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The Institute for Fermentation, Osaka publishes the IFO Research Communication on a biennial basis. Purchase orders of the Research Communication 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 each; No. 10, 1,000 yen; and Nos. 11–15, 1,300 yen each, and plus postage and consumers tax.

REPORT OF THE DIRECTOR

The Institute for Fermentation, Osaka (IFO) was established in 1944, since when it has shown steady growth. Its major functions are to collect, preserve, and distribute cultures as resources for research, education and industrial application. It also carries out basic research on microorganisms and animal cell lines, with a sophisticated understanding of recent developments in microbiology, microbial genetics and cell biology. We are striving continuously to improve culture quality to maximize the usefulness and to keep cultures as living assets into the next century.

At the Annual Meeting of Councilors in May 1991, the chairman of the Board of Trustees, Mr. Shinbei Konishi, resigned from the post after nine years in this responsible position. He was succeeded by Councilor Dr. Katsura Morita. Dr. Yukio Sugino, Director of Takeda Chemical Industries, Ltd., was nominated as a member of the Board of Trustees. With the expiry of their terms of office, Councilors Dr. Kiyoshi Yora, Professor emeritus of the University of Tokyo, and Dr. Shoichi Takao, Professor emeritus of Hokkaido University, retired from the Council, and Dr. Kumao Toyoshima, Professor of Osaka University, and Dr. Fusao Tomita, Professor of Hokkaido University, were newly nominated as Councilors of IFO.

The chairman of Board of Trustees, Dr. Katsura Morita, received new funds amounting to 175 million yen from Takeda Chemical Industries, Ltd., in March 1992. Addition of the funds to the foundation of IFO was approved at the Annual Meeting of the Board of Trustees in March 1992.

Changes in the research staff were as follows: Deputy director, Dr. I. Banno retired from the Institute in March 1992. His dedicated service for 38 years in the yeast section including two years as dupty director is much appreciated. Dr. Masao Takeuchi was promoted to the post of deputy director in July 1992. Mr. K. Mikata was raised to the position of Research Head, and Messrs. T. Nishii, T. Ito and K. Sakane were each raised to the position of Research Chief. Ms. Mariko Takeuchi and Dr. A. Nakagiri were promoted to Associate Research Head.

"IFO Research Communications" No.15 was published in March 1991. The total number of cultures preserved in the IFO culture collection amounted to 13,985 at the end of 1991 and 14,297 at the end of 1992. The newly

accepted cultures during these two years will be listed in "IFO Research Communications" No.16, which will be issued in March 1993. The total number of cultures distributed from the IFO culture collection amounted to 8,345 in 1991 and 8,270 in 1992. "IFO List of Cultures" 3rd ed. (Animal cell lines) and 9th ed. (Microorganisms) were published in March 1992 and May 1992, respectively. These listed about 10,000 cultures: 5,608 fungi, 1,709 yeasts, 2,532 bacteria, 57 bacteriophages and 126 animal cell lines. "IFO List of Cultures" 3rd ed. was compiled and printed by use of a personal computor NEC 9801, after the data base of the IFO culture collection had been transferred from a IBM/36 computor to the NEC 9801. On the other hand, the list of microbial cultures (9th ed.) was directly compiled from the data base in the IBM/36 computor for the first time.

The Committee for Confirmation of International Streptomyces Project strains in Japan conducted regular confirmatory tests of ISP strains stored at IFO in December 1991 and 1992, respectively. L-dried specimens, which were prepared for distribution to customers, were tested for viability, taxonomic characteristics and authenticity of the strains. The test specimens were confirmed to be satisfactory.

IFO has developed a simple method for the transport of non-sporulated fungal cultures stored by freezing. Frozen cultures in cryotubes at -80C are defrosted and immediately airmailed to users. By this method, more than 150 fungal cultures mailed to users in Japan and broad in a defrosted state have survived fully. This seems to be the most labor-saving and practical method for sending non-spolulated fungal cultures.

Members of IFO attended several international meetings: Dr. Masao Takeuchi attended the 1991 World Congress on Cell and Tissue Culture and presented a paper in Anaheim, U.S.A., in June 1991. After the meeting, he visited the Research Institute of Scripps Clinic, La Jolla. Dr. K. Imai attended the 2nd, 3rd and 4th meetings of ISO/TC 198 on the sterilization of health care products, in Bilthoven, Netherlands, in July 1991, in Arlington, U.S.A., in March 1992, and in London, U.K., in November 1992, respectively. Dr. Toru Hasegawa attended the 8th International Symposium on Biology of Actinomycetes in Madison, U.S.A., in August 1991. At the same time, he was able to visit the National Center for Agricultural Utilization Research in Peoria, U.S.A. Dr. A. Yokota attended the Conference of Taxonomy and Automated Identification of Bacteria in Prague, Czechoslovakia, in July 1992, and presented two papers. After the meeting,

he visited the Czechoslovak Collection of Microorganisms (CCM), Masaryk University, Brno, Deutche Sammulung von Mikroorganismen und Zellkulturen GmbH (DSM), Braunschweig, Germany, and Max-Planck Institut für Immunbiologie (MPI) in Freiburg, Germany. Dr. Toru Hasegawa was invited as a guest speaker of the UNESCO Regional Training Workshop on "Exploitation of Novel Microorganisms, Especially Actinomycetes" in Taejon, Korea, in August 1992, and he lectured on rare actinomycetes and their bioactive secondary metabolites. After the workshop, he visited the College of Engineering, Yonsei University and Chong Kun Dang Corporation in Seoul, and gave three lectures. Mr. T. Ito, Mr. K. Mikata, Dr. Masao Takeuchi and Dr. Toru Hasegawa attended the 7th International Congress for Culture Collections in Beijing, China, in October 1992, and each presented a poster. After the congress, all of these except Dr. Masao Takeuchi also visited the Institute of Microbiology, Academia Sinica in Beijing and the Tianjin Bureau of Technical Supervision, and discussed the culture collection and exchanged information on matters of mutual interest.

As cooperative activities, Dr. Masao Takeuchi visited the Food Industry Research and Development Institute (FIRDI) in Hsinchu, Taiwan, in February 1992, and demonstrated some of the newest techniques for the cultivation of animal cell lines and their quality control and gave a lecture. Dr. K. Imai visited FIRDI in June 1992, introduced some newly developed methods for preservation of bacteriphages, and lectured.

Members of IFO participated in the following forays in Japan for the past two years. Mr. T. Ito joined the 1991 foray of the Mycological Society of Japan in Hiroshima Prefecture in October 1991, and Mr. T. Ito and Dr. A. Nakagiri joined the 1992 foray of the same society in Oita Prefecture in September 1992.

Dr. Toru Hasegawa was selected as the recipient of the Society for Actinomycetes Japan Award and gave the award lecture at the University of Tokyo in July 1991.

IFO has welcomed a number of guests in the past two years. Lectures and seminars were given by the following guest speakers.

Prof. T. Kamihara, Faculty of Engineering, Kyoto University: A role of membrane-lipids in the cell morphogenesis and physiology of yeasts.

Dr. M.A. Lachance, University of Western Ontario, Canada: Ecology and taxonomy of the yeast genus Sporopachydermia.

Dr. H. Huang, Central Research, Pfizer Inc., U.S.A.: Development of the perithecium in Gnomonia comari (Diaporthaceae)

Prof. P.A. Blanz, University of Bayreuth, Germany: Phylogeny of basidiomycetous fungi as inferred from nucleic acid studies.

Dr. I. Okane, Institute of Agriculture and Forestry, University of Tsukuba: Ecological and taxonomical studies on Puccinia coronata complex parasitic on Gramineae in Japan.

Dr. Y. Nakagawa, Institute of Applied Microbiology, University of Tokyo: Phylogeny of Cytophaga and related genera.

Dr. L.K. Nakamura, National Center for Agricultural Utilization Research, U.S.A.: Advances in Bacillus taxonomy.

Dr. Chitra N. Wendakoon, Department of Fisheries, Faculty of Agriculture, Kyoto University: Biogenic amine formation in fish muscle and its inhibition by food additives.

Ms. K. Ueda, International Center of Cooperative Research in Biotechnology, Japan, Osaka University: Taxonomic study of Streptomyces spp. by using the variable region of 16S rRNA gene.

IFO also hosted guest researchers during this period: Mr. S. Kayano and Ms. M. Konyo from Miki Trading Co.; Ms. Endang Sutriswati Rahayu from Gadjah Mada University, Indonesia; Dr. H. Nishimura, from Kyoto Prefectural University of Medicine; Mr. Fwu-Ling Lee from the Department of Agricultural Chemistry, Tokyo University of Agriculture; Mr. H. Sawada from Akitsu Branch, Fruit Tree Research Station; Mr. S. Yamada from Akita Prefecture Forest Technical Center; Ms. Y. Tabata and three others from Kobe College; Messrs. O. Inoue and K. Nakamura from Nagano Research Institute for Health and Pollution; Misses T. Sawada, M. Isoda and S. Ohtani from Kansai Women's Junior College; Mr. H. Nasu from Okayama Prefectural Agricultural Experiment Station; Mr. K. Shirahige from the Medical School, Osaka University and Dr. Chitra N. Wendakoon from the Faculty of Agriculture, Kyoto Univertsity; Dr. S. Okada from the Culture Collection Center, Tokyo University of Agriculture; Dr. Y. Kanzawa from Kobe Women's University, and Mr. M. Kaida from Hikari Plant, Takeda Chemical Industries, Ltd.

(Toru Hasegawa)

Heartfelt condolences are offered on the death of Councilor Dr. Hiroshi Iizuka, who passed away on 18th March, 1992, and Honorary Member, Professor emeritus Dr. Tsunesaburo Fujino, who passed away on 15th August, 1992. They made great contributions to the establishment of the Institute for Fermentation, Osaka.

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ESTABLISHMENT OF HPRT-DEFICIENT CELL LINES FROM MOUSE NEUROBLASTOMA NEURO-2A AND RAT PHEOCHROMOCYTOMA PC12

MOTONOBU SATOH and MASAO TAKEUCHI

Summary

Hypoxanthine/guanine phosphoribosyl transferase (HPRT)-deficient cell lines, designated as Neuro-2aTG and PC12TG, were established from mouse neuroblastoma Neuro-2a and rat pheochromocytoma PC12, respectively. Both cell lines stably exhibited HPRT$^-$ phenotype, and expressed neuronal properties, i.e., constitutive expression of 200-kD neurofilament protein in Neuro-2aTG and responsiveness to NGF in PC12TG. Therefore, these cell lines will be useful as fusion partners in somatic cell hybridization with neurons.

Keywords: HPRT-deficient, neuronal cell line.

In studying the properties and functions of neurons, use of clonal immortalized neuronal cell lines has the advantage that a large number of homogeneous cells can be easily obtained (15). To immortalize neurons, three strategies have been used: oncogene transfection into cells via retrovirus vectors (1-3, 7), genetically targeted tumorigenesis in transgenic mice bearing oncogenes (10, 17), and somatic cell fusion (9, 13, 14, 18, 24). So far, oncogene transfection has been employed for mitotic neuronal progenitor cells, since mature neurons are postmitotic, and the transgene is not efficiently introduced into cells unless DNA replication occurs (4). Somatic cell fusion can effectively immortalize postmitotic, identified, mature neurons (13, 14). However, it requires fusion partners

bearing a genetic marker such as $HPRT^-$ (16). Furthermore, the partner should express some neuronal properties because hybrid cells often lose the differentiated phenotypes of parent cells when two different types of cells are fused (5). Therefore, the fusion method is limited by the parent cells. Here we describe the establishment of two HPRT-deficient cell lines with neuronal properties.

Materials and Methods

Cell lines A mouse neuroblastoma Neuro-2a (IFO 50081) (12) was once subcloned in our laboratory, and the resultant clone 29 showing high responsiveness to neurite-promoting agents such as cAMP and ganglioside G_{M1} was used. The rat pheochromocytoma PC12 line (8) used in this study was a flat-shaped, adherent variant of the original line. Rat2 (IFO 50282) (20) is a thymidine kinase-deficient mutant of rat fibroblast line. For all cells, basic culture medium was Dulbecco's modified Eagle's minimum essential medium (DMEM; Nissui) supplemented with 10% fetal calf serum (FCS; M. A. Bioproducts). For Rat2 cultures, 100 μM 5'-bromodeoxyuridine was added to eliminate revertants and was removed prior to cell fusion. All cultures were maintained in a humidified air-5% CO_2 at 37° C.

Mutagenesis and selection of HPRT-deficient mutants Mutagen used was ethylmethane sulfonate (EMS; Sigma). Cells were treated with 100-500 μg/ml EMS in DMEM supplemented with 20% FCS for 24 hr, washed with culture medium, then grown in fresh medium without EMS for several days. After an expression period, cells were replated at 0.5-1.0 x 10^5 cells/ 6-cm plastic dish (Corning), cultured with 20 μg/ml 8-azaguanine (8-AG; Wako, Japan) or 6-thioguanine (6-TG; Wako, Japan) for several weeks, and appearing colonies were isolated using cloning cylinders. In the case of PC12 cells, EMS mutagenesis was omitted because a small population of 6-TG-resistant cells had been proved to exist in the PC12 line used here. Isolated clones were propagated in nonselective medium and examined for overall growth and viability in each medium containing 6-TG and HAT (100 μM hypoxanthine, 0.4 μM aminoputerin, 16 μM thymidine; Flow) respectively. Viabilities were determined by counting colonies by crystal violet staining after seeding 200 cells/ 35-mm dish (Falcon). Some

clones were further subcloned in the presence of 6-TG by the limiting dilution method using 96-well plates (Nunc).

Estimation of reversion rate Cultures were maintained in the presence of 6-TG for at least 2 weeks and serially passaged for 15-18 days in the absence of 6-TG. Then the cells were plated at 0.5-2.0 x 10^6 cells/150-cm^2 flask (Corning) in HAT medium, and colonies were counted after 10 days. Reversion rate was estimated as [Number of colonies appearing in HAT] / [(Number of cells seeded) x (viability of wild-type cells in HAT) x (Generation in nonselective medium)].

Karyology Exponentially growing cells were treated with 0.1 μg/ml colcemid (Sigma) for 2 hr, harvested, treated with 75 mM KCl, fixed with Carnoi's fixative, and spread over a slide glass. Chromosome specimens were stained with Hoechst 33258 dye.

Somatic cell fusion Rat2 cell line and a HPRT-deficient mutant line of Neuro-2a were maintained in media containing BrdU and 6-TG, respectively, and transferred to nonselective medium for 3 days prior to fusion. The mixture of two cell lines, either in suspension or adhering to a culture dish, was rinsed with DMEM, and somatic cell fusion was performed by treating the cells with 50% polyethyleneglycol (PEG-1000; Wako, Japan) in DMEM for 1 min at 37° C. After rinsing with DMEM, the fusion products were incubated in DMEM supplemented with 10% FCS for 1 day, then transferred to HAT medium. HAT-resistant colonies appearing were isolated using cloning cylinders and propagated.

Isozyme analysis The electrophoretic migration pattern of nucleoside phosphorylase isozyme was analyzed with an isozyme profiling kit (Authentikit; Corning).

Immunoblot Cells confluent in a 35-mm culture dish were harvested with 0.2% EDTA in phosphate-buffered saline (PBS; pH 7.2), then collected by brief centrifugation. Samples were prepared by adding an equal volume of DNase I (1 mg/ml in 62.5 mM Tris (pH 7)) to the cell pellet, mixing, and extraction with 50-100 μl of extraction buffer (62.5 mM Tris, 8 M urea, 5 mM $MgCl_2$, 2% SDS, 5% 2-mercaptethanol) (22) for 10 min. Before electrophoresis, samples were appropriately diluted with Laemmli's sample buffer and heated for 2 min at 100° C. SDS-PAGE was performed using 4% stacking gel, 7.5% resolving gel for 75 min at 90 V. The separated proteins were electrically transferred from the gel onto nitrocellulose membrane in 25 mM Tris, 192 mM glycine, 20% methanol for 1 hr at 3

mA/cm^2. The membrane was then blocked with 1% BSA, 5% FCS in Tris-buffered saline (TBS, pH 7.5) overnight, treated serially with a polyclonal antibody raised against 200-kD neurofilament protein (1/300 dilution in TBS; Chemicon) for 1 hr and with HRP-conjugated anti-rabbit IgG goat antibody (1/3000 dilution in TBS; Bio-Rad) for 30 min, and developed with 4-chloro-1-naphthol (Bio-Rad).

Immunofluorescence Cells cultivated on coverslips coated with poly-L-ornithine (100 μg/ml in PBS; Sigma) were fixed with 4% paraformaldehyde in PBS for 15 min at 4°C. After rinsing with PBS, they were treated with 0.1 % Triton-X100, 1% BSA, 5% FCS in PBS (TBFP), then incubated with polyclonal anti-200-kD neurofilament antibody in TBFP (1/300 dilution) overnight at room temperature. After rinsing with PBS, immunoreactivity was visualized by incubating them with FITC-conjugated goat anti-rabbit IgG $F(ab')_2$ fragment in TBFP (1/200 dilution ;Cappel) for 30 min. After mounting in glycerol, the specimens were examined with a fluorescent microscope (Olympus BH2).

Responsiveness to nerve growth factor Cells seeded onto poly-L-ornithine-coated or type I collagen-coated (Corning) dishes were treated with 20-100 ng/ml nerve growth factor (NGF; from mouse maxillary gland; Boheringer Mannheim) and examined for neurite extension.

Results

Establishment of HPRT-deficient line from a mouse neuroblastoma Neuro-2a cells

EMS treatment was chosen to induce efficient HPRT-deficient mutation in Neuro-2a cells since it is suitable for induction of a point mutation. It is generally believed that treating the cells for a period equal to or somewhat longer than one generation time at a concentration showing 10-70% viability is adequate for EMS mutagenesis. One generation time in Neuro-2a clone 29 was 22 hr, so we set the duration of treatment at 24 hr. Viabilities of cells as determined by colony formation against varying concentration of EMS were plotted (Fig. 1), and we decided to treat the cells mainly with 200-300 μg/ml EMS. An expression period of 4-8 days was set for stabilizing a mutant phenotype. The concentration of 8-AG and 6-TG for selection of HPRT-deficient cells were determined to

be 20 μg/ml and 5 μg/ml, respectively, from the viability curve (Fig. 2). In a series of EMS mutageneses and selections by 8-AG or 6-TG, 15 clones were successfully isolated (Table 1). They were expanded and checked for resistance to 6-TG, sensitivity to HAT and reverse mutation rate to examine the HPRT$^-$ mutant phenotype. As shown in Table 2, five clones and two subclones stably expressed the HPRT-deficient phenotype. Of these, clone #8 and its subclone #8-1 exhibited morphology and doubling time most similar to those of parent cells, in either the presence or absence of 6-TG. Therefore, clone #8-1 was designated as Neuro-2aTG and its characteristics were investigated. Neuro-2a cells have large

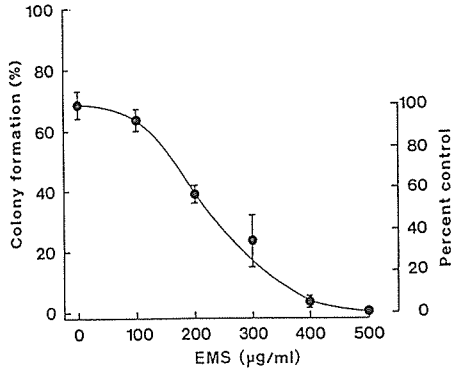

Fig. 1. Viability of Neuro-2a cells against EMS as represented by colony formation efficiency. Bars indicate standard deviation.

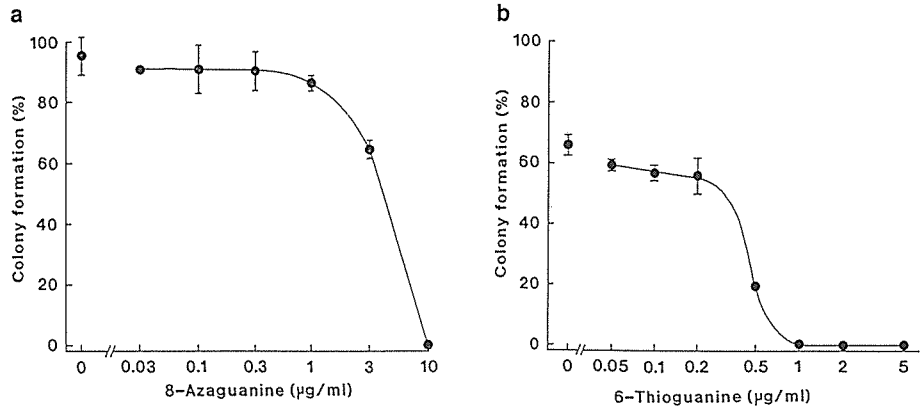

Fig. 2. Viability of Neuro-2a cells against 8-azaguanine (a) and 6-thioguanine (b) as represented by colony formation efficiency. Bars indicate standard deviation.

chromosomes with median or submedian centromeres and minute chromosomes (11). These marker chromosomes were also found in Neuro-2aTG cells (Table 3), suggesting that the clone was derived from Neuro-2a. Genetic complementation was tested between Neuro-2aTG cells and thymidine kinase-deficient rat fibroblasts (Rat2). Following somatic cell fusion, as expected, colonies appeared through HAT selection, suggesting that complementation occurred between the two cell lines. HAT-resistant clones were isolated and their hybrid natures were analyzed by NP isozyme electrophoretic migration patterns (19). NP exists as a homotrimer in normal cells and is detected as a single band in agarose gel electrophresis with a migration distance characteristic of the animal from which it was derived. In a hybrid cell, heterotrimers will form consisting of subunits from each parents locus. Fig. 3 shows NP isoenzyme profiles of HAT-resistant clones and their parents, Neuro-2aTG and Rat2. Mouse-derived

Table 1. Conditions for isolating $HPRT^-$ Neuro-2a clones

EMS (μg/ml)	Expression period (days)	Methods for selection		Number of colonies appearing	Clone No.
		Reagent	Cell number ($\times 10^6$)		
Exp. 1					
500	4	8-AG	0.5	0	
400	4	8-AG	0.5	0	
300	4	8-AG	0.5	0	
200	4	8-AG	0.5	1	#1
100	4	8-AG	0.5	0	
0	4	8-AG	0.5	0	
Exp. 2					
300	6	8-AG	3.0	12	#2-13
Exp. 3					
200	4	8-AG	4.0	1	#14
Exp. 4					
200	7	6-TG	1.2	0	
Exp. 5					
200	8	6-TG	1.6	1	#15

Neuro-2aTG and rat-derived Rat2 cells both yielded single bands. In HAT-resistant clones, hybrid-type NP bands were also detected as well as the mouse-type band, demonstrating that these clones were hybrids resulting from fusion between Neuro-2aTG and Rat2 cells. However, rat-type NP could not be detected, probably because of excessive production of mouse-

Table 2. Characteristics of isolated Neuro-2a clones

Clone	Growth characteristics (Shortly after isolation / After 2 months of passages in nonselective medium)				Reversion rate (Reversion/cell/generation)
	Overall growth		Viability (%)[a]		
	6-TG	HAT	6-TG	HAT	
#1	-/ND[b]	+/ND	ND/ND	ND/ND	ND
#2	±/ND	-/ND	1/ND	0/ND	ND
#3	±/ND	-/ND	0/ND	0/ND	ND
#4	+/+	-/-	89/86	0/0	$<4\times10^{-7}$
#5	+/+	-/-	68/99	0/0	$<2\times10^{-7}$
#6	±/ND	-/ND	0/ND	0/ND	ND
#7	±/ND	-/ND	1/ND	0/ND	ND
#8	+/+	-/-	99/123	0/0	ND
#8-1[c]	+/+	-/-	ND/93	ND/0	$<2\times10^{-7}$
#9	+/+	-/-	64/ND	1.7/ND	ND
#9-1[d]	+/+	-/-	ND/71	ND/0	$<5\times10^{-7}$
#10	+/+	-/-	85/98	0/0	$<1\times10^{-7}$
#11	+/+	-/-	78/93	0/0	$<1\times10^{-6}$
#12	±/ND	±/ND	ND/ND	ND/ND	ND
#13	ND/ND	ND/ND	34/ND	0/ND	ND
#14	+/ND	-/ND	88/ND	0.4/ND	1.7×10^{-6}
#15	±/ND	ND/ND	ND/ND	ND/ND	ND

a: Culture dishes were seeded with 200 cells/dish and appearing colonies were counted. Viability was shown as the percentage of control (nonselective) cultures.
b: ND; not determined. c: A subclone of #8. d: A subclone of #9.

Table 3. Karyotype analysis of Neuro-2aTG

Cell line	Modal chromosome number	Double minute chromosome	Metacentric or submetacentric chromosome
Neuro-2a clone 29	96-100	2-4	7-10
Neuro-2aTG	89- 91	2-4	5- 6

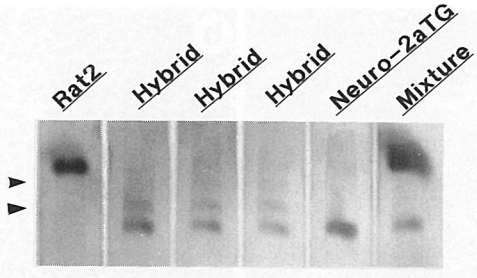

Fig. 3. Electrophoretic analysis of nucleoside phosphorylase (NP) isozymes in Neuro-2aTG x Rat2 hybrids and their parents. Mixture represents mixed enzyme sample from Neuro-2aTG and Rat2. Arrowheads indicate the bands for heterotrimeric NP isozymes.

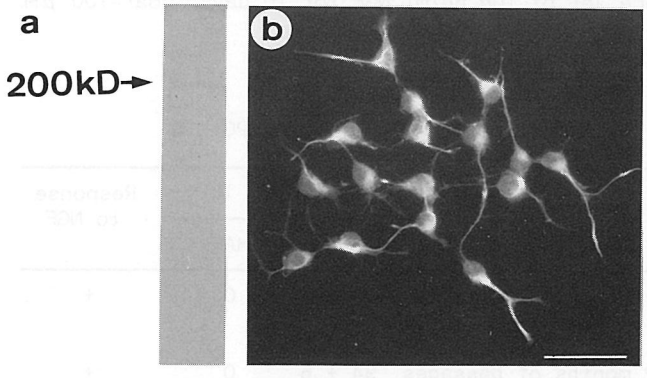

Fig. 4. Expression of 200-kD neurofilament protein (NF200) in Neuro-2aTG cells. (a) Immunoblot analysis for NF200. (b) Immunofluorescence of NF200 in Neuro-2aTG cells. Bar=50 μm.

type NP subunits in hybrid cells. Neuro-2aTG cells were examined for 200-kD neurofilament protein (NF200), a marker for neurons (21). As shown in Fig. 4a, NF200 was identified in Neuro-2aTG cells by immunoblot analysis. In immunofluorescence, NF200 was localized in neuritic processes as well as the perinuclear region of cell soma (Fig. 4b).

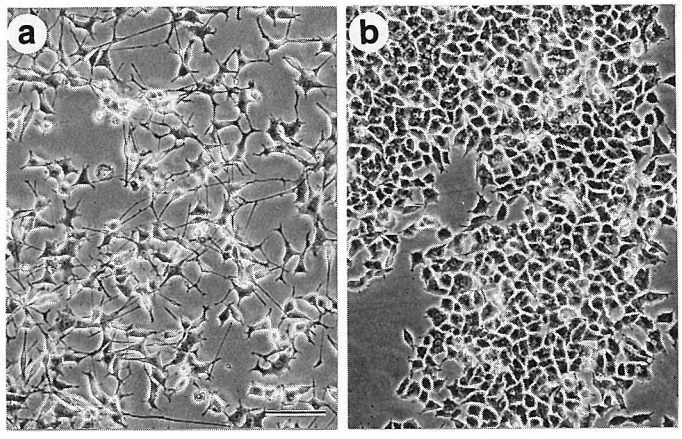

Fig. 5. Neurite extension of PC12TG cells by NGF treatment. The cells were cultured on collagen substrata in the presence (a) and absence (b) of 100 ng/ml NGF for 10 days. Bar=100 μm.

Table 4. Stability of PC12TG

	Viability (%)[a]		Response to NGF
	6-TG	HAT	
Shortly after isolation	104 ± 1	0	+
After 2 months of passages in nonselective medium	94 ± 6	0	+

a: Culture dishes were seeded with 200 cells/dish for 6-TG treatment and 10^6 cells/dish for HAT treatment, and appearing colonies were counted. Values were shown as percentage of control (nonselective) cultures.

Establishment of HPRT-deficient line from rat pheochromocytoma PC12 cells

HPRT gene is located on the X chromosome. PC12 cells have only one X chromosome, so HPRT-deficient mutations may occur spontaneously. As expected, a preliminary experiment revealed a small population of 6-TG-resistant cells in the PC12 cells used here. Thus, we isolated clones resistant to 6-TG without treating the cells with a mutagen. Of the 48 clones isolated, 7 were sensitive to HAT, and the rest were, to our surprise, HAT-resistant. One 6-TG-resistant, HAT-sensitive clone that morphologically resembled the parent cells was recloned and examined for responsiveness to NGF. The cells extended neuritic processes with increasing volume of the cell body, and ceased cell division in response to NGF (Fig. 5), which was characteristic of PC12 (8). This clone was thus designated as PC12TG. After two months of serial passages in nonselective medium, PC12TG stably exhibited $HPRT^-$ phenotype and responsiveness to NGF (Table 4).

Discussion

$HPRT^-$ is a genetic marker often used in somatic cell fusion (16). In the present study, we established the HPRT-deficient lines Neuro-2aTG and PC12TG from Neuro-2a and PC12 cells, respectively. These lines had a stable $HPRT^-$ phenotype, suggesting that the phenotype is due to mutation. Somatic cell fusion between Neuro-2aTG and a thymidine kinase-deficient fibroblast line followed by HAT selection successfully yielded hybrid cells, suggesting that introduced $HPRT^-$ was effective as a selective marker. In immortalizing neurons by somatic cell fusion, a fusion partner closely related to the neurogenic lineage is desirable because trans effects may alter the gene expression of parents in heterotypic hybrids (5). Neuro-2aTG and PC12TG derive from neuroblastoma and pheochromocytoma, respectively. Neuroblastoma is thought to derive mainly from neural crest cells which give rise to the peripheral nervous system including neurons (23). Pheochromocytoma is a tumor of the adrenal medulla which originates from a common progenitor of sympathetic neurons (6). Therefore, both cell lines may be assigned to neurogenic lineage. Moreover, we showed the constitutive expression of NF200 in Neuro-2aTG and responsiveness to NGF in PC12TG. NF200 is a component of neuron-specific

intermediate filament. The response to NGF of neurite extension is the most prominent character of parent PC12 cells, which mimics the sympathetic neuron differentiation. Thus, the established HPRT-deficient lines have neuronal properties.

Few cell lines have been established hitherto with neuronal properties bearing a genetic selection marker, and no such cell line, including the most popularly used N18TG2, is registered in a cell bank. The lines we established here will be useful as parent cells for immortalizing neurons by somatic cell fusion. Neuro-2aTG and PC12TG were deposited at the Institute for Fermentation, Osaka.

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CROSS-CONTAMINATION OF CELL LINES AS REVEALED BY DNA FINGERPRINTING IN THE IFO ANIMAL CELL BANK

MOTONOBU SATOH and MASAO TAKEUCHI

Summary

For quality control of cell lines, the Institute for Fermentation, Osaka (IFO) animal cell bank recently introduced DNA fingerprinting analysis, which enables verification of cell lines at the individual level, to detect cross-culture contamination. By using this analysis, we found two cases of cross-contamination of cell lines.

Keywords: Cross-culture contamination,
DNA fingerprinting analysis.

Reports that many human cell lines established from the 1950s, such as KB, Hep2, J-111, Chang Liver and Girardi Heart, were revealed to be HeLa cells (1, 2) have highlighted the problem of cross-contamination of cell lines. As cross-culture contamination may easily occur due to mishandling of cells, cell banks should examine the identity of the deposited cell lines as a quality control measure. For this purpose, we have been performing isoenzyme analysis (4) to characterize the species identity of cell lines. However, this method cannot effectively detect cross-culture contamination between cells originating from the same species. DNA fingerprinting analysis recentry developed has been shown to be a very potent method for the identification of individuals (6, 7), and so it has been applied to authentication of cultured cell lines (15). Here we describe two cases of cross-culture contamination that were found

in the IFO animal cell bank by using DNA fingerprinting analysis.

Materials and Methods

DNA fingerprinting Genomic DNA was isolated from cells by phenol chloroform procedures (8), and was digested with Hinf I or Alu I. DNA fragments were run on agarose gel electrophoresis (1% agarose in 20 mM Tris-acetate, 0.5 mM EDTA, pH 7.4) for 3 hr at 80 V. DNAs were denaturated by soaking the gel in 0.25 N HCl for 20 min, then in 1.5 M NaCl, 0.5 N NaOH for 30 min. After equilibration in 3 M sodium acetate, the gel was blotted to a nylon membrane (Biodin A; Pall Biosupport) in 20 x SSC (3 M NaCl, 3 M sodium citrate) overnight. DNA fragments were fixed to the membrane by baking for 2 hr at 80° C. The Southern transfer was soaked in hybridization buffer (5 x SSC, 1% SDS, 0.5% bovine serum albumin (BSA)) for 10 min at 50° C, then hybridized with an alkaline-posphatase conjugated probe that recognizes myoglobin minisatellite tandem repeats (6) (Molecular Biosystem) in hybridization buffer for 15 min at 50° C. The membrane was rinsed repeatedly with 1 x SSC, 1% SDS for 5 min at 50° C, then with 1 x SSC, 1% Triton X-100 for 5 min at 50° C, and finally with 1 x SSC for 5 min at room temperature. The hybridization bands were visualized by incubating the membrane with a color developer including nitroblue tetrazorium chloride, 5 bromo-4-chloro 3-indolylphosphate and co-factor of alkaline phosphatase (Molecular Biosystem) for 4 hr at 37° C.

Isoenzyme analysis Analysis of isoenzyme electrophoretic migration pattern was carried out using an isozyme profiling kit (Authentikit, Corning).

Immunofluorescence of glial fibrillary acidic protein (GFAP) Cells cultured on glass coverslips were either fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 15 min and treated with 0.2% Triton X-100, 1% BSA in PBS for 30 min, or fixed serially with cold acetone-acetic acid (9:1) for 20 min, cold acetone for 5 min, and cold 70 % ethanol for 5 min. Fixed cells were then incubated with a polyclonal antibody raised against cow GFAP in 1% BSA-PBS (1/100 dilution; DAKO) for 30 min. Immunoreactivity was revealed by incubating them with FITC-conjugated goat anti-rabbit IgG antibody in 1% BSA-PBS (1/100 dilution;

Miles, or 1/200 dilution; $F(ab')_2$ fragment; Cappel) for 30 min, and was examined by fluorescent microscopy (Olympus BH-2).

Results and Discussion

One case found was of cross-culture contamination of human glioma cell lines. U-87 MG and U-251 MG cell lines derive from human gliomas from a 44-year-old female and a 75-year-old male, respectively (9, 19). In the IFO cell bank, two U-87 MG-related and three U-251 MG-related strains were preserved (Table 1). During a routine isoenzyme analysis, we found a common abnormality in the electrophoretic migration pattern of lactate dehydrogenase (LDH) among the U-87 MG and two U-251 MG strains that were simultaneously deposited in IFO from the same laboratory. This abnormal LDH profile was also detected in another U-251 MG strain that was independently deposited from another laboratory. Since it has been reported that U-251 MG line expresses GFAP (18), a marker for astroglial cells, while U-87 MG does not (10), we examined the expression of GFAP. All the U-87 MG and U-251 MG strains were GFAP-positive. As a cross-culture contamination in the deposited U-87 MG was therefore suspected, another U-87 MG strain was obtained from RIKEN cell bank as a reference.

Table 1. Characteristics of U-87 MG and U-251 MG-related strains preserved in IFO and RIKEN animal cell banks.

Cell line	LDH isozyme profile	Expression of GFAP	DNA finger-print profile	Remarks
IFO				
U-87 MG	Abnormal	Positive	Identical	Deposited by the same laboratory
U-251 MG(KO) (IFO 50285)	Abnormal	Positive		
U-251 MG (IFO 50288)	Abnormal	Positive		
U-251 MG	Abnormal	Not tested		
U-87 MG-SF	Not tested	Positive		Derivative of U-87 MG deposited in IFO
RIKEN				
U-87 MG (RCB419)	Normal	Negative	Distinct from IFO strains	

Contrary to our strain, the U-87 MG from RIKEN expressed a normal LDH profile and no GFAP. Conclusive evidence came from DNA fingerprinting analysis. The suspected U-87 MG and its direct derivative, U-87 MG-SF

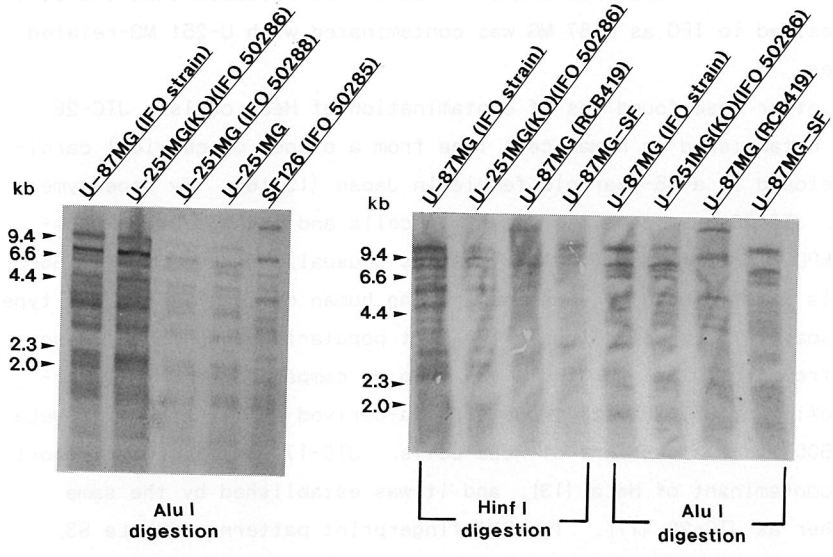

Fig. 1. DNA fingerprint profiles of U-87 MG and U-251 MG-related strains. SF126 (IFO 50286) is a human glioma cell line.

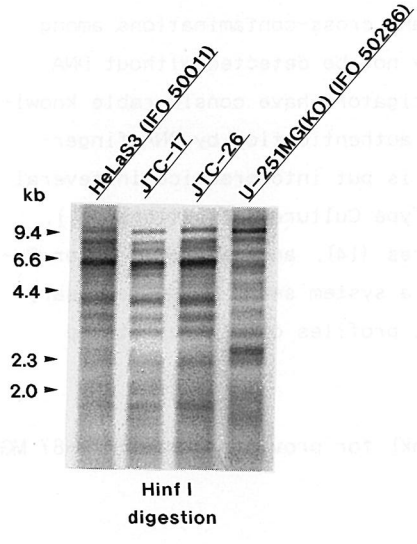

Fig. 2. DNA fingerprint profiles of Hela S3 (IFO 50011), JTC-17, JTC-26, and U-251 MG (KO) (IFO 50285)

lines exhibited the DNA fingerprint profile identical to those of U-251 MG-related lines, but distinct from the U-87 MG from RIKEN (Fig. 1), demonstrating that the suspected U-87 MG and the U-251 MG-related strains originated from the same individual. Thus it was revealed that the cell line deposited to IFO as U-87 MG was contaminated with U-251 MG-related cell lines.

The other case found was of contamination of HeLa cells. JTC-26 line was established as human cell line from a biopsy of cervical carcinoma developed in a 55-year-old female in Japan (12, 16). By isoenzyme analysis, JTC-26 was identified as human cells and its glucose-6-phosphate (G6PD) isozyme was type A. This was unusual, because type A G6PD isozyme is rarely found in Japanese. Among human cells that exhibit type A G6PD isozyme, HeLa cell line is the most popularly used, and it also derives from a cervical carcinoma (3). So we compared the DNA fingerprint profile of JTC-26 with those of HeLa-derived cells (Fig. 2). HeLa S3 (IFO 50011) is a subclone of HeLa cells. JTC-17 line has been reported as a contaminant of HeLa (13), and it was established by the same establisher as JTC-26 (17). The DNA fingerprint patterns of HeLa S3, JTC-17 and JTC-26 were identical. Thus JTC-26 line was demonstrated to originate from HeLa-related cell lines.

In both cases reported here, the cross-culture contamination was suspected before DNA fingerprinting on the basis of the other analysis such as isoenzyme profiling. However, many cross-contaminations among cell lines deriving from same species may not be detected without DNA fingerprinting analysis unless the investigators have considerable knowledge of the cells. Therefore, cell line authentication by DNA fingerprinting is required for cell banks, and is put into practice in several culture collections, including American Type Culture Collections (11), Europian Collection of Animal Cell Cultures (14), and Japanese Cancer Research Resources Bank (5). Furthermore, a system seems to be necessary that records and compares DNA fingerprint profiles of all registered cells in the IFO and other cell banks.

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INTERTIDAL MANGROVE FUNGI FROM IRIOMOTE ISLAND

AKIRA NAKAGIRI

Summary

Intertidal decomposing wood of Rhizophora stylosa and Bruguiera gymnorrhyza was collected from mangrove forests at the Shiira River and the Nakama River in Iriomote Is. and examined for higher fungi. Twenty-five species of ascomycetes and 6 species of deuteromycetes were found from 60 samples. Morphology and cultural properties of the 21 species identified are described and problems in their taxonomy are discussed. The frequency of occurrence of fungi on the two species of mangrove trees was compared. Species frequently found on R. stylosa were Caryosporella rhizophorae, Dactylospora haliotrepha, and Swampomyces triseptatus. Hypoxylon oceanicum occurred only on B. gymnorrhyza.

Keywords: mangrove fungi, Rhizophora stylosa, Bruguiera gymnorrhyza.

