus Aspergillus. Williams & Wilkins, Baltimore, Maryland.
20) Tai, F. L. 1979. Sylloge Fungorum Sinicorum. Science Press, Academia Sinica, Peking.
21) Tang, Z., and M. Zang. 1984. Additions to the key to Endogonaceae and a new species of mycorrhizal fungus. Acta Bot. Yunnan. 6: 295-304.
22) Udagawa, S. 1962. Microascus species new to the mycoflora of Japan. J. Gen. Appl. Microbiol. Tokyo 8: 39-51.
23) Ying, J. Z., X. L. Mao, Q. M. Ma, Y. C. Zong, and H. A. Wen. 1987. Icones of medicinal fungi from China. [translated into English by Wang, Y. C., H. F. Tang, and J. Z. Ying]. Science Press, Academia Sinica, Beijing.
24) Yu, Y. N. 1987. Ecology and distribution of the genus Pythium in China. Acta Mycol. Sin. 6: 20-33.
25) Yue, J. Z., and Y. Y. Pna. 1984. A new species of Candida - Candida beijingensis. Acta Mycol. Sin. 3: 157-160.
26) Zang, M., and D. G. Ji. 1985. Notes on Phallaceae from the Eastern Himalayan region of China. Acta Mycol. Sin. 4: 109-117.
27) Zeng, X. L. 1987. A study on the genus Phellinus of Jilin Province. Acta Mycol. Sin. 6: 142-146.
28) Zhao, J. D., L. W. Xu, and X. Q. Zhang. 1983. Studies on the delimitation of Trametes and the relationships with its allied genera. Acta Mycol. Sin. 2: 213-220.
29) Zheng, R. Y., and Y. N. Yu. (eds.). 1987. Erysiphales. Flora Fungorum Sinicorum, Vol. 1. Science Press, Academia Sinica, Beijing.

IFO Res. Comm. 14,
143-152, 1989(March)

QUALITY CONTROL OF THE INTERNATIONAL STREPTOMYCES PROJECT (ISP) STRAINS DEPOSITED AT THE INSTITUTE FOR FERMENTATION, OSAKA (IFO)

TŌRU HASEGAWA, TAKASHI SHOMURA*, and MASA HAMADA*

Summary

The ISP Check Committee consisting of 12 members of the Society for Actinomycetes, Japan (SAJ) was organized in 1970. Strains requiring reexamination after the Committee had checked 440 strains three times, and the newly prepared L-dried specimens were checked by the methods and criteria adopted by the Committee. As a result, the Committee confirmed the identity of the characteristics of all but two of the ISP strains with the characteristics described in the Int. J. Syst. Bacteriol.(IJSB). The L-dried specimens were confirmed to be well preserved for at least five years.

The IFO has, since 1969, been the main center in Japan responsible for the preservation and distribution of ISP strains in compliance with a request from the SAJ. In 1970, the SAJ organized the ISP Check Committee to qualify the ISP strains kept at the IFO (1).

The current members of the ISP Check Committee are:

Standing member	Masa Hamada (Institute of Microbial Chemistry)
Standing member	Takashi Shomura (Meiji Seika Kaisha, Ltd.)
	Ryuzo Enokita (Sankyo Co., Ltd.)

* ISP Check Committee, Society for Actinomycetes, Japan (SAJ)

Tamotsu Furumai (Nippon Roche Research Center)
Takashi Iwasa (Takeda Chemical Ind., Ltd.)
Isao Kawamoto (KYOWA HAKKO Kogyo Co., Ltd.)
Yoshimi Kawamura (Shionogi & Co., Ltd.)
Akihiro Matsumae (Kitasato University)
Yuzuru Mikami (University of Chiba)
Naoki Muto (TOYO JOZO Co., Ltd.)
Akio Seino (RIKEN)
Akira Shimazu (University of Tokyo)

Consultant Yoshiro Okami (Institute of Microbial Chemistry)
Consultant Eiji Higashide (Takeda Chemical Ind., Ltd.)

The members in charge of preservation and distribution of ISP strains at the IFO are:

Teiji Iijima (Director)
Tōru Hasegawa (Chief)
Isamu Asano

The ISP Check Committee has examined the selected ISP strains and confirmed the identity of key characters with the ISP descriptions available in the IJSB. As a result of the system for qualification (Fig. 1), the ISP strains at the IFO can be distributed with a high degree of confidence, depending on the activities of the ISP Committee which have been reported by Okami (4), Okami and Kusaka (5,6), and Higashide and Hamada (2).

In this paper, the results of 16 years of work by the Committee are described.

Methods

The checking methods and criteria adopted by the Committee are shown on the following flow sheet.

Methods for initiating growth

L-dried (or freeze-dried) specimens in ampoule
↓
Rehydrate in 0.2 - 0.3 ml of ISP medium 1 (Tryptone-yeast extract broth)
↓
One loopful of the rehydrated suspension
↓
Streak onto agar slants: ISP medium 4 (Inorganic salts starch agar)
JCM medium 44 (Bennett's agar)
IFO medium 231 (Maltose Bennett's agar)
↓
Incubate at 27 - 28 C for 2 weeks
↓
Count number of colonies and record as follows:
No colony.....-
Moderate numbers of colonies.....+ (number/slant)
Abundant numbers of colonies.....++

Characterization of cultures

Slant culture of ISP medium 4
↓
Prepare turbid suspension of spores or mycelial fragments in sterile NaCl solution (0.85%)
↓
Streak one loopful onto triplicated agar plates for spore formation
Agar medium used: ISP medium 2 (Yeast-extract malt extract agar)
ISP medium 3 (Oatmeal agar)
ISP medium 4
ISP medium 5 (Glycerol-asparagine agar)
JCM medium 40 (Glucose-asparagine agar)
JCM medium 42 (Yeast starch agar)
JCM medium 44
JCM medium 51 (OA-Y agar)
ATCC medium 399
IFO medium 231

Gauze's synthetic medium No. 1

JCM medium 48 (Peptone corn agar)
(for thermophilic strains only)

Incubate at 27 - 28 C for 20 days

Observe at 10 and 20 days of incubation

Check items:

1. Morphology of the spore chain
2. Ornamentation of spore surface
3. Mass color of mature (or sporulated) aerial mycelium
4. Production of melanoid pigments
5. Color of vegetative mycelium observed from the reverse side of growth
6. Color of the cultured medium, either diffusible pigment or insoluble pigment
7. The pH effect upon color of pigments

Record

Describe in the formalized card (Fig. 2)

Results and Discussion

1. The results for 1969 - 1979.

The IFO received and lyophilized 286 strains in 1969 (Group 1) and 154 strains in 1973 (Group 2). The strains of Group 1 were examined by the Committee in 1971, 1975 and 1979. The strains of Group 2 were examined in 1974 and 1979. The Committee found 3 strains in Group 1 and 7 strains in Group 2 to be inappropriate for distribution because of their extremely poor growth, poor formation of aerial hyphae, or death. Accordingly, the Committee advised the IFO to suspend the distribution of those strains to public. The Committee also recommended their replacement by strains received from other ISP repositories, including the ISP Center (Dr. Shirling, Ohio). The replacement strains were checked and, where appropriate, deposited under new IFO numbers (Table 1).

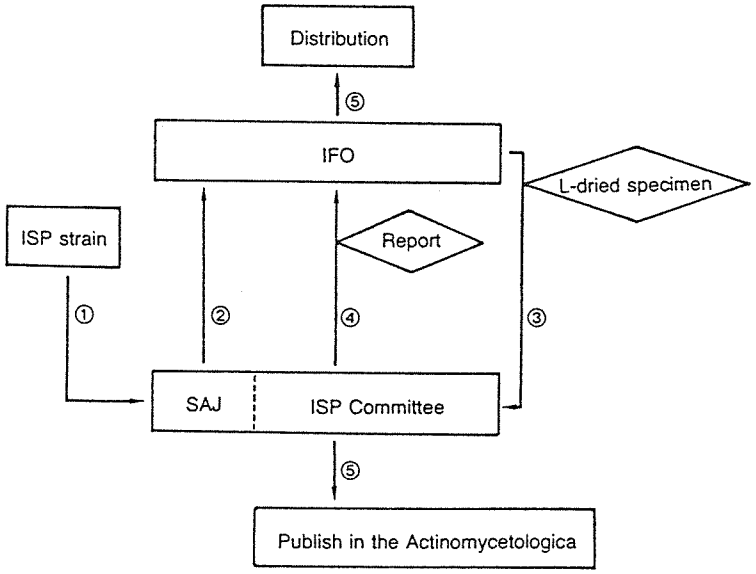


Fig. 1. Quality control system of the ISP strains deposited at the IFO.
Number shows the order of flow.

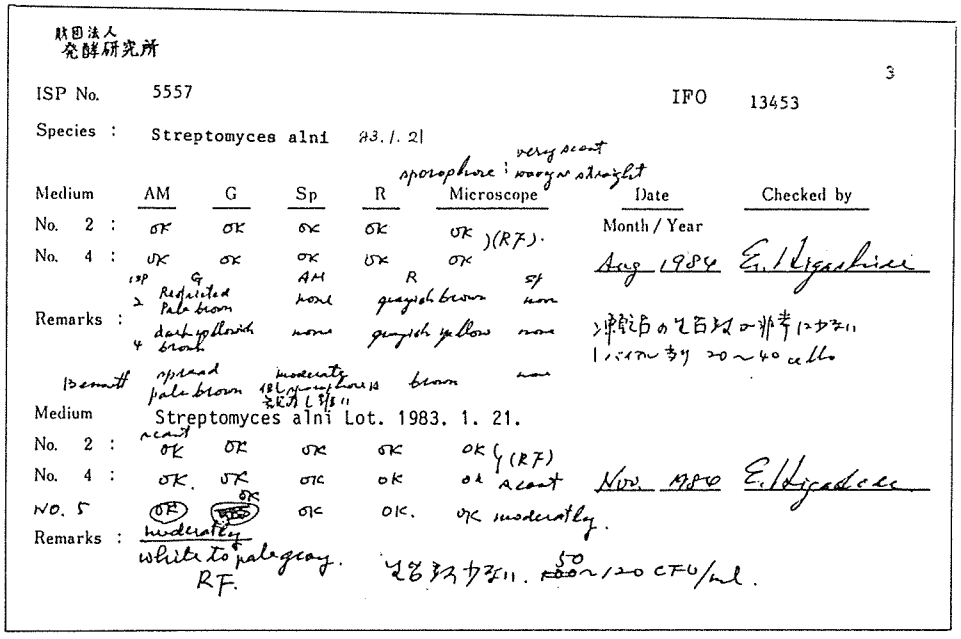


Fig. 2. An example of the formalized card.

2. The results for 1980 - 1986.

Since 1982, the IFO has preserved and distributed L-dried ampoules of bacteria, yeast and actinomycetes including ISP strains, because the L-drying method requires less labor than lyophilization and is a good method for long-term preservation of microorganisms (3,7). Therefore, the IFO has supplied to the Check Committee cultures dried by this method for confirmation study since 1983. Strains whose validity was guaranteed by the ISP Check Committee in 1985 are shown in Table 2. ISP medium 2 was most preferred for growth and sporulation of the strains guaranteed, followed by ISP medium 4 and JCM medium 42, and then by JCM medium 44 and ISP medium 3. In 1986, problems were found with the 56 degenerated strains shown in Table 3. These strains were reinoculated onto spore formation media adopted by the Committee, and were investigated. As a result, 55 strains have become available for L-drying and distribution, the exception being Streptomyces baarnensis IFO 13197.

3. The results in 1987.

In 1987, when the IFO supplied the L-dried specimens of 48 strains to the Committee for confirmation, 46 of them were approved. The remaining 2 strains, IFO 13197 (ISP 5253) and IFO 12799 (ISP 5091), were found by the Committee to have problems (as shown in Table 4) differences in their characters from those in the ISP descriptions. In future, these strains with problems will be checked again and judged by the Committee.

Culture collections maintain a wide range of organisms and should ensure the availability of the preserved strains to be used as reference cultures. Often, culture collections are not staffed by the personnel required to check detailed characters and to guarantee survival of the cultures. One possible solution to this problem is to establish a checking system by a committee such as the ISP Check Committee in Japan. The ISP Check Committee conducts a project in which experts, mostly outside the culture collection (IFO), cooperate. Accordingly, the ISP strains in the IFO, which are guaranteed by the Committee, are expected to be highly reliable for use as reference cultures of actinomycetes. Each ISP strain distributed by IFO is accompanied by a statement that the strain is authorized by the Committee of the SAJ as a qualified culture for taxonomic studies.

It is hoped that the exchange of information about the characters of ISP strains between the culture collections where other sets of ISP strains

Table 1. Strains replaced.

ISP No.	IFO No.	Taxon
5341	13076 → 13971	Streptomyces psammoticus
5252	12840 → 14035	Streptomyces arabicus
5320	13011 → 14052	Streptomyces albidus
5271	13358 → 13903	Streptomyces purpurogeniscleroticus
5302	13362 → 13902	Streptomyces niger
5269	13356 → 13904	Streptomyces sclerotialus
5443	13387 → 13905	Streptomyces thermoviolaceus subsp. thermoviolaceus
5336	13364 → 13906	Streptoverticillium kashmirense
5473	13394 → 13907	Streptoverticillium parvisporogenes
5270	13357 → 14019	Streptomyces flavisclerotícus

Table 2. Strains guaranteed (2).

	ISP No.	IFO No.	Taxon	Optimum medium for sporulation
Group 1				
	5011	12743	Nocardia autotrophica	OA-Y agar
	5107	12761	Streptomyces curacoi	ISP-3
	5061	12768	Streptomyces flaveolus	ISP-3
	5062	12771	Streptomyces flavovirens	Bennett's agar
	5155	12774	Streptomyces glaucescens	JCM 42
	5131	12790	Streptomyces limosus	ISP-2
	5016	12801	Streptomyces narbonensis	ISP-2
	5071	12802	Streptomyces nigrifaciens	ISP-2
	5088	12804	Streptomyces niveus	Gauze's No.1
	5097	12807	Streptomyces pilosus	ISP-2
	5019	12808	Streptomyces pluricolorescens	JCM 42
	5032	12820	Streptomyces sioyaensis	ISP-2
	5003	12834	Streptomyces albogriseolus	ISP-2
	5234	12838	Streptomyces antibioticus	ISP-2
	5236	12875	Streptomyces ariseus subsp. griseus	ISP-4
	5263	12877	Streptomyces humidus	Bennett's agar
	5313	13014	Streptomyces albus subsp.albus	ISP-2
	5461	13058	Streptomyces lydicus	ISP-2
	5309	13094	Streptomyces vastus	IFO 231
	5048	13193	Streptomyces parvullus	ISP-4
	5056	13199	Streptomyces bobili	JCM 40
	5021	13202	Streptomyces mediocidicus	OA-Y agar
Group 2				
	5103	13344	Streptomyces caeruleus	JCM 42
	5244	13354	Streptomyces xantholiticus	ISP-4
	5358	13368	Streptomyces novaecaesareae	-
	5475	13395	Streptomyces atrofaciens	ISP-3
	5480	13399	Streptomyces galbus	Bennett's agar
	5494	13411	Streptomyces capoamus	JCM 42
	5511	13423	Streptomyces ostreogriseus	ISP-2
	5526	13429	Streptomyces avidinii	ISP-2
	5557	13453	Streptomyces alni	IFO 231
	5560	13456	Streptomyces tauricus	ISP-4
	5578	13472	Streptomyces hygroscopicus subsp. hygroscopicus	ISP-2

-: no medium for good sporulation.

Table 3. Strains with problems (2).

ISP No.	IFO No.	Taxon	Problem				
			surv.	AM.	spo.	S.pig.	
Group 1							
5002	12754	Streptomyces chattanoogensis		poor			
5129	12759	Streptomyces collinus		poor			
5130	12766	Streptomyces felleus				none	
5093	12769	Streptverticillium flavopersicum			poor		
5152	12770	Streptomyces flavotricini				none	
5153	12772	Streptomyces flavoviridis				weak	
5066	12775	Streptomyces griseobrunneus	poor				
5053	12836	Streptomyces ambofaciens				none	
5233	12854	Streptomyces coelicolor				weak	
5145	12855	Streptomyces coeruleorubidus				weak	
5281	12873	Streptomyces griseoruber				weak	
5229	12874	Streptomyces griseoviridis				weak	
5188	12889	Streptomyces matensis				weak	
5105	12896	Streptoverticillium olivoreticulum	poor				
5386	13018	Streptomyces aureocirculatus				none	
5417	13022	Actinomyces aurigineus		none			
5262	13023	Streptomyces bottropensis					#
5300	13026	Streptomyces cavourensis		poor			
5424	13031	Actinomyces coerulatus		poor			
5427	13036	Actinomyces cyanoglomerus subsp. cellulose		poor			
5428	13038	Actinomyces flavescens		poor			
5385	13045	Streptomyces griseolavendus					#
5323	13040	Streptomyces flavogriseus		none			
5328	13048	Streptomyces heimi					#
5383	13050	Streptomyces ipomoeae		none			
5345	13051	Streptomyces karnatakensis		poor			
5321	13053	Streptomyces krainskii		poor			
5317	13056	Streptomyces lucensis	poor				
5323	13065	Streptomyces noboritoensis	poor				
5334	13066	Streptomyces olivaceoviridis	poor				
5250	13068	Streptoverticillium olivoverticillatum	poor				
5315	13070	Streptomyces paucisporogenes	poor				
5261	13090	Streptomyces tubercidicus	poor				
5278	13191	Streptomyces umbrinus	poor				
5162	13192	Streptomyces kurssanovii		poor			
5172	13194	Streptomyces roseofulvus				weak	
5045	13196	Streptomyces alboflavus		none			
5232	13197	Streptomyces baarnensis		poor			
5024	13203	Streptomyces pyridomyceticus	poor				

Table 3. (continued)

ISP No.	IFO No.	Taxon	Problem			
			surv.	AM.	spo.	S.pig.
Group 2						
5104	13345	*Streptomyces sulphureus*	poor			
5110	13347	*Streptomyces viridochromogenes*				weak
5209	13349	*Streptomyces violatus*				none
5280	13359	*Streptomyces viridiviolaceus*				none
5367	13372	*Streptomyces phaeofaciens*	poor			
5384	13374	*Streptomyces chromogenus*	poor			
5518	13427	*Streptomyces gedanensis*				#
5520	13428	*Streptomyces tropicalensis*	poor			
5527	13430	*Streptoverticillium ardum*		poor		
5539	13441	*Streptomyces durhamensis*			poor	
5567	13463	*Streptomyces paraguayensis*	poor			
5571	13466	*Streptoverticillium orinoci*			none	
5588	13477	*Streptomyces neyagawaensis*			poor	
5591	13480	*Actinomyces ochroleucus*		none		
5593	13482	*Streptomyces fulvissimus*				poor
5594	13483	*Streptomyces ochraceiscleroticus*		poor		
5271	13903	*Streptomyces purpurogeniscleroticus*		poor		

surv.: survival, AM.: aerial mycelium, spo.: sporulation, S.pig.: soluble pigment, #: others.

Table 4. Different characters found by the Committee from IJSB descriptions.

	IJSB	ISP Committee
S. baarnensis IFO 13197 (ISP 5232)		
Aerial mycelium	unclear	Gray seris
Microscopic morphology	*Rectiflexibiles*	*Retinaculiaperti* or *Spirales*
S. murinus IFO 12799 (ISP 5091)		
Growth color	grayish yellow or yellow	dark brown dots on a field of yellow

are preserved will stimulate the validation of ISP strains as precious biologic standards.

The significant activities of the Committee over the long period described above have depended on the leadership of Dr. Y. Okami (Institute of Microbial Chemistry) and the great efforts of each member of the Committee. Significant contributions by Drs. T. Hasegawa, K. Nakazawa, K. Tubaki, T. Yokoyama, T. Kusaka and T. Iijima, Director of the IFO, must also be acknowledged.

References

1) Hasegawa, T., and Y. Okami. 1973. Confirmation system for the ISP strains preserved in Japan. IFO Res. Comm. 6: 1-3.
2) Higashide, E., and M. Hamada. 1986. Studies on the preservation of characters of the ISP strains. Actinomycetologica No.49, 21-27.
3) Iijima, T., and T. Sakane. 1973. A method for preservation of bacteria and bacteriophages by drying in vacuo. Cryobiol. 10: 379-385.
4) Okami, Y. 1971. Report from the ISP Committee. Actinomycetologist No. 18, 6-7.
5) Okami, Y., and T. Kusaka. 1985. Further qualification of ISP strains deposited in IFO by the Society for Actinomycetes, Japan (SAJ). IFO Res. Comm. 12: 83-89.
6) Okami, Y., and T. Kusaka. 1985-1986. Qualification of the International Streptomyces Project (ISP) strains deposited at the Institute for Fermentation, Osaka (IFO) by the Society for Actinomycetes, Japan (SAJ). Actinomycetes 19: 2-10.
7) Sakane, T. 1982. Preservation of microorganisms by L-drying. Reitou 57: 767-775. (in Japanese)

IFO Res. Comm. 14,
153-155, 1989(March)

DESCRIPTIVE CATALOGUE OF IFO YEAST COLLECTION VI.

In the routine work of updating data on strains in the collection, interesting strains worthy of record have been found, and a few strains have been reidentified as different species. The object of this catalogue is to provide descriptions of the taxonomical characteristics of these strains. The authors of the descriptions are indicated in brackets.

45, 46 and **47.** **Saccharomyces unisporus** Jörgensen

Jörgensen, A. 1909. Die Mikroorganismen der Gärungsindustrie. 5te Auflage, P. Parey, Berlin; Yarrow, D. 1984. In The Yeasts, a taxonomic study. 3rd ed. by N. J. W. Kreger-van Rij. Elsevier, Sci Pub. B. V., Amsterdam p. 392-393.

IFO 0215, IFO 0270 and **IFO 0286**

IFO 0215 was obtained from the Government Research Institute of Formosa in 1941, originated from C. Wehmer; IFO 0270 was deposited by K. Sakaguchi, faculty of Agriculture, the University of Tokyo in 1942, who received it from Central Laboratory, South Manchuria Railway Co. Ltd.; and IFO 0286 was deposited by H. Naganishi, Faculty of Engineering, Hiroshima University in 1946, who received it from the National Research Institute of Brewing, Tokyo. The three strains have been maintained under the name Saccharomyces exiguus.

Electrophoretic karyotypes of chromosomal DNA of 10 strains designated as S. exiguus were examined by pulsed-field gel electrophoresis (PFGE). It was found that the three yeasts IFO 0215, IFO 0270 and IFO 0286 had very similar chromosome DNA band patterns to the type strain of S. unisporus, but not to that of S. exiguus as seen in Fig 1.

Morphological and cultural characteristics of the two strains closely matched the standard description of S. unisporus in The Yeasts 3rd ed. (1984), with exception that no ascus was found on corn meal agar, malt

extract agar and potassium acetate agar after 2 weeks at 20 C.

Their physiological characteristics are presented in Table 1.

Tsuchiya et al. (1974) reported that IFO 0215, IFO 0270 and IFO 0286 had antigen 23, which was specific to the species S. unisporus (Mycopath. Mycol. Appl. 53: 82 1974).

Consequently, the three strains have been reidentified as Saccharomyces unisporus Jorgensen.

[k. Mikata]

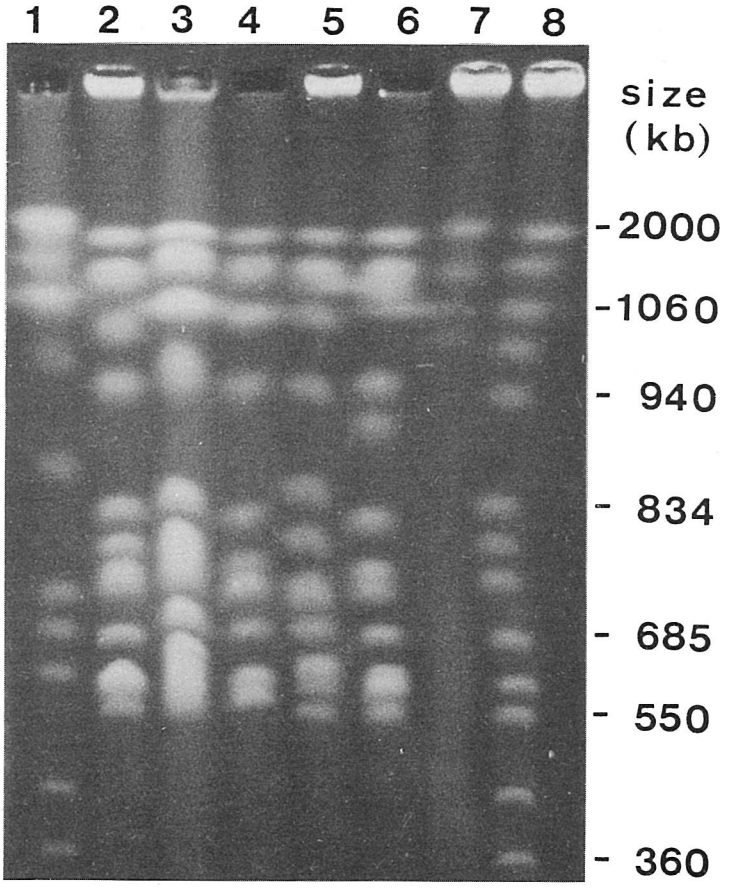


Fig. 1. The PFGE banding pattern of Saccharomyces.
1. IFO 1128 (S. exiguus type strain),
2. IFO 0215, 3. IFO 0270, 4. IFO 0286,
5. IFO 0316 (S. unisporus type strain),
6. IFO 1174 (S. unisporus),
7. IFO 0955 (Toluraspora delbrueckii type strain)
8. SH 964 (S. cerevisiae).
240V 190mA pulse time 1 min. ran time 24 hr.

Table 1. Physiological characteristics.

	IFO[T] 0316	IFO 0215	IFO 0270	IFO 0286		IFO 0316	IFO 0215	IFO 0270	IFO 0286
Fermentation:									
Glucose	+	+	+	+	Lactose	-	-	-	-
Galactose	+	+	+	+	Raffinose	-	-	-	-
Sucrose	-	-	-	-	Inulin	-	-	-	-
Maltose	-	-	-	-	Soluble Starch	-	-	-	-
Trehalose	-	-	-	-	α-Methyl-D-glucoside	-	-	-	-
Assimilation of carbon compounds:									
Glucose	+	+	+	+	D-Ribose	-	-	-	-
Galactose	+	+	+	+	Rhamnose	-	-	-	-
L-Sorbose	-	-	-	-	Ethanol	+w	-	-	-
Sucrose	-	-	-	+w	Glycerol	-	-	-	-
Maltose	-	-	-	-	Erythritol	-	-	-	-
Cellobiose	-	-	-	-	Ribitol	-	-	-	-
Trehalose	+s	+s	+s	+s	Galactitol	-	-	-	-
Lactose	-	-	-	-	D-Mannitol	-	-	-	-
Melibiose	-	-	-	-	D-Glucitol	-	-	-	-
Raffinose	-	-	-	-	α-Methyl-d-glucoside	-	-	-	-
Melezitose	-	-	-	-	Salicin	-	-	-	-
Inulin	-	-	-	-	DL-Lactic acid	-	-	-	+w
Soluble starch	-	-	-	-	Succinic acid	-	-	-	-
D-Xylose	-	-	-	-	Citric acid	-	-	-	-
L-Arabinose	-	-	-	-	Inositol	-	-	-	-
D-Arabinose	-	-	-	-	Arbutin	-	-	-	-
Assimilation of potassium nitrate						-	-	-	-
Assimilation of ethylamine hydrochloride						+	+	+	+
Growth in vitamin free-medium						-	-	-	-
Growth at 37 C						-	-	-	-
G+C content						32.1	31.2	33.7	31.9

T: Saccharomyces unisporus type strain.