In the course of studies on the fungal flora of decomposing intertidal mangrove wood from Iriomote Is., 20 species of ascomycetes and 5 species of deuteromycetes were recorded from Rhizophora stylosa Griff. and 10 species of ascomycetes and 2 species of deuteromycetes from Bruguiera gymnorrhyza. The 21 species identified are described here in terms of their morphology and cultural characteristics. Ten species remain

unidentified. Newly found characteristics and findings contradictory to earlier reports are noted and the taxonomic problems of the fungi are discussed. The frequencies of occurrence of fungi on the two species of mangrove wood are compared.

Materials and Methods

Decomposing prop roots, branches and trunks of R. stylosa and B. gymnorrhyza were collected from intertidal mangroves of the Shiira River and the Nakama River, Iriomote Is. (25° 20'N, 123° 90'E), Okinawa Pref., on 26 Nov. 1991. The wood samples were incubated in plastic boxes at 20-25 C and were examined under a dissecting microscope for fungi occurring on and in the wood. Observation was continued for 5 months. In total, 45 samples of R. stylosa and 15 samples of B. gymnorrhyza were examined. Phase-contrast light microscopy and scanning electron microscopy [JSM 5400 (JEOL)] were used for observation. Isolation of cultures was carried out with a Skerman's micromanipulator. Single-spore isolates were incubated on sea water cornmeal agar [SWCMA: 2% cornmeal extract and 1.5% agar in 20‰ salinity (S) artificial sea water (Jamarin S; Jamarin Lab., Osaka)] at 25 C.

Results and Discussion

Description of occurring fungi

Ascomycetes and deuteromycetes found on mangrove wood, most of which are newly recorded Japanese fungal flora, are described here in alphabetical order.

Ascomycetes

Aigialus grandis Kohlm. & Schatz
Trans. Br. Mycol. Soc. 85: 699 (1985)

Figs. 1, A-D

Ascocarps subglobose in frontal view, obovate in vertical section, laterally compressed, mostly immersed in a black stroma, with carbona-

ceous, black, slightly convex stroma surrounding ostiole at the surface of substrates. Bitunicate asci arising from a basal ascogenous tissue. Because the specimens examined were old, asci and hamathecia were not observed in detail. Ascospores 72-102 X 20-28 μm ($\bar{x}$=81.8 X 23.8 μm), ellipsoid, muriform, with 11-15 trans-septa and 1-3 longisepta in all but the end cells, slightly constricted at the middle septum and the end septa, yellow-brown to dark brown except for the hyaline apical cell, with an easily deciduous, gelatinous cap around the apical cells.

Colonies on CMSWA pale brown, 11 mm in diam after incubation for 5 weeks at 25 C. Single-ascospore isolates [AN-1208 (IFO 32469), AN-1209 (IFO 32470) and AN-1210] from the specimen Shi-Ya-7 (IFO H-12137) produced ascocarp initials on CMSWA.

Substrates: Submerged wood of Rhizophora stylosa and Bruguiera gymnorrhyza.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-7, and B. gymnorrhyza, Nakama River, Na-O-6.

Aniptodera limnetica Shearer

Mycologia 81: 140 (1989)

Figs. 2, A-L

Ascocarps 180-220 μm high, 170-240 μm in diam, globose to subglobose, superficial or partly immersed, ostiolate, white to cream colored; long necks 200-440 X 38-48 μm, cylindrical, with 1-2 band-like hyphal structures, which may indicate the ceased and resumed elongation of the neck. Catenophyses present. Asci 90-130 X 16-24 μm, eight-spored, clavate, pedunculate, unitunicate, thin-walled, with thick apical plate and pore, with retracted cytoplasm below the apical plate. Ascospores 20-25 X 8-10 μm ($\bar{x}$=22.5 X 8.9 μm), one-septate, ellipsoid to oblong-ellipsoid, thin-walled [not thick-walled, as described by Shearer (1989)], hyaline. Ascospores are released from asci through a fissure in the apical plate, which splits at the pore.

Colonies on CMSWA dark olive to brown, 3-4 mm in diam after incubation for one month at 25 C. Single-ascospore isolates [AN-1270 (IFO 32471), AN-1271 (IFO 32472) and AN-1272] were obtained from the specimen Shi-O-3 (IFO H-12138).

Substrate: Submerged wood of B. gymnorrhyza.

Specimen examined: Submerged wood of B. gymnorrhyza, Shiira River, Shi-O-3.

The disagreement in ascospore wall thickness with the description by Shearer (1989) is probably due to the difference in appearance under the microscope in a different mount solutions. As indicated by Shearer (1989), appearance of ascospores of A. chesapeakensis Shearer & Miller differed when they were mounted in water or lactic acid. In lactic acid, ascospores appeared thick-walled, whereas in water they appeared thin-walled [compare Figs. 2, 5 and 9 in Shearer (1989)]. Ascospores of A. limnetica mounted in water or Shear's solution in this study appeared thin-walled, like those of A. chesapeakensis in water. Other characteristics of the present fungus fit well with Shearer's description. Therefore, I identified this fungus as A. limnetica.

Aniptodera longispora Hyde

Bot. Mar. 33: 335 (1990)

Figs. 3, A-J

Ascocarps 480-500 μm long, 200-280 μm thick, pyriform to subglobose in frontal view, elliptical in vertical section, lying horizontally immersed in the substrate, coriaceous to membranous, light brown to black at the upper exposed part, hyaline at the lower immersed part, ostiolate, papilate; necks rising eccentrically from one end. Catenophyses present. Asci 184-224 X 20-28 μm, eight-spored, clavate to cylindrical, pedunculate, unitunicate, persistent, thin-walled, with a refractive pore or plug-like structure at the apex, with swollen cytoplasm below the apical refractive structure, arising from the base of ascocarp venter, I-. Ascospores 40-53 X 10-14 μm ($\bar{x}$= 47.3 X 11.8 μm), cylindrical with rounded apices, one-septate, slightly constricted at the septum, hyaline. Ascospores were released from asci through a fissure made at the refractive pore or plug-like structure. The refractive structure was observed only in mature asci, not in young ones. Scanning electron microscopy did not show any corresponding structure.

Colonies on CMSWA olive brown to dark brown, 3-4 mm in diam after incubation for two months at 25 C. Single-ascospore isolates [AN-1267 (IFO 32473), AN-1268 (IFO 32474) and AN-1269] were obtained from the specimen Shi-Ya-19 (IFO H-12139).

Substrate: Submerged wood of R. stylosa.

Specimen examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-19.

Caryosporella rhizophorae Kohlm.

Proc. Ind. Acad. Sci. (Plant Sci.) 94: 356 (1985)

Figs. 4, A-J

Ascocarps up to 1 mm in diam and height, gregarious, globose to subglobose, superficially seated on a thin black stroma, ostiolate, short papillate, carbonaceous, thick-walled, black. Hamathecia (pseudoparaphyses?) 1.5-2 μm in diam, septate, simple between asci, branched and anastomosing above asci, arising with asci from the base of ascocarp venter. Asci 170-210 X 11-14 μm, eight-spored (abnormally six-spored), cylindrical, long pedunculate, bitunicate (or multitunicate?), I-. Ascospores 20-26 X 9-11 μm ($\bar{x}$= 22.8 X 10.6 μm) [ascospores in six-spored asci; 28-34 X 10-11 μm], uniseriate, ellipsoidal, one-septate, slightly constricted at the septum, brown to dark brown, light colored or hyaline at the apices, thick-walled except for the apices, verrucose. Under SEM, two kinds of ornamentations, ca. 1 μm long flexible conical spines and smaller, 0.1-0.3 μm in diam, granules, were revealed. Small black pycnidia (spermogonia?) with short or no papilla are formed on stroma close to ascocarps. Unicellular, globose conidia (spermatia?) (1.5-2 μm in diam) are abundantly produced from phialides in the pycnidia.

Colonies on CMSWA hyaline, grow fast at 25 C. Single-ascospore isolates [AN-1179 (IFO 32475), AN-1180 (IFO 32476) and AN-1181] from the specimen Shi-Ya-5 (IFO H-12140) produced globose black pycnidia (400-480 μm in diam) with a long neck (240-260 X 56-72 μm) but without stroma on CMSWA.

Substrate: Submerged wood of R. stylosa.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-5, 7, 13, 16, 17, 19, 22, 23, 24, 26, 30, 31, 32, 33, 34.

Cultural study confirmed that the pycnidia-like structures found in natural substrates were truely of C. rhizophorae, and also showed the variablity in morphology of pycnidia.

Cucullosporella mangrovei (Hyde & Jones) Hyde & Jones in Jones and Hyde

Mycotaxon 37: 200 (1990)

Basionym: Cucullospora mangrovei Hyde & Jones, Bot. Mar. 29: 491 (1986)

Figs. 5, A-F

Ascocarps 280-400 μm high, 240-400 μm in diam, subglobose, pale brown, immersed, ostiolate; necks 200-280 X 64-80 μm, dark brown to black. Asci 220-260 X 32-52 μm, eight-spored, clavate, pedunculate, unitunicate, thin-walled, with a thickened apex and a refractive plug-like structure at the center of the thickness, with swollen cytoplasm below the refractive structure. Ascospores 50-68 X 13-20 μm, fusiform to ellipsoid, one-septate, not constricted at the septum, hyaline, with fine longitudinal striation visible under SEM, with polar appendages arising from a tube-like extension of the spore wall. Appendages composed of a coiled string (0.3-0.5 μm in diam), which elongates freely to become long thread-like polar filaments.

Colonies on CMSWA dark brown to black, 15 mm in diam after incubation for two months at 25 C. single-ascospore isolates [AN-1152 (IFO 32477) and AN-1153 (IFO 32478)] from the specimen Shi-Ya-0 (IFO H-12141) produced ascocarp initials on CMSWA.

Substrate: Submerged wood of R. stylosa.

Specimen examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-0.

Scanning electron microscopy revealed the refractive structure at the ascus apex as a disc-like (2.5-3 μm in diam) structure.

Dactylospora haliotrepha (Kohlm. & Kohlm.) Hafellner

Beih. Nova Hedwigia 62: 111 (1979)

Basionym: Buellia haliotrepha Kohlm. & Kohlm., Nova Hedwigia 9: 90 (1965)

Figs. 6, A-G

Ascocarps 200-300 μm high, up to 1mm in diam, at first globose, developed into discoid, flat or convex, apothecia-like, superficial, sessile, fleshy-leathery, dark reddish-brown, solitary or gregarious. Paraphyses 85-110 X 1.5-2 μm, at the swollen apex 3-5 μm in diam, sep-

tate, branching at the upper part, hyaline. Asci 60-86 X 16-20 μm, eight-spored, clavate, short pedunculate, bitunicate, apically thick-walled, thinner toward the base; the ectoascus secrets a gelatinous sheath that reacts blue with iodine, and the endoascus elongate upto 58 μm long through a fissure at the apex of the ectoascus and the gelatinous sheath. Ascospores 16-22 X 8-11 μm, obovoid, one-septate in the lower third, constricted at the septum, light brown; spore surface with longitudinally or obliquely woven ribs.

Colonies on CMSWA white, floccose, 15 mm in diam after incubation for three months at 25 C. Hyphae characteristically branch perpendicularly and often anastomose with each other. A four-ascospore isolate [AN-1170 (IFO 32479)] and single-ascospore isolates [AN-1171 (IFO 32480) and AN-1172] were obtained from the specimen Shi-Ya-0 (IFO H-12142).

Substrate: Submerged wood of R. stylosa and B. gymnorrhyza.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-0, 1, 7, 11, 14, 17, 19, 20, 22, 28, 30, and B. gymnorrhyza, Nakama River, Na-0-5, 6.

Halosarpheia abonis Kohlm.

Mar. Ecol. (P. S. Z. N. I.) 5: 339 (1984)

Figs. 7, A-H

Ascocarps subglobose, immersed, ostiolate, with long neck, coriaceous, hyaline at the lower immersed part, light brown at the upper exposed neck. Catenophyses present. Asci 180-264 X 32-60 μm, eight-spored, clavate, pedunculate, unitunicate, thin-walled, thicker at the apex, with swollen cytoplasm below the apex only in immature asci, I- but ascus wall, ascospores and ascospore appendages are stained brown with iodine. Ascospores 38-48 X 16-20 μm, ellipsoid, one-septate, slightly constricted at the septum, hyaline, with apical appendages; a scoop-like appendage is attached to each apex and runs along the side of the ascospore; at maturity it becomes soft and the composing fibers uncoil into long sticky filaments.

Colonies on CMSWA light brown, 26 mm in diam after incubation for 5 weeks at 25 C. Single-ascospore isolates [AN-1205 (IFO 32481), AN-1206 (IFO 32482) and AN-1207] obtained from the specimen Shi-Ya-8 (IFO H-12143) produce ascocarp initials (?) on CMSWA.

Substrates: Submerged wood of R. stylosa.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-8, 27.

Halosarpheia fibrosa Kohlm. & Kohlm.

Trans. Br. Mycol. Soc. 68: 208 (1977)

Figs. 8, A-L

Ascocarps 480-560 μm high, 480-520 μm in diam, pyriform to subglobose, immersed to partly immersed or superficial, ostiolate, with a long neck (160-700 X 96-105 μm), coriaceous, dark brown, top of necks lighter colored. Catenophyses present. Asci 152-184 X 40-48 μm, eight-spored, clavate, pedunculate, unitunicate, thin-walled, persistent, without apical apparatus or thickness but with dome-shaped thin-walled apex, I- but ascospores were densely stained brown with iodine. Ascospores 32-39 X 20-22 μm, broad ellipsoid, one-septate, not or slightly constricted at the septum, hyaline, with apical appendages; cap-like appendage is attached to each apex, at maturity the composing strings uncoil into long sticky filaments.

Colonies on CMSWA olive green, cottony, 10-20 mm in diam after incubation for 3 weeks at 25 C. Single-ascospore isolates [AN-1258 (IFO 32483), AN-1259 (IFO 32484) and AN-1260] were obtained from the specimen Shi-Ya-18 (IFO H-12144).

Substrates: Submerged wood of R. stylosa.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-18.

Under SEM, the ascospore appendage was seen to be composed of a meandering, 0.3-0.6 μm thick string. The string is attached to each pole of the ascospore and at maturity it uncoils and breaks down into many thinner, ca. 0.1 μm in diam, filaments. A similar appendage was illustrated for H. trullifera (Kohlm.) Jones et al. by Farrant (2). Outer morphology of ascus tip under SEM did not show any evidence for an apical thickening or apparatus.

Hypoxylon oceanicum Schatz

Mycotaxon 33: 413 (1988)

Figs. 9, A-G

Ascocarps 1-2 mm in diam, subglobose, solitary or united, ostiolate, short papillate, carbonaceous, black. Paraphyses 200-250 X 2-3 μm, septate, branching. Asci 160-200 X 14-20 μm, eight-spored, cylindrical, pedunculate, unitunicate, thin-walled, with apical ring stained blue with iodine (I+), with a flexible apical cushion at the apex. Ascospores 18-24 X 9-13 μm ($\bar{x}$= 20.8 X 10.6 μm), one-celled, subglobose to broad ellipsoid, with a germ slit, brown to dark brown.

Colonies on CMSWA white, sparse, 3.0-3.4 mm in diam after incubation for 2 weeks at 25 C. Multi-ascospore isolate [AN-1252 (IFO 32485)] and single-ascospore isolates [AN-1253 (IFO 32486) and AN-1254] were obtained from the specimen Na-O-1 (IFO H-12145).

Substrates: Submerged wood of B. gymnorrhyza.

Supecimens examined: Submerged wood of B. gymnorrhyza, Nakama River, Na-O-1, 4, 6.

The apical cushion of asci was seen under SEM as an undulate disc with a pore-like depression at the center.

Lineolata rhizophorae (Kohlm. & Kohlm.) Kohlm. & Volkm.-Kohlm. Mycol. Res. 94: 688 (1990)

Basionym: Didymosphaeria rhizophorae Kohlm. & Kohlm., Icones Fungorum Maris, Fasc. 4 & 5, Tabs. 62 and 62a, Figs. 1-19, J. Cramer, Weinheim (1967)

Figs. 10, A-G

Ascocarps 440-600 μm high (including a neck), 280-420 μm in diam, pyriform, partly or completely immersed, ostiolate, papillate, subcarbonaceous, dark brown to black, solitary or gregarious; necks 80-90 μm in diam at the apex, conical. Hamathecia (pseudoparaphyses?) 1.5-2 μm in diam, septate, branched and anastomosing, arising with asci from the base of the ascocarp venter. Asci 110-170 X 10-16 μm, eight-spored (infrequently four- and six-spored), cylindrical, short pedunculate, bitunicate, fissitunicate (physoclastic), with refractive apical apparatus, I-. Ascospores 26-34(-38) X 10-13 μm (four- or six-spored asci have long spores), ellipsoid, one-septate, slightly constricted at the septum, brown, with longitudinal striation of 0.2-0.3 μm wide, forking ribs.

Colonies on CMSWA olive grey to brown, 9-10 mm in diam after incu-

bation for 1 month at 25 C. Single-ascospore isolates [AN-1246 (IFO 32487), AN-1247 (IFO 32488) and AN-1248] were obtained from the specimen Shi-Ya-22 (IFO H-12146).

Substrates: Submerged wood of R. stylosa.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-1, 11, 14, 22, 28.

Massarina ramunculicola Hyde

Mycologia 83: 839 (1991)

Figs. 11, A-J

Ascocarps up to 450 μm high and 600 μm in diam, immersed or partly erumpent, with darkened areas on the wood around ascocarps, ostiolate, papillate, coriaceous, dark brown to black, solitary or gregarious. Hamathecia (pseudoparaphyses?) 1-2 μm in diam, filamentous, septate, branching, anastomosing. Asci 120-190 X 20-30 μm, eight-spored, clavate, pedunculate, bitunicate, fissitunicate, with a ring-like apical apparatus, I- but exposed endotunica was stained brown with iodine. Ascospores 34-45 X 11-13 μm, ellipsoid to fusiform, one-septate but in some spores having one more septum at each end, constricted at the septum, hyaline, surrounded by a sheath. The sheath breaks at ascospore poles, from which mucilaginous pads are released. Later the sheath and pads swells into a large mucilage surrounding whole spore.

Colonies on CMSWA light brown to light grey, 4 cm in diam after incubation for 5 weeks at 25 C. Single-ascospore isolates [AN-1199 (IFO 32489), AN-1200 (IFO 32490) and AN-1201] obtained from the specimen Shi-Ya-8 (IFO H-12147) produced black pycnidia (spermogonia?) on CMSWA, in which pyriform phialides (8-10 X 2-3 μm) produced ellipsoid, one-celled, hyaline conidia (spermatia?) (2-3 X 1 μm).

Substrates: Submerged wood of R. stylosa.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-8, 9.

In identifying this fungus, I compared material with authentic specimens of M. ramunculicola identified by Dr. K. D. Hyde and also with the holotype of M. velatospora (IMI 29770).

Quintaria lignatilis (Kohlm.) Kohlm. & Volkm.-Kohlm.
Bot. Mar. 34: 35 (1991)
Basionym: Trematosphaeria lignatilis Kohlm., Mar. Ecol. (P. S. Z. N. I.) 5: 365 (1984)

Figs. 12, A-H

Ascocarps obpyriform, immersed, ostiolate, papillate, carbonaceous, black, solitary or gregarious; black stromatic tissue surrounding neck in wood. Hamathecia (pseudoparaphyses?) 1.5-2 μm in diam, septate, branching and anastomosing. Asci 210-260 X 28-40 μm, eight-spored, cylindrical, pedunculate, bitunicate, fissitunicate, with an eccentric apical plate. The apical plate, at which ectotunica and endotunica unite, is a lid of the apical isthmus of the endotunica. When the ectotunica with the apical plate is detached from the endotunica, ascospores are released through the opened isthmus. Ascospores 52-74 X 12-20 μm ($\bar{x}$= 60.7 X 16 μm), fusiform, five-septate, constricted at the septa, hyaline to pale yellow. A chain of ascospores, which had possibly been contained in an endotunica, was often seen as having been forced out of the ostiole.

Colonies on CMSWA olive brown to dark brown, 2 cm in diam after incubation for 5 weeks at 25 C. Single-ascus isolate [AN-1193 (IFO 32491)] and single-ascospore isolates [AN-1194 (IFO 32492) and AN-1195] obtained from the specimen Shi-Ya-4 (IFO H-12148) produced ascocarp initials (?) on CMSWA.

Substrates: Submerged wood of R. stylosa.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-4, 12, 30.

Savoryella paucispora (Cribb & Cribb) Koch
Nordic J. Bot. 2: 169 (1982)
Basionym: Leptosphaeria paucispora Cribb & Cribb, Univ. Queensl. Pap., Dep. Bot. 4: 41 (1960)

Figs. 13, A-M

Ascocarps 200-240 μm high, 170-320 μm in diam, flask-shaped, ostiolate, papillate, superficial to immersed, dark brown; necks 240-330 X 50-72 μm, light brown. Catenophyses present. Asci 88-130 X 16-22 μm, two-spored, cylindrical, short pedunculate, with subapical slight retraction of cytoplasm in youg asci, with slightly thickened apex, I-. SEM observa-

tion revealed a pore or a depression at the ascus apex. Following dehiscence of the apex, ascospores are forcibly ejected. Thick ascus wall sometimes breaks laterally at the middle and cytoplasm is pushed out, suggestive of physoclastic bitunicate asci. This characteristic of asci might have led Cribb and Cribb (1960) originally to assign this fungus to Loculoascomycetes. Ascospores 42-56 X 14-18 μm ($\bar{x}$= 50.8 X 15.5 μm), three-septate, with brown central cells and hyaline end cells, smooth but with small warts close to endsepta on the central brown cells. The end cells are thin-walled and germinate hyphae.

Colonies on CMSWA brown to dark brown, 16-17 mm in diam after incubation for 6 weeks at 25 C. Single-ascospore isolates [AN-1276 (IFO 32493), AN-1277 (IFO 32494) and AN-1278] obtained from the specimen Na-O-4 (IFO H-12149) produced ascocarps on CMSWA, from around which ascospores were discharged.

Substrates: Submerged wood of B. gymnorrhyza.

Specimens examined: Submerged wood of B. gymnorrhyza, Nakama River, Na-O-4.

Presence or abscence of paraphyses is one of the taxonomic problems in this species and the genus. When Cribb and Cribb (1960) described this fungus as Leptosphaeria paucispora, they mentioned "paraphyses are poorly developed, and in some perithecia were difficult to detect;" and in Latin, "paraphysibus raris, simplicibus vel ramosis, usque ad 1 μm diam." However, Koch (1982) did not observe paraphyses in his specimens. Recently, Jones and Hyde (7) mentioned having "observed paraphyses, ---[which] are difficult to detect and may deliquesce in mature specimens," but presented no illustration of paraphyses. In this study, I observed catenophyses rather than paraphyses in ascocarps, but these do not seem to be identical with the paraphyses described by Cribb and Cribb (1960). I tried to examine the type material, but it was not available in any institution. In conclusion, further studies are neccessary to clarify whether this fungus has paraphyses or catenophyses or neither. This problem is also present in definition of the genus. The type species, S. lignicola Jones & Eaton, was first described as aparaphysate, but later Jones and Hyde (7) amended this to "paraphyses present, but sparse," but did not illustrate the paraphyses clearly in their Figures 5 and 6 (7). I examined the type material, IMI 129784, but could not observe any paraphyses on it. Further studies using fresh materials are necessary to

elucidate the presence or abscence of parphyses in S. lignicola.

Another taxonomic problem is of the apical structure of asci. Jones and Hyde (7) stated that the asci of all Savaryella species including their two new species have apical thickening containing a pore. However, the apical pore was not clearly shown in their light micrographs except for that of S. longispora Jones & Hyde. Therefore, it is not possible to confirm whether "the pores" are identical or not in all species of the genus. SEM studies on the apical structure, as shown in this study, for all species are necessary to resolve this problem.

Swampomyces triseptatus Hyde & Nakagiri

Sydowia 44: 122 (1992)

Figs. 14, A-H

Ascocarps pyriform, immersed, with long axis horizontal to oblique to the host surface, coriaceous, ostiolate, papillate, dark brown to black, solitary, developing under a darkened superficial pseudostroma; contents apricot coloured in mass; neck white to dull orange at the upper part. Paraphyses 1-2 μm in diam, branching, filamentous , hyaline. Asci 140-160 X 8-12 μm, eight-spored, cylindrical, short pedunculate, thin-walled, with ring-like structure at apex, apically thickened with a small central indentation visible under SEM, I-. Ascospores are released by splitting of ascus apex. Ascospores (14-)17-25 X 7-11 μm, uniseriate, ellispoid, three-septate, constricted at the septa, hyaline, with granular ornamentation visible under SEM.

Colonies on CMSWA white to hyaline at first, becoming dark brown with age, 4 cm in diam after incubation for 5 weeks at 25 C. Single-ascospore isolates [AN-1184 (IFO 32495), AN-1185 (IFO 32496) and AN-1186] were obtained from the specimen Shi-Ya-2 (IFO H-12150).

Substrates: Submerged wood of R. stylosa.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-1, 2, 5, 7, 8, 11, 12, 13, 14, 20, 22, 25, 28.

Verruculina enalia (Kohlm.) Kohlm. & Volkm.-Kohlm.

Mycol. Res. 94: 689 (1990)

Basionym: Didymosphaeria enalia Kohlm., Ber. Deutsch. Bot. Ges.

79: 28 (1966)

Figs. 15, A-I

Ascocarps subglobose to pyriform, partly or completely immersed, ostiolate papillate, clypeate, carbonaceous, black. Necks up to 200 X 80 μm, surrounded by black clypeus. Hamathecia (pseudoparaphyses?) 1-2 μm in diam, septate, branched. Asci 150-220 X 10-13 μm, eight-spored, cylindrical, pedunculated, bitunicate, physoclastic, with subapical thickening of endotunica, I- but ascus cytoplasm is stained orange with iodine, developing at the base of the ascocarp venter. Ascospores 18-22 X 9-11 μm ($\bar{x}$= 20.6 X 10.3 μm), uniseriate, ellipsoid, one-septate, constricted at the septum, brown, seen as methalic reddish blue under phase contrast, verrucose with 0.3-0.7 μm in diam, rounded warts on the surface, surrounded by an evanescent spore sheath or ascus cytoplasm remnant which is stained orange with iodine.

Colonies on CMSWA light brown, cottony, 15-20 mm in diam after incubation for 4 weeks at 25 C. Single-ascospore isolates [AN-1243 (IFO 32497), AN-1244 (IFO 32498) and AN-1245] obtained from the specimen Shi-Ya-17 (IFO H-12151) produced ascocarps on CMSWA, from which ascospores were ejected around.

Substrates: Submerged wood of R. stylosa and B. gymnorrhyza.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-17, 20, 21, 26, and B. gymnorrhyza, Nakama River, Na-0-5.

Deuteromycetes

Cirrenalia tropicalis Kohlm.

Mycologia 60: 267 (1968)

Figs. 16, A-B

Conidiophores 16-76 μm long, 2.5-4 μm in diam, macronematous, mononematous, light brown. Conidiogenous cells integrated, determinate. Conidia solitary, produced holoblastically, helicoid, mostly 1 to 1.5 times contorted, 7-9 septate, not or slightly constricted at the septa, brown to dark brown; spirals 32-40 μm in diam; cells increasing in diameter from base to apex; terminal cell 8-16 μm high, 11-16 μm in diam.

Colonies on CMSWA dark brown to black. Single-conidium isolates [AN-1150 (IFO 32499) and AN-1151 (IFO 32500)] obtained from the specimen

Shi-Ya-0 (IFO H-12152) produced conidia abundantly on CMSWA.

Substrates: Submerged wood of R. stylosa and B. gymnorrhyza.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-0 and B. gymnorrhyza, Nakama River, Na-0-2.

Dictyosporium elegans Corda

Weitenweber's Beitrage ...: 87 (1836)

Figs. 17, A-C

Conidiophores micronematous, mononematous, hyaline to light brown. Conidiogenous cells integrated, determinate. Conidia 32-44 μm long, 18-32 μm wide, 8-11 μm thick, solitary, produced holoblastically, branched, cheiroid, composed of 5-7 rows of cells all approximately the same length.

Colonies on CMSWA white to hyaline. Single-conidium isolates [AN-1196 (IFO 32501), AN-1197 (IFO 32502) and AN-1198] obtained from the specimen Shi-Ya-4 (IFO H-12153) produced conidia abundantly on CMSWA.

Substrates: Submerged wood of R. stylosa.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-4.

Mycocentrolobium platysporum Goos

Mycologia 62: 172 (1970)

Figs. 18, A-K

Conidiophores micronematous, mononematous, short, hyaline. Conidiogenous cells integrated, determinate. Conidia up to 90 μm long, up to 120 μm wide, up to 25 μm thick, solitary, produced holoblastically, dictyosporous, flattened in one plane, variable in shape, fanwise or lobed, shiny dark brown, composed of two muriform fans united like a pair of scallop shells. From the upper edge of matured conidium, ampulliform phialids (?) are often formed.

Colonies on CMSWA white at first, then becoming olive green, 15-17 mm in diam after incubation for 25 days at 25 C. Single-conidium isolates [AN-1249 (IFO 32503), AN-1250 (IFO 32504) and AN-1251] obtained from the specimen Shi-Ya-29 (IFO H-12154) produced conidia abundantly on and in CMSWA.

Substrates: Submerged wood of R. stylosa.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-29.

Conidium development was first observed under SEM. Clavate conidium initials first develop into a stipitate cup, then opposing sides of the cup elongate upward and attach to each other at the apex of the two lobes. The lobes continue to elongate upward and laterally until they attach to each other along their edges. Septation in the lobes continues to become muriform. The young conidia resemble a pair of scallop shells. At maturity, the two lobes unite firmly and the middle line between them becomes obscure. Observation of broken conidia revealed that the two lobes are united along the facing sides and no space is left in the conidium. The phialide-like cells come from between the two lobes in mature conidia. But it is not clear whether these cells are phialides producing secondary conidia or germinating hypha.

Phragmospathula phoenicis Subram. & Nair

Antonie van Leeuwenhoek 32: 384 (1966)

Figs. 19, A-J

Conidiophores 8-14 μm long, 6-8 μm in diam, macronematous, mononematous, arising from creeping hyphae on wood, crowded, unbranched, short, clavate to cup-shaped, light brown. Conidiogenous cells integrated, percurrent. Conidia 35-42 X 9-11 μm, solitary, holoblastically produced on 2-3 times percurrent proliferating conidiophores, spathulate, three-septate; the apical cell hyaline, shortest (2-3 μm); two middle cells brown, broadest; the basal cell hyaline, longest (13-21 μm), vacating cytoplasm at maturity. Germination occurrs from the apical cell.

Colonies on CMSWA white to light brown. Single-conidium isolates [AN-1241 (IFO 32505) and AN-1242 (IFO 32506)] obtained from the specimen Shi-Ya-14 (IFO H-12155) produced conidia abundantly on CMSWA. Conidiophores arise mostly from hyphal tufts composed of 2-3 hyphae.

Substrates: Submerged wood of R. stylosa.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-0, 14, 28.

The above conidial measurements differed from the description (25-35 X 7-10 μm) by Ellis (1). In the original description, Subramanian and

Nair (1966) recorded only the length of the apical cell (3.3-6.0 μm) and the basal cell (9.9-26.4 μm), not the total length and width of conidia, and thus it is impossible to compare the sizes with those of the present fungus. However, conidial length was found to be variable, since the isolates produced remarkably shorter conidia, 25-33 X 8-11 μm (with 1.5-2 μm long apical cell and 9-14 μm long basal cell), on CMSWA than on natural substrates, the latter being similar to Ellis's measurements. Therefore, I tentatively identified this isolate as P. phoenicis. Further studies are necessary to clarify the variation of conidial size in this species.

Stachybotrys mangiferae Misra & Srivastava
Trans. Br. Mycol. Soc. 78: 556 (1982)

Figs. 20, A-C

Conidiophores 60-120 μm long, 3-4 μm in diam, macronematous, mononematous, branched, septate, hyaline to light brown, verrucose, covered with granules. Conidiogenous cells 10-14 X 3-4 μm, discrete, phialidic, borne in groups of 6-8 at the apex of conidiophore, clavate, light brown. Conidia 5-7 X 3-4 μm, ovoid to ellispoid, verrucose, greyish brown, aggregated in a globose drop.

Colonies on CMSWA white to hyaline with black conidial drops on conidiophores, grow fast. Multi-conidia isolates [AN-1177 (IFO 32507) and AN-1178 (IFO 32508)] obtained from the specimen Shi-Ya-5 (IFO H-12156) produced conidia abundantly on CMSWA.

Substrates: Submerged wood of R. stylosa.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-5.

Trichocladium achrasporum (Meyers & Moore) Dixon in Shearer and Crane
Mycologia 63: 244 (1971)
Basionym: Culcitalna achraspora Meyers & Moore, Amer. J. Bot. 47: 349 (1960)

Figs. 21, A-B

Conidiophores nearly absent or short, mononematous, hyaline, pro-

duced laterally on the mycelium. Conidiogenous cells integrated, determinate. Conidia 20-26 X 11-13 μm, ovate to obpyriform, two to three-septate, constricted and occasionally with black bands at the septa, brown to dark brown; basal cell subhyaline.

Colonies on CMSWA olive brown to dark brown, 4 cm in diam after incubation for 5 weeks at 25 C. Single-conidium isolates [AN-1182 (IFO 32509) and AN-1183 (IFO 32510)] obtained from the specimen Shi-Ya-4 (IFO H-12157) produced conidia abundantly on CMSWA.

Substrates: Submerged wood of R. stylosa and B. gymnorrhyza.

Specimens examined: Submerged wood of R. stylosa, Shiira River, Shi-Ya-4 and B. gymnorrhyza, Shiira River, Shi-O-3.

Frequency of occurrence of fungi

Of the 45 wood samples of R. stylosa and 15 samples of B. gymnorrhyza, 34 and 9 samples, respectively, were found to be colonized by mangrove fungi. The frequency of occurrence of fungal species is summarized in Table 1.

Caryosporella rhizophorae, Dactylospora haliotrepha and Swampomyces triseptatus were commonly found. The former two have frequently been reported from mangroves in various parts of the world (3, 4, 5, 6, 8, 10). The third species is a very recently described one, but (as Sphaerulina cf. oraemaris) it was reported to be one of the common fungi on mangroves in Thailand (6).

Apparent host specificity or preference was seen in some species; e.g., C. rhyzophorae, Lineolata rhizophorae and Swampomyces triseptatus was found only on Rhizophora stylosa, while Hypoxylon oceanicum was found only on Bruguiera gymnorrhyza. This relationship is not certain, however, because of the small sample size. Host-specific fungal distribution is known on various species of mangrove trees (4). This fungal distribution may be affected by differences of substrates, e.g., chemical composition of wood, or environments where the mangrove trees and the inhabiting fungi grow. The two host trees examined here occupy different habitats in the mangrove area. Rhizophora stylosa grows mainly at river mouth close to the sea with higher salinity, while Bruguiera gymnorrhyza inhabits the middle to upper reach with lower salinity. In addition to these differences, the stage of decomposition of wood (10), the presence

Table 1. Occurrence of fungi from intertidal decomposing mangrove wood of Rhizophora stylosa and Bruguiera gymnorrhyza.

Fungal species	Substrate	
	R. stylosa	B. gymnorrhyza
Aigialus grandis	1a)	1b)
Aigialus sp. 1*	1	0
Aniptodera limnetica	0	1
Aniptodera longispora	1	0
Caryosporella rhyzophorae	15	0
Cucullosporella mangrovei	1	0
Dactylospora haliotrepha	11	2
Halosarpheia abonis	2	0
Halosarpheia fibrosa	1	0
Halosarpheia sp. 1*	1	3
Hypoxylon oceanicum	0	3
Leptosphaeria perviana?*	1	0
Lignincola sp. 1*	2	1
Lignincola sp. 2*	1	0
Lignincola sp. 3*	0	1
Lulworthia sp. 1*	0	1
Lineolata rhizophorae	5	0
Massarina ramunculicola	2	0
Quintaria lignatilis	3	0
Remispora salina?*	1	0
Savoryella paucispora	0	1
Savoryella sp. 1*	4	0
Swampomyces triseptatus	13	0
Swampomyces sp. 1*	1	0
Verruculina enalia	4	1
Cirrenalia tropicalis	0	1
Dictyosporium elegans	1	0
Mycocentrolobium platysporum	1	0
Phragmospathula phoenicis	2	0
Stachybotrys mangiferae	1	0
Trichocladium achrasporum	1	1

* Unidentified species.

a) Numbers of samples on which the fungus was observed out of 45 samples of R. stylosa.

b) Numbers of samples on which the fungus was observed out of 15 samples of B. gymnorrhyza.

or absence of bark on wood (6), season, fungal species-species interaction, and various environmental factors should be examined to clarify the factors affecting fungal distribution. Further studies, especially combined with cultural studies, and accumulation of flora data on individual mangrove tree species are necessary.

I wish to express my thanks to Dr. K. D. Hyde (Qld. D. P. I., Australia) and the International Mycological Institute (UK) for lending me authentic specimens.