+: positive, +s: slow growth, +w: weak growth, -: negative.

IFO Res. Comm. 14,
156-161, 1989(March)

DESCRIPTIVE CATALOGUE OF IFO FUNGUS COLLECTION XI.

In routine identification work on fungi newly isolated in Japan, and in checking the list of the fungi preserved in the IFO culture collection for published records of their occurrence in Japan, many fungi have been found to be taxa either new to Japan or obscurely or insufficiently described. In some cases, the first record of a fungus in Japan gives only the name of a taxon, without an adequate description of the species concerned. The object of this series is to provide descriptions of the fungi preserved or newly deposited in the IFO fungus collection and/or in the IFO herbarium and to contribute to the knowledge of the fungal flora of Japan.

New taxa will be published in other papers. The authors of the descriptions of these fungal taxa are shown in parentheses.

85. Eleutherascus lectardii (Nicot) von Arx (Figs. 1 & 2) Pezizales
Persoonia 6: 377 (1971); Huang and Schmitt, Ohio J. Sci. 73: 234 (1973); von Arx, The genera of fungi sporulating in pure culture, J. Cramer. p. 315 (1974); Huang, Mycologia 67: 293 (1975).
Syn. Arachniotus lectardii Nicot apud Nicot and Durand, Bull. Soc. mycol. Fr., 85: 319 (1969).
Hemiascosporium spinulosum Batra, Mycologia 65: 797 (1973).

Colonies on malt agar (MA) grow rapidly, floccose to arachnoid or partly with loose tufts, then creep on the medium; at first white, later pale cream to pale brown, particularly in compact tufts; surface appearance of colonies very similar to those of Mortierella spp.; reverse white to pale brown.

Colonies on cornmeal agar (CM) and potato carrot agar (PCA) are partly floccose and partly submerged in the medium; submerged mycelium pale brown to brown. Vegetative mycelium septate, 3.0-7.5 μm wide, irregularly

branched, partly swollen. Ascogonial initials coiled, 4-6 μm wide. Ascomata absent. One to three gymnocarpic asci formed directly on the aerial mycelium, at first white, light brown at maturity. Asci containing two to six ascospores, rarely eight-spored, globose to oboid, 30-35 μm; ascus wall more or less persistent, hyaline, rather thick. Ascospores globose, pale brown, covered with acute spines 2-2.5 μm long, 10-13 μm in diam excluding the spines. Anamorph has not been observed on various media.

Growth is good at 37 C.

Hab. paddy field soil. Nakatsu-cho, Ibaraki, Osaka Pref., 18 July 1976 (T. Yokoyama ZIE-2-10-4 = IFO 30531); 17 July 1978 (T. Yokoyama ZIXE-1-10-17 & ZIXE-2-5-3); 22 January 1979 (T. Yokoyama ZXI-1-10-15). Hachioji, Ikeda, Osaka Pref., 9 August 1976 (T. Yokoyama YIE-1-15-9 & YIE-4-15-6); 8 November 1976 (T. Yokoyama YIIE-4-10-4); 17 February 1977 (T. Yokoyama YIII-5-10-7 & YIIIE-3-10-6 = IFO 30534); 7 May 1977 (Y. Yokoyama YIV-1-15-7); 5 August 1977 (T. Yokoyama YV-1-10-10 & YVE-5-5-12). Shakudo, Habikino, Osaka Pref., 20 August 1976 (T. Yokoyama XI-3-15-15); 22 November 1976 (T. Yokoyama XII-4-10-15 = IFO 30532); 22 August 1977 (T. Yokoyama XVE-5-5-3); 21 November 1977 (T. Yokoyama XVI-2-15-13); 19 February 1978 (T. Yokoyama XVII-2-15-11); 21 November 1978 (T. Yokoyama XXE-1-10-9 & XXE-3-15-7). Kuragaki, Nose-cho, Toyono-gun, Osaka Pref., 13 December 1976 (T. Yokoyama WIIE-1-10-6 = IFO 30533); 12 June 1978 (T. Yokoyama WVIIIE-1-5-5).

This fungus was originally isolated from soil in Moselle, France and described by Nicot and Durand (1969) as Arachniotus lectardii J. Nicot. Arx (1971) re-examined the type strain deposited in CBS by Nicot and concluded that this fungus is not congeneric with Arachniotus and should be accommodated in the new genus Eleutherascus as E. lectardii (Nicot) von Arx, the type of the genus. This fungus was also isolated in U.S.A. by Huang and Schmitt (1973) who obtained four isolates from two soil samples collected in Ohio.

The genus Arachniotus belongs to Eurotiales, while the genus Eleutherascus belongs to Pezizales and is considered to have a close affinity to the genus Ascodesmis in having gymnocarpous asci (Arx 1974; Huang 1975).

The fungus has been isolated from soils of four selected rice paddy

fields in Osaka Prefecture; 4 isolates in Ibaraki, 8 in Ikeda, 7 in Habikino and 2 in Nose, respectively. Of the total 21 isolates obtained, 13 were isolated by the 50% (v/v) ethanol treatment method and 8 were isolated by the dilution plate method. The numbers of isolates from soil depths of 0-10 cm, 10-20 cm, and 20-30 cm were 4, 10 and 7, respectively.

(T. Ito & T. Yokoyama)

86. Zopfiella lundqvistii Shearer & Crane (Figs. 3 & 4) Eurotiales
Trans. Brit. Mycol. Soc. 70: 456 (1978).

Colonies on MA grow moderately, floccose, gray to grayish brown, partially immersed; reverse black. Cleistothecia formed in abundance, superficial, solitary to gregarious, spherical, pale brown, covered with flexuous, pale brownish mycelium, 350-550 μm in diam; peridium membraneous, consisting of five to eight layers with angular cells, pale brown, 13-24 μm thick. Asci variable in shape, clavate to pyriform, 8-spored, evanescent, 65-80 x 20-27 μm. Ascospores biseriate, upper cells triangular, slightly concave at side, smooth, thick-walled, at first olive green, becoming dark brown, one end round, distal end with a prominent germ pore of 0.1 μm in diam, basal end with a lower cell, 32-35 x 22-26 μm; basal cells hyaline, cylindrical to clavate, thin-walled, 15-18 x 4-5 μm. Ascospores were also produced in abundance on YpSs agar and modified Leonian's agar.

Growth is nil at 37 C.

Hab. paddy field soil. Nakatsu-cho, Ibaraki, Osaka Pref., 17 October 1976 (T. Yokoyama ZII-3-15-24 = IFO 30650); 16 October 1977 (T. Yokoyama ZVI-2-10-18); 16 April 1978 (T. Yokoyama ZVII-3-5-27 = IFO 30651); 16 July 1978 (T. Yokoyama ZIX-4-15-20 = IFO 30652); 25 October 1978 (T. Yokoyama ZX-1-10-30). Shakudo, Habikino, Osaka Pref., 20 August 1976 (T. Yokoyama XI-1-15-11; XI-2-10-10); 20 February 1977 (T. Yokoyama XIII-3-10-16); 23 May 1977 (T. Yokoyama XIV-1-15-20); 22 August 1977 (T. Yokoyama XV-1-15-15); 21 November 1977 (T. Yokoyama XVI-1-15-19 = IFO 30648); 21 August 1978 (T. Yokoyama XIX-2-15-26 = IFO 30649). Kuragaki, Nose-cho, Toyono-gun, Osaka Pref., 1 June 1976 (T. Yokoyama W-1-15-10); 13 September 1976 (T. Yokoyama WI-1-5-13 & WI-4-15-15); 3 December 1976 (T. Yokoyama WII-1-15-33 = IFO 30643 & WII-2-10-17); 14 March 1977 (T. Yokoyama WIII-1-10-20, WIII-2-10-21, WIII-3-15-17, WIII-4-15-12 & WIII-5-10-20); 13 June 1977 (T. Yokoyama

WIV-1-10-13 & WIV-5-5-13); 12 September 1977 (T. Yokoyama WV-3-5-24); 13 March 1978 (T. Yokoyama WVII-1-5-12 = IFO 30644, WVII-2-10-28 = IFO 30645 & WVII-2-15-30 = IFO 30646); 3 September 1978 (T. Yokoyama WIX-3-15-19 = IFO 30647); 11 December 1978 (T. Yokoyama WX-3-10-15 & WX-3-15-10).

This fungus was described by Shearer and Crane (1978) from Illinois, U. S. A., based on a culture isolated from balsa wood blocks (Ochroma pyramidale (Cav.) Urb.), submerged in a pond. This species is distinguished from other known species of Zopfiella in the shape of the ascospores, which are distinctly triangular and thick-walled.

In Osaka Prefecture, this fungus has frequently been isolated from paddy field soils; 5 isolates in Ibaraki, 7 in Habikino, and 19 in Nose, all isolated by the dilution plate method. Of these 31 isolates, 5 isolates were found at soil depths of 0-10 cm, while 11 and 15 were found at depths of 10-20 cm and 20-30 cm.

(T. Ito & T. Yokoyama)

87. Flosculomyces floridaensis Sutton (Figs. 5 & 6) Hyphomycetes
Mycologia 70: 789 (1978).

Colonies on PSA grow moderately, finely floccose, dirty white at first, then grayish salmon to pale ochraceous, often olivaceous gray; reverse hay to dark vinaceous; medium colored by livid vinaceous to vinaceous red pigment. Colonies on MA grow moderately, almost the same as on PSA. Conidiophores macronematous, mononematous, arising singly, straight or flexuous, unbranched at cylindrical, thick-walled base, branched to one to two orders above, distal ends being the conidiogenous cells, lateral branches originating just below septa, often in whorls up to three, 3-8-septate, smooth, dark brown at the base, paler or subhyaline towards the apex, 40-50 (-100) x 2-3 μm. Conidiogenous cells holoblastic, determinate, apical or lateral on the conidiophores, 8-12 μm long, pale brown, rather thick-walled, 4-5 μm wide at the base; constricted to 2-2.5 μm wide in the middle; paler, thinner-walled, inflating to 4-5 μm wide at the apex; becoming cupulate after dispersing the conidia. Conidia smooth, cruciately septate, four-celled, rarely three-celled, brown but very strongly dark brown at the conidial base, 12-16 μm high, with a distinct abscission pore at the base of one of the conidial cells.

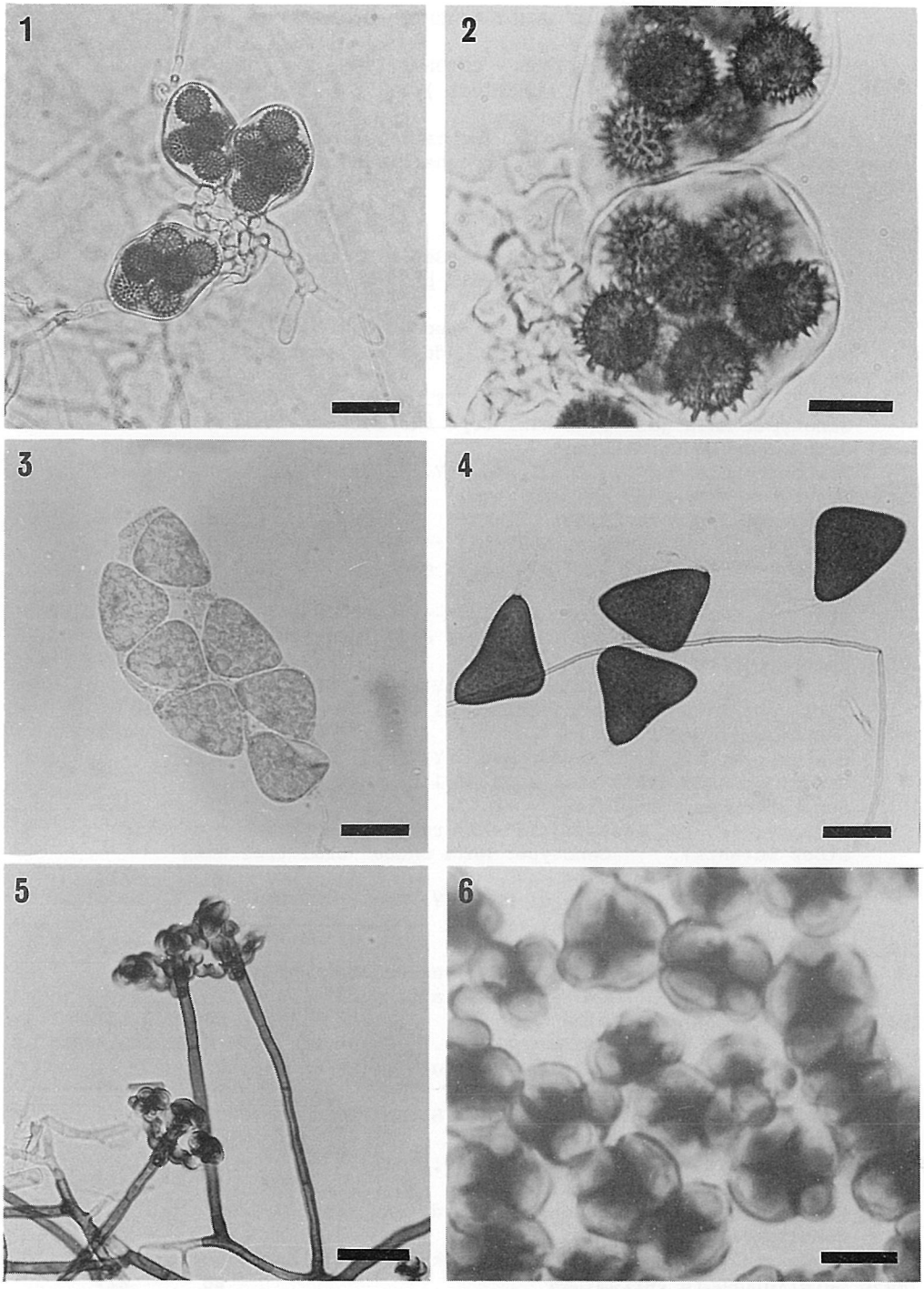
Growth is nil at 37 C.