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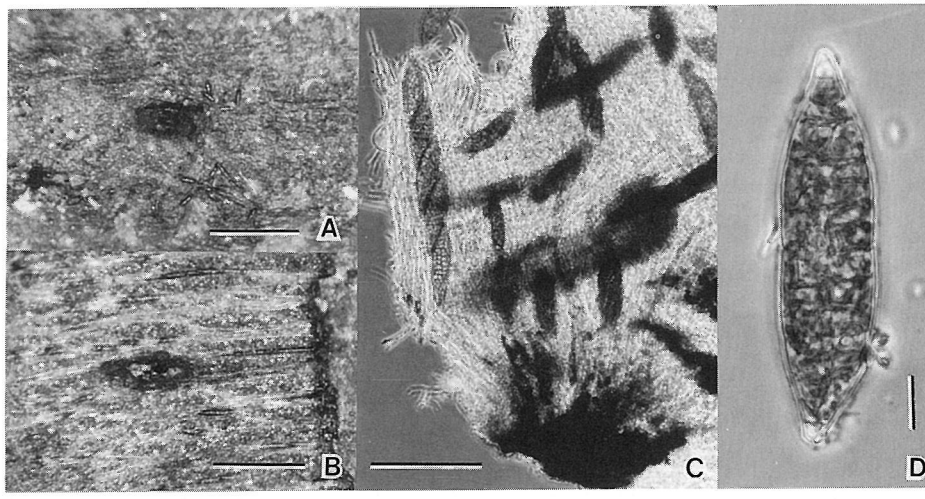

Fig. 1, A-D. Aigialus grandis. A. Front view of immersed ascocarp showing an ostiole opening. B. Surface-scraped wood showing ascocarp with thick peridium. C. Asci and pseudoparaphyses (?). D. Ascospore. (Bars: A, B = 500 μm; C = 100 μm; D = 10 μm)

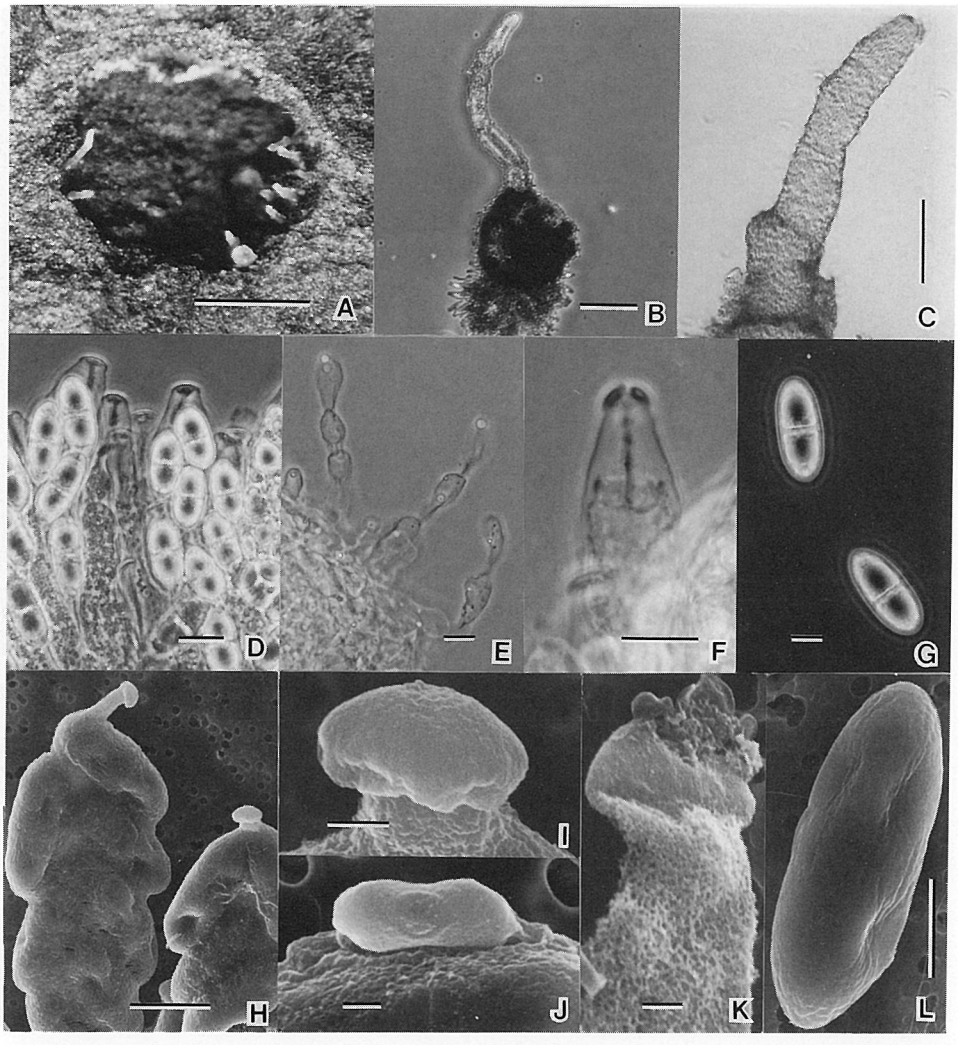

Fig. 2, A-L. Aniptodera limnetica. A. Ascocarp necks emerging from wood. B. Ascocarp. C. Ascocarp neck with band-like hyphal structures. D. Asci with a thick apical plate. E. Catenophyses. F. Fissured ascus apex. G. Ascospores. H. Asci. I-J. Apical plate with pore-like structure. K. Dehiscent ascus apex. L. Ascospore. (Bars: A = 500 μm; B = 100 μm; C = 50 μm; D-H = 10 μm; I-K = 1 μm; L = 5 μm)

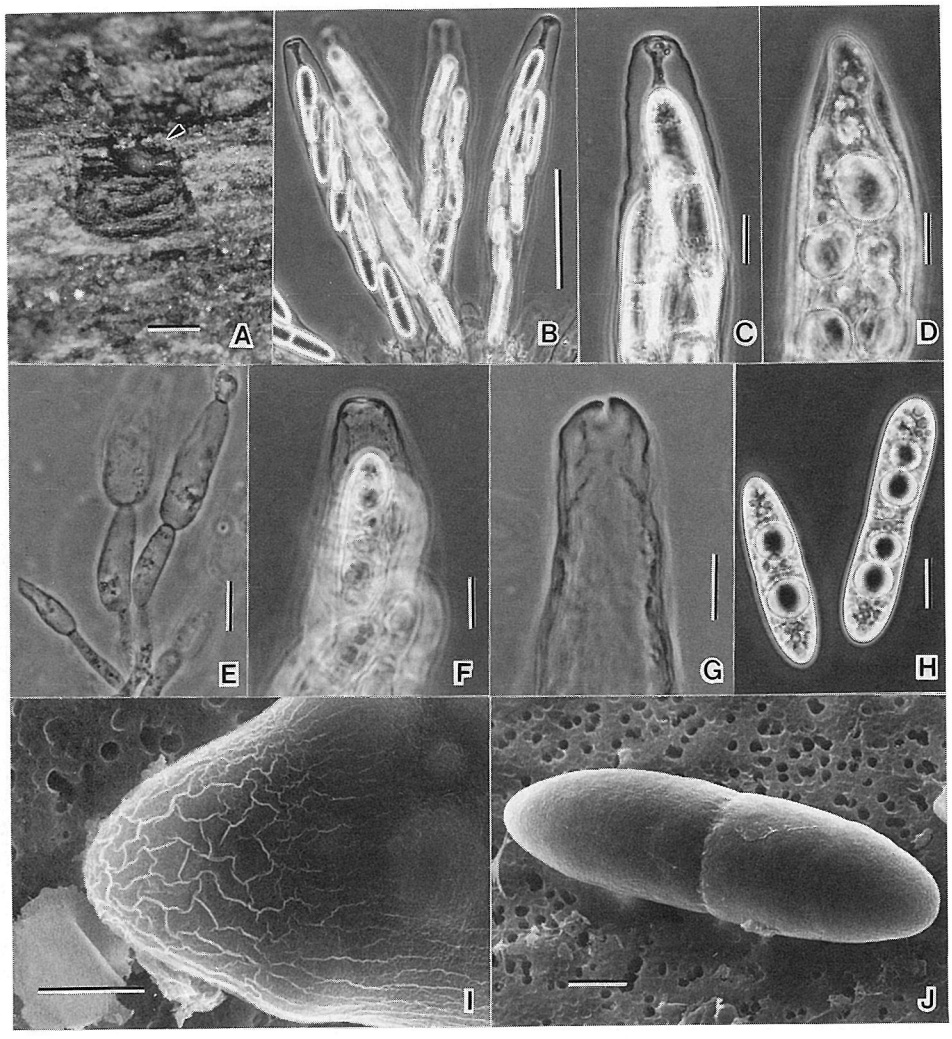

Fig. 3, A-J. Aniptodera longispora. A. Side view of immersed ascocarp lying horizontally (arrow). B. Asci. C. Apex of mature ascus with a refractive pore or plug-like structure. D. Subapex of young ascus with swollen cytoplasm. E. Catenophyses. F. A refractive structure at ascus apex. G. Dehiscent ascus apex. H. Ascospores. I. Ascus apex. J. Ascospore. (Bars: A = 500 μm; B = 50 μm; C-H = 10 μm; I, J = 5 μm)

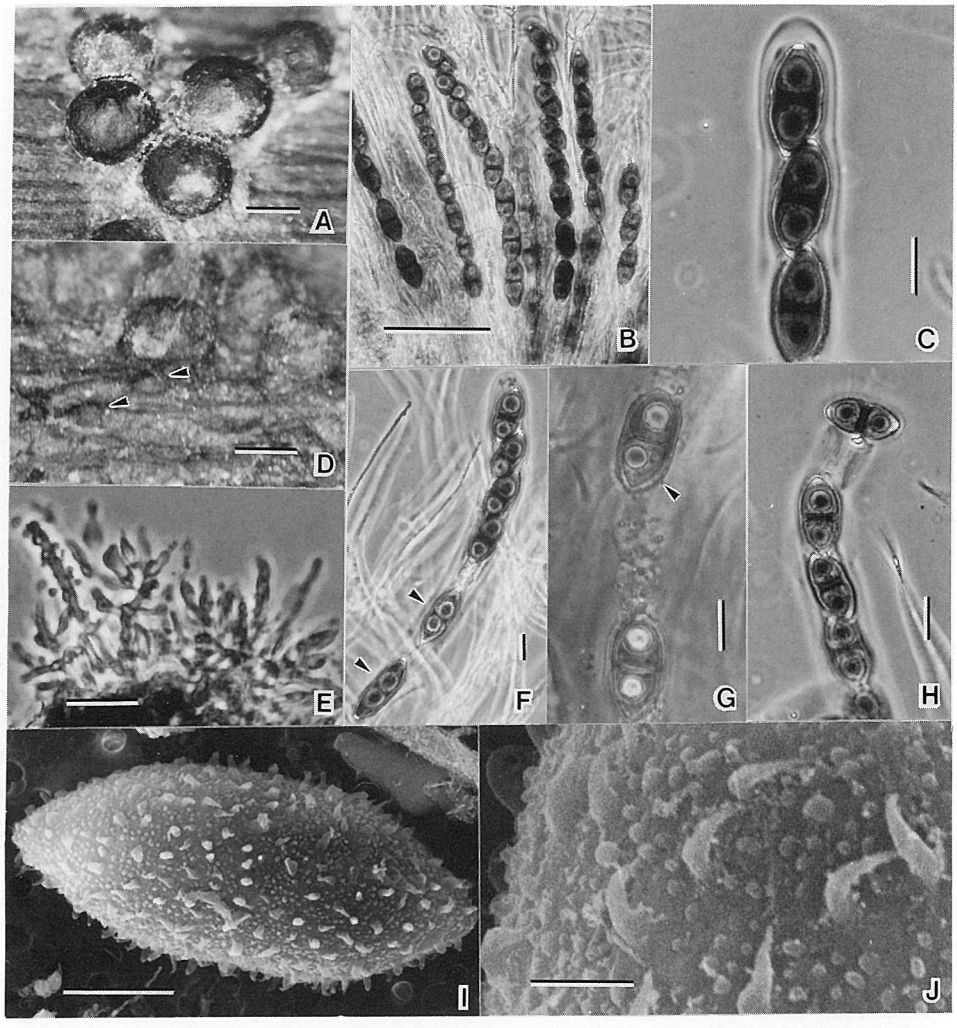

Fig. 4, A-J. Caryosporella rhizophorae. A. Front view of ascocarps on wood. B. Asci and pseudoparaphyses (?). C. Ascus apex with ectoascus. D. Pycnidia (or spermogonia?) (arrows) formed close to ascocarps. E. Phialides in pycnidia producing conidia (or spermatia?). F. Six-spored ascus with two larger spores (arrows) and four spores of ordinary size. G. Young ascospores with thick and verrucose outer wall (arrow) which is separated from inner wall. H. Ascus and released ascospore. I-J. Ascospore surface with flexible conical spines and small granules. (Bars: A, D = 500 μm; B = 50 μm; C, E-H = 10 μm; I = 5 μm; J = 1 μm)

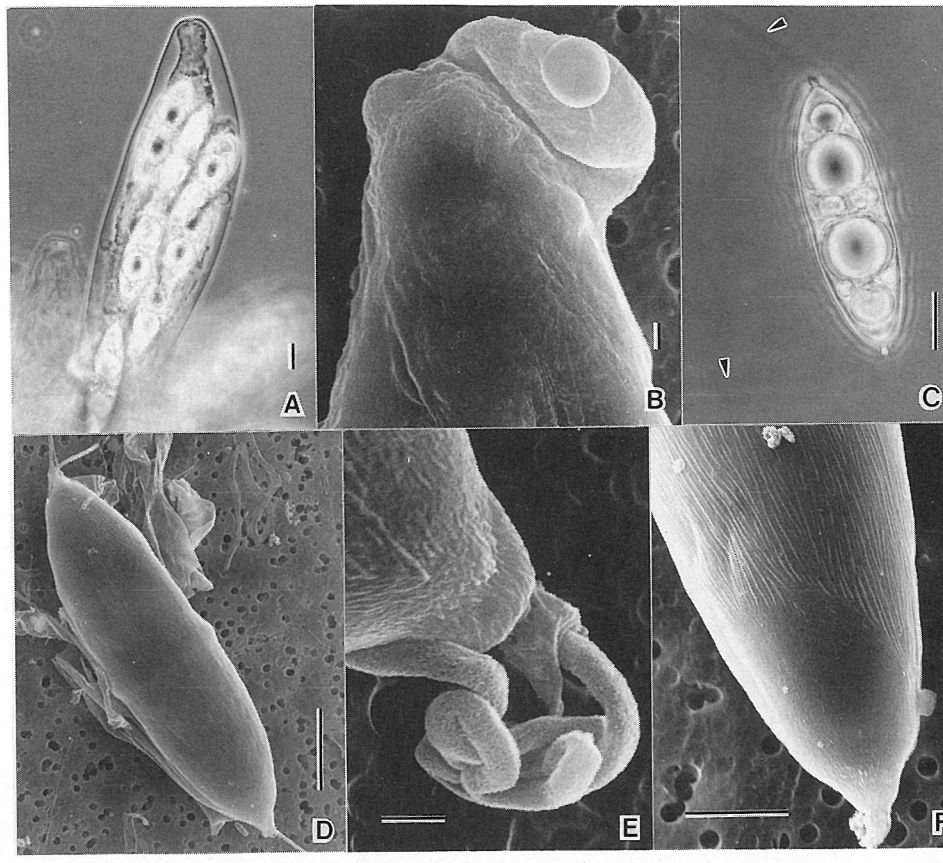

Fig. 5, A-F. Cucullosporella mangrovei. A. Ascus with thickened apex and a refractive structure and with subapical swelling of cytoplasm. B. Thickened ascus apex and a plug-like structure. C. Ascospore with thread-like appendages (arrows). D. Ascospore. E. Appendage arising from tube-like extension at the end of ascospore. F. Striation on ascospore surface. (Bars: A, C, D = 10 μm; B, E = 1 μm; F = 5 μm)

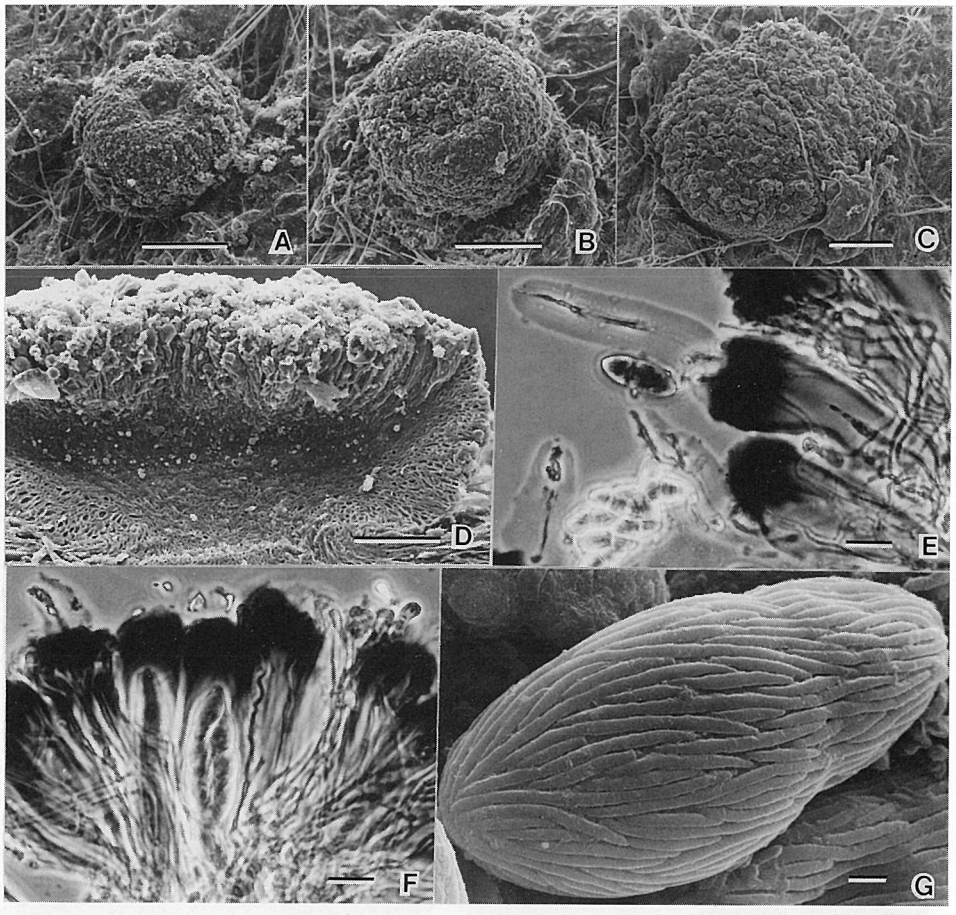

Fig. 6, A-G. Dactylospora haliotrepha. A-C. Development stages of ascocarp. D. Vertical section of ascocarp. E. Fissitunicate ascus showing elongated endotunica. F. Asci covered with gelatinous sheath which is stained blue by iodine. G. Ascospore with longitudinally woven ribs on the surface. (Bars: A-C = 100 μm; D = 50 μm; E, F = 10 μm; G = 1 μm)

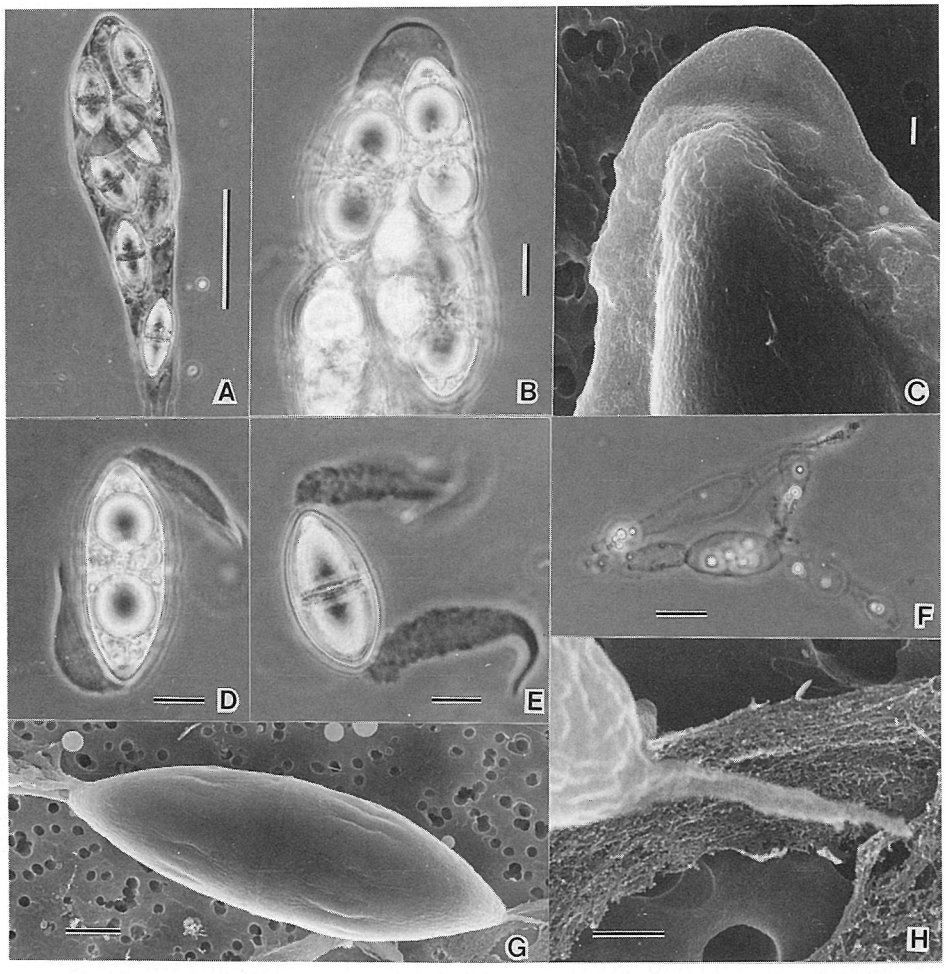

Fig. 7, A-H. Halosarpheia abonis. A. Ascus. B. Thickened ascus apex. C. Ascus apex under SEM. D. Ascospore with scoop-like appendages. E. Ascospore with uncoiled appendages. F. Catenophyses. G. Ascospore. H. Appendage composed of fine fibers. (Bars: A = 50 μm; B, D-F = 10 μm; C, H = 1 μm; G = 5 μm)

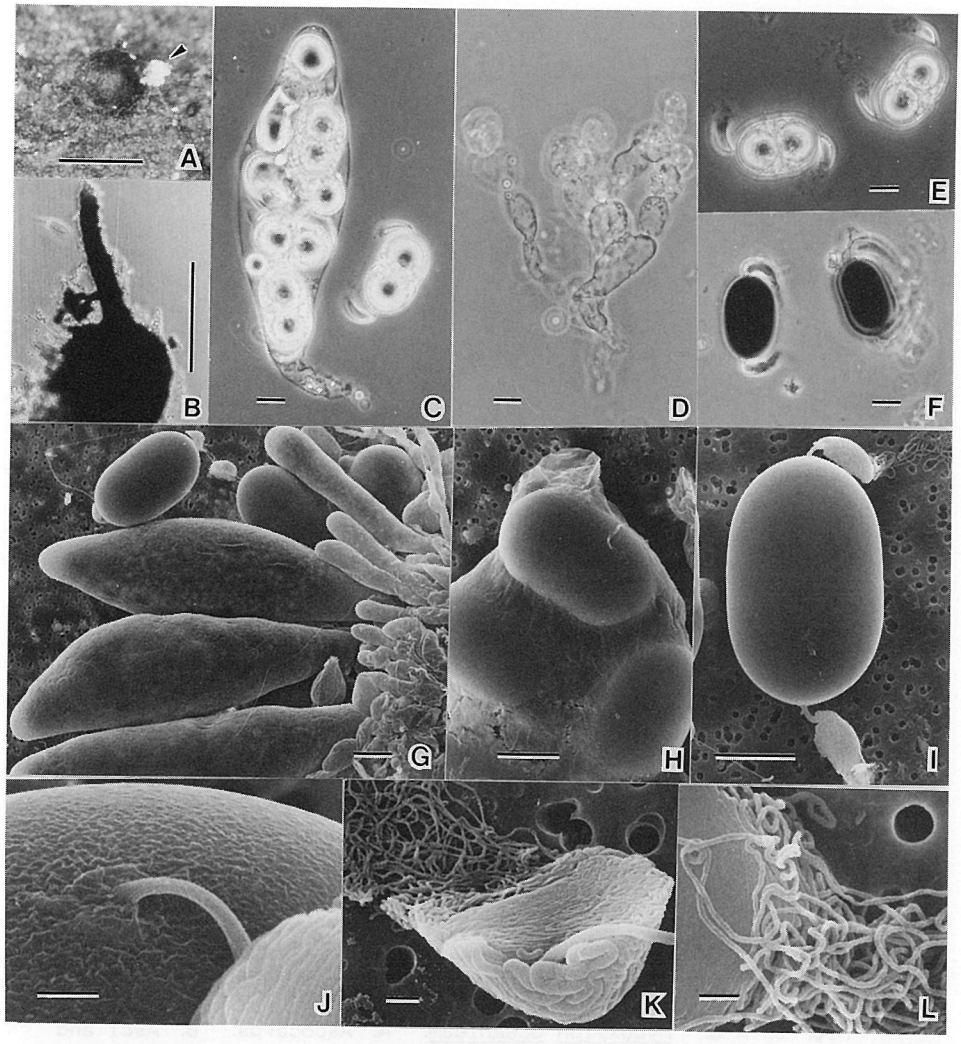

Fig. 8, A-L. Halosarpheia fibrosa. A. Ascocarp with a neck, from which asci and ascospores are ejected (arrow). B. Ascocarp with a long neck. C. Thin-walled ascus and an ascospore. D. Catenophyses. E. Ascospores with cap-like appendages. F. Ascospores densely stained brown with iodine. G. Asci. H. Thin-walled ascus apex. I. Ascospore with appendages. J. Appendage string attaching to the pole of ascospore. K. Appendage composed of filament. L. Uncoiling filament of appendage. (Bars: A, B = 500 μm; C-I = 10 μm; J-L = 1 μm)

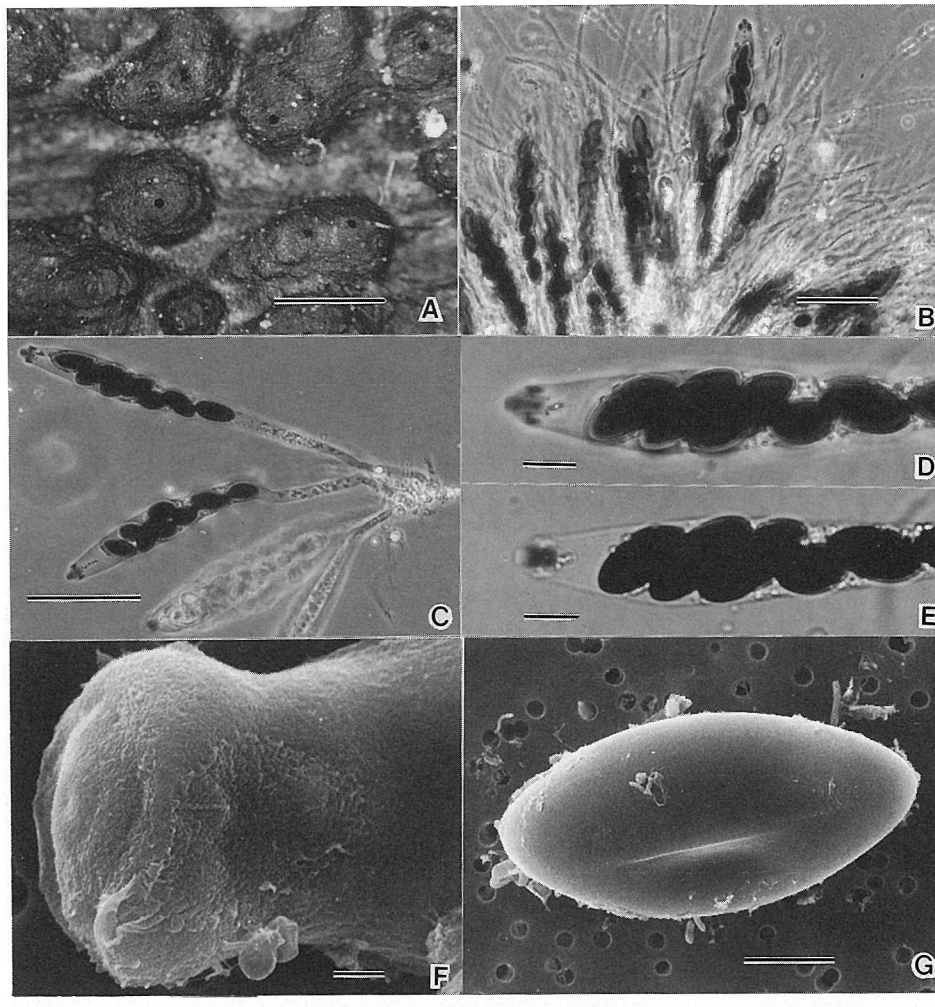

Fig. 9, A-G. Hypoxylon oceanicum. A. Ascocarps. B. Asci and paraphyses. C. Mature and immature asci. D. Ascus apex under phase-contrast light microscope. E. Apical ring of ascus stained with iodine under ordinary light microscope. F. Undulate disc with a pore-like depression at ascus apex. G. Ascospore with a germ slit. (Bars: A = 1mm; B, C = 50 μm; D, E = 10 μm; F = 1 μm; G = 5 μm)

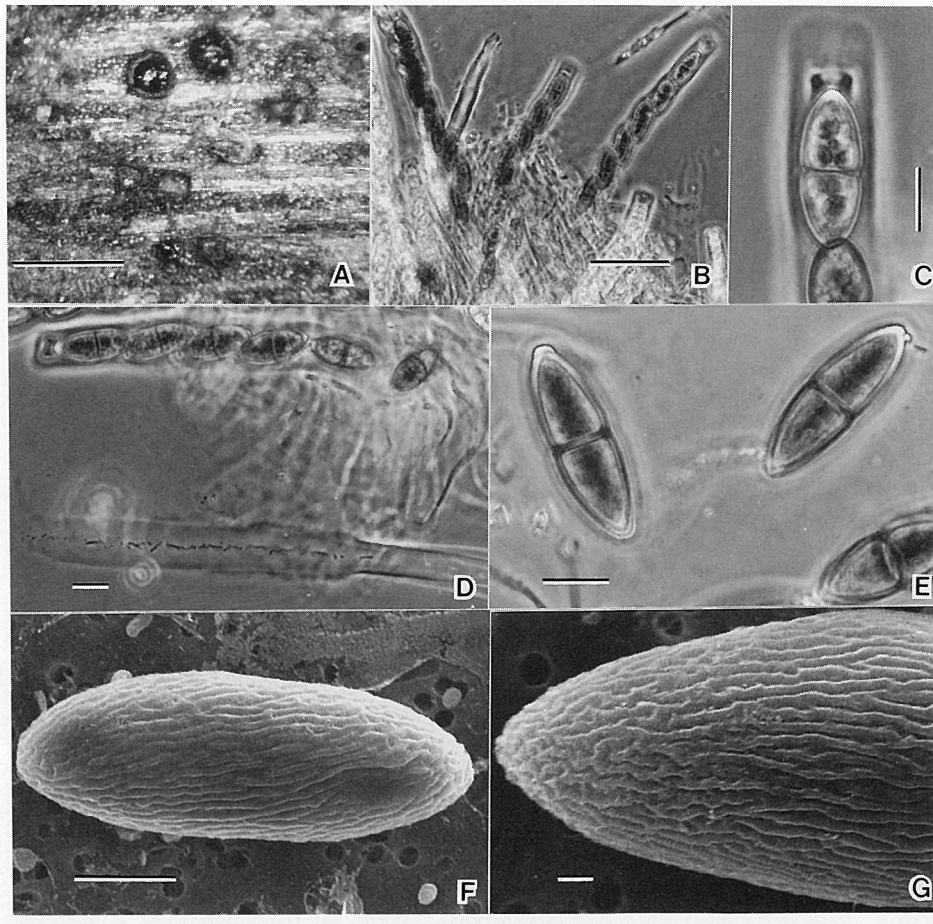

Fig. 10, A-G. Lineolata rhizophorae. A. Surface-scraped wood showing immersed ascocarps. B. Asci and pseudoparaphyses (?). C. Ascus apex with refractive apparatus. D. Physoclastic bitunicate asci. E. Ascospores. F-G. Ascospore with longitudinal striation of ribs. (Bars: A = 500 μm; B = 50 μm; C-E = 10 μm; F = 5 μm; G = 1 μm)

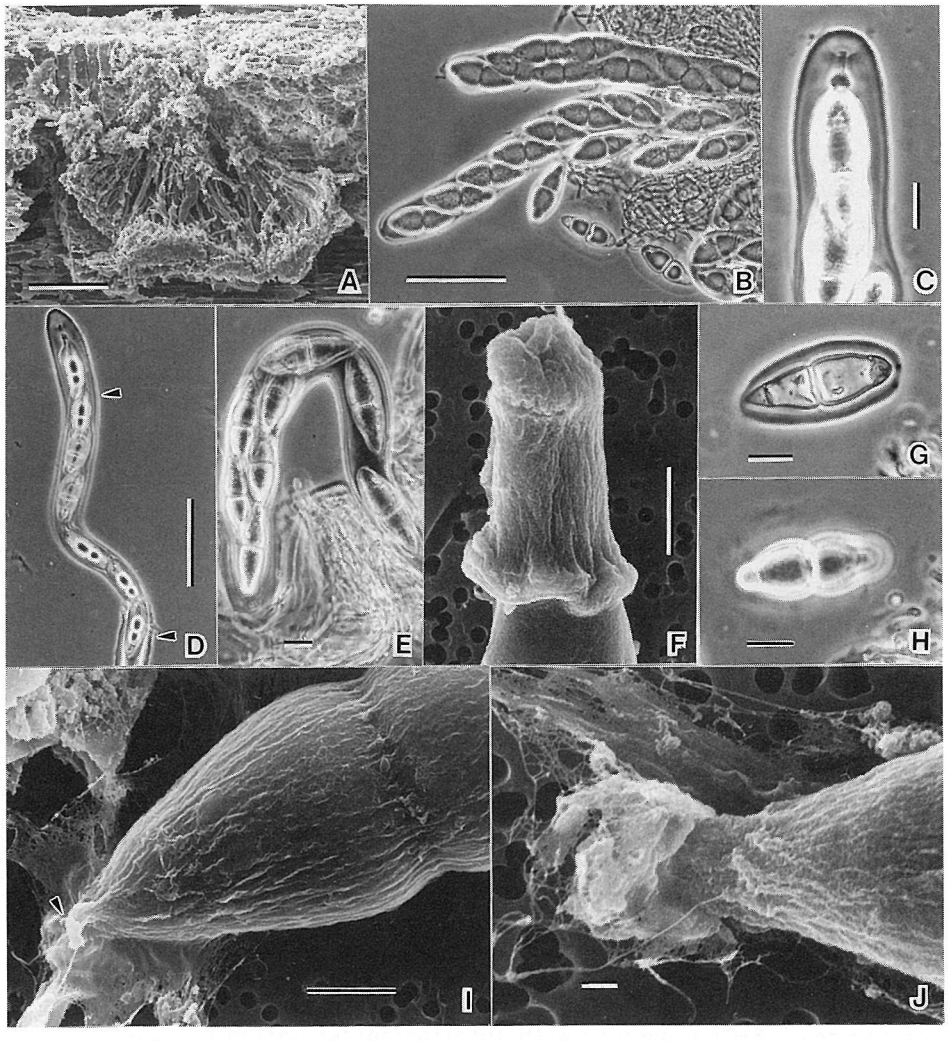

Fig. 11, A-J. Massarina ramunculicala. A. Vertical section of immersed ascocarp. B. Asci and pseudoparaphyses (?). C. Ring-like apical apparatus of ascus. D. Bitunicate ascus. Arrows shows ectotunica. E. Elongated and exposed endotunica stained brown with iodine. F. Ascus apex covered with ectotunica. G. Ascospore surrounded by sheath. H. Ascospore surrounded by mucilage. I-J. Ascospore pole releasing mucilage through tube-like structure (arrow). (Bars: A = 100 μm; B, D = 50 μm; C, E, G, H =10 μm; F, I = 5 μm; J = 1 μm)

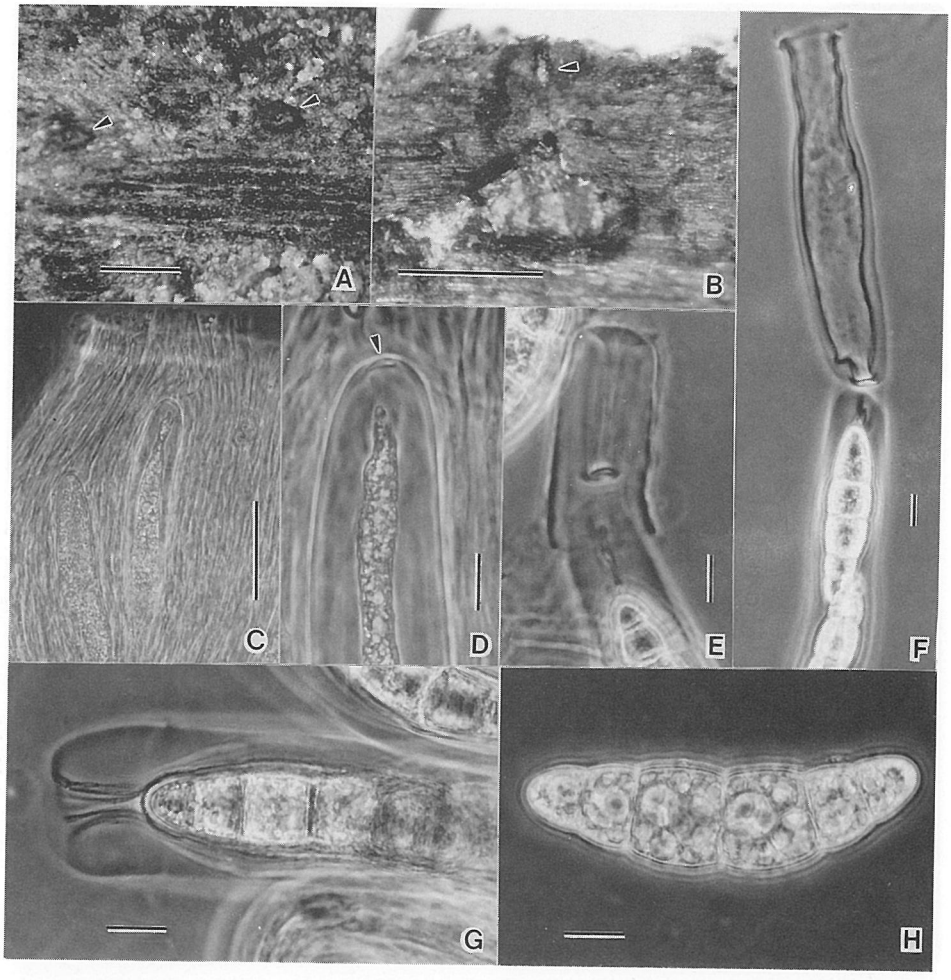

Fig. 12, A-H. Quintaria lignatilis. A. Surface view of immersed ascocarps. Arrows show ostiole openings. B. Vertical section of immersed ascocarp with an ostiole (arrow). C. Asci and pseudoparaphses (?). D. Ascus apex with apical plate (arrow). E-F. Inverting and completely inverted ectotunica attaching to endotunica at apical plate. G. Apex of endotunica releasing ascospores. H. Ascospore. (Bars: A, B = 500 μm; C = 50 μm; D-H = 10 μm)

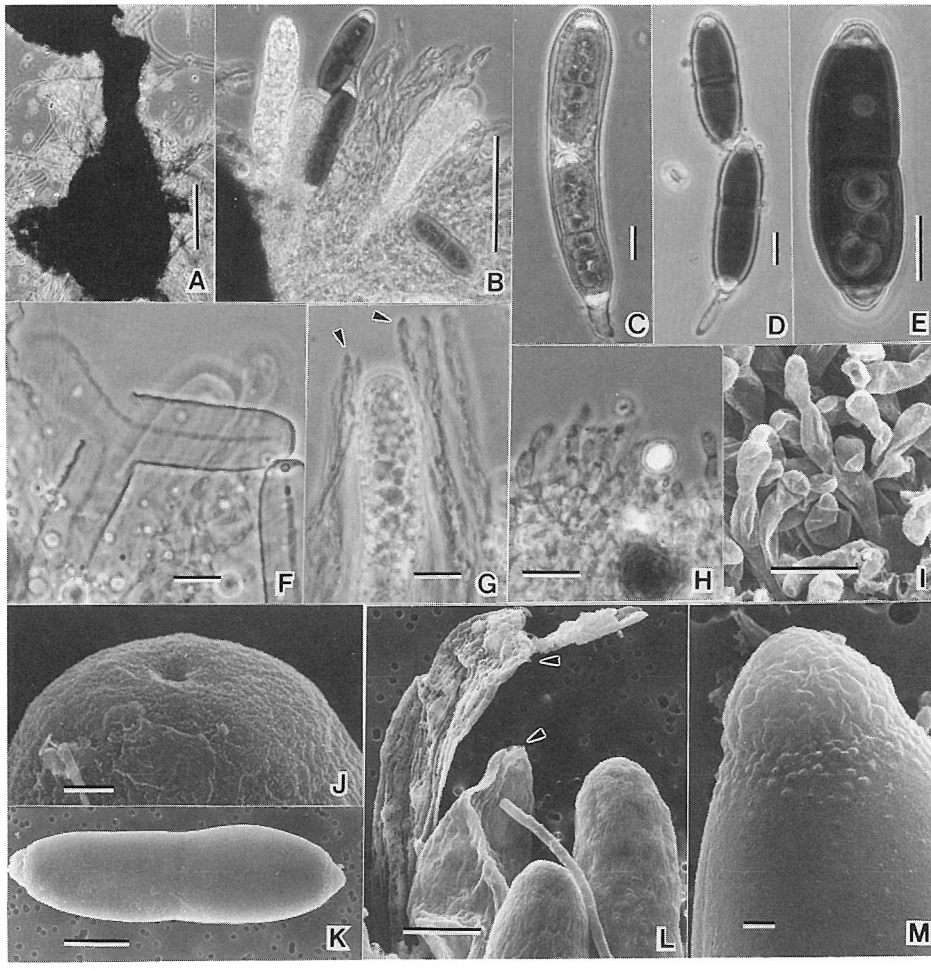

Fig. 13, A-M. Savoryella paucispora. A. Ascocarp. B. Mature and immature asci. C. Young ascus. D. Mature ascus. E. Ascospore. F. Thick-walled ascus releasing cytoplasm from the middle, resembling physoclastic bitunicate ascus. G. Spore-released asci with split apex (arrows). H-I. Catenophyses. J. Pore (or depression) of ascus apex. K. Ascospore. L. Dehiscent ascus apices (arrows). M. Spore end with small warts on the surface. (Bars: A = 100 μm; B, C = 50 μm; D-I, K, L = 10 μm; J, M = 1 μm)

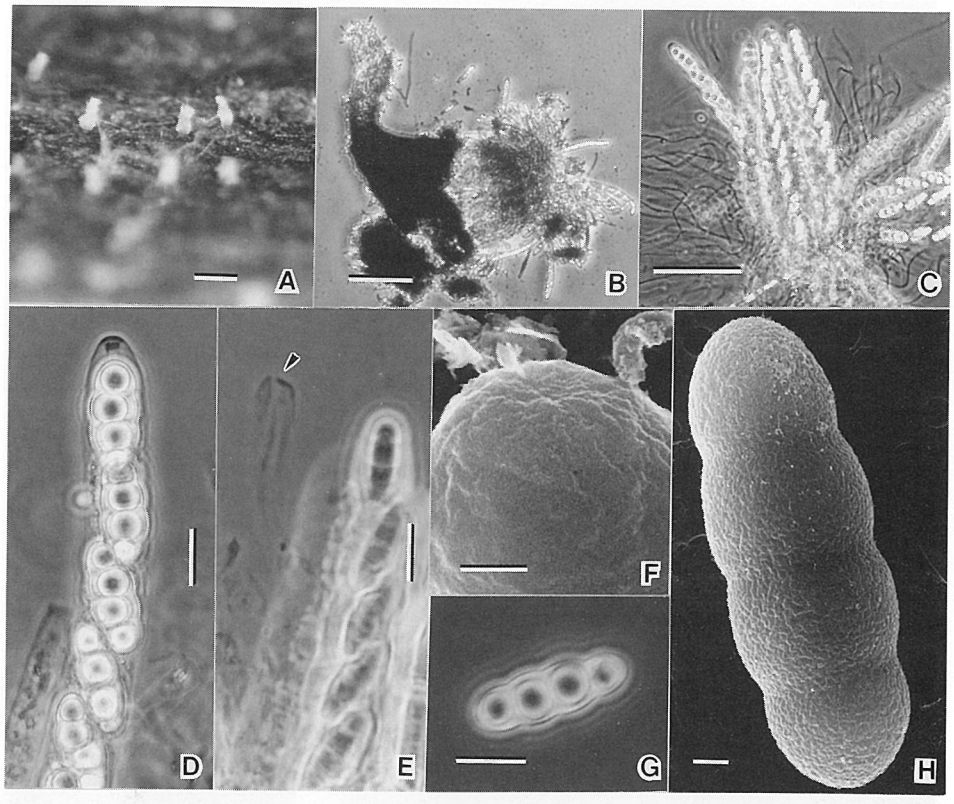

Fig. 14, A-H. Swampomyces triseptatus. A. Necks of immersed ascocarps. B. Horizontally lying ascocarp with a perpendicularly bending neck. C. Asci and paraphyses. D. Ascus apex with apical thickening and ring-like structure. E. Split apex of spore-released ascus. F. Ascus apex with central indentation. G. Ascospore. H. Ascospore with granular surface. (Bars: A, B = 100 μm; C = 50 μm; D, E, G = 10 μm; F, H = 1 μm)