Hab. on peduncle sheath of Pandanus boninensis Warb. Chichi-jima, Ogasawara-mura, Tokyo, 24 November 1972 (T. Yokoyama 4711-24-25 = IFO 30653 & 4711-24-45 = IFO 30654); on fallen leaf of Calophyllum inophyllum L. Taketomi-jima, Taketomi-cho, Yaeyama-gun, Okinawa Pref., 8 June 1973 (T. Yokoyama RI-69-2 = IFO 30655); on fallen leaf of Lithocarpus edulis Nakai. Tanzaki-bana, Oodomari, Sata-cho, Kimotsuki-gun, Kagoshima Pref., 11 November 1975 (T. Yokoyama SC'-5-10 = IFO 30656).

Based on a fungus on decayed leaves of Podocarpus sp. collected in Tampa, Florida, U.S.A., Sutton (1978) described this interesting species in his new genus Flosculomyces as monotype species. In Japan, on four occasions a fungus identical with F. floridaensis had been isolated before this publication; first on Pandanus peduncle sheath in the Bonin Islands, then on fallen leaves of Calophyllum inophyllum in Okinawa and also Lithocarpus edulis in Kagoshima. All of these locations lie between 28° N and 32° N and have a subtropical climate, as at the type locality of the species, Florida, which lies 27° N. This suggests that its distribution in the world may be wider than expected but must be confined to subtropical and border areas with the temperate zone. It is noted that Pandanus boninensis is endemic in the Bonin Islands, and Calophyllum inophyllum is endemic in the Bonin Islands and Ryukyu Islands, while Lithocarpus edulis is found in the Bonin Islands, Ryukyu Islands, Formosa, Tropical Asia, Polynesia and Madagascar.

Recently, this fungus has also been found on decaying needles of Pinus luchuensis, an endemic pine, in Haha-jima (Bonin Islands), Ogasawara-mura, Tokyo (August, 1982) and also in Ishigaki-shi, Okinawa Prefecture (October, 1985). (Personal communication from Dr. Seiji Tokumasu, Tsukuba University.)

(T. Yokoyama & T. Ito)



Figs. 1 & 2. Eleutherascus lectardii. 1. A cluster of gymnocarpic asci. 2. Asci and ascospores. 3 & 4. Zopfiella lundqvistii. 3. Immature ascus and ascospores. 4. Mature ascospores with a hyaline lower cell. 5 & 6. Flosculomyces floridaensis. 5. Conidial structures. 6. Conidia. Bars for figs. 1, 3-5. 20 μm; 2 & 6. 10 μm.

CATALOGUE OF NEWLY ACCEPTED STRAINS

NOVEMBER 1986 - OCTOBER 1988
(Numerical)

The cultures involved in the following catlogue can be distributed under the same condition as strains listed IFO LIST OF CULTURES 8TH EDITION.

IFO 10430 **Saccharomyces cerevisiae** 28 109
History: IFO (Y. Kaneko; NA74-3A; Genetic recombination).
Genotype: MATa leu2-3,112 his4-519 pho9-1 can1 rho^{+}

IFO 10444 **Saccharomyces cerevisiae** 28 109
History: IFO (Y. Kaneko; KYC 77; recombinant between genetic stock mutants). Genotype: MATa gal1 ade1 ade2 ura1 trp5

IFO 10445 **Saccharomyces cerevisiae** 28 112
History: Keio Univ., School Med. (Y. Nogi; 107-1D) -- H.C. Douglas.
Genotype: MATa gal3 ura1 his1 trp1 thr met

IFO 10446 **Saccharomyces cerevisiae** 28 109
History: Keio Univ., School Med. (Y. Nogi; N3-1A).
Genotype: MATα gal3 ade his1 trp1

IFO 10447 **Saccharomyces cerevisiae** 28 112
History: IFO (Y. Kaneko; N435-1A) -- Yeast Genetic Stock Center.
Genotype: MATa his7 lys7 met6 arg1 gal4

IFO 10448 **Saccharomyces cerevisiae** 28 109
History: IFO (Y. Kaneko; 3654-1D) -- Yeast Genetic Stock Center.
Genotype: MATα his4 trp1 ura4 ade6 gal7 leu2 lys2 thr4 tyr1 arg4

IFO 10449 **Saccharomyces cerevisiae** 28 109
History: Keio Univ., School Med. (Y. Nogi; N72-16D).
Genotype: MATa gal10 ade1 ade2 ura1 tyr1

IFO 10450 **Saccharomyces cerevisiae** 28 109
History: Keio Univ., School Med. (Y. Nogi; N3-2B).
Genotype: MATα gal10 ura1 ade2 his1 trp met thr

IFO 10453 **Saccharomyces cerevisiae** 28 112
History: IFO (Y. Kaneko; K155-2B; recombinant between genetic stock mutants). Genotype: MATa $GAL80^{S}$ ura1 trp1 his4

IFO 10454 **Saccharomyces cerevisiae** 28 112
History: Keio Univ., School Med. (Y. Nogi; 423-6A) -- H.C. Douglas.
Genotype: MATα $GAL80^{S}$ ura1 trp1

IFO 10455 **Saccharomyces cerevisiae** 28 112
History: IFO (Y. Kaneko; M60-5B; recombinant between genetic stock mutants). Genotype: MATα ade3 pho81-1 ura3

IFO 10456 **Saccharomyces cerevisiae** 28 112
History: OUT (S. Harashima; SH930). Genotype: MATa/MATα HMLα/HMLα HMRa/HMRa HO/HO ura3-52/ura3-52 leu2-3,112/leu2-3,112

IFO 13601 **Streptomyces inusitatus** 28 227
History: RTCI (T. Hasegawa; T-41575; soil).

IFO 13604 **Streptomyces inaequalis** 28 227
History: RTCI (T. Hasegawa; C-5780; soil).

IFO 14240 **Streptomyces pactum** 28 227
History: SS Pharmaceutical Co., Ltd. (K. Yano; S 12538; soil).

IFO 14495 **Catellatospora citrea** 28 227
History: Tokyo Res. Lab., Kyowa Hakko Co. (I. Kawamoto; 6183-E; soil).

IFO 14496 **Catellatospora ferruginea** 28 227
History: Tokyo Res. Lab., Kyowa Hakko Co. (I. Kawamoto; 6257-C; soil).

IFO 14550 **Catellatospora matsumotoense** 28 227
History: Tokyo Res. Lab., Kyowa Hakko Co. (K. Asano; 6393-C; soil).

IFO 14551 **Catellatospora ishikariense** 28 227
History: Tokyo Res. Lab., Kyowa Hakko Co. (K. Asano; 6432-C; soil).

IFO 14552 **Catellatospora tsunoense** 28 227

History: Tokyo Res. Lab., Kyowa Hakko Co. (K. Asano; 6420-P; soil).
IFO 14553 **Catellatospora citrea subsp. methionotrophica** 28 227
History: Tokyo Res. Lab., Kyowa Hakko Co. (K. Asano; 6257-B; soil).
IFO 14556 **Pilimelia terevasa** 28 228
History: ATCC 25603 -- W.K. Hanton, soil.
IFO 14566 **Thiobacillus delicatus** 30 260
History: IAM 12624 -- Fujimura & Kuraishi, THI 091 -- Mizoguchi, TuT-1.
IFO 14617 **Micromonospora violacea** 28 231
History: RIA 1643 (E.M. Singal; 6688-1; soil).
IFO 14618 **Micromonospora atratovinosa** 28 228
History: RIA 1644 (E.M. Singal; 32-7; soil).
IFO 14619 **Micromonospora pallidocoerulea** 28 231
History: RIA 1645 (E.M. Singal; 6734-2; soil).
IFO 14620 **Streptomyces hygroscopicus subsp. geldanus** 28 228
History: RTCI -- The Upjohn Co., UC-5208 (A. Dietz).
IFO 14622 **Excellospora viridinigra** 55 8
History: IAM (A. Shimazu; AIAM 5058) -- ATCC 33518.
IFO 14623 **Actinomadura madurae** 25 227
History: IAM (A. Shimazu; AIAM 5059) -- ATCC 19425.
IFO 14624 **Microbispora aerata** 37 231
History: IAM (A. Shimazu; AIAM 5060) -- ATCC 15448.
IFO 14625 **Nocardiopsis dasseonvillei subsp. dassonvillei** 25 227
History: IAM (A. Shimazu; AIAM 5061) -- ATCC 21944.
IFO 14626 **Nocardiopsis dasseonvillei subsp. dassonvillei** 25 227
History: IAM (A. Shimazu; AIAM 5062) -- ATCC 23218.
IFO 14642 **Streptomyces aculeolatus** 28 227
History: JCM 6055 -- Pharm. Res. Lab., Meiji Seika Kaisha, Ltd. (T. Shomura; SF 2415).
IFO 14651 **Saccharomonospora azurea** 37 229
History: Sichuan Ind. Inst., 86128 (R. Hu; NA-128; soil).
IFO 14656 **Micromonospora narashino** 28 227
History: RTCI -- KCC A-0129 -- IFM 1110 (T. Arai; 76-N3-6; soil).
IFO 14657 **Streptoverticillium album** 37 231
History: RTCI -- KCC S-0542 -- IPV 1993 -- NRRL 2401 -- Chas. Pfizer & Co., BA-3972.
IFO 14658 **Actinomadura rosea** 28 231
History: Sichuan Inst. Antibiot. (R. Hu; 805168; soil).
IFO 14660 **Streptomyces peuceticus subsp. caesius** 28 231
History: Dept. Genet. Cell. Biol., Univ. Malaya (C.C. Ho; H 3502) -- IMRU 3920 (R.E. Gordon) -- Farmitalia (B. Camerino; FI106).
IFO 14661 **Streptomyces peuceticus subsp. caesius** 28 231
History: Dept. Genet. Cell. Biol., Univ. Malaya (C.C. Ho; H 6272).
IFO 14662 **Streptomyces peuceticus subsp. caesius** 28 231
History: Dept. Genet. Cell. Biol., Univ. Malaya (C.C. Ho; H 6125).
IFO 14663 **Streptomyces peuceticus subsp. caesius** 28 231
History: Dept. Genet. Cell. Biol., Univ. Malaya (C.C. Ho; H 6271).
IFO 14664 **Streptomyces tolypophorus** 28 228
History: RTCI (M. Shibata; B-2847; soil).
IFO 14665 **Vibrio algoinfesta** 28 204
History: Kyushu Univ. (S. Ishio; S1; sediment of sea bottom).
IFO 14666 **Vibrio algoinfesta** 28 204
History: Kyushu Univ. (S. Ishio; Q1; sediment of sea bottom).
IFO 14667 **Actinomadura chengduensis** 28 227
History: Sichuan Inst. Antibiot. (R. Hu; SIA 77-5334; soil).
IFO 14668 **Streptomycoides glaucoflavus** 28 227
History: AS (G. Zhang; 80-56; soil).
IFO 14669 **Microstreptospora cinerea** 28 227
History: AS (Y. Zhang; 80-133; sewage ditch).
IFO 14671 **Pseudomonas putida** 30 201

History: IAM 1049 -- ATU (K. Arima; A-10-3).
IFO 14678 **Actinomadura citrea** 28 227
History: JCM 3295 -- KCC A-0295 -- Meiji Seika Kaisha, Ltd., MS 2135 -- ATCC 27887 -- INA 1849.
IFO 14679 **Actinomadura coerulea** 28 227
History: JCM 3320 -- KCC A-0320 -- INA 765.
IFO 14680 **Actinomadura fastidiosa** 37 227
History: JCM 3321 -- KCC A-0321 -- VKM (N.S. Agre; Ac-804; mud, hot spring.
IFO 14681 **Actinomadura helvata** 37 227
History: JCM 3143 -- KCC A-0143 -- RIFY (H. Nonomura; A-105; soil).
IFO 14682 **Actinomadura livida** 37 231
History: JCM 3387 -- DSM 43677 -- N.S. Agre.
IFO 14683 **Actinomadura malachitica** 28 227
History: JCM 3297 -- KCC A-0297 -- Meiji Seika Kaisha Ltd., MS 2138 -- ATCC 27888.
IFO 14684 **Actinomadura pusilla** 37 227
History: JCM 3144 -- KCC A-0144 -- RIFY (H. Nonomura; A-118).
IFO 14685 **Actinomadura roseola** 28 227
History: JCM 3323 -- KCC A-0323 -- N.S. Agre -- INA 1671.
IFO 14686 **Actinomadura rubra** 37 227
History: JCM 3389 -- DSM 43768 -- VKM (N.S. Agre; Ac-615).
IFO 14687 **Actinomadura salmonea** 28 227
History: JCM 3324 -- KCC A-0324 -- N.S. Agre -- INA 2488.
IFO 14688 **Actinomadura vinacea** 37 227
History: JCM 3325 -- KCC A-0325 -- N.S. Agre -- INA 1682.
IFO 14689 **Actinomadura yumaensis** 37 231
History: JCM 3369 -- NRRL 12515.
IFO 14690 **Streptomyces albosporeus subsp. albosporeus** 28 231
History: HUT 6130 -- IPV 880.
IFO 14691 **Streptomyces gibsonii** 28 227
History: HUT 6151 -- IPV 890.
IFO 14692 **Streptomyces rubrocyanodiastaticus subsp. piger** 28 231
History: HUT 6117 -- IPV 307.
IFO 14693 **Streptoverticillium baldaccii** 28 231
History: HUT 6222 -- KCC S-0272 -- IPV 174.
IFO 14694 **Streptoverticillium rubrochlorinum** 28 227
History: HUT 6225 -- KCC S-0281 -- V.A. Tyganov -- LIA 0084 (Y.E. Konev; 51-10).
IFO 14695 **Actinomadura atramentaria** 28 228
History: Meiji Seika Kaisha, Ltd. (S. Miyadoh; SF2197; soil).
IFO 14709 **Lactobacillus casei subsp. casei** 30 205
History: Nat. Food Res. Inst. (K. Kiuchi; KTB 2-6; corn silage).
IFO 14710 **Lactobacillus casei subsp. rhamnosus** 30 205
History: Nat. Food Res. Inst. (K. Kiuchi; KTD 4-1; Italian rye glass silage).
IFO 14711 **Lactobacillus plantarum** 30 205
History: Nat. Food Res. Inst. (K. Kiuchi; KTB 2-13; corn silage).
IFO 14712 **Lactobacillus plantarum** 30 205
History: Nat. Food Res. Inst. (K. Kiuchi; KTC 4-3; Italian rye glass silage).
IFO 14713 **Lactobacillus plantarum** 30 205
History: Nat. Food Res. Inst. (K. Kiuchi; KTE 2-9; Italian rye glass silage).
IFO 14714 **Streptococcus faecalis** 30 205
History: Nat. Food Res. Inst. (K. Kiuchi; KTG 4-4; Italian rye glass silage).
IFO 14715 **Halobacterium cutirubrum** 37 255
History: DSM 669 -- D. Keradjopoulos -- NRC 34001 -- A.G. Lochhead; 63-R2.
IFO 14716 **Halobacterium halobium** 37 255

History: DSM 670 -- D. Keradjopoulos -- W. Stoeckenius -- NRC.

IFO 14717 **Halobacterium saccharovorum** 37 255
History: DSM 1137 -- L.I. Hochstein, M6.

IFO 14718 **Halobacterium salinarium** 37 255
History: DSM 668 -- D. Keradjopoulos -- ATCC 19700 -- J. Stevenson -- H. Larsen, strain 1, salted fish.

IFO 14719 **Halococcus morrhuae** 37 255
History: DSM 1307 -- CCM 537 -- C.B. van Niel, strain L.D.3.1. -- B. Elazari-Volcani.

IFO 14720 **Natronobacterium pharaonis** 37 256
History: DSM 2160 -- H.G. Trüper, strain Gabara.

IFO 14727 **Streptomyces baarnensis** 28 231
History: CBS 306.55.

IFO 14739 **Halobacterium mediterranei** 30 257
History: ATCC 33500 -- Universidad de Alicante, Spain (F. Rodriquez-Valera; R-4; salt ponds).

IFO 14740 **Halobacterium sodomense** 37 258
History: ATCC 33755 -- Hebrew University of Jerusalem, Israel (A. Oren; RD-26; Dead Sea).

IFO 14741 **Halobacterium vallismortis** 37 259
History: ATCC 29715 -- Instituto Jaime Ferran de Microbiologia, Spain (C. Gonzalez; J.F. 54; salt pools).

IFO 14742 **Halobacterium volcanii** 30 257
History: ATCC 29605 -- H. Larsen, DS2, shore mud.

IFO 14743 **Micromonospora megalomicea subsp. megalomicea** 28 227
History: Schering Corp., W-847.

IFO 14744 **Micromonospora megalomicea subsp. nigra** 28 228
History: Schering Corp., W-847-21.

IFO 14745 **Nocardiopsis africana** 28 227
History: JCM 6240 -- DSM 43748 -- INA 1839.

IFO 14747 **Lactobacillus fructivorans** 30 209
History: IFO (K. Imai; OR-8; spoiled salad dressing).

IFO 14748 **Streptomyces macrosporus** 37 227
History: IACR, Rothamsted Experimental Stn. (J. Lacey; A1201; soil) -- Lomollov Univ., N.S. Agre.

IFO 14749 **Streptomyces megasporus** 37 227
History: IACR, Rothamsted Experimental Stn. (J. Lacey; A1202; soil).

IFO 14750 **Streptomyces thermolineatus** 37 227
History: IACR, Rothamsted Experimental Stn. (J. Lacey; A1484; sewage/wood chip compost).

IFO 14755 **Pimelobacter jensenii** 30 201
History: JCM 1364 (K. Suzuki; CNF 091) -- IAM 12581 -- NCIB 9770 -- C.W. Evans -- H.L. Jensen.

IFO 14756 **Mycobacterium diernhoferi** 30 201
History: Osaka City Univ. (I. Yano; MD-1) -- Nat. Chubu Hosp. (M. Tsukamura; 41005).

IFO 14757 **Caseobacter polymorphus** 30 201
History: DSM 20536 -- Crombach, Meshanger cheese.

IFO 14758 **Xanthobacter autotrophicus** 30 201
History: DSM 1618 -- J. Wiegel, ditch mud.

IFO 14759 **Xanthobacter flavus** 30 201
History: DSM 338 -- J.R. Postgate -- Kalininska, strain 301, turf podzol soil.

IFO 14760 **Rarobacter faecitabidus** 30 261
History: JCM 6097 --S. Sato, YLM-1, activated sludge.

IFO 14761 **Microtetraspora glauca** 28 228
History: JCM 3300 -- ATCC 23057 -- J.E. Thiemann T/158 soil.

IFO 14762 **Brachybacterium faecium** 30 201
History: NCIB 9860 -- H.E. Schefferle, strain 6-10, Poultry deep litter.

IFO 14763 **Exiguobacterium aurantiacum** 28 262

History: NCIB 11798 -- B.M. Lund, BL77/1, effluent treatment plant potato processing factory.

IFO 14764 **Arthrobacter sp.** 30 201
History: OUT (Y. Yamada; Y-11; soil).

IFO 14766 **Halomonas subglaciescola** 20 263
History: Univ. Tassmania (P.D, Franzmann, ACAM 12, organic lake).

IFO 14767 **Kitasatosporia cochleata** 28 227
History: IFO (T. Hasegawa; M-3; soil).

IFO 14768 **Kitasatosporia cochleata** 28 227
History: IFO (T. Hasegawa; M-5; soil).

IFO 14769 **Kitasatosporia cochleata** 28 227
History: IFO (T. Hasegawa; M-13; soil).

IFO 14771 **Actinomadura viridis** 28 245
History: Pharmaceutic. Res. Labs., Meiji Seika Kaisha, Ltd. (S. Miyadoh; SF2461).

IFO 14777 **Rubrobacter radiotolerans** 37 264
History: JCM 2153 -- IAM 12072 -- ATU (T. Yoshinaka; P-1; soil of hot spring).

IFO 14778 **Rhizobium leguminosarum biovar. viceae** 28 218
History: Dept. Biol., Toyama Univ. (H. Oyaizu) -- IAM 12609 -- ATCC 10004 -- L.W. Erdman, 3H0q18.

IFO 14779 **Rhizobium loti** 28 218
History: Dept. Biol., Toyama Univ. (H. Oyaizu) -- ATCC 33669 -- B.D.W. Jarvis, NZP2213.

IFO 14780 **Rhizobium fredii** 28 218
History: Dept. Biol., Toyama Univ. (H. Oyaizu) -- ATCC 35423 -- H.H. Keyser, USDA 205.

IFO 14781 **Bradyrhizobium sp.** 28 218
History: Dept. Biol., Toyama Univ. (H. Oyaizu) -- IAM 12610 -- ATCC 10319 -- L.W. Erdman, 3C231.

IFO 14782 **Rhizobium meliloti** 28 218
History: Dept. Biol., Toyama Univ. (H. Oyaizu) -- IAM 12611 -- ATCC 9930 -- N.R. Smith, 3D0a2.

IFO 14783 **Bradyrhizobium japonicum** 28 218
History: Dept. Biol., Toyama Univ. (H. Oyaizu) -- IAM 12608 -- ATCC 10324 -- L.W. Erdman, 3I1b6.

IFO 14784 **Rhizobium leguminosarum biovar. trifolii** 28 218
History: Dept. Biol., Toyama Univ. (H. Oyaizu) -- IAM 12613 -- ATCC 14480 -- U.M. Means, 3D1K22a.

IFO 14785 **Rhizobium leguminosarum biovar. phaseoli** 28 218
History: Dept. Biol., Toyama Univ. (H. Oyaizu) -- IAM 12612 -- ATCC 14482 -- U.M. Means, 3I6c15.

IFO 14788 **Parvopolyspora pallida** 28 227
History: Meiji Seika Kaisha, Ltd. (S. Miyadoh).

IFO 14789 **Kitasatosporia mediocidica** 28 227
History: NRRL B-16109 -- M.P. Lechevalier, LL-80 -- Waksman Inst., L. McDaniels.

IFO 14790 **Kitasatosporia mediocidica** 28 227
History: NRRL B-16110 -- M.P. Lechevalier, LL-81 -- Waksman Inst., L. McDaniels.

IFO 32065 **Aureobasidium microstictum** 24 1
History: Chutan Branch, Kyoto Pref. Inst. Agr. (M. Yoshikawa; CB-8533; leaf of Hemerocallis fulva var. kwanso).

IFO 32066 **Aureobasidium microstictum** 24 1
History: Chutan Branch, Kyoto Pref. Inst. Agr. (M. Yoshikawa; CB-8534; leaf of Hemerocallis fulva var. disticha).

IFO 32067 **Aureobasidium microstictum** 24 1
History: Chutan Branch, Kyoto Pref. Inst. Agr. (M. Yoshikawa; CB-8535; leaf of Hemerocallis fulva var. kwanso).

IFO 32068 **Aureobasidium microstictum** 24 1
History: Chutan Branch, Kyoto Pref. Inst. Agr. (M. Yoshikawa; CB-8554;

leaf of Hemerocallis fulva var. disticha).

IFO 32069 **Aureobasidium microstictum** 24 1
History: Chutan Branch, Kyoto Pref. Inst. Agr. (M. Yoshikawa; CB-8555; leaf of Hemerocallis fulva var. disticha).

IFO 32070 **Aureobasidium microstictum** 24 1
History: Chutan Branch, Kyoto Pref. Inst. Agr. (M. Yoshikawa; CB-8557; leaf of Hemerocallis fulva var. kwanso).

IFO 32071 **Aseroe arachnoidea** 24 7
History: IFO (T. Ito; T. Ito S62-1; on heaped chaff).

IFO 32072 **Pythium irregulare** 24 8
History: Coll. Agr., Univ. Osaka Pref., UOP 359 -- Kyoto Pref. Res. Inst. Agr. (T. Fukunishi; 861; root of Tulipa gesneriana).

IFO 32073 **Pythium irregulare** 24 8
History: Coll. Agr., Univ. Osaka Pref., UOP 362 -- Fac. Agr., Saga Univ. (K. Tanaka; 1-2; stem of Allium cepa).

IFO 32074 **Aspergillus sojae** 24 1
History: IAM 2703 -- ATU O-18-7 (T. Takahashi; T-23; tamari soya).

IFO 32077 **Hormographis ramirezii** 24 8
History: Dept. Biol. & Microbiol., Fac. Med., Univ. Barcelona (J. Guarro; FMR 186; soil).

IFO 32078 **Monascella botryosa** 24 8
History: Dept. Biol. & Microbiol., Fac. Med., Univ. Barcelona (J. Guarro; FMR 724; soil).

IFO 32079 **Uncinocarpus reesii** 24 8
History: Dept. Biol. & Microbiol., Fac. Med., Univ. Barcelona (J. Guarro; FMR 1513; soil).

IFO 32080 **Neosartorya fennelliae** 28 1
History: M. Takada, NHL 2951, marine sludge.

IFO 32081 **Neosartorya fennelliae** 28 1
History: M. Takada, NHL 2952, marine sludge.

IFO 32084 **Sphaeropsis visci** 20 1
History: Fac. Agr., Hirosaki Univ. (Y. Harada; 1053; Viscum album var. coloratum).

IFO 32085 **Clasterosporium degenerans** 20 1
History: Fac. Agr., Hirosaki Univ. (Y. Harada; 1155; leaf of Prunus mume).

IFO 32086 **Halocyphina villosa** 24 13
History: IFO (A. Nakagiri; AN-961) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-961; dead prop root of Rhizophora stylosa).

IFO 32087 **Halocyphina villosa** 24 13
History: IFO (A. Nakagiri; AN-965) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-965; dead prop root of Rhizophora stylosa).