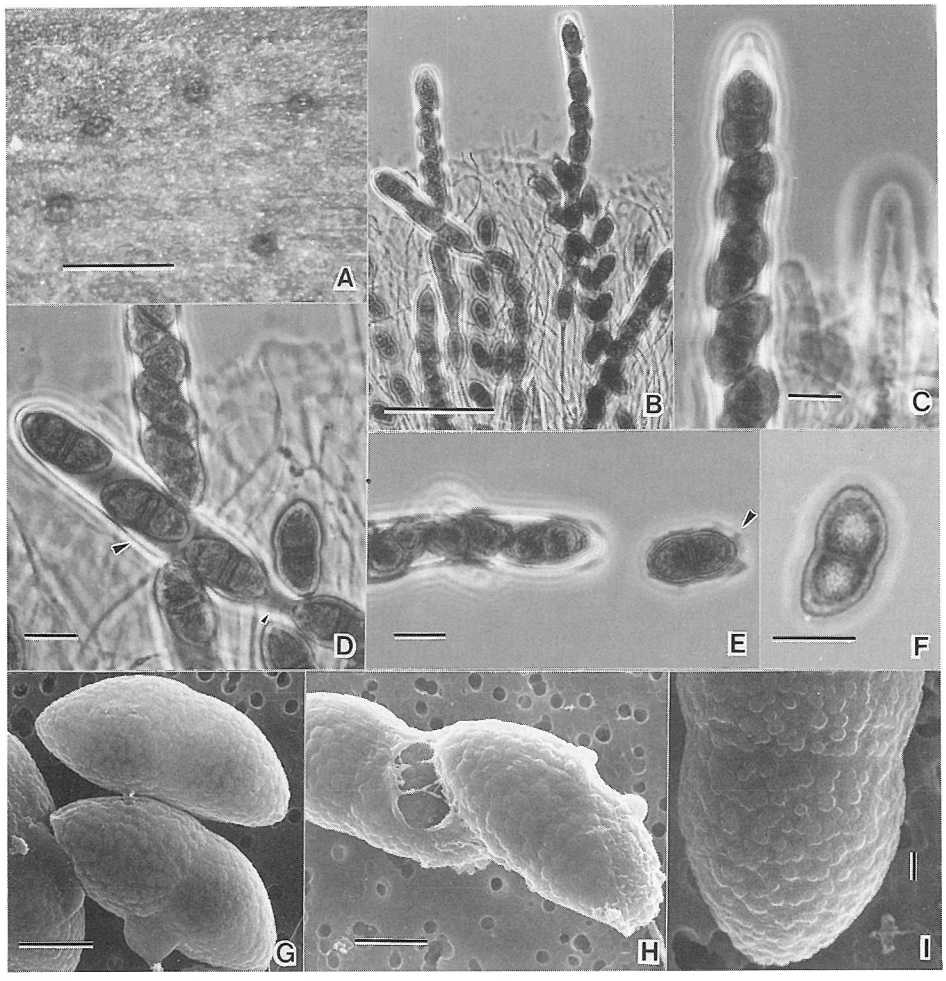

Fig. 15, A-I. Verruculina enalia. A. Surface view of immersed ascocarps showing ostiole openings. B. Asci and pseudoparaphyses (?). C. Ascus apex with subapical thickening of endotunica. D. Physoclastic bitunicate ascus showing ectotunica (large arrow) and endotunica (small arrow). E. Ascospore released from ascus apex, surrounded by mucilage (arrow). F. Ascospore. G-H. Ascospores surrounded by mucilage (or ascus cytoplasm remnant?). I. Verrucose spore surface with rounded warts. (Bars: A = 500 μm; B = 50 μm; C-F = 10 μm; G, H = 5 μm; I = 1 μm)

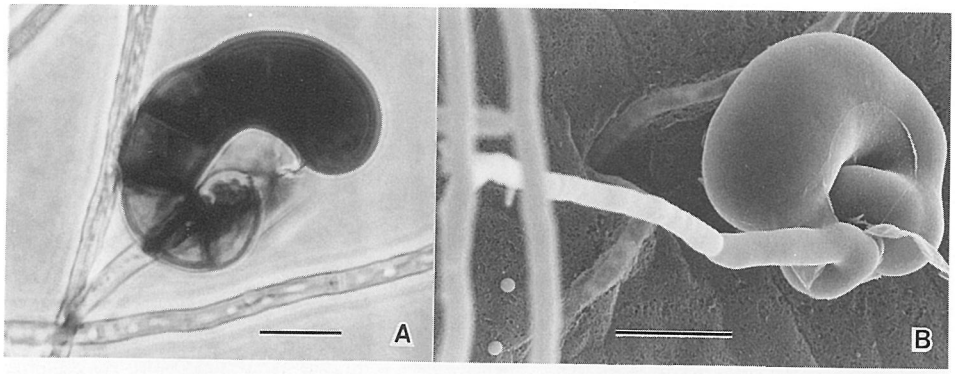

Fig. 16, A-B. Cirrenalia tropicalis. A-B. Conidium. (Bars: A, B = 10 μm)

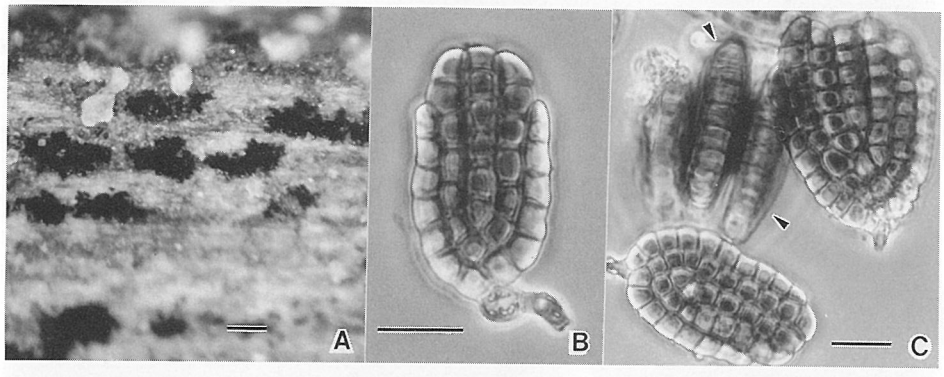

Fig. 17, A-C. Dictyosporium elegans. A. Conidial mass produced on wood. B. Conidium. C. Surface views and side views (arrows) of conidia. (Bars: A = 100 μm; B, C = 10 μm)

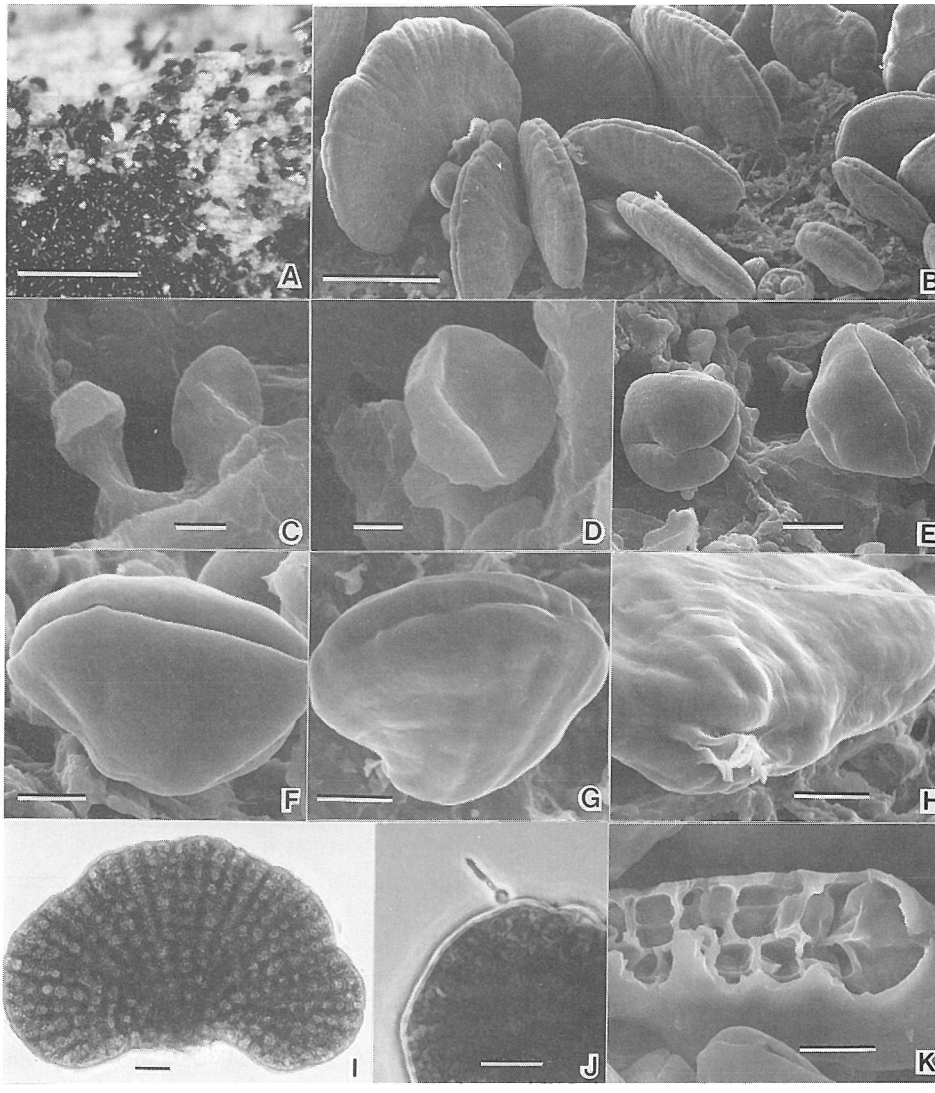

Fig. 18, A-K. Mycocentrolobium platysporum. A. Conidial mass produced on wood. B. Conidia. C-G. Development stages of conidium. H. Conidium base with a remains of conidiophore. I. Dictyosporous fan-shaped conidium. J. Phialide-like cell arising from conidium. K. Broken conidium showing the two composing lobes completely united with no space between. (Bars: A = 500 μm; B = 50 μm; C, D = 1 μm; E, F = 5 μm; G-K = 10 μm)

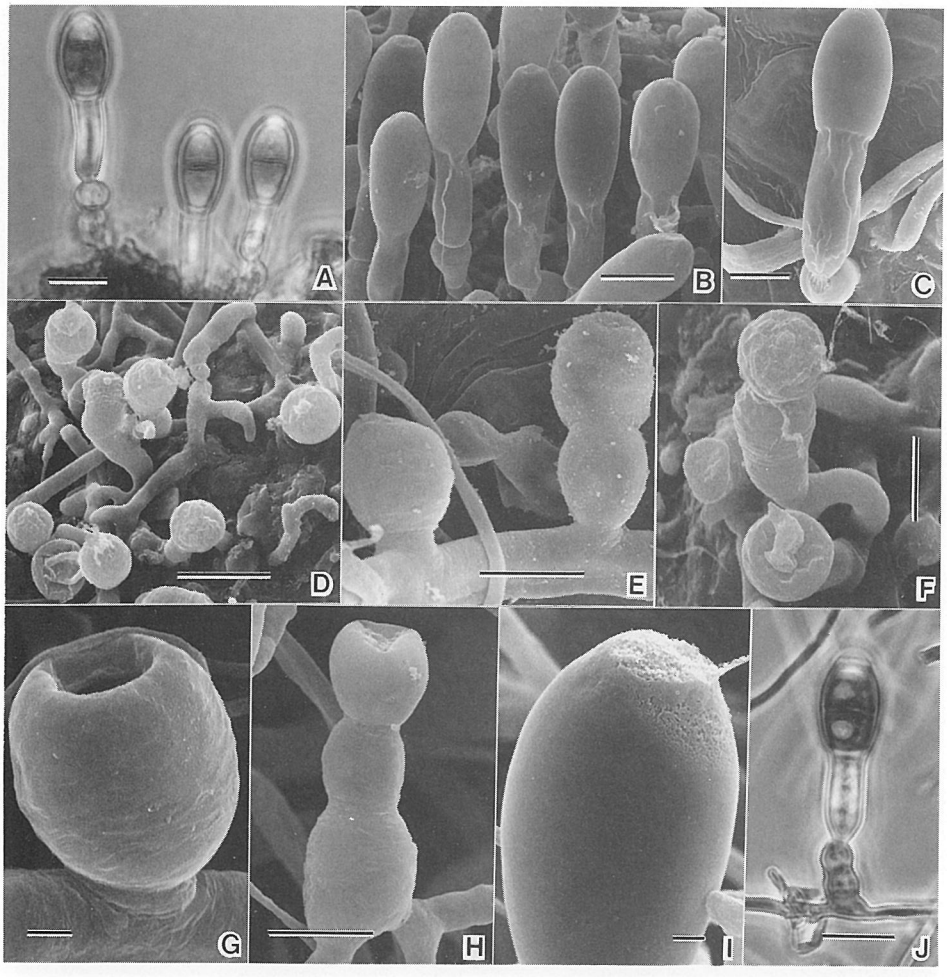

Fig. 19, A-J. Phragmospathula phoenicis. A-B. Conidia on wood. C. Conidium detaching from conidiophore. D. Conidiophores arising from creeping hyphae on wood. E-F. Percurrently proliferated conidiophores. G. Cup-shaped conidiophore. H. Three times proliferated conidiophore. I. Conidium apex with scar possibly left after detachment of a previously formed conidium. J. Conidium produced in culture. (Bars: A, B, D, J = 10 μm; C, E, F, H = 5 μm; G, I = 1 μm)

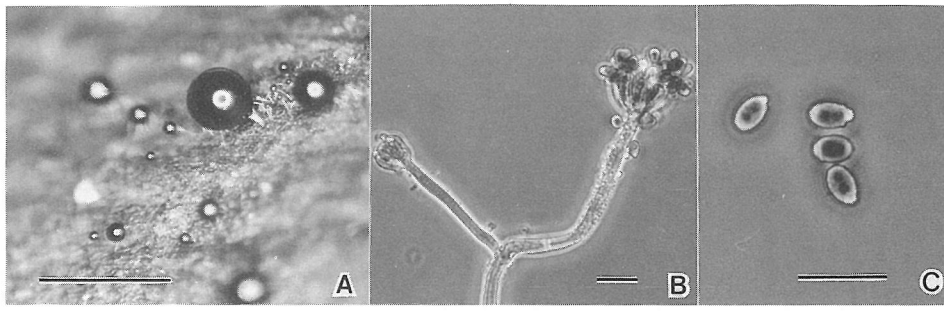

Fig. 20, A-C. Stachybotrys mangiferae. A. Habit on wood. Black drops are mass of conidia produced on conidiophores. B. Conidiophore. C. Conidia. (Bars: A = 500 μm; B, C = 10 μm)

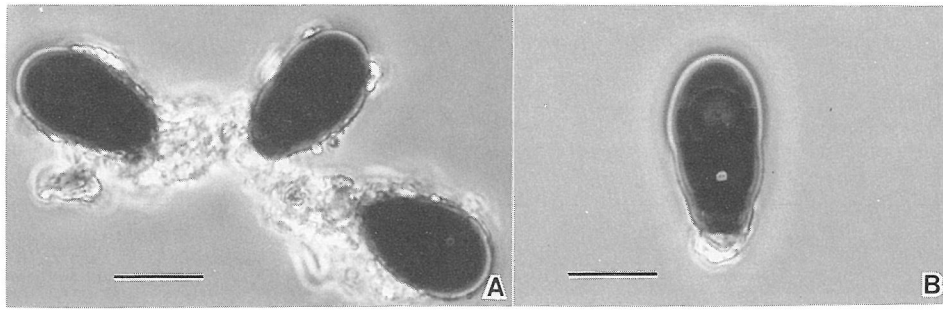

Fig. 21, A-B. Trichocladium achrasporum. A-B. Conidia. (Bars: A, B = 10 μm)

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CHANGES OF FUNGAL FLORA IN SOIL AFTER A BONFIRE

TADAYOSHI ITO

Summary

Serial changes in the fungal flora of field soil after a bonfire were surveyed by four isolation methods. Soil samples were collected vertically from two burned spots over a period of six months. The total number of fungi in the upper layer of the central spot was drastically reduced immediately after the bonfire, but recovered completely to the original number after three months. Such species as Pseudeurotium zonatum, Talaromyces flavus var. flavus and T. trachyspermus were detected in high frequency at both central and marginal spots and were unaffected by the heat of the bonfire. The thermophilic and thermotolerant species Aspergillus fumigatus, Malbranchea pulu-chella var. sulfurea and Myceliophthora thermophila were not detected in the upper layer of the central spot immediately after the bonfire, but they recovered after one month. Stachbotrys chartarum and Trichoderma harzianum were considered capable of being the first colonizers of the burned area since they recovered after three months. Eupenicillium ornatum and Gilmaniella humicola were repeatedly detected at both spots after the bonfire, suggesting that the germination of propagules in these species was promoted by the heat of the bonfire.

It was found that the fungal flora and total number of fungi were affected only in the upper layer, depending on the heat of the bonfire, and they returned to the original composition and number one to three months after the bonfire.

Keywords: Changes of fungal flora, number of fungi, bonfire, burnt soil, first colonizer.

Soils of burned ground provide a particular ecological niche for pyrophilous fungi (1, 2, 6, 7, 11, 18, 21). These fungi occur because the competition of microorganisms is excluded, their germination is promoted, and they assimilate the residual chemical compounds of bonfires (3, 13, 22, 23). In the case of forest fires, it has been reported that the changes in fungal flora of soils are not affected by the heat of the fire since the fire burns only the organic layer (L horizon), humus layer (H horizon) and surface soil (A horizon), but does not burn the accumulation horizon (B horizon) (3, 14, 24). To inhibit the pathogenic fungi, steam sterilization of soil has been tested in greenhouse cultivation, and its effects on planting are well known. Little is known, however, about the total number of fungi and the change of fungal flora in burned field soils. If bonfires have an impact on field soils, the fungal flora seems likely to be affected. To investigate the change of fungal flora in the field before and after a bonfire, a small bonfire was made of disused timber. Soil fungi were isolated by four methods: heat incubation at 45 C, alcohol treatment for 15 min, heat treatment at 70 C for 15 min, and the normal dilution plate method. Total numbers of fungi were assessed by the dilution plate method.

Materials and methods

Locality of sampling spots. The field investigated is located at 1-20, Hata, Ikeda City, Osaka Pref., Japan (ca. 34° 50'N, 135° 25'E) (Fig. 1) and has been grown with various vegetables for more than fifty years. It is about 400 m^2 in area and was covered with Vicia sativa Linn. before

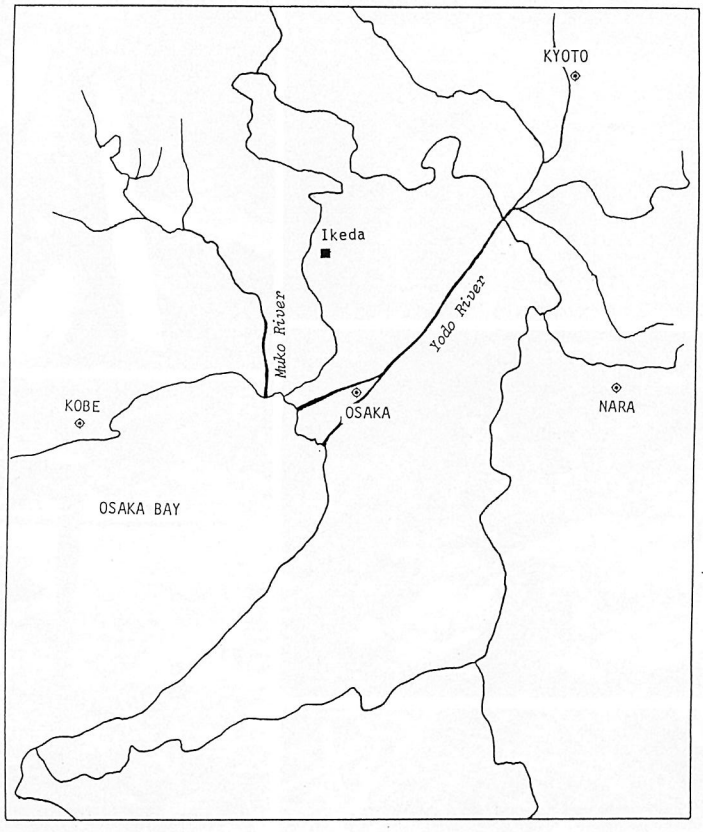

Fig. 1. Map of Osaka prefecture showing the study spot.

the bonfire. The surrounding area was grown with various vegetables and rice. The bonfire burned for about one hour on April 22, 1990, and the diameter of the burned area was about 1 m (Figs. 2-6).

Sampling method. Soil samples were collected from the central (spot A) and marginal spots (spot B) of burned area just before the bonfire as a control, and immediately, 1, 3, and 6 months after the bonfire. Soil was withdrawn vertically five times from each site using a sterilized stainless-steel soil sampler with longitudinal slit. Samples were divided into three fractions (upper, middle and lower layers) representing depths of 0-10 cm, 10-20 cm and 20-30 cm from the soil surface,

Figs. 2-6. General views of the field tested. 2. A view of the field before burning. 3. The bonfire. 4. A view of field immediately after burning. 5. Sampling with a soil sampler. 6. A view of the field after sampling.

and samples of 1 g, 2 g and 3 g were taken from the center of each fraction in our laboratory.

Isolation and identification. To isolate various fungal species, the following four isolation methods were used. Final soil samples were suspended in 5 ml of sterilized water.

Heat incubation: 0.2 ml of the original suspension was spread onto YMA medium (see below) in two 9-cm Petri dishes.

Alcohol treatment: 2 ml of the original suspension was treated for 15 min with the same volume of ethanol (99.5%), and 0.2 ml of the mixture was spread onto two plates of the same medium.

Heat treatment: 2 ml of the original suspension was heated at 70 C for 15 min in a water bath, then 0.2 ml of the treated suspension was spread onto two plates of the same medium.

Dilution plate: the original suspension was diluted 10-fold and 100-fold with sterilized water, then 0.1 ml of each dilute suspension was spread onto five plates of the same medium.

The medium used for isolation is as follows. YMA medium: glucose, 20 g; peptone, 5 g; malt extract, 3 g; yeast extract, 3 g; agar, 20 g; tetracycline, 50 ug/ml; distilled water, 1000 ml adjusted to pH 5.6. Plates for heat incubation and the other three methods were incubated respectively at 45 C and 24 C for three to four days.

Colonies were picked up from the plates under a dissecting microscope and transferred to malt agar slants. A representative strain was used to identify each species isolated from each sample, and each strain was incubated on suitable medium.

The isolates were mainly identified after Domsch et al. (5), Evans (8), Raper & Fennell (17), Samson et al. (19), Stolk and Samson (20), and IFO strains preserved were used for comparison.

Results

Changes in the total number of fungi

Fig. 7 shows the total number of fungi detected in each soil layer at the central and marginal spots by the dilution plate method. The numbers were: 1.1×10^5/g in the upper layer, 6.3×10^4/g in the middle

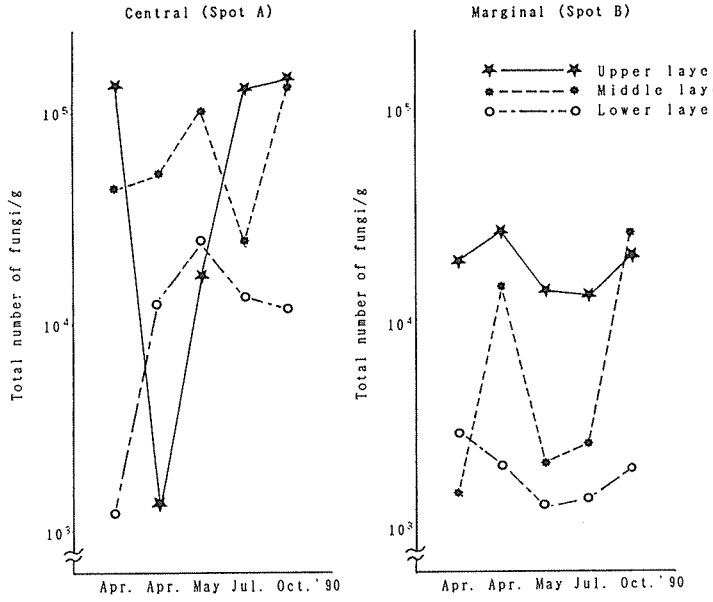

Fig. 7. Total number of fungi detected by the dilution plate method.

layer, and 1.3 x 10^3/g in the lower layer of the central spot; and 3.2 x 10^4/g, 2.0 x 10^3/g, and 4.7 x 10^3/g in the respective layers of the marginal spot. The numbers differed by about the order of 10 between the spots. The colony count in the upper layer of the central spot immediately after the bonfire decreased drastically to 1.0 x 10^3/g, but it recovered 2.2 x 10^4/g after one month, and returned to 1.3 x 10^5/g, close to the original number, after 3 months. The fungal population in the middle and lower layers of the central spot increased from immediately after to one month after the bonfire, but it was decreased by 3 months in both layers. After 6 months, the population in the middle layer increased, but that in the lower layer decreased somewhat. The pattern of variation of colony count in the upper and lower layers of the marginal spot resembled each other, while that in the middle layer was oscillatory.

Kinds of fungi detected

Tables 1 to 3 show the fungi detected from the two spots by four isolation methods and their distribution at each depth, together with their frequencies. Frequency expresses the percentage of the number of samples in which the fungus was detected to the total number of samples taken at a given spot.

A total of 103 species and 64 genera were isolated and identified. They were 5 species belonging to Zygomycotina, 33 to Ascomycotina, 63 to Deuteromycotina, and 2 to Basidiomycotina. No Mastigomycotina were detected.

The dominant fungi at both spots were Pseudeurotium zonatum v. Beyma, Talaromyces flavus (Klocker) Stolk & Samson var. flavus and Aspergillus fumigatus Fres., having frequencies of 80.0-100.0%. Less common fungi with frequencies of 6.7-13.3% were Chaetomium globosum Kunze : Fr., Dichotomomyces cejpii (Milko) Scott var. cejpii, Preussia minima (Auersw.) v. Arx, Acremonium butyri (v. Beyma) W. Gams, Dactylaria ampliforme (Tubaki) Bhatt & Kendrick, Fusarium moniliforme Sheld., Geomyces pannorum (Link) Sigl. & Carm., Gliomastix cerealis (Karst.) Dickin., Gliomastix murorum (Corda) Hughes var. felina (March.) Hughes, Metarhizium anisopliae (Metschn.) Sorok., Myrothecium roridum Tode : Fr. The genera Eupenicillium and Thielavia belonging to Ascomycotina were detected with more than 40% frequency at both spots. The genera represented by the greatest number of species were Talaromyces, Fusarium and Trichoderma. Many species detected at both spots had frequencies of 20.0-73.3%.

The Zygomycotina were represented by the genus Mortierella, the most abundant in Zygomycotina was M. alpina Peyronel, with Mucor hiemalis Whemer f. hiemalis next in frequency.

The Ascomycotina were represented by 18 genera at the central spot and 16 at the marginal site. Thirteen genera of these isolates were common to both spots. Of 33 species of Ascomycotina, 6 were ascosporic Aspergilli and Penicillia belonging in the genera Emericella, Eurotium, Neosartorya, Eupenicillium, Hamigera and Talaromyces. Members of Ascomycotina isolated included 3 species of Emericellopsis, 2 species of Pseudeurotium, and 2 species of Westerdykella. The species of Neosartorya fischeri (Whemer) Malloch & Cain var. glabra (Fennell & Raper) Malloch & Cain, Pseudeurotium zonatum, Talaromyces flavus var. flavus, Talaromyces trachyspermus (Shear) Stolk & Samson and Thielavia terricola (Gilm. &

Table 1. List of fungi isolated from burnt soils by four isolation methods.

Spot A (Central)	Method*	Spot B (Marginal)	Method
ZYGOMYCOTINA		**ZYGOMYCOTINA**	
Cunninghamella echinulata (Thaxt.) Thaxt. var. echinulata	D		
Cunninghamella echinulata (Thaxt.) Thaxt. var. elegans (Lendner) Shipton & Lunn	D		
Gongronella butleri (Lendner) Peyronel & Dal Vesco	D		
Mortierella alpina Peyronel	D	Mortierella alpina Peyronel	D
Mortierella spp.	D	Mortierella spp.	D
Mucor hiemalis Whemer f. hiemalis	D	Mucor hiemalis Whemer f. hiemalis	D
ASCOMYCOTINA		**ASCOMYCOTINA**	
Anixiella reticulata (Booth & Ebben) Cain	E, T	Anixiella reticulata (Booth & Ebben) Cain	T
Anixiopsis fulvescens (Cooke) de Vries var. fulvescens	D		
Chaetomium globosum Kunze : Fries	D	Chaetomium globosum Kunze : Fries	D
Corynascus sepedonium (Emmons) v. Arx	H		
Dichotomomyces cejpii (Milko) Scott var. cejpii	E, T	Dichotomomyces cejpii (Milko) Scott var. cejpii	E, T
		Echinopodospora jamaicensis Robison	D
Eleutherascus lectardii (Nicot) v. Arx	E		
Emericella nidulans (Eidam) Vuill. var. echinulata (Fennell & Raper) Subram.	H		
Emericella nidulans (Eidam) Vuill. var. nidulans	H		
Emericellopsis microspora Buckus & Orpurt	D		
Emericellopsis minima Stolk	D		
Emericellopsis terricola v. Beyma	D	Emericellopsis terricola v. Beyma	D
Eupenicillium javanicum (v. Beyma) Stolk & Scott	E, T, D	Eupenicillium javanicum (v. Beyma) Stolk & Scott	E, T, D
Eupenicillium ornatum Udagawa	E, D	Eupenicillium ornatum Udagawa	E, T, D
Eupenicillium spp.	E, T, D	Eupenicillium spp.	E, T, D

*: H; heat incubation. E; alcohol treatment. T; heat treatment. D; dilution plate.

(continued).

Spot A (Central)	Method*	Spot B (Marginal)	Method
Eurotium amstelodami Mangin	E		
Eurotium chevalieri Mangin	E, T, D	Eurotium chevalieri Mangin	E, D
		Hamigera avellanea Stolk & Samson	E
		Microascus cinereus (Emile-Weil & Gaudin) Curzi	D
Neosartorya fischeri (Wehmer) Malloch & Cain var. fischeri	H, E, T	Neosartorya fischeri (Wehmer) Malloch & Cain var. fischeri	E, T
Neosartorya fischeri (Wehmer) Malloch & Cain var. glabra (Fennell & Raper) Malloch & Cain	H, T, D	Neosartorya fischeri (Wehmer) Malloch & Cain var. glabra (Fennell & Raper) Malloch & Cain	H, E, T
Petriellidium boydii (Shear) Malloch	D		
Preussia minima (Auersw.) v. Arx	E	Preussia minima (Auersw.) v. Arx	D
Pseudeurotium ovale Stolk	E	Pseudeurotium ovale Stolk	E, D
Pseudeurotium zonatum v. Beyma	E	Pseudeurotium zonatum v. Beyma	E, D
Talaromyces flavus (Klöcker) Stolk & Samson var. flavus	H, E, T, D	Talaromyces flavus (Klöcker) Stolk & Samson var. flavus	H, E, T, D
		Talaromyces flavus (Klöcker) Stolk & Samson var. macrosporus	T
Talaromyces trachyspermus (Shear) Stolk & Samson	H, E, T, D	Talaromyces trachyspermus (Shear) Stolk & Samson	H, E, T, D
		Talaromyces ucrainicus Udagawa apud Stolk & Samson	T
Talaromyces wortmannii C.R. Benjam. apud Stolk & Samson	E	Talaromyces wortmannii C.R. Benjam. apud Stolk & Samson	E
Thermoascus aurantiacus Miehe	H	Thermoascus aurantiacus Miehe	H
Thielavia terricola (Gilm. & Abbott) Emmons	H, E	Thielavia terricola (Gilm. & Abbott) Emmons	H
		Westerdykella dispersa (Clum) Cejp & Milko	D
Westerdykella multispora (Saito & Minoura) Cejp & Milko	D	Westerdykella multispora (Saito & Minoura) Cejp & Milko	H, D
DEUTEROMYCOTINA		**DEUTEROMYCOTINA**	
		Acremonium alabamense Morgan-Jones	H
Acremonium butyri (v. Beyma) W. Gams	D	Acremonium butyri (v. Beyma) W. Gams	D
Acremonium strictum W. Gams	D	Acremonium strictum W. Gams	D
Acremonium spp.	D	Acremonium spp.	D

*: H; heat incubation. E; alcohol treatment. T; heat treatment. D; dilution plate.

(continued).

Spot A (Central)	Method*	Spot B (Marginal)	Method
Aspergillus fumigatus Fres.	H, D	Aspergillus fumigatus Fres.	H, D
Aspergillus niger v. Tiegh.	H, D	Aspergillus niger v. Tiegh.	H, D
Aspergillus terreus Thom	H, D	Aspergillus terreus Thom	H
		Aureobasidium pullulans (de Bary) Arnaud	E, D
Chloridium virescens (Pers.:Fr.) W. Gams & Hol.-Jech. var. chlamydosporum (v. Beyma) W. Gams & Hol.-Jech.	D	Chloridium virescens (Pers.:Fr.) W. Gams & Hol.-Jech. var. chlamydosporum (v. Beyma) W. Gams & Hol.-Jech.	D
Chrysosporium merdarium (Link ex Grev.) Carm.	D		
Cladosporium cladosporioides (Fres.) de Vries	D	Cladosporium cladosporioides (Fres.) de Vries	D
		Cladosporium oxysporum Berk. & Curt.	D
		Codinaea parva Hughes & Kendrick	D
Dactylaria ampulliformis (Tubaki) Bhatt & Kendrick	D	Dactylaria ampulliformis (Tubaki) Bhatt & Kendrick	D
Fusarium fusarioides (Frag. & Cif.) Booth	D		
Fusarium merismoides Corda	D	Fusarium merismoides Corda	D
Fusarium moniliforme Sheld.	D	Fusarium moniliforme Sheld.	D
Fusarium oxysporum Schlecht. emend. Sny. & Hans.	T, D	Fusarium oxysporum Schlecht. emend. Sny. & Hans.	D
Fusarium solani (Mart.) Appel & Wollenw. emend. Sny. & Hans.	D	Fusarium solani (Mart.) Appel & Wollenw. emend. Sny. & Hans.	D
Fusarium spp.	D	Fusarium spp.	D
Geomyces pannorum (Link) Sigl. & Carm.	D	Geomyces pannorum (Link) Sigl. & Carm.	D
Gilmaniella humicola Barron	E, T, D	Gilmaniella humicola Barron	E, T
Gliocladium catenulatum Gilm. & Abbott	D	Gliocladium catenulatum Gilm. & Abbott	D
Gliocladium roseum (Link) Bain.		Gliocladium roseum (Link) Bain.	
Gliocladium virens Miller et al.	D	Gliocladium virens Miller et al.	D
Gliomastix cerealis (Karst.) Dickin.	D	Gliomastix cerealis (Karst.) Dickin.	D
Gliomastix murorum (Corda) Hughes var. felina (March.) Hughes	D	Gliomastix murorum (Corda) Hughes var. felina (March.) Hughes	D
Gonytrichum macrocladum (Sacc.) Hughes	D	Gonytrichum macrocladum (Sacc.) Hughes	D
Humicola fuscoatra Traaen	D	Humicola fuscoatra Traaen	D
Humicola grisea Traaen var. grisea	D	Humicola grisea Traaen var. grisea	D

*: H; heat incubation. E; alcohol treatment. T; heat treatment. D; dilution plate.

(continued).

Spot A (Central)	Method*	Spot B (Marginal)	Method
Humicola grisea Traaen var. thermoidea Cooney & Emerson	H		
Humicola insolens Cooney & Emerson	H		
Leptodiscella africana (Papend.) Papend.	D		
Malbranchea pulchella Sacc. & Penzig var. sulfurea (Miehe) Cooney & Emerson	H	Malbranchea pulchella Sacc. & Penzig var. sulfurea (Miehe) Cooney & Emerson	H
Metarhizium anisopliae (Metschn.) Sorok.	D	Metarhizium anisopliae (Metschn.) Sorok.	D
Monocillium mucidum W. Gams	D		
Myceliophthora thermophila (Apinis) v. Oorschot	H	Myceliophthora thermophila (Apinis) v. Oorschot	H
Myrothecium cinctum (Corda) Sacc.	D	Myrothecium cinctum (Corda) Sacc.	D
Myrothecium roridum Tode : Fr.	D	Myrothecium roridum Tode : Fr.	D
Oidiodendron truncatum Barron	D	Oidiodendron truncatum Barron	D
Paecilomyces lilacinus (Thom) Samson	D	Paecilomyces lilacinus (Thom) Samson	D
Paecilomyces marquandii (Mass.) Hughes	D		
Penicillium funiculosum Thom	H, D	Penicillium funiculosum Thom	D
Penicillium piceum Raper & Fennell	H, D	Penicillium piceum Raper & Fennell	H, D
Penicillium spp.	D	Penicillium spp.	D
Phialophora cyclaminis v. Beyma	D		
Phialophora fastigiata (Lagerb. & Melin) Conant	D	Phialophora fastigiata (Lagerb. & Melin) Conant	D
Phialophora malorum (Kidd & Beanum.) McColloch	D		
Phoma eupyrena Sacc.	D		
		Phoma herbarum Westend.	D
Phoma spp.	D	Phoma spp.	D
		Pseudobotrys terrestris (Timonin) Subram.	D
Pyrenochaeta terrestris (Hansen) Gorenz et al.	D	Pyrenochaeta terrestris (Hansen) Gorenz et al.	D
Scolecobasidium humicola Barron & Busch	D	Scolecobasidium humicola Barron & Busch	D
		Scolecobasidium terreum Abbott	D
Stachybotrys chartarum (Ehrenb.) Hughes	D	Stachybotrys chartarum (Ehrenb.) Hughes	D
Staphylotrichum coccosporum Meyer & Nicot	D		

*: H; heat incubation. E; alcohol treatment. T; heat treatment. D; dilution plate.

(continued).

Spot A (Central)	Method*	Spot B (Marginal)	Method
Thermomyces lanuginosus Tsiklinsky	H	Thermomyces lanuginosus Tsiklinsky	H
		Trichocladium opacum (Corda) Hughes	D
Trichoderma aureoviride Rifai	T, D		
		Trichoderma hamatum (Bonord.) Bain.	D
Trichoderma harzianum Rifai	D	Trichoderma harzianum Rifai	D
Trichoderma koningii Oudem.	D	Trichoderma koningii Oudem.	D
Trichoderma polysporum (Link : Pers.) Rifai	D		
Trichoderma pseudokoningii Rifai	D	Trichoderma pseudokoningii Rifai	D
Trichoderma spp.	D	Trichoderma spp.	D
		Tripospermum myrti (Lind) Hughes	T
Volutina concentrica Penzig & Sacc.	D	Volutina concentrica Penzig & Sacc.	D
BASIDIOMYCOTINA		**BASIDIOMYCOTINA**	
		Limnoperdon incarnatum Escob. apud Escob. et al.	D
Psathyrella obtusata (Fr.) A.H. Smith	D		

*: H; heat incubation. E; alcohol treatment. T; heat treatment. D; dilution plate.

Table 2. List of fungi isolated from burnt soils in spot A by four isolation methods.

Spot A (Central)	Bonfire before U* M L	immed. U M L	1 Mon. U M L	3 Mon. U M L	6 Mon. U M L	Freq. (%)**
ZYGOMYCOTINA						
Cunninghamella echinulata				+		6.7
Cunninghamella elegans			+			6.7
Gongronella butleri				+	+	13.3
Mortierella alpina	+	+	+ +	+ + +	+	53.3
Mucor hiemalis f. hiemalis			+ +	+		20.0
ASCOMYCOTINA						
Anixiella reticulata			+	+ +		20.0
Anixiopsis fulvescens var. fulvescens	+					6.7
Chaetomium globosum			+		+	13.3
Corynascus sepedonium				+		6.7
Dichotomomyces cejpii var. cejpii	+ +					13.3
Eleutherascus lectardii		+				6.7
Emericella nidulans var. echinulata				+		6.7
Emericella nidulans var. nidulans	+			+		13.3
Emericellopsis microspora		+				6.7
Emericellopsis minima				+		6.7
Emericellopsis terricola		+	+		+	20.0
Eupenicillium javanicum	+ +	+ +	+ + +	+ +	+ + +	80.0
Eupenicillium ornatum			+ + +	+ + +	+ +	53.3
Eurotium amstelodami				+		6.7
Eurotium chevalieri		+	+ +	+	+	33.3
Neosartorya fischeri var. fischeri	+		+	+ + +	+ +	46.7
Neosartorya fischeri var. glabra	+	+ + +	+ + +	+ + +	+ +	80.0
Petriellidium boydii			+	+		13.3
Preussia minima	+					6.7
Pseudeurotium ovale	+	+			+ +	26.7
Pseudeurotium zonatum	+ + +	+ +	+ + +	+ +	+ + +	86.7
Talaromyces flavus var. flavus	+ + +	+ + +	+ + +	+ + +	+ + +	100.0
Talaromyces trachyspermus	+ + +	+ + +	+ + +	+ + +	+ + +	100.0
Talaromyces wortmannii	+		+ +	+	+	33.3
Thermoascus aurantiacus	+	+		+	+	26.7
Thielavia terricola	+	+	+	+ +	+ + +	53.3
Westerdykella multispora	+	+ +	+ + +		+	46.7

*: U; upper layer. M; middle layer. L; lower layer.

**: Number of positive samples / total number of samples.

(continued).

Spot A (Central)	Bonfire before U* M L	immed. U M L	1 Mon. U M L	3 Mon. U M L	6 Mon. U M L	Freq. (%)**
DEUTEROMYCOTINA						
Acremonium butyri	+					6.7
Acremonium strictum		+	+	+ +	+	33.3
Aspergillus fumigatus	+ + +	+ +	+ + +	+ + +	+ + +	93.3
Aspergillus niger	+ +		+ +	+ + +	+ +	60.0
Aspergillus terreus	+ + +	+ +	+ +	+ + +	+ + +	86.7
Chloridium virens var. chlamydosporum	+		+			13.3
Chrysosporium merdarium		+				6.7
Cladosporium cladosporioides			+	+		13.3
Dactylaria ampulliformis		+				6.7
Fusarium fusarioides					+	6.7
Fusarium merismoides			+ +	+ +	+	33.3
Fusarium moniliforme				+		6.7
Fusarium oxysporum	+		+ +	+ +		33.3
Fusarium solani	+	+		+ +		26.7
Geomyces pannorum	+		+			13.3
Gilmaniella humicola	+	+	+ + +	+ + +	+ + +	73.3
Gliocladium catenulatum	+			+	+	20.0
Gliocladium roseum	+	+	+ +	+	+ + +	53.3
Gliocladium virens		+	+			13.3
Gliomastix cirealis					+	6.7
Gliomastix murorum var. felina	+					6.7
Gonytrichum macrocladum	+		+	+	+	26.7
Humicola fuscoatra		+	+ + +	+		33.3
Humicola grisea var. grisea	+	+			+ +	26.7
Humicola grisea var. thermoidea	+			+		13.3
Humicola insolens				+	+	13.3
Leptodiscella africana	+					6.7
Malbranchea pulchella var. sulfurea	+	+	+ +	+ + +	+	53.3
Metarhizium anisopliae					+	6.7
Monocillium mucidum					+	6.7
Myceliophthora thermophila	+ +	+	+ +	+ + +	+	60.0
Myrothecium cinctum			+	+ +	+	26.7
Myrothecium roridum				+ +		13.3
Oidiodendron truncatum		+ +	+			20.0
Paecilomyces lilacinus	+ +	+	+		+	33.3
Paecilomyces marquandii				+		6.7
Penicillium funiculosum			+	+	+	20.0

*: U; upper layer. M; middle layer. L; lower layer.

**: Number of positive samples / total number of samples.

(continued).

Spot A (Central)	Bonfire before U*M L	immed. U M L	1 Mon. U M L	3 Mon. U M L	6 Mon. U M L	Freq. (%)**
Penicillium piceum	+ +		+	+ +	+	40.0
Phialophora cyclaminis		+				6.7
Phialophora fastigiata		+ +		+ +		26.7
Phialophora malorum					+	6.7
Phoma eupyrena					+	6.7
Pyrenochaeta terrestris	+					6.7
Scolecobasidium humicola	+ +	+		+ + +		40.0
Stachybotrys chartarum	+ +	+	+	+ +	+ +	53.3
Staphylotrichum coccosporum		+		+	+	20.0
Thermomyces lanuginosus				+		6.7
Trichoderma aureoviride				+ +		13.3
Trichoderma harzianum	+ + +	+	+ +	+ +	+ + +	73.3
Trichoderma koningii	+	+ +	+ +		+ +	46.7
Trichoderma polysporum					+ +	13.3
Trichoderma pseudokoningii			+	+ +		20.0
Volutina concentrica	+	+	+		+	26.7
BASIDIOMYCOTINA						
Psathyrella obtusata					+	6.7
Total number of species	28, 22, 12/11, 26, 14/20, 30, 26/28, 32, 35/24, 24, 28					

*: U; upper layer. M; middle layer. L; lower layer.
**: Number of positive samples / total number of samples.

Table 3. List of fungi isolated from burnt soils in spot B by four isolation methods.

Spot B (Marginal)	Bonfire before U* M L	immed. U M L	1 Mon. U M L	3 Mon. U M L	6 Mon. U M L	Freq. (%)**
ZYGOMYCOTINA						
Mortierella alpina	+	+		+	+	26.7
Mucor hiemalis f. hiemalis			+			6.7
ASCOMYCOTINA						
Anixiella reticulata		+		+		13.3
Chaetomium globosum		+		+		13.3
Dichotomomyces cejpii var. cejpii				+	+	13.3
Echinopodospora jamaicensis	+					6.7
Emericellopsis terricola	+		+	+		20.0
Eupenicillium javanicum	+	+ +	+	+ +		40.0
Eupenicillium ornatum			+	+ + +	+ + +	46.7
Eurotium chevalieri				+	+	13.3
Hamigera avellanea			+			6.7
Microascus cinereus		+				6.7
Neosartorya fischeri var. fischeri		+			+	13.3
Neosartorya fischeri var. glabra	+	+ +	+	+ +	+ +	53.3
Preussia minima	+					6.7
Pseudeurotium ovale	+ +	+ + +	+ +	+	+ +	66.7
Pseudeurotium zonatum	+ + +	+ +	+ + +	+ + +	+ +	86.7
Talaromyces flavus var. flavus	+ + +	+ + +	+ + +	+ + +	+ + +	100.0
Talaromyces flavus var. macrosporus					+	6.7
Talaromyces trachyspermus	+ +	+ +	+	+ + +	+ + +	73.3
Talaromyces ucrainicus				+		6.7
Talaromyces wortmannii	+				+ +	20.0
Thermoascus aurantiacus	+	+			+	20.0
Thielavia terricola	+	+ +	+	+ +	+ +	53.3
Westerdykella dispersa			+		+	13.3
Westerdykella multispora	+ +	+ +	+	+ +	+	53.3
DEUTEROMYCOTINA						
Acremonium alabamense		+				6.7
Acremonium butyri					+	6.7
Acremonium strictum	+ +	+			+ +	33.3
Aspergillus fumigatus	+ + +	+ + +	+ + +	+ +	+ +	86.7
Aspergillus niger	+		+	+	+ +	33.3
Aspergillus terreus	+		+	+ + +	+	40.0

*: U; upper layer. M; middle layer. L; lower layer.

**: Number of positive samples / total number of samples.

(continued).

Spot B (Marginal)	Bonfire before U* M L	immed. U M L	1 Mon. U M L	3 Mon. U M L	6 Mon. U M L	Freq. (%)**
Aureobasidium pullulans		+	+			13.3
Chloridium virens var. chlamydosporum	+ +	+				20.0
Cladosporium cladosporioides		+	+ +	+ +	+	40.0
Cladosporium oxysporum					+ +	13.3
Codinaea parva	+		+			13.3
Dactylaria ampulliformis		+				6.7
Fusarium merismoides			+	+	+ +	26.7
Fusarium moniliforme				+		6.7
Fusarium oxysporum		+	+	+ +		26.7
Fusarium solani	+	+ +	+			26.7
Geomyces pannorum		+				6.7
Gilmaniella humicola				+ +	+ + +	33.3
Gliocladium catenulatum			+	+	+	20.0
Gliocladium roseum	+ +		+ +	+	+ + +	53.3
Gliocladium virens				+ +	+	20.0
Gliomastix cirealis			+			6.7
Gliomastix murorum var. felina				+		6.7
Gonytrichum macrocladum		+			+ +	20.0
Humicola fuscoatra	+	+				13.3
Humicola grisea	+	+		+ +		26.7
Malbranchea pulchella var. sulfurea				+ +		13.3
Metarhizium anisopliae		+		+		13.3
Myceliophthora thermophila		+ +	+			20.0
Myrothecium cinctum			+			6.7
Myrothecium roridum		+	+			13.3
Oidiodendron truncatum	+ +	+ +	+	+ +	+ +	60.0
Paecilomyces lilacinus	+	+	+	+		26.7
Penicillium funiculosum				+		6.7
Penicillium piceum	+	+		+		20.0
Phialophora fastigiata				+	+	13.3
Phoma violacea	+	+ +	+	+		33.3
Pseudobotrys terrestris		+				6.7
Pyrenochaeta terrestris	+ +	+			+	26.7
Scolecobasidium humicola			+			6.7
Scolecobasidium terreum		+ +				13.3
Stachybotrys chartarum			+	+		13.3
Thermomyces lanuginosus		+		+	+	20.0
Trichocladium opacum	+	+				13.3
Trichoderma hamatum			+			6.7

*: U; upper layer. M; middle layer. L; lower layer.
**: Number of positive samples / total number of samples.

(continued).

Spot B (Marginal)	Bonfire before U* M L	immed. U M L	1 Mon. U M L	3 Mon. U M L	6 Mon. U M L	Freq. (%)**
Trichoderma harzianum	+ + +	+		+ + +	+ +	60.0
Trichoderma koningii	+	+ + +		+	+	40.0
Trichoderma pseudokoningii	+ +		+ +	+	+	40.0
Tripospermum myrti			+			6.7
Volutina concentrica		+	+ +	+	+	33.3
BASIDIOMYCOTINA						
Limnoperdon incarnatum	+	+	+	+ +		33.3
Total number of species	24, 12, 15/26, 23, 13/17, 13, 19/21, 27, 21/20, 27, 13					

*: U; upper layer. M; middle layer. L; lower layer.
**: Number of positive samples / total number of samples.

Abott) Emmons were isolated with frequencies of more than 50% at both spots. The species Talaromyces flavus var. flavus was detected with 100% frequency at both spots.

Nearly 60% of the total of 103 species isolated can be assigned to the Deuteromycotina. Isolates of Deuteromycotina dominated in frequency at both spots. Aspergillus fumigatus, Gliocladium roseum (Link) Bain. and Trichoderma harzianum Rifai had frequencies of more than 50%, while fungi with frequencies of more than 40% included Aspergillus terreus Thom and Trichoderma koningii Oudem.. The species Gilmaniella humicola Barron was reisolated from 3 months after the bonfire. The two species Psathyrella obtsusata (Fr.) A. H. Smith and Limnoperdon incarnatum Escob. apud Escob. et al., belonging to Basidiomycotina, were rarely detected.

Differences in fungal flora between the two spots

Seventy-eight species in 54 genera were detected at the central spot, and 77 species in 57 genera at the marginal spot. The total numbers of species and genera at the two spots were closely similar. The species Mortierella alpina in Zygomycotina, Eupenicillium javanicm (v. Beyma) Stolk & Scott, and Eupenicillium ornatum Udagawa in Ascomycotina, and Aspergillus niger v. Tiegh., Aspergillus terreus, Gilmaniella humicola, Malbranchea pulchella Sacc. & Penzig var. sulfurea Cooney & Emerson, Myceliophthora thermophila (Apinis) v. Oorschot and Stachybotrys chartarum (Ehrenb.) Hughes in Deuteromycotina appeared with more than 50% frequency only at the central spot. On the other hand, the species Pseudeurotium ovale Stolk and Westerdykella multispora (Saito & Minoura) Cejp & Milko in Ascomycotina, and Oidiodendron truncatum Barron in Deuteromycotina occurred with more than 50% frequency only at the marginal spot.

Vertical distribution of the fungal flora

The total number of species in the upper layer of the central spot, as shown in Tables 2 and 3, was 28, 11, 20, 28 and 24 just before, and immediately, 1, 3, and 6 months after the bonfire, respectively, while, at the marginal spot, 24, 26, 17, 21 and 20 species were detected. At the central spot, the number of species detected in the upper layer immediately after the bonfire was less than half of that before. In the middle layer, the number of species detected was 22, 26, 30, 32 and 24 at

the central spot, and 12, 23, 13, 27, and 27 at the marginal spot. In the lower layer, it was 12, 14, 26, 35 and 28 species at the central spot and 15, 13, 19, 21 and 13 at the marginal spot. Almost all the species detected were mainly distributed in the upper and middle layers.

Seasonal fluctuation

As shown in Fig. 2, the total number of fungi was higher in spring and autumn than summer. The colony count in summer, namely, the three months after the bonfire, was lowest in the middle layer of the central spot and the upper layer of the marginal spot.

Effect of isolation method

The species detected by each isolation method are shown in Table 1. Of the 103 fungal species isolated and identified, 61 were isolated only by the dilution plate method, 9 only by the heat incubation method, 4 only by the alcohol treatment method, and 2 only by the heat treatment method. Twenty-three fungi were isolated by two to four isolation methods. The unidentified species of Mortierella in Zygomycotina, and Acremonium, Fusarium, Penicillium, Phoma and Trichoderma in Deuteromycotina were detected only by the dilution plate method. Eupenicillium species in Ascomycotina were obtained by the three isolation methods other than the heat incubation method. Of the 21 fungi obtained by the heat incubation method, the species Thermoascus aurantiacus Miehe in Ascomycotina and Acremonium alabamense Morgan-Jones, Humicola grisea Traaen var. thermoidea Cooney & Emerson, Humicola insolense Cooney & Emerson, Malbranchea pulchella var. sulfurea, Myceliophthora thermophila and Thermomyces lanuginosus Tsiklinsky were true thermophiles in the sense of Cooney and Emerson (4).

Discussion

The total number of fungi in burnt soil decreased drastically in the upper layer of the central spot immediately after the bonfire. However, they recovered to half of the original number (before the bonfire) after one month and recovered completely three months after the bonfire. Keim et al. (10) reported that the number of fungi in paddy

field soil in the USA was 1.7 x 10^5/g at 5 cm depth, 8.0 x 10^4/g at 10 cm depth and 9.5 x 10^3/g at 20 cm depth. The total number of fungi at the central spot in this experiment agreed with this report and that of Min et al. (16). Although Ahlgren and Ahlgren (2) reported that the number of bacteria in soil after forest fire recovered 12 to 14 months later, the fungi recovered their original number three months after the bonfire. Thus, the total number of fungi recovered faster than the population of bacteria. The number of fungal propagules per gram of soil has been shown to tend to decrease with increasing depth (16). This tendency was recognized in our experiment.

As shown in Table 1, the fungal species detected were almost the same at both spots. These fungi might be typical soil fungi, as mentioned by Domsch et al. (5), and the findings in our experiment closely agreed with the fungal flora in burnt soil at Mt. Daisen in Japan (13). The fungal flora at the burnt aerea is considered to have recovered from one to three months after the bonfire as shown in Tables 2 and 3, because some species undetected immediately after the bonfire were reisolated from the upper layer. Of the species detected in high frequency at the central spot, Eupenicillium javanicum, Pseudeurotium zonatum belonging to Ascomycotina, and Aspergillus fumigatus, Aspergillus terreus, and Trichoderma harzianum in Deuteromycotina were not detected in the upper layer immediately after the bonfire. However, the species Pseudeurotium zonatum and Aspergillus fumigatus were detected in the upper layer of the marginal spot. This result seems to reflect the susceptibility of these species to heat and might correlate with the temperature of the fire, as suggested by Wicklow (23).

The species Aspergillus fumigatus and Trichoderma harzianum, undetected in the upper layer immediately after the bonfire, were again isolated after one month. Therefore these species are considered to be the first colonizers of a burnt area. Little difference in the distribution of fungal flora between the two spots was observed. This means that the heat of the bonfire does not affect the lower layer or the marginal site of the bonfire, as mentioned by Widden and Parkinson (24). However, it is difficult to interpret the vertical distribution of fungal flora in this experiment, as population is known to vary in inverse portion to depth (10, 12, 16).

Of the four isolation methods used, the dilution plate method has

been the most widely used to survey the fungal flora of soil. Almost all the fungi belonging to Deuteromycotina were frequently detected by this method. Alcohol and heat treatment methods were more selective than the dilution plate method to isolate the ascomycetous fungi. The effect of these methods has been reported by mycologists (9, 13, 16).

The heat incubation method allowed the detection of thermophiles and thermotolerant fungi in soil. The results obtained agree with the reports of Huang and Schmitt (9), Ito & Yokoyama (12, 13) and Mahoney (15). The species Gilmaniella humicola seemed to be induced by the heat of the bonfire, as this species dominated the central spot and was isolated much more from one month after the bonfire. Furthermore, it occupied a frequency of 70% of colonies appearing on each isolation plate. The species Dichotomomyces cejpii var. cejpii was less dominant in this experiment, and it is considered to be susceptible to heat because it was detected only before the bonfire. However, only two species of the pyrophilous fungi, Anixiella reticulata (Booth & Ebben) Cain and Gilmaniella humicola, were isolated. This seems to be due to the short time of burning.

Many species of Mortierella, Eupenicillium, Acremonium, Fusarium, Penicillium, Phoma and Trichoderma were detected in this experiment, but they could not all be identified as to species name. The two species Psathyrella obtsusata and Limnopredon incarnatum were isolated less dominantly. The former species is recorded for the first time in soil, and the latter is famous as a " floating gasteroid. "

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FURTHER STUDIES ON THE CELLULAR FATTY ACID COMPOSITION OF *RHIZOBIUM* AND *AGROBACTERIUM* SPECIES

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Summary

Cellular fatty acid composition of an additional 20 strains belonging to the genera *Rhizobium* and *Agrobacterium* was examined. *Rhizobium* and *Agrobacterium* species could be separated into eight clusters based on their fatty acid profiles, mainly on their 2- and 3-hydroxy fatty acid composition. The combination of cellular fatty acid profile and another simple biochemical test, 3-ketolactose-forming activity, can be used as criteria to differentiate species and biovar groups of *Rhizobium* and *Agrobacterium*.

Keywords: *Rhizobium*, *Agrobacterium*, Cellular fatty acid

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Attempts to differentiate strains of Rhizobiaceae by cellular fatty acids have been reported (2,8). We have also reported fatty acid profiles of Rhizobiaceae (8,9). However, with recent reports of the new species Rhizobium huakuii (1,4) and Rhizobium tropicii (3) in the genus Rhizobium, new species Agrobacterium vitis (5), and new types (probably new species) of Agrobacterium tumefaciens strains (6), additional studies have become necessary to obtain more information on the fatty acid profiles in order to establish criteria to distinguish fully between species of Rhizobiaceae.

This communication reports additional data on the cellular fatty acid composition of strains of Rhizobiaceae.

Materials and Methods

Microorganisms. The bacterial strains used in this study are listed in Table 1. Rhizobium huakuii IFO 15244 (My-3) was isolated from root nodules of Astragalus sinicus cultivar Japan. Strains of Agrobacterium sp. IFO 15296 (K-Ag-3) and IFO 15297 (K-Ag-4) were isolated from crown galls of kiwifruit in Hiroshima Pref. Japan, and IFO 15292 (Ch-Ag-4), IFO 15293 (Ch-Ag-5), IFO 15294 (Ch-Ag-7) and IFO 15295 (Ch-Ag-8) were isolated from crown galls of cherry trees in Okayama Pref. Japan. Agrobacterium vitis (biovar 3) strains IFO 15140-IFO 15145 are isolates from crown galls of grapevine from countries outside Japan (5), and strains K-Ag-1, G-Ag-19, G-Ag-26 and G-Ag-60 are isolates from grapevines in Japan (7). Type strains are indicated by the letter "T" after the strain numbers.

Cultivation of microorganisms. The medium (YEM) and conditions for cultivation of strains were as described previously (8,9).

Analysis of fatty acids. Cellular fatty acids were transmethylated by heating with 5% HCl-methanol. Non-polar, 2-hydroxy and 3-hydroxy fatty acids were separated by TLC and analyzed with a Shimadzu GC-9A gas chromatograph as described previously (8,9). Fatty acid methyl esters were identified by gas-liquid chromatography-mass spectrometry (GLC-MS) using a Hewlett Packard 5890A mass spectrometer equipped with a capillary OV-101 fused silica column (25 m x 0.25 mm i.d.).

Table 1. Bacterial strains studied.

Taxon	Strain	Source and Comments
Genus Rhizobium		
R. huakuii	IFO 15243 T	
R. huakuii	IFO 15244	My-3, Root nodule of Astragalus sinicus, Yokota
R. tropici	IFO 15247 T	
R. loti	IFO 14779 T	
Genus Agrobacterium		
Agrobacterium sp.	IFO 15292	Ch-Ag-4, Cherry, Sawada
Agrobacterium sp.	IFO 15293	Ch-Ag-5, Cherry, Sawada
Agrobacterium sp.	IFO 15294	Ch-Ag-7, Cherry, Sawada
Agrobacterium sp.	IFO 15295	Ch-Ag-8, Cherry, Sawada
Agrobacterium sp.	IFO 15296	K-Ag-3, Kiwifruit, Sawada
Agrobacterium sp.	IFO 15297	K-Ag-4, Kiwifruit, Sawada
A. vitis (biovar 3)	NCPPB 2562	Grapevine
A. vitis (biovar 3)	IFO 15140	K-309, Grapevine, Ophel
A. vitis (biovar 3)	IFO 15141	K-377, Grapevine, Ophel
A. vitis (biovar 3)	IFO 15142	K-306, Grapevine, Ophel
A. vitis (biovar 3)	IFO 15143	AG-57, Grapevine, Ophel
A. vitis (biovar 3)	IFO 15144	CG-49, Grapevine, Ophel
A. vitis (biovar 3)	IFO 15145	CG-484, Grapevine, Ophel
A. vitis (biovar 3)	K-Ag-1	Kiwifruit, Sawada
A. vitis (biovar 3)	G-Ag-19	Grapevine, Sawada
A. vitis (biovar 3)	G-Ag-26	Grapevine, Sawada
A. vitis (biovar 3)	G-Ag-60	Grapevine, Sawada

T, type strain

Fatty acid nomenclature. Fatty acids are designated by the total number of their carbon atoms. Figures following the colon indicate the number of double bonds. The prefixes i and ai indicate iso- and anteiso-branched acid. Cyclopropane rings are indicated by cyc. The prefix OH indicates a hydroxy group at the position indicated.

Results and Discussion

The distribution of fatty acids in whole cells of 20 strains of Rhizobium and Agrobacterium examined in this study are shown in Tables 2 and 3. The distribution of non-polar fatty acids in Rhizobium and Agrobacterium strains reported previously (8,9) was also re-examined, and the results of those strains are included in the tables.

Table 2. Cellular fatty acid composition of *Rhizobium* strains

Strain		Non-hydroxylated fatty acid (%)[a]							3-Hydroxy fatty acid (%)[b]									2-Hydroxy fatty acid			
		16:0	16:1	i17:0	18:0	18:1	19:0	21:1	10:0	12:0	i13:0	14:0	i15:0	ai15:0	16:0	16:1	18:0	18:1	16:0	18:1	i19:0	19:0
Rhizobium																					
R. huakuii	IFO 15243 T	15	tr	6	8	39	tr	22	-	12	33	tr	-	-	-	-	tr	55	-	-	-	-
R. huakuii	IFO 15244	17	tr	7	8	62	tr	6	-	15	39	5	-	-	-	-	tr	41	-	-	-	-
R. tropici	IFO 15247 T	10	tr	tr	3	55	tr	22	-	-	-	34	31	-	23	-	12	-	-	52	10	38
R. leguminosarum	IFO 14778 T	10	-	-	16	42	17	15	-	-	-	39	-	17	14	-	30	-	-	-	-	-
R. leguminosarum	IFO 14785	9	-	-	22	36	23	11	-	-	-	58	-	3	8	-	32	-	-	-	-	-
R. leguminosarum	IFO 14784	8	-	-	15	55	14	9	-	-	-	50	-	7	11	-	32	-	-	-	-	-
R. meliloti	IFO 14782 T	14	-	-	7	51	15	12	-	-	-	63	-	-	6	-	31	-	-	-	-	-
R. fredii	IFO 14780 T	8	1	-	10	76	-	4	-	-	-	66	-	-	3	-	31	-	-	-	-	-
R. galegae	IFO 14965 T	24	-	-	4	18	36	18	-	-	-	41	-	-	38	-	14	7	-	-	-	-
R. loti	IFO 14779 T	14	-	5	5	70	6	5	-	10	34	15	-	-	12	10	20	-	-	-	-	-
R. loti	IFO 13336	10	-	6	7	66	5	7	-	19	47	2	-	-	9	9	15	-	-	-	-	-
R. loti	IFO 14996	13	3	3	3	76	1	2	-	11	37	3	-	-	24	8	18	-	-	-	-	-
R. loti	IFO 14997	11	-	5	8	70	2	3	-	17	45	8	-	-	8	6	17	-	-	-	-	-
R. loti	IFO 14998	13	-	5	10	58	4	10	-	19	46	4	-	-	8	8	15	-	-	-	-	-
R. loti	IFO 14999	13	-	5	10	58	6	7	-	22	48	9	-	-	6	4	12	-	-	-	-	-

tr, trace; -, absent.

[a] Percentages of the total non-hydroxylated acids.

[b] Percentages of the total 3-hydroxy acids.

Table 3. Cellular fatty acid composition of Agrobacteium strains

Strain		Non-hydroxylated fatty acid (%)[a]										3-Hydroxy fatty acid (%)[b]										2-Hydroxy fatty acid			
		16:0	16:1	i17:0	18:0	18:1	19:0	19:1	19cyc	21:1	22:0	10:0	12:0	i13:0	14:0	i15:0	ai15:0	16:0	16:1	18:0	18:1	16:0	18:1	i19:0	19:0
Genus Agrobacterium																									
[Biovar 1]																									
A. radiobacter	IFO 13532 T	20	-	-	-	36	-	-	26	15	-	-	-	-	62	-	-	31	-	-	7	-	-	-	-
A. radiobacter	IFO 13533	12	2	-	tr	69	2	1	4	8	2	-	-	-	60	-	-	38	-	2	-	-	-	-	-
A. tumefaciens	IFO 12667	17	-	-	-	51	-	-	23	9	-	-	-	-	64	-	-	30	-	-	6	-	-	-	-
A. tumefaciens	IFO 13264	11	3	-	1	74	1	1	3	5	1	-	-	-	61	-	-	38	-	1	-	-	-	-	-
A. rhizogenes	IFO 14554	20	-	-	-	25	-	-	40	5	-	-	-	-	65	-	-	29	-	2	4	-	-	-	-
A. rhizogenes	IFO 14555	20	-	-	-	29	-	-	38	14	-	-	-	-	65	-	-	29	-	2	4	-	-	-	-
[Biovar 2]																									
A. tumefaciens	IFO 14793	15	-	3	4	19	-	-	35	25	-	-	-	-	22	32	-	28	-	18	-	-	30	65	4
A. rhizogenes	IFO 13257 T	21	-	4	8	22	2	3	14	23	4	-	-	-	10	36	-	28	-	25	-	-	49	24	16
[Biovar 3]																									
A. vitis	NCPPB 2562	9	9	-	2	76	-	-	2	2	-	tr	-	-	53	-	-	14	-	22	11	-	79	12	9
A. vitis	IFO 15140	11	10	-	2	76	-	-	1	1	-	-	-	-	69	-	-	13	-	11	7	-	100	-	-
A. vitis	IFO 15141	8	10	-	2	80	-	-	-	1	-	tr	-	-	71	-	-	12	-	7	10	-	100	-	-
A. vitis	IFO 15142	10	9	-	2	77	-	-	2	1	-	-	-	-	70	-	-	11	-	9	11	-	100	-	-
A. vitis	IFO 15143	10	7	-	3	79	-	-	1	1	-	-	-	-	72	-	-	12	-	10	6	-	100	-	-
A. vitis	IFO 15144	6	6	-	2	78	-	-	1	1	-	-	-	-	72	-	-	13	-	11	4	-	100	-	-
A. vitis	IFO 15145	14	11	-	1	65	-	-	5	4	-	-	-	-	71	-	-	14	-	8	6	-	95	5	-
A. vitis	MAFF 06-63001	6	7	-	-	83	-	-	1	1	-	-	-	-	51	-	-	16	-	26	7	-	89	11	-
A. vitis	K-Ag-1	10	7	-	4	74	-	-	3	2	-	-	-	-	46	-	-	13	-	33	8	-	96	-	4
A. vitis	G-Ag-26	10	6	-	5	72	-	-	5	5	-	tr	-	-	55	-	-	13	-	24	8	-	71	10	19
A. vitis	G-Ag-19	9	6	-	5	71	-	-	5	5	-	-	-	-	51	-	-	12	-	25	12	-	71	12	17
A. vitis	G-Ag-60	6	9	-	2	75	-	-	2	2	-	tr	-	-	41	-	-	15	-	32	11	-	89	-	11
[A. rubi]																									
A. rubi	IFO 13260	21	7	-	2	59	-	1	3	6	1	-	-	-	72	-	-	26	-	2	-	-	-	-	-
A. rubi	IFO 13261 T	16	11	-	1	57	-	1	4	8	1	-	-	-	74	-	-	26	-	tr	-	-	-	-	-
[Probably new species]																									
Agrobacterium sp.	IFO 15292	25	-	2	3	20	-	-	16	28	5	-	-	-	17	32	-	33	-	18	-	-	59	21	19
Agrobacterium sp.	IFO 15293	25	-	2	3	20	-	-	17	28	5	-	-	-	17	33	-	33	-	18	-	-	62	19	19
Agrobacterium sp.	IFO 15294	26	-	2	3	18	-	-	16	29	5	-	-	-	17	33	-	32	-	19	-	-	92	6	28
Agrobacterium sp.	IFO 15295	21	-	2	3	27	tr	-	9	31	7	-	-	-	17	30	-	32	-	21	-	-	96	4	-
Agrobacterium sp.	IFO 15296	21	-	2	3	14	tr	-	36	21	4	-	-	-	27	27	-	25	-	21	-	2	27	16	54
Agrobacterium sp.	IFO 15297	29	-	1	3	17	tr	-	23	29	5	-	-	-	26	27	-	28	-	18	-	4	28	28	39

tr, trace; -, absent.
[a,b] See Table 2.

Fatty acid profile of Rhizobium strains

The non-polar fatty acid composition of a strain of the newly reported species R. tropici was very similar to the composition of strains of Rhizobium and Agrobacterium species, but it does not contain the fatty acid i-17:0. Its hydroxy fatty acid profile differed significantly from those of Rhizobium strains but was the same as those of strains of Agrobacterium biovar 2 (Table 3): The 2-hydroxy acids present were 2-OH 18:1, 2-OH i-19:0, and 2-OH 19:0; and the 3-hydroxy acids were 3-OH 14:0, 3-OH i-15:0, 3-OH 16:0 and 3-OH 18:0.

The non-polar and 3-hydroxy fatty acid profiles of R. huakuii strains were almost the same as those of R. loti strains. They contained 3-OH 12:0, 3-OH i-13:0 and 3-OH 18:1. Both species contained i-17:0 as a characteristic non-polar fatty acid. Strains of other Rhizobium species did not contain this non-polar fatty acid (Table 2).

One of the non-polar fatty acids of Rhizobium and Agrobacterium strains, i-17:0, was assigned for the strain R. loti IFO 14779^T from its electron impact (EI)-mass spectrum and ECL value on GLC by a similar method described previously (8). EI-MS of the fatty acids showed intensive fragments at m/z 74 $[CH_3\underset{\underset{OH}{|}}{O}C{=}CH_2]$, m/z 87 $[CH_3\overset{\overset{O}{||}}{C}(CH_2)_2]^+$, m/z 143 $[CH_3\overset{\overset{O}{||}}{C}(CH_2)_6]^+$, and m/z 241 (M-43). The molecular weight deduced from EI-MS was 284. Based on ECL value on GLC and EI-MS, the fatty acid was assigned as iso-heptadecanoic acid (i-17:0).

Fatty acid profile of Agrobacterium strains

The cellular fatty acid composition of 11 strains of A. vitis and 6 strains of Agrobacterium sp. is shown in Table 3. The strains IFO 15296 (K-AG-3) and IFO 15297 (K-AG-4) isolated from crown galls of kiwi-fruit, and the strains IFO 15292 (Ch-Ag-4), IFO 15293 (Ch-Ag-5), IFO 15294 (Ch-Ag-7) and IFO 15295 (Ch-Ag-8) isolated from crown galls of cherry trees, were tentatively identified as A. tumefaciens but are considered to belong to a new species of the genus Agrobacterium on the basis of their physiological characteristics, DNA-DNA hybridization and 16S rRNA sequencing studies (6 and Sawada et al., in preparation). The fatty acid profiles of these strains were similar to those of the biovar 2 strains (Table 3). They contained both 2-hydroxy and 3-hydroxy fatty acids. A

Table 4. Summary of the cellular fatty acid profiles in Rhizobiaceae and related species

Genera and species	Major fatty acid	i-17:0	2-Hydroxy fatty acids	3-Hydroxy fatty acid						
				12:0	i-13:0	14:0	i-15:0	ai-15:0	16:0	18:0
Bradyrhizobium	18:1	-	-	+	-	+	-	-	-	-
Rhizobium										
R. leguminosarum	18:1 19cyc	-	-	-	-	+	-	+	+	+
R. meliloti R. fredii	18:1	-	-	-	-	+	-	-	±	+
R. galegae	16:0 19cyc	-	-	-	-	+	-	-	+	+
R. loti R. huakuii	18:1	+	-	+	+	+	-	-	+	+
[Agrobacterium biovar 1] A. rubi	18:1 19cyc	-	-	-	-	+	-	-	+	-
[Agrobacterium biovar 2] Kiwifruit strains Cherry strains R. tropici	18:1 19cyc 21:1	+	+	-	-	+	+	-	+	+
[Agrobacterium biovar 3]	18:1	-	+	-	-	+	-	-	+	+

small amount of a characteristic non-polar fatty acid, i-17:0, was also present.

The non-polar, 2-hydroxy and 3-hydroxy fatty acid compositions of A. vitis strains was similar to that of Agrobacterium biovar 2 strains and the strains from kiwifruit and cherry. The 2- and 3-hydroxy acid profiles were the same, but A. vitis strains were different from other Agrobacterium biovar strains in having non-polar acid composed mainly of 18:1, the absence of the characteristic acid i-17:0, the presence of 3-OH 18:1, and the absence of i-15:0.

The cellular fatty acid profiles of the species belonging to Bradyrhizobium, Rhizobium and Agrobacterium obtained in this and the previous studies (8,9) are summarized in Table 4. This study shows that analysis of cellular fatty acids may be a good method for identifying new isolates of Rhizobiaceae.

Table 5 shows the differential characteristics of the species in Rhizobiaceae based on a combination of a biochemical test and hydroxy fatty

Table 5. Differential characteristics of the species in Rhizobiaceae

Group	Genus or species	3-Ketolactose formation	i-17:0	2-OH fatty acids	3-OH 12:0	3-OH i-13:0	3-OH i-15:0	3-OH ai-15:0	3-OH 16:0	3-OH 18:0
1	Bradyrhizobium	-	-	-	+	-	-	-	-	-
2	R. leguminosarum	-	-	-	-	-	-	+	+	+
3	R. meliloti, R. fredii	-	-	-	-	-	-	-	±	+
4	R. galegae	-	-	-	-	-	-	-	+	+
5	R. loti, R. huakuii	-	+	-	+	+	-	-	+	+
6	Agrobacterium biovar 1	+	-	-	-	-	-	-	+	-
7	Agrobacterium biovar 2	-	+	+	-	-	+	-	+	+
	Kiwifruit strains									
	Cherry strains									
	R. tropici									
8	A. vitis (biovar 3)	-	-	+	-	-	-	-	+	+
9	A. rubi	-	-	-	-	-	-	-	+	-

acid profile. On this basis, the species in Rhizobiaceae can be divided into 9 groups. Agrobacterium biovar 2 strains showed the same response in 3-ketolactose test and had the same hydroxy acid profile as those of R. tropici. Kiwifruit and cherry strains of A. tumefaciens were also included in the same group as biovar 2. R. loti and R. huakuii showed the same fatty acid profile. However, in other cases, the combination of these chemotaxonomic characteristics can be used as criteria to differentiate among genera and species of Rhizobiaceae.

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CELL-WALL SUGAR COMPOSITION IN THE GENERA MICROCOCCUS AND DEINOCOCCUS

MARIKO TAKEUCHI and AKIRA YOKOTA

Summary

Cell-wall sugar composition was examined in 21 strains (12 species) of the genus Micrococcus and 5 strains (5 species) of the genus Deinococcus. The neutral sugars galactose, rhamnose, mannose, fucose and/or xylose were contained in the cell walls of all strains of the genera Micrococcus and Deinococcus except for 6 strains of "Micrococcus aquaticorosans." The sugar profiles were not characteristic of the genus in either case, but sugar composition was uniform at an intraspecies level. Cell walls of "M. aquaticorosans" strains are composed of arabinose and galactose, characteristic sugar components found in the new genus of Gram-positive cocci represented by "Kineococcus aurantiacus," which strongly suggested that these strains be excluded from the genus Micrococcus.

Keywords: Cell-wall sugar composition, genus Micrococcus, genus Deinococcus

Cell wall amino acid composition has proven to be a useful taxonomic criterion at the generic level for classifying Gram-positive bacteria (5), but cell-wall sugar composition has not been established as a valuable criterion, and its taxonomic significance remains to be resolved (10). Previously (19, 20), we reported the taxonomic significance of cell-wall sugar composition in coryneform bacteria, and we clarified that the kinds of cell-wall polymer are useful within the genus, and that sugar composi-

tion and molar ratio of the cell-wall polymer are uniform at an intra-species level.

A comparative analysis of 16S ribosomal RNA (rRNA) sequences suggests that the genus Micrococcus (11) is more closely related to the genus Arthrobacter than to other genera of Gram-positive cocci, such as Staphylococcus, Planococcus (12, 18) and Sporosarcina (3), and that Micrococcus luteus and Arthrobacter globiformis strains should be placed in a single taxon (13). Micrococcus species should be regarded as a degenerated form of arthrobacters (9), locked into the coccoid stage of the Arthrobacter life cycle. Amino acid composition of the peptidoglycan of the genera Micrococcus has been studied well (8, 11, 15-17), but data on the cell-wall sugar composition of the genus Micrococcus are still incomplete (8).

The genus Deinococcus (14) is believed to be a Gram-positive bacteria and near to Micrococcaceae (1, 2), but its superficial phenotype is more closely allied to Gram-negative bacteria. Recently, it was reported that the genus Deinococcus appears relatively close to the genus Thermus based on the comparative analysis of 16S rRNA sequences (22). The profile of the cell wall of Deinococcus species includes an outer membrane, which is structurally complex and layered (17), and the peptidoglycan does not seem to be associated with another polymer (21).

In this paper, we describe the results of analysis of sugar composition of cell walls in the genera Micrococcus and Deinococcus.

Materials and Methods

Bacterial strains. The strains used are shown in Table 1. Deinococcus erythromyxa may in future be excluded from the genus Deinococcus (4, 6) but is tentatively included there in the present study. For comparison of cell-wall sugar composition, "Kineococcus aurantiacus," which was newly proposed by Yokota et al. (23) as an aerobic Gram-positive, motile coccus with meso-diaminopimelic acid and arabinogalactan in the cell wall, was also used. All strains were cultured at 28°C with shaking in PY medium containing 1% peptone, 0.2% NaCl and 0.2% glucose, pH 7.0.

Preparation of cell walls and chemical analysis. Cell walls were prepared by disrupting the cells (wet wt. ca. 10 g) with glass beads (0.11

Table 1. Bacterial strains studied.

Genus	Species	IFO	Other strain designations
Micrococcus	M. agilis	15260^T	ATCC 966, DSM 20550
	M. agilis	15343$_T$	CCM 2688, JCM 2587
	M. halobius	15353$_T$	ATCC 21727, DSM 20541, CCM 2591
	M. kristinae	15354$_T$	ATCC 27570, DSM 20032, CCM 2690
	M. lylae	15355$_T$	ATCC 27566, CCM 2693
	M. luteus	3333^T	ATCC 4698
	M. luteus	12708$_T$	ATCC 9341, IAM 1099
	M. nishinomiyaensis	15356$_T$	ATCC 29093, DSM 20448, CCM 2140
	M. roseus	3768$_T$	ATCC 186
	M. sedentarius	15357$_T$	ATCC 14392, DSM 20547, CCM 314
	M. varians	15358^T	ATCC 15306, DSM 20033, CCM 884
	"M. aquaticorosans"	15324	ATCC 27221, CCM 2583
	"M. aquaticorosans"	15325	ATCC 27219, CCM 2584
	"M. aquaticorosans"	15349	ATCC 27222, CCM 2585
	"M. aquaticorosans"	15350	ATCC 27220, CCM 2586
	"M. aquaticorosans"	15351	ATCC 27218, CCM 2587
	"M. aquaticorosans"	15352$_T$	ATCC 27223, CCM 2588
	"M. aurantiacus"	15364^T	ATCC 11731
	"M. conglomeratus"	15266	CCM 2136, IAM 1448
	"M. conglomeratus"	15267$_T$	CCM 2137, IAM 1480
Deinococcus	D. erythromyxa*	15344$_T$	ATCC 187, CCM 706
	D. proteolyticus	15345^T	ATCC 35074, DSM 20540, CCM 2703
	D. radiodurans	15346^T	ATCC 13939, DSM 20539, CCM 1700
	D. radiophilus	15347$_T$	ATCC 27603, DSM 20551, CCM 2564
	D. radiopugnans	15348$_T$	ATCC 19172, CCM 2785
"Kineococcus"	"K. aurantiacus"	15268^T	

T: type strain.
Abbreviations for culture collections: ATCC, American Type Culture Collections, Rockville, Maryland, U.S.A.; DSM, Deutsche Sammulung von Mikroorganismen, Mascheroder Weg 1b, D-3300, Braunschweig, F.R.G.; IAM, Institute of Applied Microbiology, The University of Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan.
* See text.

- 0.12 mm in diameter) using a Kubota sonic oscillator at 180 W for 30 min, followed by treatment with sodium dodecyl sulfate, RNase A and pronase E, and then lyophilized as described by Schleifer and Kandler (16).

For quantitative determination of sugar composition, cell wall (dry wt. ca. 2 mg) was hydrolyzed with 2N HCl at 100°C for 3 hr. After removal

of HCl, the hydrolysate was analyzed using a high performance liquid chromatography (HPLC) apparatus (Shimadzu LC-5A) equipped with a Shim-pack ISA 07/S2504 column (250 x 4 mm) and a Shimadzu RE-530 spectrofluorometer as descrived previously (19).

Results and Discussion

Table 2 shows the molar ratio of cell-wall sugars relative to galactose. Cell walls of M. luteus, M. roseus and "M. conglomeratus" were composed of galactose only, and those of M. agilis, M. lylae, M. nishinomiyaensis and M. varians were composed of galactose and a small amount of glucose. Rhamnose and mannose were detected respectively in the cell walls of M. halobius and M. kristinae as diagnostic sugar components besides galactose, and these sugars are characteristic of these species in the genus Micrococcus. In the cell wall of M. sedentarius, neutral sugar was not detected. On the other hand, cell walls of six strains of "M. aquaticorosans" were composed of arabinose and galactose (molar ratio of 1.2-2.0 : 1.0) as diagnostic sugar and glucose as a minor component. Cell-wall polymer of "M. aquaticorosans" seems to be arabinogalactan, and therefore, this species could be excluded from the genus Micrococcus. Recently, Yokota et al. proposed a new genus Kineococcus with one species, Kineococcus aurantiacus, for aerobic gram-positive, motile coccus (23). K. aurantiacus is characterized by the presence of meso-diaminopimelic acid (DAP) and arabinogalactan in the cell wall, menaquinone MK-9(H_2) and G+C content of DNA of 73.9 mol%, but does not have mycolic acid. On the other hand, "M. aquaticorosans" has the following characteristics: major respiratory quinone is menaquinone MK-8(H_2), G+C content of DNA is 68.9 - 69.7 mol%, and meso-DAP, arabinogalactan and mycolic acid are present (data not shown). These chemotaxonomic characteristics indicate that "M. aquaticorosans" dose not belong to the genus Kineococcus. To clarify the taxonomic position of this species, further studies are progress in our laboratory and will be published elsewhere. Cell wall of "M. aurantiacus" was composed of glucose only and that of "M. conglomeratus" was composed of galactose only.