IFO 32088 **Nia vibrissa** 24 13
History: IFO (A. Nakagiri; AN-713) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-713; sea foam).

IFO 32089 **Nia vibrissa** 24 13
History: IFO (A. Nakagiri; AN-826) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-826; sea foam).

IFO 32090 **Nia vibrissa** 24 13
History: IFO (A. Nakagiri; AN-1023) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-1023; wood in sands of seashore).

IFO 32091 **Cercophora coprophila** 24 8
History: IFO (T. Ito; T. Ito S61E-3-2; burned soil).

IFO 32092 **Gilmaniella subornata** 24 8
History: IFO (T. Ito; T. Ito S61E-17-2; burned soil).

IFO 32093 **Gilmaniella subornata** 24 8
History: IFO (T. Ito; T. Ito S6170-9-1; burned soil).

IFO 32094 **Wardomyces humicola** 24 8
History: IFO (T. Ito; T. Ito S61-1-1; burned soil).

IFO 32095 **Arenariomyces trifurcatus** 24 13
History: IFO (A. Nakagiri; AN-391) -- Inst. Biol. Sci., Univ.

Tsukuba (A. Nakagiri; AN-391; sea foam).

IFO 32096 **Arenariomyces trifurcatus** 24 13
History: IFO (A. Nakagiri; AN-485) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-485; sea foam).

IFO 32097 **Carbosphaerella leptosphaerioides** 24 13
History: IFO (A. Nakagiri; AN-625) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-625; sea foam).

IFO 32098 **Ceriosporopsis halima** 24 13
History: IFO (A. Nakagiri; AN-548) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-548; immersed wood).

IFO 32099 **Ceriosporopsis halima** 24 13
History: IFO (A. Nakagiri; AN-528) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-528; drift bamboo wood).

IFO 32100 **Corollospora angusta** 24 13
History: IFO (A. Nakagiri; AN-759) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-759; sea foam).

IFO 32101 **Corollospora angusta** 24 13
History: IFO (A. Nakagiri; AN-421) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-421; sea foam).

IFO 32102 **Corollospora angusta** 24 13
History: IFO (A. Nakagiri; AN-422) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-422; sea foam).

IFO 32103 **Corollospora colossa** 24 13
History: IFO (A. Nakagiri; AN-569) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-569; sea foam).

IFO 32104 **Corollospora colossa** 24 13
History: IFO (A. Nakagiri; AN-780) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-780; sea foam).

IFO 32105 **Corollospora colossa** 24 13
History: IFO (A. Nakagiri; AN-1012) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-1012; sea foam).

IFO 32106 **Corollospora filiformis** 24 13
History: IFO (A. Nakagiri; AN-802) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-802; sea foam).

IFO 32107 **Corollospora fusca** 24 13
History: IFO (A. Nakagiri; AN-724) -- TKB-C-1456 (A. Nakagiri; AN-724; sea foam).

IFO 32108 **Corollospora fusca** 24 13
History: IFO (A. Nakagiri; AN-691) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-691; sea foam).

IFO 32110 **Corollospora gracilis** 24 13
History: IFO (A. Nakagiri; AN-515) -- TKB-C-1457 (A. Nakagiri; AN-515; sea foam).

IFO 32111 **Corollospora gracilis** 24 13
History: IFO (A. Nakagiri; AN-470) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-470; sea foam).

IFO 32112 **Corollospora pseudopulchella** 24 13
History: IFO (A. Nakagiri; AN-844) -- TKB-C-1458 (A. Nakagiri; AN-844; beach sand).

IFO 32113 **Corollospora pseudopulchella** 24 13
History: IFO (A. Nakagiri; AN-554) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-554; sea foam).

IFO 32114 **Corollospora quinqueseptata** 24 13
History: IFO (A. Nakagiri; AN-711) -- TKB-C-1459 (A. Nakagiri; AN-711; sea foam).

IFO 32115 **Corollospora quinqueseptata** 24 13
History: IFO (A. Nakagiri; AN-753) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-753; dead thallus of Sargassum sagamianum).

IFO 32116 **Corollospora quinqueseptata** 24 13
History: IFO (A. Nakagiri; AN-467) -- Inst. Biol. Sci., Univ.

Tsukuba (A. Nakagiri; AN-467; sea foam).

IFO 32117 **Corollospora maritima** 24 13
History: IFO (A. Nakagiri; AN-381) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-381; sea foam).

IFO 32118 **Corollospora maritima** 24 13
History: IFO (A. Nakagiri; AN-815) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-815; sea foam).

IFO 32119 **Corollospora intermedia** 24 13
History: IFO (A. Nakagiri; AN-851) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-851; sea foam).

IFO 32120 **Corollospora intermedia** 24 13
History: IFO (A. Nakagiri; AN-474) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-474; sea foam).

IFO 32121 **Corollospora lacera** 24 13
History: IFO (A. Nakagiri; AN-686) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-686; sea foam).

IFO 32122 **Corollospora lacera** 24 13
History: IFO (A. Nakagiri; AN-722) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-722; sea foam).

IFO 32123 **Corollospora pulchella** 24 13
History: IFO (A. Nakagiri; AN-794) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-794; sea foam).

IFO 32124 **Corollospora pulchella** 24 13
History: IFO (A. Nakagiri; AN-870) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-870; beach sand around a buried drift wood).

IFO 32127 **Halosphaeriopsis mediosetigera** 24 13
History: IFO (A. Nakagiri; AN-821) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-821; sea foam).

IFO 32128 **Halosphaeriopsis mediosetigera** 24 13
History: IFO (A. Nakagiri; AN-778) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-778; sea foam).

IFO 32129 **Lignincola laevis** 24 13
History: IFO (A. Nakagiri; AN-593) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-593; submerged wood).

IFO 32130 **Lignincola laevis** 24 13
History: IFO (A. Nakagiri; AN-597) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-597; submerged wood).

IFO 32131 **Lindra thalassiae** 24 13
History: IFO (A. Nakagiri; AN-646) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-646; sea foam).

IFO 32132 **Lindra thalassiae** 24 13
History: IFO (A. Nakagiri; AN-678) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-678; sea foam).

IFO 32133 **Lulworthia crassa** 24 13
History: IFO (A. Nakagiri; AN-744) -- TKB-C-1382 (A. Nakagiri; AN-744; sea foam).

IFO 32134 **Lulworthia crassa** 24 13
History: IFO (A. Nakagiri; AN-790) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-790; sea foam).

IFO 32135 **Lulworthia lignoarenaria** 24 13
History: IFO (A. Nakagiri; AN-669) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-669; sea foam).

IFO 32136 **Lulworthia lignoarenaria** 24 13
History: IFO (A. Nakagiri; AN-745) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-745; sea foam).

IFO 32137 **Lulworthia uniseptata** 24 13
History: IFO (A. Nakagiri; AN-900) -- TKB-C-1383 (A. Nakagiri; AN-900; submerged wood).

IFO 32138 **Lulworthia uniseptata** 24 13
History: IFO (A. Nakagiri; AN-903) -- Inst. Biol. Sci., Univ.

Tsukuba (A. Nakagiri; AN-903; submerged wood).

IFO 32139 **Dendryphiella salina** 24 13
History: IFO (A. Nakagiri; AN-537) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-537; sea foam).

IFO 32140 **Dendryphiella arenaria** 24 13
History: IFO (A. Nakagiri; AN-341) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-341; drift wood).

IFO 32141 **Asteromyces cruciatus** 24 13
History: IFO (A. Nakagiri; AN-636) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-636; dead thallus of Ecklonia cava).

IFO 32142 **Asteromyces cruciatus** 24 13
History: IFO (A. Nakagiri; AN-651) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-651; sea foam).

IFO 32143 **Pleospora gaudefroyi** 24 13
History: IFO (A. Nakagiri; AN-940) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-940; dead culm of Salicornia herbacea).

IFO 32144 **Pleospora gaudefroyi** 24 13
History: IFO (A. Nakagiri; AN-937) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-937; dead culm of Salicornia herbacea).

IFO 32145 **Torpedospora radiata** 24 13
History: IFO (A. Nakagiri; AN-864) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-864; sea foam).

IFO 32146 **Torpedospora radiata** 24 13
History: IFO (A. Nakagiri; AN-873) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-873; dead culm of grass).

IFO 32147 **Halosphaeria appendiculata** 24 13
History: IFO (A. Nakagiri; AN-688) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-688; submerged balsa wood).

IFO 32148 **Halosphaeria appendiculata** 20 13
History: IFO (A. Nakagiri; AN-603) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-603; submerged balsa wood).

IFO 32149 **Kohlmeyeriella tubulata** 20 13
History: IFO (A. Nakagiri; AN-698) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-698; sea foam).

IFO 32150 **Kohlmeyeriella tubulata** 20 13
History: IFO (A. Nakagiri; AN-885) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-885; sea foam).

IFO 32151 **Marinospora calyptrata** 24 13
History: IFO (A. Nakagiri; AN-742) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-742; submerged wood).

IFO 32152 **Marinospora longissima** 24 13
History: IFO (A. Nakagiri; AN-729) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-729; submerged wood).

IFO 32153 **Marinospora longissima** 24 13
History: IFO (A. Nakagiri; AN-770) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-770; sea foam).

IFO 32154 **Cirrenalia macrocephara** 24 13
History: IFO (A. Nakagiri; AN-530) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-530; drift wood).

IFO 32155 **Humicola alopallonella** 24 13
History: IFO (A. Nakagiri; AN-913) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-913; submerged drift wood).

IFO 32156 **Humicola alopallonella** 24 13
History: IFO (A. Nakagiri; AN-954) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-954; drift wood).

IFO 32157 **Orbimyces spectabilis** 24 13
History: IFO (A. Nakagiri; AN-582) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-582; sea foam).

IFO 32158 **Orbimyces spectabilis** 24 13
History: IFO (A. Nakagiri; AN-952) -- Inst. Biol. Sci., Univ.

Tsukuba (A. Nakagiri; AN-952; drift wood).

IFO 32159 **Sigmoidea marina** 24 13
History: IFO (A. Nakagiri; AN-708) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-708; sea foam).

IFO 32160 **Sigmoidea marina** 24 13
History: IFO (A. Nakagiri; AN-931) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-931; sea foam).

IFO 32161 **Trichocladium achrasporum** 24 13
History: IFO (A. Nakagiri; AN-549) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-549; submerged wood).

IFO 32162 **Trichocladium achrasporum** 24 13
History: IFO (A. Nakagiri; AN-631) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-631; drift wood).

IFO 32163 **Varicosporina ramulosa** 24 13
History: IFO (A. Nakagiri; AN-808) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-808; sea foam).

IFO 32164 **Zalerion maritimum** 24 13
History: IFO (A. Nakagiri; AN-674) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-674; submerged beech wood).

IFO 32166 **Pythium torulosum** 24 1
History: College of Agric., Univ. Osaka Pref. (T. Ichitani; UOP 365; soil of golfgreen).

IFO 32167 **Pythium torulosum** 24 1
History: College of Agric., Univ. Osaka Pref. (T. Ichitani; UOP 366; stem of Zoysia matrella).

IFO 32168 **Pythium torulosum** 24 1
History: College of Agric., Univ. Osaka Pref. (T. Ichitani; UOP 367; stem of Zoysia matrella).

IFO 32169 **Pythium vanterpoolii** 24 1
History: College of Agric., Univ. Osaka Pref. (T. Ichitani; UOP 368; soil of golfgreen).

IFO 32170 **Pythium vanterpoolii** 24 1
History: College of Agric., Univ. Osaka Pref. (T. Ichitani; UOP 369; basal segment of newly developing leaf of Zoysia matrella).

IFO 32171 **Pythium vanterpoolii** 24 1
History: College of Agric., Univ. Osaka Pref. (T. Ichitani; UOP 370; stem of Zoysia matrella).

IFO 50154 **FBHE** 37 535
History: RTCI (M. Sakaguchi) -- ATCC (CCL 1395).

ABSTRACTS 1987 - 1988

Structural characteristics of *PHO8* gene encoding repressible alkaline phosphatase in *Saccharomyces cerevisiae*

Y. Kaneko, N. Hayashi*, A. Toh-e**, I. Banno and Y. Oshima*

Gene 58: 137-148 (1987)

The nucleotide sequence of a 3694-bp DNA fragment bearing the *PHO8* gene encoding nonspecific repressible alkaline phosphatase (rALPase; EC3.1.3.1) of *Saccharomyces cerevisiae* was determined. The sequence contains a 1698 bp open reading frame (ORF), and the major *PHO8* transcription start point at 32 bp upstream from the ATG codon; several minor transcription start points are located between the major start point and ATG. The major start point is most responsive to the phosphate signals. The amino acid (aa) sequence deduced from the ORF contains several homologous regions in common with alkaline phosphatases of *Escherichia coli* and human placenta. A *PHO8* DNA fragment previously isolated [Kaneko et al., Mol. Cell. Biol. 5 (1985) 248-252] was found to be truncated for the region encoding the 22 aa residues at the C terminus of the enzyme, which were replaced with 17 aa encoded by a pBR322 DNA. The modified gene could produce significant rALPase activity without the function of proteinase A which is required for the maturation of rALPase from its precursor.

* Department of Fermentation Technology, Faculty of Engineering, Osaka University.

** Department of Fermentation Technology, Faculty of Engineering, Hiroshima University.

Taxonomic studies of the genus *Corollospora* (Halosphaeriaceae, Ascomycotina) with descriptions of seven new species

A. Nakagiri* and R. Tokura**

Trans. mycol. Soc. Japan 28: 413-436 (1987)

Seven new species of the genus *Corollospora* (Halosphaeriaceae, Ascomycotina) isolated from the Japanese coast are described: *C. angusta*, sp. nov., *C. colossa*, sp. nov., *C. filiformis*, sp. nov., *C. fusca*, sp. nov., *C. gracilis*, sp. nov., *C. pseudopulchella*, sp. nov., *C. quinqueseptata*, sp. nov.

* Institute of Biological Sciences, University of Tsukuba; present address, Institute for Fermentation, Osaka
** Laboratory of Biology, Kyoto University of Education

A new pyrrole-amidine antibiotic TAN-868 A

M. Takizawa*, S. Tsubotani*, S. Tanida*, S. Harada* and Toru Hasegawa

J. Antibiot. 40: 1220-1230 (1987)

A new pyrrol-amidine antibiotic TAN-868 A was isolated from the culture broth of *Streptomyces idiomorphus* sp. nov. Its chemical structure was determined by spectroscopic analyses and degradation studies to be 4-[(2S,4*R*)-4-hydroxy-5-iminoprolyl]amino-*N*-(2-amidinoethenyl)-2-pyrrolecarboxamide. The antibiotic is active against bacteria, fungi and a protozoan, and has cytotoxic activity against murine tumor cells. DNA thermal denaturation studies suggest that TAN-868 A preferentially interacts with AT rich regions of double-stranded DNA.

* Applied Microbiology Laboratories, Central Research Division, Takeda Chemical Industries Ltd.

***Prototheca*, isolated from the sewage treatment plant**

K. Tubaki*, T. Hosoya*, A. Nakagiri** and Y. Tokiwa***

J. Antibact. Antifung. Agents 15: 487-490 (1987)

A microorganism in the sewage treatment plant of the pickles industry was isolated and identified to be a member of the genus *Prototheca*. The genus is composed of microscopic achlorophyllous organisms with a life cycle similar to that of *Chlorella*, a green algae, and has been assigned to be a nonpigmented organism related by loss of chlorophyll to the Chlorellaceae. The present isolate fitted in all respects with the description of *Prothotheca zopfii* Krüger.

* Institute of Biological Sciences, University of Tsukuba
** Institute of Biological Sciences, University of Tsukuba; present address, Institute for Fermentation, Osaka
*** Fermentation Research Institute, Ministry of International Trade & Industry

Hasegawaea gen. nov., an ascosporogenous yeast genus for the organisms whose asexual reproduction is by fission and whose ascospores have smooth surfaces without papillae and which are characterized by the absence of coenzyme Q and by the presence of linoleic acid in cellular fatty acid composition

Y. Yamada* and I. Banno

J. Gen. Appl. Microbiol. 33: 295-298 (1987)

New genus *Hasegawaea* is proposed for the fission yeast characterized by ascospores of smooth surfaces without papillae, absence of coenzyme Q and presence of linoleic fatty acid. The genus includes *H. japonicus* var. *japonicus* comb. nov. and *H. japonicus* var. *versatilis* comb. nov..

* Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Shizuoka university

An electrophoretic comparison of enzymes in strains of species in the fission yeast genera _Schizosaccharomyces_, _Octosporomyces_, and _Hasegawaea_

Y. Yamada*, K. Aizawa*, A. Matsumoto*, Y. Nakagawa*, and I. Banno

J. Gen. Appl. Microbiol. 33: 363-369 (1987)

Taxonomic study below the generic or at the specific level was made of the electrophoretic patterns of five enzymes in fourteen strains of *Schizosaccharomyces*, *Octosporomyces*, and *Hasegawaea* species. The five enzymes were glucose-6-phasphate dehydrogenase, 6-phosphogluconate dehydogenase, hexokinase, phosphoglucomutase, and fumarase. All of the six strains of *S. pombe* examined were linked to each other with a similarity value of 40% or more. The type strain of *S. malidevorans* was closely related to that of *S. pombe* with a similarity value of 60%. The similarity values of three strains of *O. octosporus* were 80% or more. All the three

strains of *H. japonica* var. *japonica* examined had a uniform electrophoretic enzyme pattern. The similarity value between the strains of *H. japonica* var. *japonica* and *H. japonica* var. *versatilis* was 60%. The three species, *S. pombe*, *O. octosporus*, and *H. japonica* had quite different electorphoretic enzyme patterns. Their similarity values were all 0%. The Co-Q systems of the strains were reinvestigated. These data are discussed from the taxonomic point of view.

* Laboratory of Applied Microbiology, Department of Agricultural, Chemistry, Shizuoka University.

Lipopolysaccharides of chemolithotrophic bacteria *Thiobacillus versutus* and a related *Thiobacillus* species

A. Yokota, S. Schlecht* and H. Mayer*

FEMS Microbiol. Letters 44: 197-201 (1987)

The lipopolysaccharides (LPSs) of two strains of *Thiobacillus versutus* and a *Thiobacillus* sp. strains were isolated and chemically analyzed. They contained neutral sugars, glucosamine, 2-keto-3-deoxyoctonate (KDO), and phosphorus, but were devoid of heptose. The fatty acids were characterized as amide-linked 3-OH-14:0 and 3-oxo-14:0, and ester-linked 3-OH-10:0. The lipid A backbones contain glucosamine as the only amino sugar. Deoxycholate-polyacrylamide gel electrophoresis (DOC-PAGE) showed that the LPSs have R-type character. The presence of amide-linked 3-OH-14:0 and 3-oxo-14:0 and of ester-linked 3-OH-10:0, the lack of heptose, and the R-type character of LPSs, indicate a structure of LPSs of *T. versutus* and *Thiobacillus* sp. strains similar to those found for the phylogenetically related *Rhodobacter* species and *Paracoccus denitrificans*.

* Max-Planck-Institut für Immunbiologie, Freiburg i. Br., Federal Republic of Germany

Lipopolysaccharides of *Thiobacillus* species containing lipid A with 2,3-diamino-2,3-dideoxyglucose

A. Yokota, M. Rodriguez*, Y. Yamada, K. Imai, D. Borowiak**, and H. Mayer**

Arch. Microbiol. 149: 106-111 (1987)

Lipopolysaccharides were isolated from two strains of *Thiobacillus ferrooxidans* and one strain each of *Thiobacillus thiooxidans*, *Thiobacillus novellus* and *Thiobacillus* sp. IFO 14570. Neutral sugars, 2-keto-3-deoxyoctonate, fatty acids and the rare 2,3-diamino-2,3-dideoxyglucose were detected in all lipopolysaccharides. Lipopolysaccharides of both *T. ferrooxidans* strains contained L-glycero-D-*manno*-heptose, whereas that of *T. thiooxidans* contained both L-glycero-D-*manno*-heptose and D-glycero-D-*manno*-heptose. On the other hand, heptoses were absent in lipopolysaccharides of *T. novellus* and *Thiobacillus* sp. IFO 14570. Lipid A of *T. ferrooxidans* and *T. thiooxidans* contained both glucosamine and 2,3-diamino-2,3-dideoxyglucose, in contrast, lipid A of *T. novellus* and *Thiobacillus* sp. IFO 14570 most likely contain only 2,3-diamino-2,3-dideoxyglucose as backbone sugar. Deoxycholate polyacrylamide gel electrophoresis revealed S-type character for all lipopolysaccharides studied. The significance of the lipopolysaccharide composition for taxonomic and phylogenetic questions with regard to thiobacilli is discussed.

* Microbiology Laboratory, Biological Sciences Faculty, Ponteficia Universidad Catolica de Chile, Santiago, Chile

** Max-Planck-Institut für Immunbiologie, Freiburg i. Br., Federal Republic of Germany

Leaf blight of day lily caused by *Aureobasidium microstictum* (Bubák) W. B. Cooke

M. Yoshikawa* and T. Yokoyama

Ann. Phytopath. Soc. Japan 53: 606-615 (1987)

Brown spots and yellow stripes were observed on leaves of *Hemerocallis fulva* var. *kwanso* at the herbal garden of Kyoto Prefectural Research Institute of Agriculture in Ayabe in the late June of 1985. In the beginning, small brown spots appeared on both sides of the leaves and then bright yellow stripes occurred and brownings expanded gradually from the leaf tips. These symptoms had been observed until mid-November. No pathogen was generally observed on diseased leaves, while acervuli composed of the tightly interwoven hyphae were formed on host plants in moist chamber. Conidiogenous cells were clavate to subcylindrical. Conidia were blastic, ellipsoidal to fusoid, hyaline, smooth, one-celled. Inoculation

tests indicated that isolates were pathogenic on injured leaves of *H. fulva* var. *kwanso*, *H. fulva* var. *disticha*, and *H. fulva* var. *fulva*. An isolate from naturally infected *H. fulva* var. *disticha* showed the same pathogenicity as those from *H. fulva* var. *kwanso*. On PDA medium, single conidium isolate formed creamy and yeastlike colonies at first, then after formed white hyphae, and eventually turned dark brown to black in color. Growth on PSA medium occurred in the range from 8 to 30 C and optimum growth at between 20 to 24 C. Conidia formed on PDA medium were blastic, ellipsoidal to fusoid, hyaline, smooth, one-celled. Secondary conidia were formed and yeastlike growth appeared. On the basis of its morphological characteristics and pathogenicity on the plants of the genus *Hemerocallis*, the present fungus was identified as *Aureobasidium microstictum* (Bubák) W. B. cooke, and a common name, leaf blight, was proposed.

* Chutan Branch, Kyoto Prefectural Research Institute of Agriculture

Actinokineospora*: a new genus of the *Actinomycetales

Toru Hasegawa

Actinomycetologica 2: 31-45 (1988)

A new genus *Actinokineospora* is described. It is characterized by forming chains of zoospores originating from aerial mycelium, and has type IV/A cell walls and a type PII phospholipid pattern. The major menaquinone is MK-10. No mycolic acids are present. The guanine-plus-cytosine content of the deoxyribonucleic acid is 72.0 mol%. The type strain of *A. riparia* is C-39162 (IFO 14541).

Maintenance of bacteriophages for *Pseudomonas aeruginosa* by L-drying

K. Imai

Japan. J. Freez. Dry. 34: 66-68 (1988)

L-dried specimens of eighteen bacteriophage strains for *Pseudomonas aeruginosa* were prepared, and their viability was examined. The results obtained by the accelerated storage test predicted that the shelf life of dried specimens that are preserved below 5 C is more than thirty years.

Structure and function of conidia of *Varicosporina* species (marine Hyphomycetes)

A. Nakagiri*

Trans. Br. mycol. Soc. 90: 265-271 (1988)

Conidia of *Varicosporina ramulosa* and *V. prolifera* were examined in culture and classified into four types of branching system. Sedimentation rates of polymethyl methacrylate models of conidia were measured in a silicone oil column. This experiment indicated that the four types of conidium had advantages in decreasing the sedimentation rate and in receiving water action equally from all directions in turbulent natural waters.

* Institute of Biological Sciences, University of Tsukuba; present address, Institute for Fermentation, Osaka

Effect of ethylenediamine on the survival of L-dried Aquaspirillia

T. Sakane and K. Imai

Japan. J. Freez. Dry. 34: 60-65 (1988)

It was known that the viability of L-dried specimens of *Aquaspirillum metamorphum* IFO 13960 was increased by addition of magnesium sulfate into the rehydration medium, and that the organism might be susceptible to the damage of the cell membrane, as a result of L-drying. Compounds which protect the cell membrane from damage were screened using IFO 13960, and ethylene-diamine dihydrochloride was found to be markedly effective. Addition of this compound into a suspending fluid gave ten-fold higher viability than that using the suspending fluid alone. The effect of this compound on the viability was enhanced by addition of sorbitol into the fluid. Neither incubation of the bacterial cells with ethylenediamine dihydrochloride before submitting to L-drying nor addition of this compound into the rehydration medium increased the viability. The release of cell components, such as RNA, proteins, and lipids, into the medium after rehydration was reduced by this compound. These findings indicate that ethylenediamine dihydrochloride prevents damage of the bacterial cell membrane during L-drying.

(in Japanese)

Lipopolysaccharides of iron-oxidizing *Leptospirillum ferrooxidans* and *Thiobacillus ferrooxidans*

A. Yokota, Y. Yamada, and K. Imai

J. Gen. Appl. Microbiol. 34: 27-37 (1988)

Lipopolysaccharides were isolated from one strain of *Leptospirillum ferrooxidans* and three strains of *Thiobacillus ferrooxidans*. All strains contained 2-keto-3-deoxyoctonate (KDO), heptose(s) and glucosamine besides neutral sugars, but were devoid of phosphorus. Lipopolysaccharides from iron-grown cells and from sulfur-grown cells had a similar chemical composition. Thus, the chemical properties of the lipopolysaccharide was unaffected by the energy sources utilized.

Chemical analysis of lipopolysaccharide from *Thiobacillus ferrooxidans* IFO 14262 revealed the presence of L-rhamnose, D-glucose, L-glycero-D-*manno*-heptose, KDO, D-glucosamine, and a lipophilic sugar identified as L-acofriose (3-O-methyl-L-rhamnose). 3-Hydroxymyristic acid was the main fatty acid. By hydrolysis in weak acid, the lipopolysaccharide has been separated into the polysaccharide part ("degraded polysaccharide") and lipid A. Presumably the lipid A contains a glucosamine backbone.