In the genus Deinococcus, cell walls of all the strains except for D. radiophilus and D. erythromyxa were composed of galactose and glucose as

Table 2. Molar ratio of cell-wall sugars in species of the genera Micrococcus, Deinococcus and "Kineococcus"

Genus	Species	IFO	Molar ratio of sugar composition[a]						
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc
Micrococcus	M. agilis	15260^T	-	-	-	-	-	1.0	0.15
	M. agilis	15343$_T$	-	-	-	-	-	1.0	0.05
	M. halobius	15353^T	1.03	-	-	-	-	1.0	1.40
	M. kristinae	15354^T	-	-	-	-	3.1	1.0	-
	M. lylae	15355^T	-	-	-	-	-	1.0	0.29
	M. luteus	3333^T	-	-	-	-	-	1.0	-
	M. luteus	12708$_T$	-	-	-	-	-	1.0	-
	M. nishinomiyaensis	15356$_T$	-	-	-	-	-	1.0	0.30
	M. roseus	3768^T	-	-	-	-	-	1.0	-
	M. sedentarius	15357^T	-	-	-	-	-	-	-
	M. varians	15358^T	-	-	-	-	-	1.0	0.30
	"M. aquaticorosans"	15324	-	-	1.2	-	-	1.0	0.43
	"M. aquaticorosans"	15325	-	-	1.2	-	-	1.0	0.37
	"M. aquaticorosans"	15349	-	-	2.2	-	-	1.0	0.35
	"M. aquaticorosans"	15350	-	-	2.0	-	-	1.0	0.17
	"M. aquaticorosans"	15351	-	-	2.0	-	-	1.0	0.29
	"M. aquaticorosans"	15352$_T$	-	-	2.0	-	-	1.0	0.26
	"M. aurantiacus"	15364^T	-	-	-	-	-	-	1.0
	"M. conglomeratus"	15266	-	-	-	-	-	1.0	-
	"M. conglomeratus"	15267$_T$	-	-	-	-	-	1.0	-
Deinococcus	D. erythromyxa*	15344^T	-	1.14	-	0.20	-	1.0	0.41
	D. proteolyticus	15345^T	-	-	-	-	-	1.0	0.37
	D. radiodurans	15346^T	0.10	-	-	-	-	1.0	0.55
	D. radiophilus	15347^T	-	-	-	-	-	tr	-
	D. radiopugnans	15348^T	-	-	-	-	-	1.0	0.13
"Kineococcus"	"K. aurantiacus"	15268^T	-	-	0.35	-	-	1.0	0.81

[a] Abbreviations for sugars: Rha, rhamnose; Fuc, fucose; Ara, arabinose; Man, mannose; Xyl, xylose; Gal, galactose; Glc, glucose; tr, trace.

diagnostic sugar components and a small amount of rhamnose was detected in D. radiodurans. In the cell wall of D. erythromyxa, fucose and xylose, which are characteristic of this species, were detected in addition to galactose and glucose. But this species is reported to be excluded from the genus Deinococcus based on the lipid profile and the analysis of cell wall amino acid composition (4, 6). In the cell wall of D. radiophilus, only amino sugars were detected. Thus, although Bergey's Manual of Systematic Bacteriology Vol. 2 (14) notes that the peptidoglycan of the genus Deinococcus does not seem to be associated with another polymer (21), all

the species except for D. radiophilus were confirmed to contain sugar polymer in their cell walls.

From the results described here, it was clarified that the cell-wall polymer of the genera Micrococcus and Deinococcus is a neutral polysaccharide composed of rhamnose, mannose, galactose and glucose, and that although cell-wall sugar composition was characteristic of the species, it is neither uniform within either genus nor characteristic of the genus in the sugar profile. This was also true of the genus Arthrobacter and other actinobacteria (7, 19, 20) except for the genera Corynebacterium, Mycobacterium and Rhodococcus, which have arabinose and galactose as diagnostic sugars.

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DISTRIBUTION OF MADUROSE, AN ACTINOMYCETE WHOLE-CELL SUGAR, IN THE GENUS *STREPTOMYCES*

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Summary

The distribution of madurose, an actinomycete whole-cell sugar, in the strains of genus *Streptomyces* was examined. Among 111 strains (103 species) studied, madurose was detected in 25 strains (22 species) of *Streptomyces*. Most of the species of the 'setae group' of *Streptomyces* contained this sugar. The taxonomic value of madurose in this genus is discussed.

Keywords: Madurose, *Streptomyces* strains, whole-cell sugar, chemotaxonomy of actinomycetes.

Cell wall chemotype and whole-cell sugar pattern have become widely used as chemotaxonomic markers to differentiate distinct genera of actinomycetes (1). Twelve genera of aerobic actinomycetes with *meso*-2, 4-diaminopimelic acid (A_2pm) are known to possess madurose as whole-cell sugar (cell wall chemotype III, whole-cell sugar pattern B). Recently, it has been reported that *Micromonospora carbonacea* subsp. *carbonacea*, *Micromonospora chalcea* subsp. *izumensis* and *Micromonospora rosaria* (2) having whole-cell sugar pattern D (cell wall chemotype II), and *Kibdelosporangium aridum* (9) having whole-cell sugar pattern A (cell wall chemotype IV) possessed madurose in their whole cells, but we found madurose to be absent in all of these strains except for *M. rosaria* (4). Furthermore, we have found that seven *Streptomyces* species which were considered to contain no characteristic sugars in the whole cells (cell wall chemotype

I) contained madurose (4, 8). Therefore, we examined the distribution of madurose among *Streptomyces* species. For precise detection of even a small amount of madurose, we used an enzymatic HPLC method (6, 7).

The present paper describes the distribution of strains with madurose as whole-cell sugar in the genus *Streptomyces*.

Materials and Methods

Bacterial strains. The strains used in this study were selected based on the classification by Williams *et al.* (10) and are shown in Table 1. The superscript 'T' indicates type strain.

Whole-cell sugar analysis. Cells used for whole-cell sugar analysis were obtained by incubation at 28 C for 3 days in yeast extract-glucose broth (pH 7.1) containing (per liter) 10 g of yeast extract and 10 g of glucose. Whole-cell sugars were analyzed by the HPLC method of Yokota and Hasegawa (6, 7) with modification. HPLC was performed by stepwise elution with borate buffers at concentrations of 0.15 M (pH 9.0) for 20 min, 0.3 M (pH 9.0) for 10 min, and 0.4 M (pH 9.0) for 15 min.

Results and Discussion

Analysis of whole-cell sugar by HPLC.

Figure 1 shows the HPLC chromatogram of whole-cell hydrolysate of *Streptomyces cochleatus* IFO 14767. The hydrolysate contained glucose, galactose, mannose and madurose. The authenticity of peak ascribed to madurose was confirmed by its retention time and its disappearance after incubation with D-galactose oxidase (6, 7).

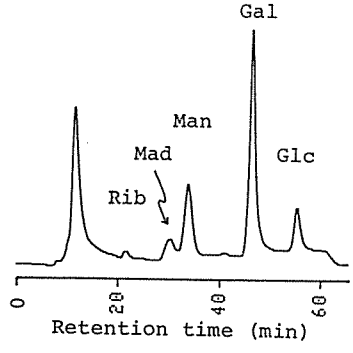

Fig 1. HPLC chromatogram of whole-cell hydrolysate of *Streptomyces cochleatus* IFO 14767.
Peak identification: Rib, ribose; Mad, madurose; Man, mannose; Gal, galactose; Glc, glucose.

Selection of bacterial strains.

Williams *et al.* (10) classified *Streptomyces* species into 23 major, 20 minor, and 25 single-member clusters consisting of 219, 46, and 25 validly described species, respectively, based on the results of numerical classification studies. The three groups of clusters were named category I, II and III, respectively. Clusters in each category were represented by the earliest validly described species in each cluster, and the other species in a cluster are listed as subjective synonyms or allied species. To determine whether the presence or absence of madurose is related to the classification by Williams *et al.*, we examined whole-cell sugars of species in categories I, II, III and the setae group (3, 5). Furthermore, the results of invalidly published species are summarized in Table 1.

Distribution of madurose in the genus *Streptomyces*

Nine of the species in category I, *S. violaceus*, *S. rochei*, *S. albus* subsp. *albus*, *S. cacaoi* subsp. *cacaoi*, *S. anbofaciens*, *S. lydicus*, *S. libani*, *S. platensis*, and *S. albofaciens*, were found to contain madurose in their whole cells (Table 1).

S. lydicus has five species of subjective synonyms and one allied species. Two of the species of subjective synonyms, *S. libani* and *S. platensis* were found to contain madurose, while the other three species of subjective synonyms, *S. griseoplanus*, *S. nigrescens* and *S. sioyaensis* and one allied species, *S. albulus*, did not.

The whole-cell sugars of three strains of *S. platensis* were examined, and all strains were found to contain madurose. One invalidly published subspecies of *S. platensis*, '*S. platensis* subsp. *malvinus*' was also found to contain it.

The type species of the genus *Streptomyces*, *S. albus* subsp. *albus* IFO 13014^{T} was found to contain madurose, but its content was affected by unknown factors of the culture conditions, and it was sometimes completely absent. While the other five strains of *S. albus* subsp. *albus* and another subspecies of *S. albus*, *S. albus* subsp *pathocidicus*, did not contain madurose, the invalidly published subspecies '*S. albus* subsp. *coleimyceticus*' contained it.

In category II, five species, *S. aureofaciens*, *S. chattanoogensis*, *S. catenulae*, *S. albolongus*, *S. misakiensis*, were found to contain madurose. However, *S. griseoluteus* and *S. xanthochromogenes*, which are the subjective synonyms of *S. catenulae* and *S. albolongus*, respectively, did not contain madurose.

Table 1. The Distribution of madurose in the genus *Streptomyces*

Strains	IFO No.	Madurose
Category I		
S. albidoflavus	13010^T	-
S. rutgersensis subsp. *rutgersensis*	12819^T	-
S. anulatus	13369^T	-
S. halstedii	12783^T	-
S. exfoliatus	13919^T	-
S. violaceus	13103^T	+
S. fulvissimus	13482^T	-
S. longispororuber	13448^T	-
S. rochei	12908^T	+
S. chromofuscus	12851^T	-
S. argenteolus	12841^T	-
S. albus subsp. *albus*	3195	-
S. albus subsp. *albus*	3418	-
S. albus subsp. *albus*	3422	-
S. albus subsp. *albus*	3710	-
S. albus subsp. *albus*	3711	-
S. albus subsp. *albus*	13014^T	+
S. albus subsp. *pathocidicus*	13812^T	-
S. cacaoi subsp. *cacaoi*	12748^T	+
S. griseoviridis	12874^T	-
S. cyaneus	13346^T	-
S. diastaticus	13412^T	-
S. achromogenes subsp. *achromogenes*	12735^T	-
S. olivaceoviridis	13066^T	-
S. griseoruber	12873^T	-
S. lydicus	13058^T	+
S. griseoplanus	12779^T	-
S. libani	13452^T	+
S. nigrescens	12894^T	-
S. platensis	12901^T	+
S. platensis	14007	+
S. platensis	14008	+
S. sioyaensis	12820^T	-
S. albulus	13410^T	-
S. violaceusniger	13459^T	-
S. endus	12859^T	-
S. hygroscopicus subsp. *hygroscopicus*	13472^T	-
S. hygroscopicus subsp. *angustmyceticus*	14017^T	-
S. griseoflavus	13044^T	-
S. phaeochromogenes	12898^T	-
S. rimosus	12907^T	-
S. albofaciens	12833^T	+
S. kanamyceticus	13414^T	-
S. microflavus	13062^T	-
S. antibioticus	12838^T	-
S. lavendulae subsp. *lavendulae*	12789^T	-
S. purpureus	13927^T	-
Category II		
S. aburaviensis	12830^T	-
S. californicus	12750^T	-
S. cellulosae	13027^T	-
S. aureofaciens	12843^T	+
S. flaveolus	12768^T	-
S. filipinensis	12860^T	-
S. noboritoensis	13065^T	-
S. chattanoogensis	12754^T	+
S. thermovulgaris	13089^T	-
S. longisporoflavus	12886^T	-

Table 1. continued

Strains	IFO No.	Madurose
S. griseoluteus	13375^T	-
S. catenulae	12848^T	+
S. pactum	13433^T	-
S. aurantiacus	13017^T	-
S. luridus	12793^T	-
S. xanthochromogenes	12828^T	-
S. albolongus	13465^T	+
S. misakiensis	12891^T	+
S. psammoticus	13971^T	-
S. fradiae	12773^T	-
S. poonensis	13485^T	-
S. atroolivaceus	12741^T	-
Category III		
S. prunicolor	13075^T	-
S. canus	12752^T	-
S. graminofaciens	13455^T	-
S. viridochromogenes	13347^T	-
S. glaucescens	12774^T	-
S. nogalater	13455^T	-
S. prasinosporus	13419^T	-
S. ochraceiscleroticus	13483^T	-
S. aurantiogriseus	12842^T	-
S. bambergiensis	13479^T	-
S. gelaticus	12866^T	-
S. amakusaensis	12835^T	-
S. varsoviensis	13093^T	-
S. tubercidicus	13090^T	-
S. badius	12745^T	-
S. ramulosus	12812^T	-
S. sulphureus	13345^T	-
S. yerevanensis	12517^T	-
S. massasporeus	12796^T	+
S. alboflavus	13196^T	-
S. bikiniensis	14598^T	-
S. fragilis	12862^T	-
S. lateritius	12788^T	-
S. finlayi	13201^T	-
S. novaecaesareae	13368^T	-
Setae group		
S. setae	14216^T	+
S. griseolosporeus	14371^T	+
S. phosalacinea	14372^T	+
S. mediocidica	14789^T	-
S. atroaurantiacus	14327^T	+
S. cystargineus	14836^T	+
S. kifunensis	15206^T	-
S. cochleatus	14767	+
S. cochleatus	14768^T	+
S. paracochleatus	14769^T	+
S. azaticus	13803^T	-
Others		
Streptomyces sp.	14834	-
Streptomyces sp.	14835	-
'*S. albus* subsp. *coleimyceticus*'	13840^T	+
'*S. platensis* subsp. *malvinus*'	13827^T	+
'*S. platensis* subsp. *robigocidicus*'	13818^T	-
'*S. hygroscopicus* subsp. *aabomyceticus*'	13815^T	-

The presence of madurose was shown by the mark '+'.

In category III, only one species, *S. massasporeus*, was found to contain madurose.

We have proposed classifying *Streptomyces* species into two groups, the setae group and the original *Streptomyces* group (3, 5). Seven of the ten species in the setae group, *S. setae, S. griseolosporeus, S. phosalacinea, S. atroaurantiacus, S. cystargineus, S. cochleatus* and *S. paracochleatus* were found to contain madurose. The other three species in this group, *S. mediocidicus, S. kifunensis* and *S. azaticus*, did not contain madurose.

As described above, the distribution of madurose in the *Streptomyces* species does not seem to correlate well with the grouping of categories of subjective synonyms by Williams *et al.* (10). Therefore, the present study suggests that madurose cannot be used as a criterion to differentiate species of the genus *Streptomyces*. However, since seven of the ten species in the setae group contained madurose, this sugar may be helpful in classifying strains belonging to the setae group in the genus *Streptomyces*.

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TAXONOMIC STUDIES ON TWO SOIL ISOLATES AND FOUR ACTINOMYCETE STRAINS IN IFO CULTURE COLLECTION

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Summary

Taxonomic positions of two strains, N-36 and N-37, isolated from soil in Nepal and four strains in the IFO culture collection, '*Streptomyces hofunensis*' IFO 13810^T, '*Micropolyspora roseoalba*' IFO 14337^T, '*Micropolyspora cinereoflava*' IFO 14338^T and '*Kitasatosporia clausa*' IFO 15240^T, were studied. Morphological, physiological and chemotaxonomic characteristics of the isolates indicated that strains N-36 and N-37 might be new species in the genera *Actinomadura* and *Microtetraspora*, respectively. Taxonomic characteristics of the strains IFO 13810^T, IFO 14337^T, IFO 14338^T and IFO 15240^T indicated that all the strains belong to the genus *Microtetraspora*. On the basis of DNA-DNA homology studies, '*M. roseoalba*' IFO 14337^T and '*M. cinereoflava*' IFO 14338^T were classified as *Microtetraspora fastidiosa*. '*K. clausa*' IFO 15240^T was identified as *Microtetraspora helvata* based on the similarity of their taxonomic characteristics. '*S. hofunensis*' IFO 13810^T was suggested to be a new species of the genus *Microtetraspora*.

Keywords: Taxonomy of actinomycetes, soil isolates in Nepal, *Streptomyces hofunensis*, *Micropolyspora roseoalba*, *Micropolyspora cinereoflava*, *Kitasatosporia clausa*, *Microtetraspora fastidiosa*, *Micro-*

tetraspora helvata.

In the course of screening of rare actinomycetes, strains N-36 and N-37 were isolated from soil in Nepal. Based on their morphological, physiological and chemotaxonomic characteristics, these strains were ascribed to the genera *Actinomadura* and *Microtetraspora*, respectively.

The genus *Micropolyspora* was established by Lechevalier *et al.* with the type species *Micropolyspora brevicatena* (13). However, *M. brevicatena* was transferred to the genus *Nocardia* (6). The genus *Kitasatosporia* established by Omura *et al.* (29) was unified with the genus *Streptomyces* at the proposal of Wellington *et al.* (39). While the genera *Micropolyspora* and *Kitasatosporia* thus no longer exist, however, strains IFO 14337^T, IFO 14338^T and IFO 15240^T have been left under the names of '*Micropolyspora roseoalba*' (15), '*Micropolyspora cinereoflava*' (15) and '*Kitasatosporia clausa*' (14).

'*Streptomyces hofunensis*' IFO 13810^T proposed by Nara *et al.* (24) was found not to contain LL-A_2pm in the cell wall, but to contain *meso*-A_2pm (23) at our laboratory. Therefore, it obviously does not belong to the genus *Streptomyces*.

This paper deals with the results of morphological, physiological and chemotaxonomic studies on two isolates from soil and four IFO strains of which the taxonomic positions are uncertain.

Materials and Methods

Bacterial strains. The strains used in this study are summarized in Table 1. *Catellatospora ferruginea* IFO 14496^T was used as a reference for the DNA relatedness study. The superscript 'T' indicates type strain.

Morphological observations. For morphological observations under an optical microscope and a scanning electron microscope (SEM) (JSM 5400, JEOL Ltd.), the strains were grown on media recommended by Shirling and Gottlieb (32). The samples for SEM were prepared by dehydrating agar blocks of the culture media through a graded ethanol series without fixation, then in a critical point drying apparatus (Hitachi HCP-2).

Cultural observations. Cultural characteristics were recorded after 14 days of incubation at 28 C or 37 C based on the method of the Interna-

Table 1. Strains used in this study

Soil isolate and species	Strain	Other strain designations	Source and comment
Soil isolate	N-36		soil, this study
Soil isolate	N-37		soil, this study
'*Streptomyces hofunensis*'	IFO 13810^T	ATCC 21970, FERM P-2216	soil,Yamamoto (25)
'*Micropolyspora roseoalba*'	IFO 14337^T	NRRL B-16241	soil, Lu (15)
'*Micropolyspora cinereoflava*'	IFO 14338^T	NRRL B-16242	soil, Lu (15)
'*Kitasatosporia clausa*'	IFO 15240^T	IMAS 4.1223	soil, Liu (14)
Actinomadura livanotica	IFO 14095^T	ATCC 35576, NRRL B-16097	
Actinomadura madurae	IFO 13909^T	ATCC 19425	
Microtetraspora fastidiosa	IFO 14680^T	ATCC 33516, DSM 43674	
Microtetraspora helvata	IFO 14681^T	ATCC 27295, DSM 43142	
Microtetraspora recticatena	IFO 14525^T	VKM Ac 940, DSM 43937	

ATCC, American Type Culture Collection, Rockville, U.S.A.; DSM, Deutsche Sammlung von Mikroorganismen, Mascheroder Weg 1b, D-3300, Braunschweig, FRG; FERM, Fermentation Research Institute, Agency of Industrial, Sciences and Technology, Tsukuda, Japan; IMAS, Institute of Microbiology, Academia Sinica, Beijing, China; NRRL, ARS Culture Collection, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, U.S.A.; VKM, All-Union Collection of Microorganisms, Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Science, Pushino, Moscow Region, Russia.

tional Streptomyces Project (ISP) (32). The Color Harmony Manual (9) was used to identify the color designation of strains.

Physiological tests. Carbohydrate utilization was determined by the method of Pridham and Gottlieb (31) and Nonomura and Ohara (26). Temperature range for growth was determined on ISP medium No. 2 agar slants at temperatures of 5-50 C. Tolerance to NaCl was measured using nutrient agar medium with various concentrations of NaCl. Hydrolysis of starch and milk and decomposition of adenine, L-tyrosine, xanthine, hypoxanthine and esculin were evaluated using the media of Gordon *et al.* (7). Reduction of nitrate and production of melanoid pigment were determined by the methods of ISP (32). Liquefaction of gelatin and decomposition of calcium malate were evaluated by the method of Waksman (34). All cultures were incubated at 28 C and their characteristics were recorded after 14 days.

Chemotaxonomic analysis. Cells used for chemotaxonomic analysis were obtained by incubation at 28 C for 3 days in yeast extract-glucose broth (pH 7.1) containing (per liter) 10 g of yeast extract and 10 g of glucose. Isomers of A_2pm were analyzed by thin-layer chromatography (TLC) by the method of Hasegawa *et al.* (8) and high-performance liquid chromatography (HPLC) by the method of Takahashi *et al.* (34). Amino acid compositions of cell walls were analyzed by gas-liquid chromatography (GLC) (28). Whole-cell sugars were analyzed by HPLC by the method of Mikami and Ishida (18). Phospholipid compositions were determined by the method of Minnikin *et al.* (20). Mycolic acids were analyzed by the method of Minnikin *et al.* (19). Menaquinones were extracted and purified by the method of Collins *et al.* (1) and analyzed by HPLC (2). The guanine-plus-cytosine (G+C) contents of DNAs were determined by HPLC (35).

Determination of DNA relatedness. DNA homologies between strains were determined fluorometrically by the modified method of Ezaki *et al.* (3), as described in the previous paper (24).

Results and Discussion

I. Taxonomic characteristics

Cultural, physiological and chemotaxonomic characteristics of the six strains are summarized in Tables 2, 3 and 4, respectively.

Strain N-36

Aerial mycelia of the strain N-36 were rectiflexible, and formed partly hooks or spirals with one turn. Mature spore chains on aerial mycelia consisted of spherical spores with interspore pads. Spore was smooth, 1.1 to 1.3 μm in diameter and 1.0 to 1.1 μm in length. No synnema, sporangium, zoospore or sclerotium was observed. This strain grew moderately on yeast extract-malt extract agar, Bennett's agar and nutrient agar. On yeast extract-malt extract agar and Bennett's agar, pinkish white colored aerial mycelia were observed. The cell walls consisted of glutamic acid, alanine and *meso*-A_2pm. It contained glucose, galactose, mannose, ribose and madurose as whole-cell sugars. These data indicated that strain N-36 had a chemotype III cell wall and a type B whole-cell sugar pattern. As phospholipids, it contained phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), but not phosphatidylethanolamine (PE), phosphatidylcholine (PC), and unidentified phospholipid containing glucosamine (NPG) (type PI). Mycolic acids were absent. MK-9(H_4), MK-9(H_6) and MK-9(H_8) were detected as the predominant menaquinones in the ratio of 1:2:1. 15-Methylpentadecanoic acid (*iso*-16:0) and 10-methyloctadecanoic acid (10Me-18:0) were contained as predominant fatty acids. Hydroxy fatty acid was absent. The G+C content of DNAs was 70.2 mol%.

Strain N-37

Strain N-37 formed rectiflexible aerial mycelia. Mature spore chains on aerial mycelia consisted of cylindrical spores. Spore was smooth in surface, 0.8 to 0.9 μm in diameter and 1.1 to 1.3 μm in length. No synnema, sporangium, zoospore or sclerotium was observed. This strain showed good growth on yeast extract-malt extract agar, Czapek's agar and Bennett's agar, and white aerial mycelia were observed. The cell walls consisted of glutamic acid, alanine and *meso*-A_2pm (cell wall chemotype

Table 2. Cultural characteristics of strains N-36 and N-37, '*Streptomyces hofunensis*' IFO 13810^T, '*Micropolyspora roseoalba*' IFO 14337^T, '*Micropolyspora cinereoflava*' IFO 14338^T and '*Kitasatosporia clausa*' IFO 15240^T.

Media	N-36	N-37	IFO 13810^T	IFO 14337T	IFO 14338^T	IFO 15240^T
Yeast extract-malt extract agar (ISP-medium 2)	G:moderate, colonial yellow (2ga)-maple(4le) AM:moderate, shell pink(5ba) -fresh pink(6ca) SP:none	G:moderate, wrinkled lt amber(3ic) AM:moderate, slight yellowish white SP:none	G:moderate, wrinkled, bamboo(2gc) AM:moderate, white SP:none	G:moderate, wrinkled cinnamon(3le) AM:good, sand(3cb)-5cb SP:none	G:moderate wrinkled topaz(3ne) AM:poor, white SP:none	G:moderate, wrinkled, lt amber (3ic)-camel(3ie) AM:poor, white SP:none
Oatmeal agar (ISP-medium 3)	G:none AM:none SP:none	G:moderate, flat colorless AM:poor, white SP:none	G:moderate, flat canary yellow (1ea) AM:poor, white SP:none	G:moderate honey gold(2ic) AM:moderate, pearl(3ba) SP:none	G:moderate, lt antique gold (11/2ic) AM:poor, white SP:none	G:poor, lt ivory(2ca) -lt wheat(2ea) AM:none SP:none
Inorganic salts-starch agar (ISP-medium 4)	G:poor, flat colorless AM:poor, white SP:none	G:moderate, lt yellow (11/2ea) AM:poor, lt wheat(2ea) SP:none	G:moderate, flat canary yellow (1ea) AM:moderate, white SP:none	G:moderate, flat, lt antique gold(11/2ic) AM:poor, pearl(3ba) SP:none	G:moderate, flat lt ivory(2ca) AM:poor, white SP:none	G:moderate, lt ivory(2ca) AM:poor, white SP:none
Glycerol-asparagine agar (ISP-medium 5)	G:poor, flat colorless AM:poor, white SP:none	G:moderate, lt wheat(2ea) -camel(3ie) AM:poor, white SP:none	G:moderate, flat, squash yellow (2ia) AM:poor, white SP:none	G:moderate, flat lt wheat(2ea) AM:poor, white SP:none	G:moderate, flat honey gold(2ic) AM:none SP:none	G:poor, lt ivory(2ca) AM:none SP:none
Peptone-yeast extract-iron agar (ISP-medium 6)	G:moderate, lt wheat(2ea) -lt amber(3ic) AM:moderate, white SP:none	G:poor, lt wheat(2ea) -amber(3nc) AM:none SP:none	G:poor, elevated lt tan(3gc) -camel(3ic) AM:none SP:none	G:poor, wrinkled cinnamon(3le) AM:none SP:none	G:poor, wrinkled topaz(3ne) AM:none SP:none	G:poor, wrinkled, lt amber(3ic) -cinnamon(3le) AM:none SP:none
Tyrosine agar (ISP-medium 7)	G:poor, flat, colorless AM:poor, white SP:none	G:moderate, lt wheat(2ea) -camel(3ie) AM:poor, white SP:none	G:moderate, flat bamboo(2gc) AM:poor, white SP:none	G:moderate, honey gold(2ic) AM:poor, pearl(3ba) SP:none	G:moderate, flat, honey gold(2ic) AM:none SP:none	G:poor, amber(3pe) AM:none SP:none
Czapek's agar	G:poor, colorless AM:poor, white SP:none	G:moderate, dusty yellow(11/2gc) AM:moderate, white -lt ivory(2ca) SP:none	G:moderate lt yellow(11/2ea) AM:none SP:none	G:thin, colorless -lt wheat(2ea) AM:poor,white SP:none	G:thin, colorless -lt wheat(2ea) AM:none SP:none	G:no data AM:no data SP:no data
Nutrient agar	G:moderate, colorless -lt wheat(2ea) AM:poor, white SP:none	G:moderate, lt wheat(2ea) -honey gold(2ic) AM:none SP:none	G:moderate, glittering honey gold(2ic) AM:none SP:none	G:moderate, honey gold(2ic) AM:poor, white SP:none	G:moderate, amber(3pc) AM:none SP:none	G:moderate camel(3ie) AM:none SP:none
Bennett's agar	G:moderate, lt yellow(11/2ea) -lt tan(3gc) AM:moderate, shell pink(5ba) -fresh pink(6ca) SP:none	G:good, amber (3nc)-golden olive(11/2pq) AM:moderate, white SP:none	G:moderate, lt tan(3gc) -camel(3ie) AM:good, white SP:none	G:moderate, wrinkled, topaz(3ne) AM:poor, white SP:none	G:moderate, honey gold(2ic)-mustard gold(2ne) AM:poor, white SP:none	G:moderate, wrinkled, cinnamon(3le) AM:none SP:none

Abbreviations: G, growth of vegetative mycelia; AM, aerial mycelia; SP, soluble pigments; lt, light.

Table 3. Physiological characteristics of strains N-36 and N-37, '*Streptomyces hofunensis*' IFO 13810^T '*Micropolyspora roseoalba*' IFO 14337^T, '*Micropolyspora cinereoflava*' IFO 14338^T and '*Kitasatosporia clausa*' IFO 15240^T.

Characteristics	N-36	N-37	IFO 13810^T	IFO 14337^T	IFO 14338^T	IFO 15240^T
Temperature for growth (C)	14-36	17-37	22-38	14-51	15-51	17-42
NaCl tolerance (%)	2>	2>	2>	5>	5>	4>
Liquefaction of gelatin	-[a]	-	-	-	-	+
Coagulation of milk	-	-	-	-	-	-
Peptonization of milk	-	-	+	+	+	+
Reduction of nitrate	+	+	-	+	+	+
Hydrolysis of starch	-	-	-	-	-	-
Decomposition of calcium malate	-	-	-	-	-	-
Formation of melanoid pigment	-	-	-	-	-	-
Decomposition of						
adenine	-	-	-	+	+	+
L-tyrosine	-	+	-	+	+	-
xanthine	-	-	-	+	-	-
hypoxanthine	-	+	+	+	+	-
Utilization of [b]						
L-arabinose	++	++	±	++	+	++
D-fructose	±	++	++	++	++	-
inositol	-	+	+	++	++	+
D-mannitol	-	++	++	+	+	++
raffinose	±	-	±	-	-	+
L-rhamnose	±	++	++	++	++	++
sucrose	±	-	++	-	-	++
D-xylose	++	++	++	++	++	++
D-glucose	++	++	++	++	++	++

[a] +, Positive; -, negative.
[b] ++, Strongly positive; +, positive; ±, doubtful; -, negative.

III). The strain contained galactose, mannose, ribose and madurose as whole-cell sugars (whole-cell sugar pattern B). As phospholipids, it contained PG, PE, DPG, but not PC and NPG (type PIV). Mycolic acids were absent. MK-9(H_2) and MK-9(H_4) were detected as the predominant menaquinones in the ratio of 2:5. *Iso*-16:0 and 10Me-18:0 were contained as predominant fatty acids. α-Hydroxy-14-methylpentadecanoic acid (2-OH *iso*-16:0) as hydroxylated fatty acid was contained notably. The G+C content of DNA was 69.1 mol%.

'*Streptomyces hofunensis*' IFO 13810^T

'*S. hofunensis*' IFO 13810^T formed rectiflexible aerial mycelia. Mature spore chains on aerial mycelia consisted of cylindrical spores. Spore was smooth in surface, 0.8 to 0.9 μm in diameter and 1.1 to 1.3 μm in length. No synnema, sporangium, zoospore or sclerotium was observed. This strain grew moderately on almost all media used. Aerial mycelia were observed on inorganic salts-starch agar and Bennett's agar, but poorly or not on other media. Composition of cell wall amino acids, whole-cell sugars, and cellular fatty acid and phospholipid patterns were the same as those of the strain N-37. MK-9(H_4) and MK-9(H_6) were detected

Table 4. Chemotaxonomic characteristics of strains N-36 and N-37, '*Streptomyces hofunensis*' IFO 13810^T, '*Micropolyspora roseoalba*' IFO 14337^T, '*Micropolyspora cinereoflava*' IFO 14338^T and '*Kitasatosporia clausa*' IFO 15240^T.

Characteristics	N-36	N-37	IFO 13810^T	IFO 14337^T	IFO 14338^T	IFO 15240^T
Amino acid (cell wall)	Glu Ala meso	Glu Ala meso	Glu Ala meso	Glu Ala meso	Glu Ala meso	Glu Ala meso
Whole-cell sugar	Glc Rib Man Mad	Glc Rib Man Mad	Glc Gal Man Mad	Glc Rib Man Mad	Glc Rib Man Mad	Glc Rib Man Mad
Phospholipid	DPG PE PI	DPG PE PI NPG	DPG PE PI NPG	DPG PE PI NPG	DPG PE PI NPG	DPG PE PI NPG
Phospholipid pattern	PI	PIV	PIV	PIV	PIV	PIV
Menaquinone	9(H4) 9(H6) 9(H8)	9(H2) 9(H4) 9(HO)t 9(H6)t	9(H4) 9(H6) 9(H2)t	9(H4) 9(H6) 9(H2)t 9(H8)t	9(H4) 9(H6) 9(H2)t 9(H8)t	9(H4) 9(H6) 9(H2)t 9(H8)t
Fatty acid compositions*						
(1) Non-polar fatty acids (%)						
iso 14:0	0.0	2.0	0.3	0.4	0.3	1.2
iso 16:0	18.3	17.9	15.1	33.6	27.5	35.4
iso 18:0	4.1	0.0	0.5	2.0	1.7	0.7
iso,anteiso 15:0	1.6	3.2	0.4	8.6	7.5	6.2
iso,anteiso 17:0	3.3	1.8	0.3	11.7	10.2	3.6
14:0	1.8	1.9	1.0	0.5	0.7	1.1
15:0	3.0	4.6	15.1	4.3	2.8	3.8
16:0	15.1	4.0	12.8	5.9	4.8	5.5
17:0	1.5	4.8	14.7	9.6	6.7	1.9
18:0	0.0	5.2	0.7	0.7	0.0	0.0
iso 16:1	0.0	1.4	0.0	0.0	0.0	0.0
cis 16:1	12.8	1.2	2.3	0.5	0.0	0.6
cis9 17:1	5.3	4.8	7.0	1.9	1.7	2.2
iso 18:1	0.0	0.0	0.8	0.7	0.8	1.0
cis9 18:1	13.1	5.7	1.5	1.4	3.5	1.7
10Me 16:0	5.8	1.7	4.4	2.0	2.4	6.7
10Me 17:0	3.8	33.0	22.3	14.4	25.3	26.0
10Me 18:0	10.3	6.5	2.3	1.6	3.1	2.4
(2) Hydroxylated fatty acids (%)						
2OH *iso* 15:0	0.0	2.0	0.0	8.8	6.4	0.0
2OH *iso* 16:0	0.0	68.0	36.1	70.4	69.7	70.0
2OH *iso* 17:0	0.0	0.0	0.0	12.1	14.6	0.0
2OH 15:0	0.0	13.3	14.3	4.7	4.0	0.0
2OH 16:0	0.0	14.0	36.2	3.9	5.1	14.0
2OH 17:0	0.0	0.0	13.5	0.0	0.0	0.0
G+C content (mol%)	70.2	69.6	71.0	71.0	70.9	68.9

Abbreviations: Glu, glutamic acid; Ala, alanine; meso, *meso*-diaminopimelic acid; Glc, glucose; Rib, ribose; Gal, galactose; Man, mannose; Mad, madurose; DPG, diphosphatidylglycerol; PE, phophatidylethanolamine; PI, phosphatidylinositol; NPG, ninhydrin and sugar positive phospholipids; t, trace.
*Abbreviations exemplified by: *iso*-15:0, 14-methyltetradecanoic acid; 16:0, hexadecanoic acid; 2OH *iso*-16:0, 2-hydroxy-14-methyl-pentadecanoic acid; 10-Me 18:0,10-methyloctadecanoic acid.

as the predominant menaquinones in the ratio of 5:4. The G+C content of DNA was 71.0 mol%.

'*Micropolyspora roseoalba*' IFO 14337^T and '*Micropolyspora cinereoflava*' IFO 14338^T

'*M. roseoalba*' IFO 14337^T and '*M. cinereoflava*' IFO 14338^T' had similar properties to each other. These strains formed rectiflexible or hooked aerial mycelia. Mature spore chains on aerial mycelia consisted of spherical to cylindrical spores. Spores were smooth in surface, 0.8 to 0.9 μm in diameter and 0.7 to 0.8 μm in length. No synnema, sporangium, zoospore or sclerotium was observed. '*M. roseoalba*' IFO 14337^T grew a little poorly to moderately on all media used, and white aerial mycelia were observed on yeast extract-malt extract agar and oatmeal agar. '*M. cinereoflava*' IFO 14338^T did not form aerial mycelia, but grew moderately, in the same way as '*M. roseoalba*' IFO 14337^T. Because no growth was observed on the basal medium of Shirling and Gottlieb in the presence of any carbohydrate, and vigorous growth occurred in the basal medium C-2 of Nonomura and Ohara (26) even in absence of carbohydrate, carbohydrate utilization of these strains could be not determined. Composition of the cell wall amino acids, whole-cell sugars, and cellular fatty acids and phospholipid patterns were the same as those of strain N-37. As predominant menaquinones, '*M. roseoalba*' IFO 14337^T and '*M. cinereoflava*' IFO 14338^T contained MK-9(H_4) and MK-9(H_6) in the ratios of 4:1 and 3:1, respectively. The G+C contents of DNAs of IFO 14337^T and IFO 14338^T were 71.0 mol% and 70.9 mol%, respectively.

'*Kitasatosporia clausa*' IFO 15240^T

'*K. clausa*' IFO 15240^T formed rectiflexible, partly 2-3 turned coiled aerial mycelia. Mature spore chains on aerial mycelia consisted of spherical spores with unclear septums. Spore was smooth in surface, 0.9 to 1.2 μm in diameter and 0.7 to 1.4 μm in length. No synnema, sporangium, zoospore or sclerotium was observed. This strain grew poorly to moderately on almost all media used. Only on the inorganic salts-starch agar were white aerial mycelia slightly observed. Carbohydrate utilization of this strain was not determined, because it did not grow on either of the basal media with any carbohydrate tested. Composition of the cell wall amino acids, whole-cell sugars, and cellular fatty acids and phospholipid patterns were the same as those of strain N-37. MK-9(H_4) and MK-9(H_6) were detected as the predominant menaquinones in the ratio of 2:1. The G+C content of DNA was 68.9 mol%.

Table 5. Comparison of taxonomic characteristics[a] among *Actinomadura* strains.

Species or subspecies and strains	Reduction of nitrate	Production of soluble pigments	Peptonization of milk	Hydrolysis of starch	Decomposition of hypoxanthine	Decomposition of tyrosine	Spore chains	Spore surface	Spore mass color	Comments	References
A. atramentaria IFO 14695^T	+[b]	+	+	-	N	N	sc	sm	wh		(5)
A. citrea IFO 14678^T	+	+	+	+	+	+	h	u	wh-c-p		(5)
A. coerulea IFO 14679^T	+	-	+	-	+	+	h-s	w	p-bl		(5)
A. cremea subsp. *cremea* IFO 14182^T	+	-	+	-	-	-	h-s	w	wh-c-y-p		(5)
A. cremea subsp. *rifamycini* IFO 14183^T	+	-	+	-	-	-	h-s	w	wh-c-y-p		(5)
A. fulvescens IFO 14347^T	-	+	N	N	N	N	s	sm	wh		(5)
A. kijaniata IFO 14229^T	-	+	+	+	+	+	sp	sm	tr		(5)
A. libanotica IFO 14095^T	+	-	-	-	-	-	h	f	wh-p	interspore pads	(5)
A. livida IFO 14682^T	+	+	+	-	-	+	h-s	u	tr		(5)
A. luteofluorescens IFO 13057^T	+	+	+	+	+	-	h	w	wh-y-bl		(5)
A. macra IFO 14102^T	+	+	-	-	N	+	h-s	sm	wh-c-p-gy		(5)
A. madurae IFO 13909^T	+	-	N	+	+	-	h-s	w	tr		(5)
A. oligospora	-	+	N	N	-	N	h	sm	tr		(5)
A. pelletieri IFO 13910^T	+	-	-	N	+	+	h-s	w	tr		(5)
A. spadix IFO 14099^T	+	+	+	-	-	N	psp	sm	y-b		(5)
A. umbrina IFO 14346^T	N	+	N	N	N	N	h	sm	wh	black-brown colony	(5)
A. verrucosospora IFO 14100^T	-	-	+	+	+	+	h-s	w	wh-p-bl		(5)
A. vinacea IFO 14688^T	-	+	+	-	-	-	str	u	wh-gy		(5)
A. yumaensis IFO 14689^T	+	+	+	+	N	+	h	sm	wh-y-gy		(5)
A. hibisca	+	+	+	-	+	+	str	sm	wh		(36)
A. fibrosa	N	-	+	+	+	+			wh	non-septum into spore	(16)
A. rubrobrunea IFO 15275^T	N	N	N	N	N	N	sc			reddish brown colony	(5)
A. echinospora IFO 14042^T	-	-	+	-	N	N	sc	sn	p		(5)
A. rugatobispora IFO 14382^T	-	-	+	+	N	N	sc	r	g		(21)
A. viridis IFO 15238^T	-	-	N	+	N	N	sc	sm	g-gy		(5)
N-36	+	-	-	-	-	-	h	f	wh-p	interspore pads	

[a] Data from references.
[b] Abbreviations: +, positive; -, negative; N, not determined; h, hooks or curled; psp, pseudosporangia; s, spirals of 1-2 turns; sp, spirals of 2-4 turns; str, straight; sc, short chains; f, folded; sm, smooth; u, irregular, uneven; w, warty; sn, spiny; r, rugose; b, brown; bl, blue; c, cream; g, green; gy, gray; p, pink; tr, traces of aerial mycelium, only microscopically visible; wh, white; y, yellow; e.p., effective publication.