Comparative studies on the sensitivity of three methods for detecting mycoplasmal contamination in cell cultures

T. Yoshida, M. Kawase*, K. Sasaki*, H. Mizusawa*, M. Ishidate*, and Masao Takeuchi

Bull. JFCC 4: 9-15 (1988)

Three methods-DNA staining, culture, and cytotoxicity-for detecting mycoplasmal contamination in animal cell lines were compared. First, the sensitivity of the three methods was determined using six different mycoplasma strains: *Mycoplasma arginini* G230, *Mycoplasma orale* CH19299, *Mycoplasma salivarium* PG20, *Acholeplasma laidlawii* PG8, *Mycoplasma hyorhinis* BTS7, and *Mycoplasma hyorhinis* ("non-cultivable"). The last mentioned strain was detected by the DNA staining and cytotoxicity methods. The DNA staining method was 10^3-fold more sensitive than the cytotoxicity method. This strain was not detected by the culture method. For the other five strains, the sensitivity of the DNA staining method was similar to

that of the culture method, however, the sensitivity of the cytotoxicity method was 10^1- 10^4-fold lower. Second, 33 animal cell lines were examined using the three methods. The rate of detection was DNA staining (70%) > culture (55%) > cytotoxicity (39%) method. Finally, 168 animal cell lines were examined using the DNA staining and culture methods. Of 168 lines, 39 (23%) were positive by the DNA staining method and 33 (20%) were positive by the culture method. Six lines (3%) were positive by the DNA staining method but were not detected by the culture method. These results show that, among the three methods employed, the DNA staining method has the highest sensitivity for detecting mycoplasmal contaminations in cell lines.

* Natinal Institute of Hygienic Sciences

Cryopreservation of animal cell lines: cooling velocity and survival

T. Yoshida, N. Yanai, and Masao Takeuchi

Bull. JFCC 4: 16-20 (1988)

The effect of cooling velocities on the survival of cryopreserved animal cells was studied. Ten cell lines were cooled from 5 C to -40 C at 0.2, 1, 5, or 10 C/min using a programmable controlled rate freezing unit. The frozen cells were thawed at about 200 C/min and their survival rates were estimated by a dye exclusion method. It was found that high survival rates of the ten cell lines were obtained by cooling them at 1 C/min. The effects of cooling velocities on survival rates exhibited various patterns among the ten cell lines. This result suggests that the degree of injury caused mainly by intracellular freezing and solution effects varied among the ten cell lines.

(in Japanese)

PRESENTATION OF PAPERS AT SCIENTIFIC MEETINGS 1987-1988

Agricultural Chemical Society of Japan (April, 1987, Tokyo)

Mariko Takeuchi, A. Yokota, K. Imai and A. Misaki[*1]

The chemical structure of cell wall polysaccharide of two species of the genus *Microbacterium*.

M. Takizawa[*2], S. Tsubotani[*2], S. Tanida[*2], S. Harada[*2] and Tōru Hasegawa

A new pyrrole-amidine antibiotic TAN-868A.

A. Yokota and K. Imai

Production of sedoheptulose by mutants of *Bacillus subtilis* deficient in transketolase.

Gentner Symosium on "Biology of Complex Carbohydrates" (April, 1987, Tel-Aviv, Israel)

J. H. Krauss[*3], A. Yokota and H. Mayer[*3]

Structural profiling of lipopolysaccharides by detergent-gel electrophoresis.

Mycological Society of Japan (May, 1987, Tsukuba)

T. Ito and T. Yokoyama

Preservation of basidiomycete cultures by freezing.

---

*1 Faculty of the Science of Living, Osaka City University

*2 Applied Microbiology Laboratories, Central Research Division, Takeda Chemical Industries, Ltd.

*3 Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-7800 Freiburg, F.R.G.

T. Nakanishi[*1], A. Nakagiri[*2] and K. Tubaki[*1]
Ascocarp formation of arenicolous marine ascomycete, *Corollospora maritima*.

J. Takachi[*3], M. Shibata[*3], H. Kuraishi[*3], A. Nakagiri[*2] and K. Tubaki[*1]
Distribution of ubiquinone systems in marine fungi.

T. Yokoyama
Three new species of Hyphomycetes from Japan.

Japanese Tissue Culture Association (June, 1987, Tokyo)

M. Kawase[*4], K. Sasaki[*4], H. Mizusawa[*4], T. Yoshida, Masao Takeuchi and M. Ishidate[*4]
Standarization of mycoplasma detection methods in cell lines.

Japanese Society of Mycoplasmology (June, 1987, Tokyo)

M. Kawase[*4], H. Mizusawa[*4], T. Yoshida, Masao Takeuchi and R. Harasawa[*5]
Detection of mycoplasmal contamination in animal cell lines (III): DNA hybridization method.

T. Yoshida, N. Yanai, M. Kawase[*4], H. Mizusawa[*4], K. Yamamoto[*6] and Masao Takeuchi
Detection of mycoplasmal contamination in animal cell lines (II): Identification of mycolplasmas by immunoblot method.

---

*1 Institute of Biological Science, University of Tsukuba
*2 Institute of Biological Science, University of Tsukuba; present adress, IFO
*3 Faculty of Agriculture, Tokyo University of Agriculture and Technology
*4 Division of Mutagenesis, National Institute of Hygienic Sciences
*5 Faculty of Agriculture, Miyazaki University
*6 Faculty of Medicine, University of Tokyo

2nd Conference on Taxonomy and Automatic Identification of Bacteria (June, 1987, Praque, Czechoslovakia)

A. Yokota, S. Schlecht[*1] and H. Mayer[*1]

Lipopolysaccharide of a chemolithotrophic bacterium *Thiobacillus versutus*: Its phylogenetic significance.

Lithoautotrophy, A Centenary Meeting in Memory of S. N. Winogradsky (August, 1987, Göttingen, F.R.G.)

A. Yokota, M. Rodriguez[*2], Y. Yamada, K. Imai and H. Mayer[*1]

Lipopolysaccharide (LPS) of chemolithotrophic bacteria *Thiobacillus* species: Its phylogenetic significance.

Japanese Cancer Association (September, 1987, Tokyo)

Masao Takeuchi, T. Yoshida, H. Mizusawa[*3] and M. Kawase[*3]

Mycoplasmal contamination in animal cell lines.

The genetic Society of Japan (October, 1987, Tsukuba)

Y. Kaneko and I. Banno

Genetic analysis of galactose metabolism in *Saccharomyces bayanus* type strain.

Agricultural Chemical Society of Japan (April, 1988, Nagoya)

K. Imai and A. Yokota

Production of 2,7-anhydro-ß-D-*ido*-heptulopyranose by microorganisms.

M. Kakimoto[*4], Y. Sumino[*4], K. Imai, S. Akiyama[*4] and Y. Nakao[*4]

Microorganisms which produce acid urease and its properties.

---

*1 Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-7800 Freiburg, F.R.G.

*2 Microbiologie Laboratory, Biological Sciences Faculty, Ponteficia Universidad Catolica de Chile, Santiago, Chile

*3 Division of Mutagenesis, National Institute of Hygienic Sciences

*4 Applied Microbiology Laboratories, Central Research Division, Takeda Chemical Industries, Ltd.

I. Nogami[*1], H. Shirafuji[*1], T. Yamaguchi[*1], M. Oka[*1], T. Sakane and K. Imai

Production of 2-keto-L-gulonic acid by fermentation: Microorganisms and oxidation of L-sorbose.

A. Yokota and H. Mayer[*2]

Lipopolysaccharides of *Thiobacillus* species: Its phylogenetic significance.

Japanese Society for Research of Freezing and Drying (April, 1988, Tokyo)

K. Imai

Maintenance of bacteriophages for *Pseudomonas aeruginosa*.

T. Sakane and K. Imai

Effect of ethylenediamine on the survival of L-dried Aquaspirilla.

International Symposium on Endotoxin (May, 1988, Oyama)

H. Mayer[*2], J. H. Krauss[*2], A. Yokota and J. Weckesser[*3]

Natural variants of lipid A.

Japanese Society of Mycoplasmology (May, 1988, Tokyo)

H. Mizusawa[*4], M. Kawase[*4], T. Yoshida and Masao Takeuchi

A DNA probe assay for the detection of mycoplasmas contaminating cell cultures.

---

*1 Applied Microbiology Laboratories, Central Research Division, Takeda Chemical Industries, Ltd.

*2 Max-Planck-Institut für Immunbiologie, Stübeweg 51, D-7800 Freiburg, F.R.G.

*3 Institut für Biologie II, Mikrobiologie, der Universität D-7800 Freiburg, F.R.G.

*4 Division of Mutagenesis, National Insitute of Hygienic sciences

T. Yoshida, N. Yanai, Y. Kaneko, I. Banno, M. Kawase[*1], H. Mizusawa[*1] and Masao Takeuchi

Detection of mycoplasmal contamination in animal cell lines (IV): Characterization of "non-cultivable" *Mycoplasma hyorhinis* isolated from contaminated cell lines.

Japanese Tissue Culture Association (May, 1988, Oita)

Masao Takeuchi, N. Yanai, T. Yoshida, Y. Aso[*2] and K. Akai[*3]

Establishment of mouse GFAP-positive cells.

The 7th International Symposium on Biology of Actinomycetes (May, 1988, Tokyo)

Tōru Hasegawa

Aktinokineospora: a new genus of the *Actinomycetales*.

Tōru Hasegawa, T. Shomura[*4] and M. Hamada[*4]

Quality control of the International *Streptomyces* Project (ISP) strains deposited at the Institute for Fermentation, Osaka (IFO) by the ISP Committee of the Society for Actinomycetes, Japan (SAJ).

The 8th Yeast Symposia Japan (May, 1988, Kyoto)

Y. Kaneko and I. Banno

A genetic study of classification in *Saccharomyces* yeasts.

The 7th International Symposium on Yeasts (August, 1988, Perugia, Italy)

I. Banno and Y. Kaneko

A genetic analysis of taxonomic relation between *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

---

*1 Division of Mutagenesis, National Institute of Hygienic Sciences

*2 National Nishi Saitama Central Hospital

*3 Faculty of Medicine, Kyorin University

*4 ISP Committee, Society for Actinomycetes, Japan

The 5th International Congress of Plant Pathology (August, 1988, Kyoto)

T. Yokoyama

Present status of culture collections of plant pathogenic fungi in Japan.

The Society for Actinomycetes, Japan (September, 1988, Osaka)

Y. Nakagaito and Tōru Hasegawa

A new species of the genus *Kitasatosporia*.

H. Tsujibo[*1], K. Miyamoto[*1], Y. Inamori[*1] and Tōru Hasegawa

Purification and characterization of alkaline protease produced by an alkalophilic actinomycete.

A. Yokota and Tōru Hasegawa

Analysis of madurose by enzymatic-HPLC.

The Japanese Biochemical Society (October, 1988, Tokyo)

S. Kadowaki[*2], K. Yamamoto[*3], M. Fujisaki[*3], H. Kumagai[*3], T. Tochikura[*3] and T. Yokoyama

A novel endo-ß-N-acethylglucosaminidase acting on complex oligosaccharides of glycoproteins in a fungus.

The 6th International Congress of Culture Collections (October-November, 1988, College Park, MD, USA)

Tōru Hasegawa

Quality control of the International *Streptomyces* Project (ISP) cultures deposited at the Institute for Fermentation, Osaka (IFO).

T. Iijima

Activities of JFCC.

---

*1 Osaka University of Pharmaceutical Sciences

*2 Kacho College

*3 Department of Food Science and Technology, Kyoto University

T. Iijima
The culture collection at the Institute for Fermentation, Osaka (IFO).

Mycological Society of Japan (November, 1988, Okinawa)

A. Nakagiri and R. Tokura[*1]
Taxonomic review of the genus *Corollospora*.

K. Yamanaka[*2] and T. Yokoyama
Anamorph morphology and cultural characteristics of *Pleurotus dryinus* (Pers.: Fr.) Kummer.

M. Yoshikawa[*3] and T. Yokoyama
Thedgonia ligustrina on *Ligustrum japonicum* and *Cercospora* sp. on *Hydrangea serrata* var. *thunbergii*.

---

*1 Biology Laboratory, Kyoto Kyoiku University
*2 Nara Forest Experimental Station
*3 Chutan Branch, Kyoto Prefectural Research Insitute of Agriculture

MISCELLANEOUS SCIENTIFIC PAPERS

Y. Kaneko. 1987. Progress in the physical analysis of chromosome structure by pulsed-field gel electrophoresis. Hakkokogaku Kaishi 65: 538-539. [in Japanese]

A. Nakagiri[*1] and K. Tubaki[*2]. 1987. Pleomorphy in marine fungi: Teleomorph-anamorph connections in the Halosphaeriaceae. *In* J. Sugiyama (ed.) Pleomorphic fungi: The diversity and its taxonomic implications, p. 79-101. Kodansha, Tokyo & Elsevier, Amsterdam.

Masao Takeuchi. 1988. A detection method of mycoplasmas. *In* Japanese Tissue Culture Association (ed.) Soshikibaiyo no Gijutsu, p. 62-65. Asakura Shoten, Tokyo. [in Japanese]

---

*1 Institute of Biological Sciences, University of Tsukuba; present adress, IFO

*2 Institute of Biological Sciences, University of Tsukuba

CORRECTIONS

In the issue of IFO Research Communications No. 13, the following corrections should be made.

Page	Line	Type	Should read
22	48	*Gonytrichella olivacea*	delete
123	1	PAPAERS	PAPERS

発酵研究所研究報告　第14号

平成元年3月1日　印刷
平成元年3月6日　発行

定価1,300円

編集者　竹　内　昌　男
発行人　飯　島　貞　二
発行所　財団法人 発 酵 研 究 所
大阪市淀川区十三本町2丁目17番85号
Tel. 06—302—7281
印刷所　日 本 印 刷 出 版 株 式 会 社
大阪市福島区吉野1丁目2番7号