II. Classification

Strain N-36

Formation of spore chain, a chemotype III cell wall, a type B whole-cell sugar pattern, a type I phospholipid pattern, and the absence of hydroxylated fatty acids placed strain N-36 in the genus *Actinomadura*. Already, 25 validly published species (containing 1 subspecies) of the genus *Actinomadura* have been described. A comparison of taxonomic characteristics of the strain N-36 with those of *Actinomadura* species described previously indicated that it is closely related to *Actinomadura livanotica* (17) based on interspore pad, white to pink aerial mycelia, hooked spore chain with folded spore, and the ability to reduce nitrate (Table 5). However, DNA-DNA homology value between these strains was lower than 5%, and therefore, strain N-36 was considered to belong to a different species from *A. livanotica* (Table 6).

In this study, we compared taxonomic data of strain N-36 with those of validly published species in the literature. Though this might not be enough to select related species correctly, our data suggest that the strain N-36 is probably a new species of the genus *Actinomadura*.

Table 6. DNA-DNA homology between strain N-36, *Actinomadura livanotica* IFO 14095^T and *Actinomadura madurae* IFO 13909^T.

	% Hybridization with labeled DNA		
Plate-bound DNA	N-36	IFO 14095^T	IFO 13909^T
Strain N-36	100	5	3
Actinomadura livanotica IFO 14095T	0	100	0
Actinomadura madurae IFO 13909T	1	1	100

Strain N-37 and '*Streptomyces hofunensis*' IFO 13810^T

Strain N-37 and '*S. hofunensis*' IFO 13810^T were characterized by the formation of long spore chains with non-motile spores, chemotype III cell wall, a type B whole-cell sugar pattern, a type IV phospholipid pattern and hydroxylated fatty acids. These properties indicated that these two strains belong to the genus *Microtetraspora*. In the genus *Microtetraspora*, 19 validly published species are known. A comparison of taxonomic characteristics of strain N-37 and '*S. hofunensis*' IFO 13810^T with those of the 19 species indicated that strain N-37 and '*S. hofunensis*' IFO 13810^T are related to *Microtetraspora recticatena* (4) based on the rectiflexible spore chain consisting of smooth spores in surface, white aerial

Table 7. Comparison of taxonomic characteristics[a] among *Microtetraspora* strains.

Species and strains	Reduction of nitrate	Production of soluble pigments	Peptonization of milk	Hydrolysis of starch	Decomposition of hypoxanthine	Decomposition of tyrosine	Optical growth of 40-45 C	Spore chains	Spore surface	Spore mass color	Comments	References
M. africana IFO 14745^T	N[b]	N	N	N	N	N	N	str	sm	bl	colony color: orange	(5)
M. fastidiosa IFO 14680^T	+	-	+	-	+	-	+	sp	u	d-wh-p		(5)
M. ferruginea IFO 14094^T	+	-	+	+	+	+	-	h-s	f	wh-o-p		(5)
M. flexuosa IFO 14349^T	+	-	+	+	-	+	N	h-s	w	wh-y		(5)
M. helvata IFO 14681^T	+	-	-	-	-	-	+	psp	sm	wh-c-y		(5)
M. niveoalba IFO 15239^T	+	-	N	+	N	N	+	sc	sm	wh		(5)
M. polychroma IFO 14345^T	N	N	N	N	N	N	N	psp	sm	wh	colony color: blue-green	(5)
M. recticatena IFO 14525^T	N	-	N	N	N	N	N	str	f	c		(5)
M. roseola IFO 14685^T	+	-	-	-	+	+	N	str	f	p		(5)
M. roseoviolacea IFO 14098^T	+	+	-	-	+	-	+	psp	sm	wh-p-v		(5)
M. rubra IFO 14070^T	+	+	-	+	+	+	N	h-s	u	tr		(5)
M. salmonea IFO 14687^T	+	-	+	-	+	+	N	h-s	w	c-p		(5)
M. spiralis IFO 14097^T	+	-	-	-	-	+	-	sp	f	wh-y		(5)
M. fusca IFO 13915^T	-	-	N	-	N	N	-	sc	sm	gy		(5)
M. glauca IFO 14761^T	+	-	N	+	N	N	-	sc	sm	bl-gy		(5)
M. pusilla IFO 14684^T	+	-	-	-	+	+	-	psp	sm	wh-c		(5)
M. tyrrhenii	+	-	+	N	+	+	-	h-s	f	wh		(37)
M. turkmeniaca IFO 14348^T	N	+	N	N	N	N	N	str-s	sm	wh	soluble pigment of pink-violet	(5)
M. angiospora IFO 13155^T	N	N	N	N	N	N	-	h	w	wh	spore surface spines enveloped in a translucent capsule	(40)
N-37	+	-	-	-	+	+	-	str-h	sm	wh-c		
'*S. hofunensis*' IFO 13810^T	-	-	+	-	+	-	-	str	sm	wh		
'*M. roseoalba*' IFO 14337^T	+	-	+	-	+	+	+	h-s	sm	p		
'*K. clausa*' IFO 15240^T	+	-	-	-	-	-	+	sp	sm	wh		

[a] Data from references.
[b] Abbreviations: +, positive; -, negative; N, not determined; h, hooks or curled; psp, pseudosporangia; s, spirals of 1-2 turns; sp, spirals of 2-4 turns; str, straight; sc, short chains; f, folded; sm, smooth; u, irregular, uneven; w, warty; b, brown; bl, blue; c, cream; g, green; gy, gray; o, orange; p, pink; v, violet; wh, white; y, yellow; d, colorless; tr, traces of aerial mycelium, only microscopically visible mycelium; e.p., effective publication.

mycelia, the production of neither melanoid pigment nor soluble pigment and no growth at 40 C (Table 7). However, the levels of DNA-DNA relatedness between strain N-37, '*S. hofunensis*' IFO 13810^T and *M. recticatena* IFO 14525^T were lower than 30% (Table 8).

In this study, we compared taxonomic data of the two strains with those of validly published species in the literature. Though this might not be enough to select related species correctly, our data suggest that strains N-37 and '*S. hofunensis*' IFO 13810^T are probably new species of the genus *Microtetraspora*.

Table 8. DNA-DNA homology between strain N-37, '*Streptomyces hofunensis*' IFO 13810^T, '*Micropolyspora roseoalba*' IFO 14337^T, '*Kitasatosporia clausa*' IFO 15240^T and *Microtetraspora* strains.

Plate-bound DNA		% Hybridization with labeled DNA						
		N-37	IFO 13810^T	IFO 14337^T	IFO 15240^T	IFO 14525^T	IFO 14680^T	IFO 14681^T
Strain N-37		100	6	3	9	30	7	10
'*Streptomyces hofunensis*'	IFO 13810^T	2	100	0	5	7	5	6
'*Micropolyspora roseoalba*'	IFO 14337^T	2	5	100	24	8	87	17
'*Kitasatosporia clausa*'	IFO 15240^T	3	5	12	100	7	18	55
Microtetraspora recticatena	IFO 14525^T	12	1	1	5	100	5	6
Microtetraspora fastidiosa	IFO 14680^T	3	6	133	28	8	100	23
Microtetraspora helvata	IFO 14681^T	1	3	10	42	5	13	100

'*Micropolyspora roseoalba*' 14337^T and '*Micropolyspora cinereoflava*' IFO 14338^T

'*M. roseoalba*' 14337^T and '*M. cinereoflava*' IFO 14338^T were indicated to belong to the genus *Microtetraspora* based on their taxonomic characteristics. Furthermore, the similarity of taxonomic characteristics and high DNA-DNA homology value (Table 9) between the two strains showed that these strains were synonymous. These strains were characterized by growth at relatively high temperature. Among the 19 validly published species of the genus *Microtetraspora*, *Microtetraspora fastidiosa* (33) and *Microtetraspora helvata* (27) were characterized by the same properties (Table 7, 10). In DNA-DNA hybridization studies, a high level of DNA-DNA related-

Table 9. DNA-DNA homology between '*Micropolyspora roseoalba*' IFO 14337^T and '*Micropolyspora cinereoflava*' IFO 14338^T

Plate-bound DNA		% Hybridization with labeled DNA		
		IFO 14337^T	IFO 14338^T	IFO 14525^T
'*Micropolyspora roseoalba*'	IFO 14337^T	100	96	5
'*Micropolyspora cinereoflava*'	IFO 14338^T	88	100	1
Microtetraspora recticatena	IFO 14525^T	2	0	100

ness as shown between '*M. roseoalba*' 14337^T and *M. fastidiosa* IFO 14680^T (Table 8). Based on the results described above, strains 14337^T and IFO 14338^T were identified as *M. fastidiosa*.

The genus *Micropolyspora* was established by Lechevalier *et al.* (13). The type species of the genus, *Micropolyspora brevicatena*, was subsequently transferred to the genus *Nocardia*. Since then, almost all species in the genus have been transferred to other genera, as follows: *Micropolyspora rectivirgula* and *Micropolyspora faeni* were re-classified as *Saccharopolyspora rectivirgula* (10), '*Micropolyspora coerulea*' was transferred to the genus *Saccharomonospora* (12), '*Micropolyspora internatus*' and '*Micropolyspora caesia*' were re-identified as *Saccharomonospora viridis* (12), '*Micropolyspora rubrobrunea*' (11) and '*Micropolyspora viridinigra*' were re-identified as *Actinomadura rubrobrunea*, '*Micropolyspora angiospora*' (11) was transferred to the genus *Microtetraspora*, and '*Micropolyspora*

Table 10. Comparison of characteristics among '*Micropolyspora roseoalba*' IFO 14337^T, *Microtetraspora fastidiosa* IFO 14680^T, '*Kitasatosporia clausa*' IFO 15240^T and *Microtetraspora helvata* IFO 14681^T.

Characteristics	IFO 14337^T	IFO 14680^T	IFO 15240^T	IFO 14681^T
Color of aerial mycelia	wh-p	wh-p	wh	wh
Soluble pigments	none	none	none	none
Temperature of growth(C)	14-51	23-55	17-42	N
NaCl tolerance (%)	5>	5>	4>	3>
Reduction of nitrate	+	+	+	+
Liquefuefaction of gelatin	-	-	-	-
Decomposition of calcium malate	-	-	-	-
Peptonization of milk (plate)	+	+	+	+
Coagulation of milk	-	-	-	-
Hydrolysis of starch	+	+	-	-*
Formation of melanoid pigments	-	-	-	-
Decomposition of				
adenine	+	+	-	-
L-tyrosine	+	-	-	-
xanthine	+^{w}	+^{w}	-	-
hypoxanthine	+	+	+	+
esculin	+	+	+	+
Utilization of				
L-arabinose	+	+	N	N
D-fructosee	+	+	N	N
inositol	+	+	N	N
D-mannitol	-	-	N	N
raffinose	-	-	N	N
L-rhamnose	+	+	N	N
sucrose	-	-	N	N
D-xylose	+	+	N	N
D-glucose	+	+	N	N

Abbreviations: wh, white; p, pink; w, weak; *, data from reference (25); N, not determined; +, positive; -, negative.

fascifera' was excluded from the genus *Micropolyspora* because of containing mycolic acid (30). '*Micropolyspora thermovirida* ' no longer exists. The remaining strains, '*M. roseoalba*' 14337^T and '*M. cinereoflava*' IFO 14338^T, were re-identified as *M. fastidiosa*, and all strains previously named *Micropolyspora* species have now been transferred to other genera. Therefore, there are no longer any species belonging to the genus *Micropolyspora*.

'*Kitasatosporia clausa*' IFO 15240^T

Taxonomic properties of '*K. clausa*' IFO 15240^T indicated that it belongs to the genus *Microtetraspora*. The strain was characterized by spiral aerial mycelia, an unclear septum between spores, and a lump consisting of spores. These properties and some physiological characteristics were common with those of *Microtetraspora helvata* in the literature (Table 6). Therefore, we compared '*K. clausa*' IFO 15240 directly with *M. helvata* IFO 14681^T. The DNA-DNA hybridization studies showed a moderate level (about 50%) of DNA-DNA relatedness between them. However, almost all taxonomic characteristics were common (Table 10). Therefore, '*K. clausa*' was re-identified as *M. helvata*.

As a result of this study, there are no longer any species belonging to the genus *Kitasatosporia*.

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L-DRYING PRESERVATION OF A *SACCHAROMYCES CEREVISIAE* KILLER STRAIN

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Summary

A killer strain of *Saccharomyces cerevisiae* has been subjected to L-drying preservation. Its killer character was completely maintained after L-drying and in an accelerated preservation test at 37°C, supporting that L-drying is a useful method for long-term storage of killer strains.

Keywords: Yeast, Killer, L-drying, Preservation.

Killer strains of *Saccharomyces cerevisiae* secrete a protein toxin that is lethal to killer-sensitive strains. Two families of double-stranded RNA (dsRNA) plasmids (M and L-A) are responsible for this killer phenomenon (for review see references 8, 12 and 13). The dsRNA plasmids are linear in form and reside in intracellular particles like a virus. These particles are not infectious but are transferred by cell-to-cell fusion as non-Mendelian genetic elements. M dsRNA encodes a 32-kDa killer toxin/immunity precursor protein (3). The K_1 killer toxin encoded by M_1 dsRNA binds to sensitive cells and kills them by inducing the release of cellular ATP and potassium into the medium. L-A dsRNA, on the other hand, has two long open frames (ORFs) in the (+) strand. ORF1 encodes an 80-kDa major coat protein for M and L-A dsRNAs (5,6). ORF2 overlaps ORF1 in the -1 frame by 130 bases and encodes a 180-kDa minor ssRNA binding protein/RNA polymerase (6). These dsRNA genomes depend on the host genome for their replication and gene expression (8,12).

The killer systems provide useful models for the study of the control of viral replication, protein processing and secretion, and toxin action and receptors (8,13).

Mitotic stability of the killer character is high, but prolonged storage of the killer strains on nutrient agar slants leads to a significant increase in nonkiller segregants (4). In addition, M dsRNA plasmids are easily eliminated by growing host strains at elevated temperature (10) or in low concentrations of cycloheximide (4). We preserve various yeast strains as L-dried specimens and distribute them on request. Therefore, it is important to confirm that the killer character is stable on L-drying preservation. In this communication, we report that L-drying can be used for long-term storage of killer strains without increase in nonkiller cells in their cell population.

Materials and Methods

Strains. Strains of *S. cerevisiae* used are shown in Table 1.

Media. YPAD contains 1% Dried Yeast Extract-S (Nihon Pharmaceutical Co. Ltd., Japan), 2% Polypepton (Nihon Pharmaceutical Co. Ltd., Japan), 0.02% adenine, and 2% glucose. For agar plate, 2% agar is added. MB medium is YPAD agar plate buffered at pH 4.7 with 0.1 M sodium citrate and containing 0.003% methylene blue (10). The suspending medium for L-drying is 0.1 M potassium phosphate buffer (pH 7.0) containing 5% monosodium glutamate, 5% lactose, 0.1% Actocol 31-56 (Takeda Chemical Industries Ltd., Japan) and 6% polyvinylpyrolidone (2).

Table 1. Yeast strains used in this study.

Strain	Genotype	Source
IFO 10153	*MAT***a** *pho3-1* [KIL-0]	Our stock
P-28-24C	*MAT***a** *pho3-1* [KIL-b]	A. Toh-e
AX66-10D	*MAT***a** *pho3-1 leu2 ura3 trp1 lys1* [KIL-b]	(2)
KYC74	*MATα pho3-1 arg6* [KIL-b]	Our stock
KYC352	*MAT***a**/*MATα pho3-1*/*pho3-1 +*/*arg6 leu2*/+ *ura3*/+ *trp1*/+ *lys1*/+ [KIL-0]	This study

Assay of killing and resistance. The ability of a strain to kill was assayed as described

by Wickner and Leibowitz (14). Colonies grown on YPAD were streaked on MB medium that had been previously inoculated with a lawn of sensitive cells. Positive phenotype of killing is indicated by a clear zone of growth inhibition surrounding the streak. Resistance to killing was examined by streaking a cell suspension of the strain to be tested on MB medium and cross-streaking with a killer strain.

Preparation of L-dried specimens. Cells grown on YPAD at 28°C for 3 days were suspended in the suspending medium and subjected to L-drying essentially as described by Banno *et al.* (2).

Rehydration of L-dried specimens. After opening an ampule containing L-dried cells, 0.5 ml of YPAD broth was added to the ampule and the contents were carefully mixed to make a homogeneous suspension. After appropriate dilution, the cell suspension were spread on a YPAD plate and cultivated at 28°C.

Results and Discussion

Isolation of killer-sensitive diploid strain for killing assay

Since growth arrest of a cell caused by mating factors can be confused for assay of killer toxin (4), killer-sensitive diploid clones from a diploid strain constructed by crossing of AX66-10D and KYC74 were used as the killer-sensitive strain in the killing assay. It was reported that growth of killer cells in low concentrations of cycloheximide or at elevated temperatures resulted in loss of M dsRNA but retention of L dsRNA (4,10). Therefore, curing the diploid of the killer plasmid was achieved by growth on YPAD at 37°C. Six independent colonies grown at 37°C on YPAD plate were picked and purified on YPAD at 28°C. Four clones out of the six isolates showed no killing ability toward a sensitive haploid strain, IFO 1136 (*i.e.*, S288C)(4). One of four nonkiller clones, named KYC352, was chosen as test strain in the subsequent killing assays.

Preservation of killer strain by L-drying

We tested the killing ability of several strains preserved in our institute. One of them, IFO 10153, showed no killing ability. IFO 10153 was deposited by Y. Oshima (Osaka University) with original strain number of P-28-24C and reported to be a killer strain (9).

To resolve this inconsistency, we tested three fresh strains labeled with P-28-24C from different laboratories for their killer character. One strain each from Y. Oshima and H. Mitsuzawa (Institute of Applied Microbiology, University of Tokyo) were nonkillers, but one from A. Toh-e (University of Tokyo) showed killing ability as reported previously. This fact suggests that IFO 10153 might have been a nonkiller strain before deposition in our collection. However, we could not exclude the possibility that L-drying leads to conversion of a killer trait to nonkiller.

The killer strain P-28-24C from A. Toh-e was subjected to L-drying. Killing ability of 100 clones selected randomly as independent colonies was assayed using KYC352 as a lawn of sensitive cells and population of killer cells was estimated. Before L-drying operation, 97% of cells showed the killing ability. Before and just after L-drying, the killer population remained at the same level as before L-drying operation (Table 2). In addition, nonkiller population did not increased after one month preservation at 37°C as shown in Table 2. These results indicate that L-drying preservation does not affect on the maintenance of killing ability of the killer strains and is a better method than strain preservation by serial transfer on nutrient agar slant.

It is known that L-drying preservation leads to no significant decrease of cells harboring recombinant plasmids (7) and no significant increase of petite mutants (data not shown). In view of these results, we consider that cytoplasmic genetic elements can be stably maintained by L-drying preservation for a long period.

Table 2. Survival of P-28-24C and population of killer cells in the L-dried specimen.

	Survival and population of killer cells (%)*		
Character	Before	After	1 month at 37°C
Survival	100	55	14
Killer	97	96	99

* Cell samples were examined before (Before) and after (After) L-drying, and after 1 month preservation at 37°C (1 month at 37°C). Before L-drying, viable cells were 1.3×10^8 cfu/ampule.

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SURVIVAL OF FROZEN CULTURES OF OOMYCETOUS FUNGI AFTER 12 AND 24 MONTHS' STORAGE IN LIQUID NITROGEN

TADASHI NISHII and AKIRA NAKAGIRI

Summary

The viability of 171 strains of oomycetous fungi preserved in liquid nitrogen was examined serially for two years. Immediately after freezing and after 6 months' storage, 165 strains and 164 strains were recovered respectively. After 12 months' storage, 164 of 168 strains examined were found to have survived. After 24 months' storage, 143 of 149 strains were recovered. Overall, survival rates of more than 96% were achieved in the serial recovery tests.

Keywords: cryopreservation, liquid nitrogen, Oomycetes

The viability of 171 frozen cultures of oomycetous fungi (mainly Phytophthora and Pythium species) maintained in the IFO culture collection were previously examined immediately after freezing and after 6 months' storage in liquid nitrogen (2). In the present report, the survival of the preserved cultures was further examined after 12 months' and 24 months' storage. Survival rates in the serial examinations were evaluated and the effectiveness of freezing storage in liquid nitrogen was discussed for long-term preservation of oomycetous fungi.

Materials and Methods

Strains examined and procedures for preculturing, preparation of agar discs with mycelium, freezing tubes and cryoprotectant, and pro-

grammed freezing were the same as those described for Experiment 2 in the previous report (2).

One handred and seventy-one strains of oomycetous fungi preserved in IFO were used for the examination. All strains were precultured on the appropriate agar media at a suitable temperature one to two weeks. Agar discs containing mycelium were removed from the edge of the fungal colony with plastic tubes 8 mm in diamteter. Two agar discs were put in a cryotube containing 0.7 ml of 10% glycerol. Four tubes were prepared for each strain. The tubes were frozen in a programmable freezer at the cooling rate 1 C/min until -40 C and at 2 C/min from -40 to -80 C. Following the previous examinations immediately after freezing and after 6 months' storage, survival was examined after 12 months' and 24 months' strage. Thawing of frozen cultures was carried out by immersing the tubes in water at 30 C for 5 min. The thawed agar discs were incubated on agar plates of the appropriate media at a suitable temperature for one to two weeks.

Results and Discussion

Viability of the frozen cultures after 12 and 24 months' storage was compiled with the survival data immediately after freezing and after 6 months' storage (Table 1).

After 12 months' storage, 4 of 168 strains, namely Phytophthora sp. IFO 30635, Py. porphyrae IFO 30347, IFO 30800, and IFO 30801, failed to survive. One of the two agar discs in a frozen tube survived in 5 strains: Py. graminicola IFO 31997, Py. periplocum IFO 31933, Pythium sp. IFO 32197, Py. sylvaticum IFO 32198, and Saprolegnia parasitica IFO 8978. After 24 months' storage, 6 of 149 strains, namely, Phy. infestans IFO 9173, Phytophthora sp. IFO 30635, Py. aristosporum IFO 32219, Py. porphyrae IFO 30800, IFO 30801, Py. zingiberum IFO 30817, were completely dead; and 4 strains, Phy. syringae IFO 31089, Phy. infestans IFO 9174, Py. graminicola IFO 31997, Py. porphyrae IFO 30347, were partly lost (Table 1).

In the serial recovery tests, Phytophthora sp. IFO 30635 and Py. porphyrae IFO 30347, IFO 30800, IFO 30801 showed comparatively low viability in liquid nitrogen storage, but there was no strain that was com-

Table 1. Viability of oomycetous fungi immediately after freezing and after storage for 6 months, 12 months, and 24 months in liquid nitrogen.

Species	IFO No.	0 M[a]	6 M[b]	12M[c]	24M[d]	Med.No.	Temp.(C)
Phytophthora cactorum (Lebert & Cohn) Schroter	30474	+ +	+ +	+ +	+ +	1	24
Phy. cactorum	31084	+ +	+ +	+ +	+ +	1	24
Phy. cactorum	31151	+ +	+ +	+ +	+ +	1	24
Phy. cactorum	32191	+ +	+ +	+ +	ND	1	24
Phy. cactorum	32192	+ +	+ +	+ +	+ +	1	24
Phy. cactorum	32193	+ +	+ +	+ +	+ +	1	24
Phy. cactorum	32194	+ +	+ +	+ +	+ +	1	24
Phy. cambivora (Petri) Buisman	30471	+ +	+ +	+ +	+ +	1	24
Phy. cambivora	30472	+ +	+ +	+ +	+ +	1	24
Phy. cambivora	30714	+ +	+ +	+ +	+ +	1	24
Phy. cambivora	30715	+ +	+ +	+ +	+ +	1	24
Phy. capsici Leonian	8386	+ +	+ +	+ +	+ +	1	24
Phy. capsici	9752	- -	+ +	+ +	+ +	1	24
Phy. capsici	30696	+ +	+ +	+ +	+ +	1	24
Phy. capsici	30697	+ +	+ +	+ +	+ +	1	24
Phy. capsici	30698	+ +	+ +	+ +	+ +	1	24
Phy. capsici	30699	+ +	+ +	+ +	+ +	1	24
Phy. capsici	31400	+ +	+ +	+ +	ND	1	24
Phy. capsici	31402	+ +	+ +	+ +	+ +	1	24
Phy. citricolor Sawada	31017	+ +	+ +	+ +	+ +	1	24
Phy. citrophthora (Smith & Smith) Leonian	31408	+ +	+ +	+ +	+ +	1	24
Phy. citrophthora	31410	+ +	+ +	+ +	+ +	1	24
Phy. colocasiae Raciborski	30695	+ +	+ +	+ +	+ +	1	24
Phy. cryptogea Pethybridge & Lafferty	31411	+ +	+ +	+ +	+ +	1	24
Phy. cryptogea	31412	+ +	+ +	+ +	+ +	1	24
Phy. cryptogea	31622	+ +	+ +	+ +	ND	1	24
Phy. drechsleri Tucker	31085	+ +	+ +	+ +	+ +	1	24
Phy. drechsleri	31153	+ +	+ +	+ +	+ +	1	24
Phy. drechsleri	31154	+ +	+ +	+ +	+ +	1	24
Phy. erythroseptica Pethybridge	31152	+ +	+ +	+ +	+ +	1	24
Phy. fragariae Hickman	31086	+ +	+ +	+ +	+ +	1	24
Phy. infestans (Montagne) de Bary	9173	+ +	+ +	+ +	- -	1	24
Phy. infestans	9174	+ +	+ +	+ +	+ -	1	24
Phy. katsurae Ko & Chang	9753	+ +	+ +	+ +	+ +	1	24
Phy. katsurae	30433	+ +	+ +	+ +	+ +	1	24
Phy. katsurae	30434	+ +	+ +	+ +	+ +	1	24
Phy. katsurae	30435	+ +	+ +	+ +	+ +	1	24
Phy. macrospora (Saccardo) Ito & Tanaka	9049	+ +	+ +	+ +	+ +	1	24
Phy. megasperma Drechsler	31623	+ +	+ +	+ +	+ +	1	24
Phy. megasperma	31624	+ +	+ +	+ +	ND	1	24
Phy. megasperma	32174	+ +	+ +	+ +	+ +	1	24
Phy. megasperma	32175	+ +	+ +	+ +	+ +	1	24
Phy. megasperma	32176	+ +	+ +	+ +	ND	1	24
Phy. megasperma Drechsler var. sojae Hildebrand	31014	+ +	+ +	+ +	+ +	1	24
Phy. megasperma var. sojae	31015	+ +	+ +	+ +	+ +	1	24
Phy. megasperma var. sojae	31016	+ +	+ +	+ +	+ +	1	24

Table 1. (continued)

Species	IFO No.	0 M[a]	6 M[b]	12M[c]	24M[d]	Med.No.	Temp.(C)
Phytophthora melonis Katsura	31413	+ +	+ +	+ +	+ +	1	24
Phy. melonis	31414	+ +	+ +	+ +	+ +	1	24
Phy. melonis	31415	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae van Breda de Haan var. nicotianae	4873	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae van Breda de Haan var. parasitica (Dastur) Waterhouse	30595	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	30716	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	30810	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	30811	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31018	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31019	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31020	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31021	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31416	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31419	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31423	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31425	+ +	+ +	+ +	+ +	1	24
Phy. palmivora (Butler) Butler	9755	+ +	+ +	+ +	+ +	1	24
Phy. palmivora	30285	+ +	+ +	+ +	+ +	1	24
Phy. palmivora	30812	- -	+ +	+ +	ND	1	24
Phy. palmivora	30813	+ +	+ +	+ +	+ +	1	24
Phy. palmivora	31428	+ +	+ +	+ +	+ +	1	24
Phy. porri Foister	30416	+ +	+ +	+ +	+ +	1	24
Phy. porri	30417	+ +	+ +	+ +	+ +	1	24
Phy. porri	30418	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30635	+ -	- -	- -	- -	1	24
Phy. sp.	30636	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30637	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30638	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30639	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30640	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30641	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30642	+ +	+ +	+ +	+ +	1	24
Phy. syringae Klebahn	31087	+ +	+ +	+ +	+ +	1	24
Phy. syringae	31088	+ +	+ +	+ +	+ +	1	24
Phy. syringae	31089	+ +	+ +	+ +	+ -	1	24
Phy. vignae Purss	30473	+ +	+ -	+ +	+ +	1	24
Phy. vignae	30613	+ +	+ +	+ +	+ +	1	24
Phy. vignae	31026	+ +	+ +	+ +	+ +	1	24
Phy. vignae	31027	+ +	+ +	+ +	+ +	1	24
Phy. vignae	31028	+ +	+ +	+ +	+ +	1	24
Phy. vignae	31029	+ +	+ +	+ +	+ +	1	24

Table 1. (continued)

Species	IFO No.	0 M[a]	6 M[b]	12M[c]	24[d]	Med.No.	Temp.(C)
Pythium afertile Kanouse & Humphrey	32195	+ +	+ +	+ +	ND	1	24
Py. aphanidermatum (Edson) Fitzpatrick	7030	+ +	+ +	+ +	+ +	8	24
Py. aristosporum Vanterpool	32219	+ +	+ +	+ +	- -	1	24
Py. butleri Subramaniam	31214	+ +	+ +	+ +	+ +	1	24
Py. debaryanum	7211	+ +	+ +	+ +	+ +	1	24
Py. debaryanum Hesse var. pelargonii H. Braun	5919	+ +	+ +	+ +	+ +	8	24
Py. dissotocum Drechsler	32196	+ +	+ +	+ +	ND	1	24
Py. gracile Schenk	30819	+ +	+ +	+ +	+ +	1	37
Py. graminocola Subramaniam	31996	+ +	+ +	+ +	+ +	1	24
Py. graminocola	31997	+ +	+ -	+ -	+ -	1	24
Py. graminocola	31998	+ +	+ -	+ +	+ +	1	24
Py. irregulare Buisman	7220	+ +	+ +	+ +	+ +	8	24
Py. irregulare	30346	+ +	+ +	+ +	+ +	8	24
Py. irregulare	32072	+ +	+ +	+ +	+ +	8	24
Py. irregulare	32073	+ +	+ +	+ +	+ +	8	24
Py. iwayamai S. Ito	31990	+ +	+ +	+ +	+ +	1	24
Py. iwayamai	31991	+ -	- -	+ +	ND	1	24
Py. iwayamai	31992	+ +	+ +	+ +	+ +	1	24
Py. myriotylum Drechsler	31022	- -	+ +	+ +	+ +	1	24
Py. oedochilum Drechsler	7218	+ +	+ +	+ +	+ +	1	24
Py. okanoganense Lipps	31921	+ +	+ +	+ +	+ +	1	24
Py. okanoganense	31922	+ +	+ +	+ +	+ +	1	24
Py. okanoganense	31941	+ +	+ +	+ +	+ +	1	24
Py. paddicum Hirane	31993	+ +	+ +	+ +	+ +	1	24
Py. paddicum	31994	+ +	+ +	+ +	+ +	1	24
Py. paddicum	31995	+ +	+ +	+ +	+ +	1	24
Py. periplocum Drechsler	31933	- -	+ +	+ -	ND	1	24
Py. porphyrae Takahashi & Sasaki apud Takahashi et al.	30347	+ +	- -	- -	+ -	12	24
Py. porphyrae	30800	+ +	+ -	- -	- -	12	24
Py. porphyrae	30801	+ +	- -	- -	- -	12	24
Py. sp.	32197	+ +	+ +	+ -	ND	1	24
Py. spinosum Sawada	7031	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7193	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7194	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7195	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7196	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7197	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7198	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7199	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7200	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7201	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7202	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7203	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7204	+ +	+ +	+ +	+ +	8	24

Table 1. (continued)

Species	IFO No.	0 M[a)]	6 M[b)]	12M[c)]	24M[d)]	Med.No.	Temp.(C)
Pythium spinosum Sawada	7205	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7206	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7207	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7208	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7209	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7210	+ +	+ +	+ +	+ +	8	24
Py. spinosum	32212	+ +	+ +	+ +	ND	8	24
Py. spinosum	32213	+ +	+ +	+ +	ND	8	24
Py. spinosum	32214	+ +	+ +	+ +	+ +	8	24
Py. sylvaticum Campbell & Hendrix	31942	+ +	+ +	+ +	+ +	1	24
Py. sylvaticum	31943	+ +	+ +	+ +	+ +	1	24
Py. sylvaticum	32198	+ +	+ +	+ -	ND	1	24
Py. torulosum Coker & Patterson	32166	+ +	+ +	+ +	+ +	1	24
Py. torulosum	32167	+ +	+ +	+ +	+ +	1	24
Py. torulosum	32168	+ +	+ +	+ +	+ +	1	24
Py. ultimum Trow	7212	+ +	+ +	+ +	+ +	8	24
Py. ultimum	7213	+ +	+ +	+ +	+ +	8	24
Py. ultimum	7214	+ +	+ +	+ +	+ +	8	24
Py. ultimum	7215	+ +	+ +	+ +	ND	8	24
Py. ultimum	7216	+ +	+ +	+ +	+ +	8	24
Py. ultimum	7217	+ +	+ +	+ +	+ +	8	24
Py. ultimum Trow var. ultimum	32210	+ +	+ +	+ +	ND	8	24
Py. ultimum	32211	+ +	+ +	+ +	ND	8	24
Py. vanterpoolii V. Kouyeas & H. Kouyeas	31923	+ +	+ +	+ +	+ +	1	24
Py. vanterpoolii	31924	+ +	+ +	+ +	+ +	1	24
Py. vanterpoolii	31925	+ +	+ +	+ +	+ +	1	24
Py. vanterpoolii	32169	+ +	+ +	+ +	+ +	1	24
Py. vanterpoolii	32170	+ +	+ +	+ +	+ +	1	24
Py. vanterpoolii	32171	+ +	+ +	+ +	+ +	1	24
Py. vexans de Bary	7221	+ +	+ +	+ +	+ +	1	24
Py. volutum Vanterpool & Truscott	31926	+ +	+ +	+ +	+ +	1	24
Py. volutum	31927	+ +	+ +	+ +	+ +	1	24
Py. volutum	31928	+ +	+ +	+ +	+ +	1	24
Py. zingiberum Takahashi	30817	+ +	+ +	+ +	- -	1	34
Py. zingiberum	30818	- -	+ +	+ +	ND	1	34
Saprolegnia parasitica Coker	8978	+ +	+ +	+ -	ND	1	24
Aphanomyces iridis Ichitani & Kodama	31934	+ +	+ +	ND	ND	14	24
Ap. iridis	31935	+ +	+ +	ND	ND	14	24
Ap. iridis	31936	+ +	+ +	ND	ND	14	24
Halophytophthora vesicula Anastasiou & Churchland	32216	- -	+ +	+ +	+ +	1	24

+ : viable
- : non-viable (signs indicate the viability of each of two agar discs)
a) : immediately after freezing
b) : 6 months after freezing
c) : 12 months after freezing
d) : 24 months after freezing
ND : No Data

pletely lost in the all recovery tests. The total survival rate in each recovery test was calculated (Table 2) and found to be constant at higher than 96% during two years of storage.

Table 2. The survival rates of frozen oomycetous cultures examined serially for two years.

	Storage period (months)			
	0	6	12	24
Strains examined	171	171	168	149
Strains surviving[a)]	165	164	164	143
Survival rate (%)	96.5	96.0	97.6	96.0

a) : numbers include both completely and partly surviving strains.

More than 96% of 149 strains of oomycetous fungi were succesfully stored for 24 months in liquid nitrogen. This suggests that cryopreservation in liquid nitrogen is equally as effective for oomycetous fungi as for other fungal groups (1, 4, 5). Of the unsuccessful strains, some survived freezing but did not survive or only partly survived 6 months, 12 months, 24 months of storage, while others that had shown no or partial survival immediately after freezing or after 6 months' or 12 months' storage showed good recovery in later tests. The former phenomenon, which was found in 12 strains, may indicate that storage period affects the viability of frozen cultures. On the other hand, the latter was observed for 10 strains and may suggest that the conditions of the agar discs for freezing may affect their survival rate (2, 3), rather than the period of storage. Further studies are necessary to clarify whether the freezing storage of oomycetous fungi in liquid nitrogen is effective for long-term preservation. However, since high survival rates were achieved over at least two years in this examination, it appears that with further improvements, e.g., in preculturing, cryoprotectants, freezing method etc., in addition to those already acheived by the authors in the freezing rate and the freezing tube case (3), liquid nitrogen storage

has the potential to be practically useful for the safe and long-term preservation of a wide range of oomycetous cultures.

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DESCRIPTIVE CATALOGUE OF IFO FUNGUS COLLECTION XIII.

In routine identification work on fungi newly isolated in Japan, and in checks of the list of fungal taxa preserved in the IFO culture collection for published records of their occurrence in Japan, many taxa have been found to be 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.

The author(s) of the descriptions of these fungal taxa are shown in parentheses.

92. Cladosporium oxysporum Berkeley & Curtis (Figs. 1-3) Hyphomycetes J. Linn. Soc., 10: 362 (1868); Ellis, Dematiaceous Hyphomycetes p. 312 (1971).

Colonies on malt extract agar grow moderately, floccose, gray to grayish brown; reverse greenish black to black. Conidiophores macronematous, mononematous, straight or slightly flexuous, with distinct nodose on the stalk, pale brown, smooth, 500-1200 um long, often proliferate, 3-5 μm thick, swelling 6-8 μm diam. Conidia pale brown to olivaceous brown, ovoid to ellipsoid, smooth to scarcely rough, 5-12 x 3-6 μm.

Growth on PSA rapid, floccose, with linear sectors, grayish green; reverse greenish black to black. Conidium formation abundant on the sector. Growth on PCA rapid, velvety, immersed at the margin, pale gray; reverse gray. Conidium formation abundant at the central part. Growth on OA rapid, floccose, immersed at the margin, grayish green, with sector; reverse grayish green to black. Conidium formation abundant on the

sector.

Growth is nil at 37 C.

Hab. field soil, Hata, Ikeda, Osaka Pref. (T. Ito D6-15-6 = IFO 32511)

This species is characterized by intercalary nodose and proliferation of conidiophores. This fungus is known from leaves, dead plants and wood in tropical countries.

(T. Ito)

93. Phialophora cyclaminis Beyma (Fig. 4 & 5) Hyphomycetes Antonie van Leeuwenhoek 8: 115 (1942); Schol-Schwarz, Persoonia 6: 70 (1970).

Colonies on potato sucrose agar grow rapidly, floccose, velvety at the margin, raised at the center, brown; reverse brown to dark brown. Conidiophores micronematous, mononematous, scarcely branched, straight, pale brown; Phialides 10-40 μm long, 2.5-4.0 μm thick, laterally or terminally on the hyphae, simple or branched, two to three proliferate, cylindrical to pyriform, often polyphialidic, collarette distinct, 3 μm wide. Conidia hyaline, globose, truncate at the base, 1.5-2.5 μm diam. Chlamydospores ellipsoid to cylindrical, dark brown, terminal or intercalary, often catenate, 8-10 x 4-5 μm.

Growth on PCA moderate, floccose, immersed at the margin, grayish brown; reverse dark brown. Conidium formation abundant. Growth on MEA slow, velvety, immersed at the margin, grayish brown, with sector; reverse grayish brown to brown. No conidium formation but chlamydospores abundant. Growth on OA moderate, floccose, velvety at the margin; reverse grayish brown to dark brown. No conidium formation but chlamydospores abundant.

Growth is nil at 37 C.

Hab. field soil, Hata, Ikeda, Osaka Pref. (T. Ito C-15-13 = IFO 32512).

This species is characterized by small, globose to napiform conidia. This fungus was originally described by Beyma isolated from Cyclamen

persicum Miller in The Netherlands. Other strains were found in water in an aquarium, soil, dung and wood in Europe.

(T. Ito)

94. Phoma eupyrena Saccard (Figs. 6-8) Coelomycetes
Michelia 1: 525 (1879); Dorenbosch, Persoonia 6: 1 (1970); Boerema, Trans. Br. mycol. Soc. 67: 298 (1976); Sutton, The Coelomycetes p. 383 (1980).

Colonies on potato sucrose agar grow rapidly, floccose, grayish brown to dark brown, often with sector; reverse dark brown. Pycnidia formed in abundance, submerged, solitary, sometime two or three ostiolate, spherical to conical, at first hyaline, later becoming dark brown to black, 320-600 x 100-320 μm. Peridium membranaceous, consisting of two to four layers with angular cells, dark brown, 8-10 μm thick. Conidia cylindrical to ellipsoid, hyaline, straight to slightly curved, often biguttulate, 4.0-6.0 x 2.5-3.0 μm. Chlamydospores formed in abundance, globose to ellipsoid or cylindrical, dark brown to black, terminal or intercalary, often catenate, 7-12 x 5-7 μm.

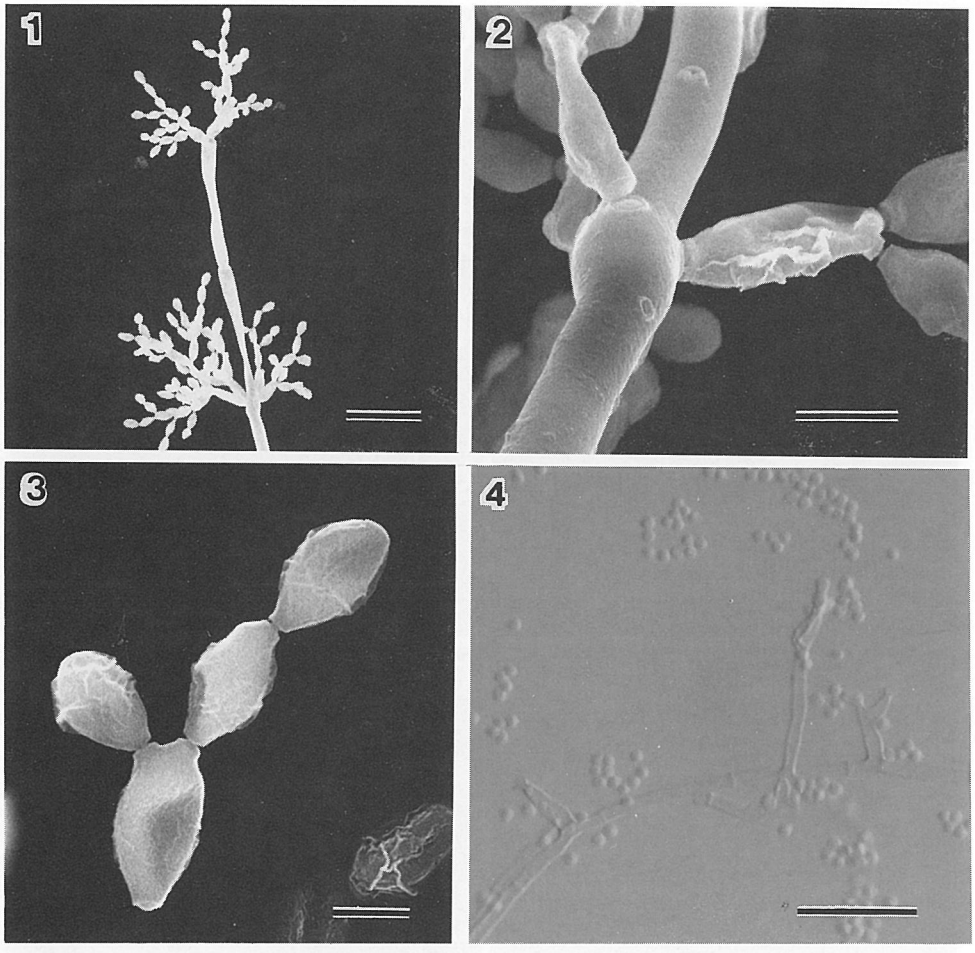
Growth on PCA rapid, floccose, dark brown; reverse pale brown. Pycinidium formation scare. Growth on MEA moderate, floccose, dark brown; reverse dark brown. No pycnidium formation. Growth on OA rapid, floccose, dark brown; reverse dark brown. Pycnidium formation scare.

Growth is nil at 37 C.

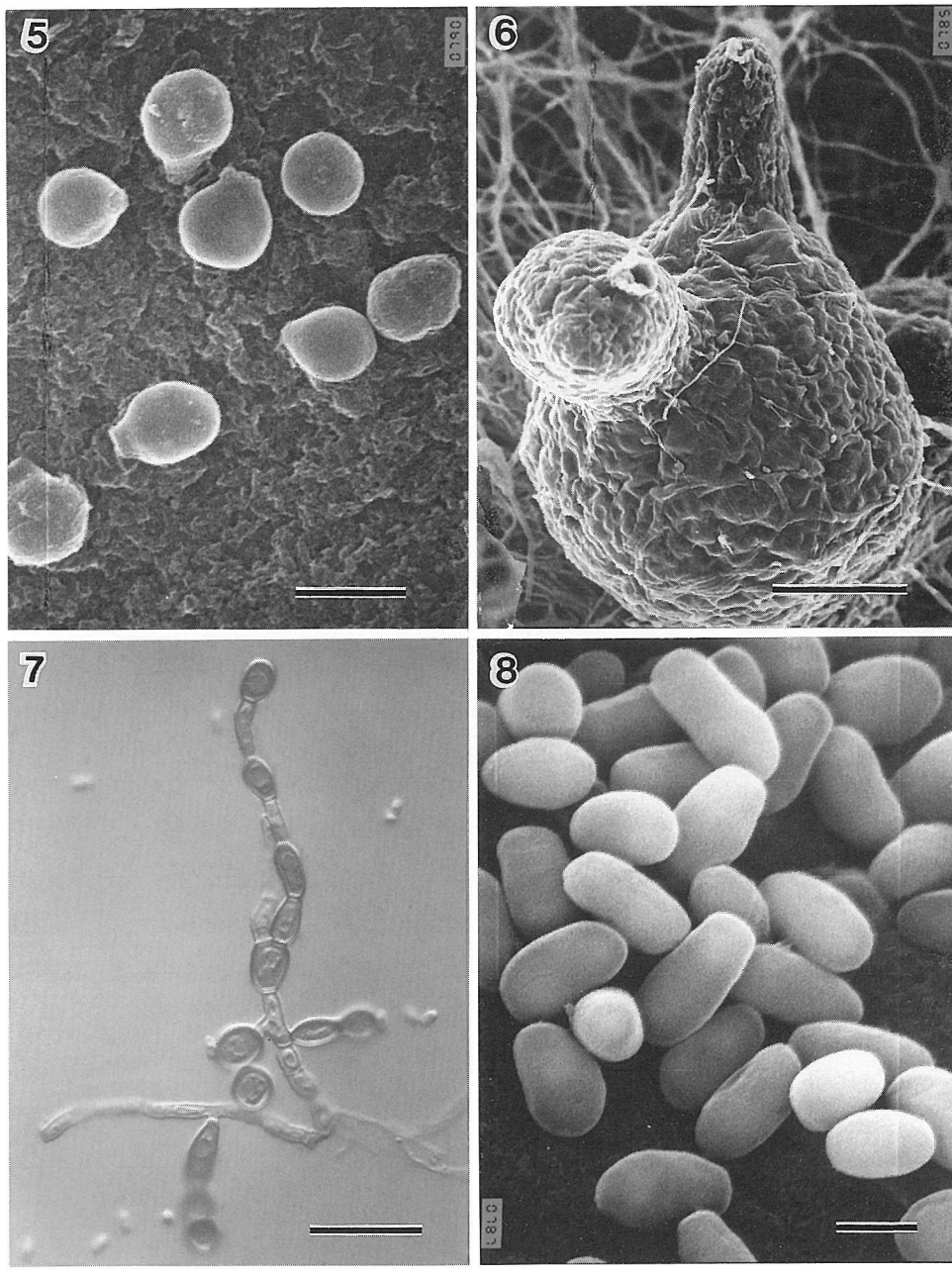
Hab. field soil, Hata, Ikeda, Osaka Pref. (T. Ito C6-5-32 = IFO 32153)

This species is distinguished by abundant chlamydospores in terminal or intercalary. According to Sutton, this fungus has been reported on 30 different host genera, and in soil, sewage, sand and veterinary sources in tropical to temperate regions of Asia, Africa, Europe and Oceania.

(T. Ito)



Figs. 1-4. Cladosporium oxysporum (IFO 32511). 1. Conidiophore. 2. Intercalary swelling. 3. Conidia. 4. Phialophora cyclaminis (IFO 32512). Phialides and conidia.
(Bars: Fig. 1 = 200 μm; Fig. 2 = 5 μm; Fig. 3 = 2 μm; Fig. 4 = 20 μm)



Figs. 5. Conidia of Phialophora cyclaminis. 6-8. Phoma eupyrena (IFO 32153). 6. Pycnidium. 7. Chlamydospores. 8. Conidia.
(Bars: Fig. 5 = 2 μm; Fig. 6 = 50 μm; Fig. 7 = 20 μm; Fig. 8 = 2 μm)

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DESCRIPTIVE CATALOGUE OF IFO ACTINOMYCETES COLLECTION II.

The purpose of this catalogue is to describe the taxonomic properties of strains which have been reidentified as different species in routine identification work on the IFO actinomycetes collection. The authors of the descriptions are shown in parentheses.

5. *Promicromonospora sukumoe* Takahashi *et al.*
Takahashi, Y. *et al.* 1987. J. Gen. Appl. Microbiol. 33: 507-519; Int. J. Syst. Bacteriol. 38: 449 (1988); Nakagaito, Y., and T. Hasegawa. 1991. IFO Res. Commun. 15: 52-56.
IFO 15062

Strain IFO 15062 was isolated from *Buruguiera gymnorrhiza* Lamk. fallen leaf collected in the mangrove region of Iriomote Island in 1989 and entered in the IFO List of Cultures under the name of *Promicromonospora citrea* Krassilnikov *et al.* The taxonomic properties of the strain were reexamined. It grew moderately, and formed bright yellow vegetative mycelia on agar media. In broth culture, the vegetative mycelia broke up into fragments of various sizes and shapes. White aerial mycelia were observed poorly on oatmeal agar, Czapek's agar and glucose-asparagine agar in the early phase of cultivation, but disappeared with growth. The spore chains on aerial mycelia consisted of cylindrical spores. Spores were smooth-surfaced, 0.8 to 0.9 μm in diameter and 1.1 to 1.3 μm in length. No synnema, sporangium, zoospore or sclerotium was observed. Melanoid pigments and soluble pigments were not produced. The temperature range for growth was 8 to 36 C. The concentration of NaCl at which growth occurs was less than 9%. Nitrate was not reduced, starch was hydrolyzed, gelatin was not liquefied, and milk was peptonized and coagulated. D-Glucose, D-fructose, D-mannitol, D-xylose, L-rhamnose, raffinose, sucrose and L-arabinose were utilized, but inositol was not utilized. The cell wall contained lysine, alanine and glutamic acid. Glucose, ribose and

rhamnose were detected as whole-cell sugars. The phospholipid pattern was type V. MK-9(H_4) and MK-9(H_6) were detected as the predominant menaquinones in the ratio of 2:1. 12-Methyltetradecanoic acid (*anteiso*-15:0) was contained as the predominant fatty acid. Hydroxylated fatty acids were absent. The G+C content of DNA was 72.7 mol%. The level of DNA-DNA relatedness between strains IFO 15026 and *P. citrea* IFO 12397^T was lower than 50%, while that between strains IFO 15026 and *Promicromonospora sukumoe* IFO 14650^T was more than 70%. Therefore, strain IFO 15026 was reidentified as *Promicromonospora sukumoe*.

(Y. Nakagaito)

6. ***Streptomyces rutgersensis*** **subsp.** ***rutgersensis*** (Waksman and Curtis) Waksman and Henrici

Shirling, E.B., and D. Gottlieb. 1968. Int. J. Syst. Bacteriol. 18: 69-189.

IFO 13029

Strain IFO 13029 was deposited under the name of '*Streptomyces citreus*' Waksman and Henrici, but the strain has been shown to have taxonomic characteristics common to *Streptomyces rutgersensis* subsp. *rutgersensis*: Nitrate was not reduced, gelatin was not liquefied, and milk was peptonized but not coagulated. Adenine, tyrosine, xanthine and hypoxanthine were decomposed. D-Glucose, D-fructose, D-mannitol, D-xylose, sucrose and L-arabinose were utilized, but inositol, L-rhamnose and raffinose were not utilized. G+C contents of of strains IFO 13029 and *S. rutgersensis* subsp. *rutgersensis* IFO 12819^T were 74.5 mol% and 74.1 mol%, respectively. DNA-DNA hybridization study showed a high level (more than 60%) of DNA-DNA relatedness between strain IFO 13029 and *S. rutgersensis* subsp. *rutgersensis* IFO 12819^T. Although strain IFO 13029 was reported to be synonymous with *Streptomyces griseus* (Ettlinger *et al.* 1958. Arch. Mikrobiol. 31: 326-358), the level of DNA-DNA relatedness between these species was around 20%. Therefore, the strain IFO 13029 was reidentified as *Streptomyces rutgersensis* subsp. *rutgersensis*.

(Y. Nakagaito)

CATALOGUE OF NEWLY ACCEPTED STRAINS
FEBRUARY 1992 - OCTOBER 1992

The cultures involed in the follwing catalogue can be distributed under the same condition as strains published in IFO LIST OF CULTURES (1992).

IFO	NAME	TEMP	MED
10575	***Ballistosporomyces ruber***	**17**	**108**
	JCM 6884 (T. Nakase; NB-258; dead leaf of *Vitis ficifolis* var. *lobata*). Type		
10576	***Ballistosporomyces xanthus***	**17**	**108**
	JCM 6885 (T. Nakase; NB-206; dead leaf of *Acer rufinerve*). Type		
10577	***Bensingtonia ciliata***	**17**	**108**
	JCM 6865 -- CBS 7514, T. Boekhout, *Auricularia auricula-judae* var. *lactea*. Type		
10578	***Bensingtonia ingoldii***	**17**	**108**
	JCM 7445 (T. Nakase; NZ-3; leaf of *Knightia excelsa* infected by sooty molds). Type		
10579	***Bensingtonia miscanthi***	**17**	**108**
	JCM 5733 (T. Nakase; NB-146; dead leaf of *Miscanthus sinensis*). Type		
10580	***Bensingtonia naganoensis***	**17**	**108**
	JCM 5978 (T. Nakase; NB-186; dead leaf of *Sasa* sp.). Type		
10581	***Bensingtonia phylladus***	**17**	**108**
	JCM 7476 -- Y. Yamada -- J.P.van der Walt, leaf of *Sclerocarya caffra*. Type		
10582	***Bensingtonia subrosea***	**17**	**108**
	JCM 5735 (T. Nakase; NB-150; dead leaf of *Miscanthus sinensis*). Type		
10583	***Bensingtonia yamatoana***	**17**	**108**
	JCM 2896 (T. Nakase; NB-26; dead leaf of *Miscanthus sinensis*). Type		
10584	***Geotrichum eriense***	**24**	**108**
	JCM 3912 -- CBS 694.83, water Lake Erie. Type		
10585	***Sporobolomyces albo-rubescens***	**24**	**108**
	JCM 5352 -- CBS 482 -- H.G. Derx, rusted leaves of bush. Type		
10586	***Sporobolomyces elongatus***	**24**	**108**
	JCM 5354 -- CBS 8080 -- J.F. Brown, leaves of *Callistemon viminalis*. Type		
10587	***Sporobolomyces falcatus***	**17**	**108**
	JCM 6838 (T. Nakase; NB-264; dead leaf of *Miscanthus sinensis*). Type		
10588	***Sporobolomyces foliicola***	**24**	**108**

JCM 5355 -- CBS 8075 -- J.F. Brown, leaves of *Banksia collina*. Type

10589 ***Sporobolomyces griseoflavus*** **17** **108**

JCM 5653 (T. Nakase; NB-176; dead leaf of *Sasa* sp.). Type

10590 ***Sporobolomyces inositophilus*** **17** **108**

JCM 5654 (T. Nakase; NB-172; dead leaf of *Sasa* sp.). Type

10591 ***Sporobolomyces kluyveri-nielii*** **17** **108**

JCM 6356 -- CBS 7168 --J.P.van der Walt, leaf of *Dombeya rotundifolia*. Type

10592 ***Sporobolomyces salicinus*** ***17*** **108**

JCM 2959 -- CBS 6983 -- R.J. Bandoni, leaf of *Salix* sp. Type

10593 ***Sporobolomyces sasicola*** **17** **108**

JCM 5979 (T. Nakase; NB-189; dead leaf of *Sasa* sp.). Type

10594 ***Sporobolomyces singularis*** **24** **108**

JCM 5356 -- CBS 5109 -- UCD 60-79, H.J. Phaff, frass of *Scolytus tsugae* in *Tsuga heterophylla*. Type

10595 ***Spoorobolomyces tsugae*** **24** **108**

JCM 2960 -- CBS 5038 -- UCD 60-71, H.J. Phaff, frass under bark of *Tsuga heterophylla*. Type

10596 ***Bensingtonia yuccicola*** **17** **108**

JCM 6251 -- R.J. Bandoni, B-74, old dead leaf of *Yucca* sp. Type

10597 ***Kluyveromyces aestuarii*** **24** **108**

CBS 4438 -- J.W. Fell, estuarine mud. Type

10598 ***Kluyveromyces aestuarii*** **24** **108**

CBS 4904 -- N. van Uden, sea.

10599 ***Kluyveromyces blattae*** **24** **108**

CBS 6284 -- S. Windisch, cockroach *Blatta orientalis*. Type

10600 ***Kluyveromyces blattae*** **24** **108**

CBS 6285 -- S. Windisch, cockroach *Blatta orientalis*.

10601 ***Kluyveromyces cellobiovorus*** **24** **108**

CBS 7153 -- NRRL Y-12509.

10602 ***Kluyveromyces delphensis*** **24** **108**

CBS 2170 -- J.P. van der Walt, sugary deposit on dried figs. Type

10603 ***Kluyveromyces dobzhanskii*** **24** **108**

CBS 2104 -- H.J. Phaff, *Drosophila pseudobscura*. Type

10604 ***Kluyveromyces dobzhanskii*** **24** **108**

CBS 5061 -- J. Boidin, fungus.

10605 ***Kluyveromyces lodderae*** **24** **108**

CBS 2563 -- J.P. van der Walt, soil.

10606	***Kluyveromyces lodderae***	**24**	**108**
	CBS 2757 -- J.P. van der Walt, soil. Type		
10607	***Kluyveromyces yarrowii***	**24**	**108**
	CBS 2684 -- J. Boidin, tanning fluid. Mating type α		
10608	***Kluyveromyces yarrowii***	**24**	**108**
	CBS 8242 -- J.P. van der Walt, CBS 2684 α x CBS 6070 a. Type		
10609	***Saccharomyces paradoxus***	**28**	**108**
	CBS 432 -- A. Guilliermond. Neotype		
10610	***Saccharomyces pastorianus***	**28**	**108**
	CBS 1503 -- H. Schnegg. Type strain of *Saccharomyces monacensis*		
15266	***Micrococcus conglomeratus***	**30**	**203**
	CCM 2136 -- IAM 1448.		
15267	***Micrococcus conglomeratus***	**30**	**203**
	CCM 2137 -- IAM 1480.		
15268	***Kineococcus aurantiacus***	**30**	**227**
	IFO (T. Tamura; RA 333; soil). Type		
15269	***Acidianus brierleyi***	**70**	**286**
	DSM 1651 -- W. Zillig & K.O. Stetter -- C.L. Brierley, thermal spring drainage. Type		
15270	***Acidianus infernus***	**88**	**287**
	DSM 3191 -- K.O. Stetter, So4a, mud from solfatara crater. Type		
15271	***Magnetospirillum gryphiswaldense***	**30**	**288**
	DSM 6361 -- D. Scher, MSR-1, river sediment. Type. Microaerophilic		
15272	***Magnetospirillum magnetotacticum***	**30**	**289**
	DSM 3856 -- ATCC 31632 -- R.P. Blakemore, MS-1, freshwater sediment. Type. Microaerophilic		
15273	***Streptomyces olivoverticillatus***	**28**	**227**
	ATCC 25480 -- F.B. Shirling -- R. Shinobu, soil.		
15274	***Tsukamurella wratislaviensis***	**28**	**227**
	Newcastle Univ. (M. Goodfellow; N800; "*aurantiaca*" group) -- R.E. Gordon, IMRU 469 (*aurantiaca* group) -- R.L. Starkey, 213 (*Nocardia* sp.), soil. Type		
15275	***Actinomadura rubrobrunea***	**55**	**245**
	DSM 43750 -- N.S. Agre, 2991, soil. Type		
15277	***Roseobacter denitrificans***	**20**	**290**
	Ocean Res. Inst., Univ. Tokyo (T. Shiba; OCh114; *Enteromorpha linza*). Type		
15278	***Roseobacter litoralis***	**20**	**290**
	Ocean Res. Inst., Univ. Tokyo (T. Shiba; OCh149; seaweed). Type		
15284	***Corynebacterium cystitidis***	**30**	**203**

JCM 3715 -- R. Yanagawa, strain 42 Hukuya. Type

15285 ***Corynebacterium pilosum*** **30 203**

JCM 3714 -- R. Yanagawa, strain 46 Hara. Type

15286 ***Corynebacterium variabilis*** **30 203**

JCM 2154 -- IAM 12346 -- CCM 1565 -- G. Mler, FK31. Type

15287 ***Corynebacterium xerosis*** **30 203**

JCM 1971 -- ATCC 373 -- J.G. Fitzgerald, ear discharge of child. Type

15288 ***Corynebacterium kutscheri*** **37 253**

ATCC 15677 -- C.H. Pierce-Chase, lung abscess of Swiss mouse. Type

15289 ***Corynebacterium mycetoides*** **37 203**

ATCC 43995 -- NCTC 9864 -- A. Castellani. Type

15290 ***Corynebatcerium renale*** **37 253**

ATCC 19412 -- NCTC 7448 -- B. Weitz, strain Charita-a, cow. Type

15291 ***Corynebacterium striatum*** **30 254**

ATCC 6940 -- NCTC 764 -- F.C. Minnett. Type

15292 ***Agrobacterium tumefaciens*** **30 203**

Fruit Tree Res. St. Akitsu Branch, Minist. Agric. Forest. Fish. (H. Sawada; Ch-Ag-4; cherry).

15293 ***Agrobacterium tumefaciens*** **30 203**

Fruit Tree Res. St. Akitsu Branch, Minist. Agric. Forest. Fish. (H. Sawada; Ch-Ag-5; cherry).

15294 ***Agrobacterium tumefaciens*** **30 203**

Fruit Tree Res. St. Akitsu Branch, Minist. Agric. Forest. Fish. (H. Sawada; Ch-Ag-7; cherry).

15295 ***Agrobacterium tumefaciens*** **30 203**

Fruit Tree Res. St. Akitsu Branch, Minist. Agric. Forest. Fish. (H. Sawada; Ch-Ag-8; cherry).

15296 ***Agrobacterium tumefaciens*** **30 203**

Fruit Tree Res. St. Akitsu Branch, Minist. Agric. Forest. Fish. (H. Sawada; K-Ag-3; kiwi fruit).

15297 ***Agrobacterium tumefaciens*** **30 203**

Fruit Tree Res. St. Akitsu Branch, Minist. Agric. Forest. Fish. (H. Sawada; K-Ag-4; kiwi fruit).

15299 ***Sphingobacterium*** sp. **28 203**

Inst. Appl. Microbiol., Univ. Tokyo (J. Sugiyama; KS 0470; faeces of *Bos sprunigenius taurus*).

15300 ***Aureobacterium*** sp. **30 203**

IFO (T. Sakane; A-1).

15301 ***Aureobacterium*** sp. **30 203**
IFO (T. Sakane; A-2).

15302 ***Aureobacterium*** sp. **30 203**
IFO (T. Sakane; A-3).

15303 ***Aureobacterium*** sp. **30 203**
IFO (T. Sakane; A-4).

15304 ***Bacillus brevis*** **30 203**
JCM 2503 -- DSM 30 -- ATCC 8246 -- N.R. Smith, 604 -- J.R. Porter -- NCTC 2611 -- W.W. Ford, 27B. Type

15305 ***Bacillus cereus*** **30 203**
JCM 2152 -- IAM 12605 -- NCIB 9373 -- R.E. Gordon. Type

15306 ***Bacillus firmus*** **30 203**
JCM 2512 -- CCM 2213 -- NCIB 9366 -- R.E. Gordon. Type

15307 ***Bacillus macerans*** **30 203**
JCM 2500 -- CCM 2012 -- R.E. Gordon. Type

15308 ***Bacillus megaterium*** **30 203**
JCM 2506 -- CCM 2007 -- R.E. Gordon. Type

15309 ***Bacillus polymyxa*** **30 203**
JCM 2507 -- CCM 1459 -- BUCSAV 162. Type

15310 ***Bacillus cycloheptanicus*** **45 291**
DSM 4006 -- K. Poralla & W.A. Kig, strain SCH, soil. Type

15311 ***Bacillus smithii*** **55 203**
DSM 4216 -- NRRL NRS-173 --N.R. Smith, cheese. Type

15312 ***Bacillus tusciae*** **50 294**
DSM 2912 -- M. Aragno, pond in solfatara. Type

15313 ***Bacillus*** sp. **70 203**
DSM 405 -- W. Heinen, YT-P, hot natural pool.

15314 ***Bacillus*** sp. **70 203**
DSM 406 -- W. Heinen, YT-G, superheated pool water.

15315 ***Bacillus*** sp. **70 203**
DSM 411 -- W. Heinen, YT-F, superheated pool water.

15316 ***Bacillus*** sp. **60 203**
DSM 730 -- L.G. Loginova, 178, a slimy bloom in hot gas well of the Yangan-Tau mountain.

15317 ***Bacillus*** sp. **60 203**
DSM 2641 -- W. Heinen, hot spring.

15318 ***Micrococcus agilis*** **24** **203**
CCM 2131 -- ATCC 998 -- R.S. Breed -- Pribram Collection -- Piorkowski.

15319 ***Micrococcus agilis*** **24** **203**
CCM 2390 -- L. Jeffries, W.O. 219 -- NCTC 7509 -- ATCC 966 -- M. Levine, 28(3). Type

15320 ***Micrococcus agilis*** **24** **203**
CCM 2539 -- K.H. Schleifer -- Inst. Pasteur, strain R-27.

15321 ***Micrococcus agilis*** **24** **203**
CCM 2687 -- K.H. Schleifer -- W.E. Kloos, KE-J11, human skin.

15322 ***Micrococcus agilis*** **24** **203**
CCM 3405 -- K. Kosar, 366, barley.

15323 ***Micrococcus agilis*** **24** **203**
CCM 3941 -- A.K. Mishra, strain 12.

15327 ***Aquaspirillum autotrophicum*** **30** **293**
LMG 4326 -- ATCC 29984 -- M. Aragno, SA 32, eutrophic lake water. Type

15328 ***Aquaspirillum dispar*** **30** **293**
LMG 4329 -- ATCC 27510 -- N. Krieg, VPI 1 -- H. Jannasch, 104, fresh water. Type

15330 ***Bacillus glucanolyticus*** **30** **203**
DSM 5162 -- F.G. Priest -- J.R. Norris, B 0030. Type

15331 ***Sulfolobus solfataricus*** **70** **281**
DSM 1616 -- W. Zillig, volcanic hot spring. Type

15334 ***Pseudomonas fluorescens*** **30** **203**
Cent. Res. Labs., Hokko Chemical Ind. Co., Ltd. (S. Kobayashi; KS-2; mutant of NIHJ B-254 =IFO 12180) -- NIHJ. Phenotype: highly susceptible to kasugamycin

15335 ***Salmonella*** sp. **37** **203**
Max-Planck-Inst. Immunbiologie (H. Mayer; SF 1111). Serotype minnesota. Parent strain of IFO 15182-IFO 15187

15336 ***Sphingobacterium*** sp. **30** **203**
IAM (J. Sugiyama; KS 0427) -- AJ 2515 (K-61-10).

15337 ***Sphingobacterium*** sp. **30** **203**
IAM (J. Sugiyama; KS 0463; faeces of *Hippopotamus amphibius*).

15338 ***Sphingobacterium*** sp. **30** **203**
IAM (J. Sugiyama; KS 0482; soil).

15339 ***Sphingobacterium*** sp. **30** **203**
IAM (J. Sugiyama; KS 0506) -- K. Kodama, S-1, activated sludge.

15340 ***Sphingobacterium*** sp. **30** **203**
IAM (J. Sugiyama; KS 0507) -- K. Kodama, S-3 3-1, activated sludge.

15342 ***Zoogloea ramigera*** **30 802**
Enviroment Products Division, Takeda Chem. Ind., Ltd. (T. Kusaka) -- IAM 12136 -- ATCC 19544 -- N.C. Dondero, 106. Type

15343 ***Micrococcus agilis*** **30 203**
JCM 2587 -- M.V. Hao, HK964 -- CCM 2688 -- K.H. Schleifer -- W.O. Back, D5.

15344 ***Deinococcus erythromyxa*** **30 203**
CCM 706 -- ATCC 187 -- R.S. Breed, KSE -- Král Collection -- W. Migula. Type

15345 ***Deinococcus proteolyticus*** **30 203**
CCM 2703 -- M. Kobatake, MRP, faeces of *Lama glama*. Type

15346 ***Deinococcus radiodurans*** **30 203**
CCM 1700 -- R.G.E. Murray -- A.W. Anderson, irradiated ground pork and beef. Type

15347 ***Deinococcus radiophilus*** **30 203**
CCM 2564 -- N.F. Lewis, RBD, Bombay duck (*Harpodon nehereus*). Type

15348 ***Deinococcus radiopugnans*** **30 203**
CCM 2785 -- B.E. Moseley, irradiated haddock. Type

15353 ***Micrococcus halobius*** **30 273**
CCM 2591 -- H. Onishi, 28-3, unrefined solar salt. Type

15354 ***Micrococcus kristinae*** **37 203**
CCM 2690 -- W.E. Kloos, PM 129, human skin. Type

15355 ***Micrococcus lylae*** **37 203**
CCM 2693 -- W.E. Kloos, JL 178, human skin. Type

15356 ***Micrococcus nishinomiyaensis*** **30 203**
CCM 2140 -- OUT 8094. Type

15357 ***Micrococcus sedentarius*** **30 203**
CCM 314 -- C.E. ZoBell, 541, sea water. Type

15358 ***Micrococcus varians*** **37 203**
CCM 884 -- NCTC 7564 -- T. Gibson, G 33, milk. Type

15359 ***Corynebacterium callunae*** **30 203**
ATCC 15991 -- NRRL B-2244 -- International Mineral and Chemical Corp. Type

15360 ***Corynebacterium matruchotii*** **37 203**
ATCC 14266 -- M. Gilmour, 47. Type

15361 ***Corynebacterium minutissimum*** **37 203**
ATCC 23348 -- D. Taplin, trunk of adult female. Type

15362 ***Corynebacterium pseudodiphtheriticum*** **37 203**
ATCC 10700 -- J.M. Coffey, throat culture. Type

15363 ***Corynebacterium pseudotuberculosis*** **37 203**
ATCC 19410 -- NCTC 3450 -- G. Petrie -- J. Buxton, infected gland of sheep. Type

15364 ***Micrococcus aurantiacus*** **37 203**
ATCC 11731 -- Chas. Pfizer Co., 34140-25. Type

15366 ***Pseudomonas putida*** **30 203**
Lab. Appl. Microbiol., Yamaguchi Univ. (O. Adachi; AYU 92311; soil).

15375 ***Bacillus alginolyticus*** **30 802**
NRRL (L.K. Nakamura; NRS-1347) -- N.R. Smith Bacillus Collection, Rutgers Univ. -- F.E. Clark, 3, soil. Type. Formerly *B. circulans* DNA homology group C

15376 ***Bacillus chondroitinus*** **30 802**
NRRL (L.K. Nakamura; NRS-1351) -- N.R. Smith Bacillus Collection, Rutgers Univ. -- F.E. Clark, 12. Type. Formerly *B. circulans* DNA homology group D

15377 ***Bacillus circulans*** **30 802**
NRRL (L.K. Nakamura; NRS-381) -- N.R. Smith Bacillus Collection, Rutgers Univ. *B. circulans* DNA homology group G

15378 ***Bacillus circulans*** **37 802**
NRRL (L.K. Nakamura; NRS-1434) -- N.R. Smith Bacillus Collection, Rutgers Univ. *B. circulans* DNA homology group B

15379 ***Bacillus circulans*** **30 802**
NRRL (L.K. Nakamura; NRS-1173) -- N.R. Smith Bacillus Collection, Rutgers Univ. *B. circulans* DNA homology group F

15380 ***Bacillus lautus*** **30 802**
NRRL (L.K. Nakamura; NRS-666) -- N.R. Smith Bacillus Collection, Rutgers Univ. Type. Formerly *B. circulans* DNA homology group H

15381 ***Bacillus psychrophilus*** **24 802**
NRRL (L.K. Nakamura; NRS-1530) -- N.R. Smith Bacillus Collection, Rutgers Univ. -- Washington State Univ. (J.L. Stokes; W16A; soil). Type

15382 ***Bacillus validus*** **30 802**
NRRL (L.K. Nakamura; NRS-1000) -- N.R. Smith Bacillus Collection, Rutgers Univ. -- J.R. Porter -- G. Bredemann. Type. Formerly *B. circulans* DNA homology group I

15384 ***Brachybacterium nesterenkovii*** **30 203**
IMV Ac-752 (T.M. Nogina; IMV 34; cheese). Type

15385 ***Micrococcus*** sp. **30 203**
CCM 2142 -- OUT (M. Oda).

15386 ***Streptomyces albosporeus*** subsp. ***albosporeus*** **28 266**
JCM 4135 -- KCC S-0135 -- R. Shinobu -- H. Nishimura -- ATCC 3003 -- S.A. Waksman, 367, soil. Type

15387 ***Streptomyces albosporeus*** subsp. ***labilomyceticus*** **28 296**
JCM 3383 -- NIHJ (A955-Y3). Type

15388 ***Streptomyces atrovirens*** **28** **296**
JCM 6913 -- INA 1551. Type

15389 ***Streptomyces caniferus*** **28** **296**
JCM 6914 -- INMI 377. Type

15390 ***Streptomyces carpaticus*** **28** **296**
JCM 6915 -- INA 8851. Type

15391 ***Streptomyces cavourensis*** subsp. ***washingtonensis*** **28** **266**
JCM 4967 -- KCC S-0967 -- J.D. Skarbek, AUW-83, contaminant in a marine fungal culture. Type

15392 ***Streptomyces champavatii*** **28** **266**
JCM 5066 -- KCC S-1066 -- MS 1482 -- NRRL B-5682 --N. Uma, 1033. Type

15393 ***Streptomyces chrysomallus*** subsp. ***chrysomallus*** **28** **245**
JCM 4296 -- KCC S-0296 -- ATCC 11523 -- IMRU 3657. Type

15394 ***Streptomyces chrysomallus*** subsp. ***fumigatus*** **28** **245**
JCM 3371 -- NRRL B-2289. Type

15395 ***Streptomyces cinereorectus*** **28** **266**
JCM 6916 -- INA 5202. Type

15396 ***Streptomyces cinereoruber*** subsp. ***fructofermentans*** **28** **266**
JCM 4956 -- KCC S-0956 -- DSM 40692 -- H. Zner, T2 -- ETH 6143, soil. Type

15397 ***Streptomyces cinereospinus*** **28** **268**
JCM 6917 -- INA 1719. Type

15398 ***Streptomyces clavifer*** **28** **268**
JCM 5059 -- KCC S-1059 -- MS 1479 -- CBS 101.27 -- W.A. Millard. Type

15399 ***Streptomyces coelicoflavus*** **28** **268**
JCM 6918 -- INA 9630. Type

15400 ***Streptomyces coeruleoprunus*** **28** **2**
JCM 6919 -- INA 1655. Type

15401 ***Streptomyces crystallinus*** **28** **266**
JCM 5067 -- KCC S-1067 -- MS 1483 -- NRRL B-3629. Type

15402 ***Streptomyces diastaticus*** subsp. ***ardesiacus*** **28** **296**
JCM 5815 -- E.M.H. Wellington -- CBS 100.56 -- E. Baldacci, IPV 755. Type

15403 ***Streptomyces erumpens*** **28** **268**
JCM 5060 -- KCC S-1060 -- MS 1475 -- ATCC 23266 -- A.P. Cercós, IMIA 17732, soil. Type

15404 ***Streptomyces flavidofuscus*** **28** **296**
JCM 6920 -- INA 15719. Type

15405 ***Streptomyces floridae*** **28** **266**

JCM 5068 -- KCC S-1068 -- MS 1484 -- NRRL 2423. Type

32439 ***Rhizoctonia solani*** **24 1**

Saga Univ. (F. Nonaka; R.S AG 2-2 88-11-02 (3); Oryza sativa). Anastomosis group AG 2-2

32440 ***Pythium aphanidermatum*** **24 14**

Coll. Agr., Univ. Osaka Pref. (T. Ichitani; UOP 344; leaf sheath of *Agrostis palustris*).

32441 ***Pythium vanterpoolii*** **24 14**

Coll. Agr., Univ. Osaka Pref. (T. Ichitani; UOP 314; basal segment of newly developing leaf of *Zoysia matrella*).

32444 ***Halophytophthora vesicula*** **20 15**

IFO (A. Nakagiri; AN-1063; fallen leaf of *Bruguiera gymnorrhyza*).

32445 ***Halophytophthora vesicula*** **20 15**

IFO (A. Nakagiri; AN-1132; fallen leaf of *Rhizophora stylosa*).

32446 ***Halophytophthora spinosa*** var. ***lobata*** **24 15**

IFO (A. Nakagiri; AN-1134; fallen leaf of *Bruguiera gymnorrhyza*).

32447 ***Halophytophthora spinosa*** var. ***lobata*** **24 15**

IFO (A. Nakagiri; AN-1135; fallen leaf of *Brugiera gymnorrhyza*).

32448 ***Tetracladium apiense*** **24 5**

Dept. Biolog. Sci. Hatherly Labs., Univ. Exeter (J. Webster; PRUM 3218; submerged leaf).

32449 ***Prototheca eriobotryae*** **28 5**

Coll. of Pharm., Nihon Univ. (K. Tubaki; PCNB 107) -- Inst. of Microb., Academia Sinica, Beijing, China, M.X. Li, S 124, loquat fruit. A culture derived from the holotype

32452 ***Heterocephalum aurantiacum*** **24 8**

IFO (T. Ito; soil).

32453 ***Pholiota lubrica*** **24 1**

IFO (T. Ito; T. Ito H3-55; decayed wood).

32454 ***Pholiota malicola*** var. ***macropoda*** **24 7**

IFO (T. Ito; T. Ito H3-56; decayed wood).

32455 ***Polyporus tuberaster*** **24 1**

IFO (T. Ito; T. Ito H3-52; decayed wood).

32459 ***Corticium salmonicolor*** **24 1**

FFPRI, Kansai Res. Cent. (M. Tabata; CS 1; *Ilex aquifolium*).

32460 ***Exobasidium pentasporium*** **24 1**

A. Ezuka, E-20 -- Nat. Inst. Agro-Environmental Sci. (T. Sato; *Rhododendron kaempferi*) Isolated from single conidium

32461 ***Epidermophyton floccosum*** **28 6**

Pub. Health Res. Inst., Kobe City (N. Toyazaki; 1; skin).

32462 ***Microsporum gypseum*** **28 6**

Pub. Health Res. Inst., Kobe City (N. Toyazaki; 2; skin).

32463 ***Sabouraudites canis*** **28 6**

Pub. Health Res. Inst., Kobe City (N. Toyazaki; 3; skin).

32464 ***Sabouraudites canis*** **28 6**

Pub. Health Res. Inst., Kobe City (N. Toyazaki; 4; skin).

32465 ***Pisolithus tinctorius*** **24 1**

Takeda Chem. Ind., Ltd. (T. Kumada; Pt; ground in pine forest).

32469 ***Aigialus grandis*** **24 16**

IFO (A. Nakagiri; AN-1208; submerged wood of *Rhizophora stylosa*).

32470 ***Aigialus grandis*** **24 16**

IFO (A. Nakagiri; AN-1209; submerged wood of *Rhizophora stylosa*).

32471 ***Aniptodera limnetica*** **24 16**

IFO (A. Nakagiri; AN-1270; submerged wood of *Bruguiera gymnorrhyza*).

32472 ***Aniptodera limnetica*** **24 16**

IFO (A. Nakagiri; AN-1271; submerged wood of *Bruguiera gymnorrhyza*).

32473 ***Aniptodera longispora*** **24 16**

IFO (A. Nakagiri; AN-1267; submerged wood of *Rhizophora stylosa*).

32474 ***Aniptodera longispora*** **24 16**

IFO (A. Nakagiri; AN-1268; submerged wood of *Rhizophora stylosa*).

32475 ***Caryosporella rhizophorae*** **24 16**

IFO (A. Nakagiri; AN-1179; submerged wood of *Rhizophora stylosa*).

32476 ***Caryosporella rhizophorae*** **24 16**

IFO (A. Nakagiri; AN-1180; submerged wood of *Rhizophora stylosa*).

32477 ***Cucullosporella mangrovei*** **24 16**

IFO (A. Nakagiri; NA-1152; submerged wood of *Rhizophora stylosa*).

32478 ***Cucullosporella mangrovei*** **24 16**

IFO (A. Nakagiri; AN-1153; submerged wood of *Rhizophora stylosa*).

32479 ***Dactylospora haliotrepha*** **24 16**

IFO (A. Nakagiri; AN-1170; submerged wood of *Rhizophora stylosa*).

32480 ***Dactylospora haliotrepha*** **24 16**

IFO (A. Nakagiri; AN-1171; submerged wood of *Rhizophora stylosa*).

32481 ***Halosarpheia abonis*** **24 16**

IFO (A. Nakagiri; AN-1205; submerged wood of *Rhizophora stylosa*).

32482 ***Halosarpheia abonis*** **24 16**

IFO (A. Nakagiri; AN-1206; submerged wood of *Rhizophora stylosa*).

32483 ***Halosarpheia fibrosa*** **24** **16**
IFO (A. Nakagiri; AN-1258; submerged wood of *Rhizophora stylosa*).

32484 ***Halosarpheia fibrosa*** **24** **16**
IFO (A. Nakagiri; AN-1259; submerged wood of *Rhizophora stylosa*).

32485 ***Hypoxylon oceanicum*** **24** **16**
IFO (A. Nakagiri; AN-1252; submerged wood of *Bruguiera gymnorrhyza*).

32486 ***Hypoxylon oceanicum*** **24** **16**
IFO (A. Nakagiri; AN-1253; submerged wood of *Bruguiera gymnorrhyza*).

32487 ***Lineolata rhizophorae*** **24** **16**
IFO (A. Nakagiri; AN-1246; submerged wood of *Rhizophora stylosa*).

32488 ***Lineolata rhizophorae*** **24** **16**
IFO (A. Nakagiri; AN-1247; submerged wood of *Rhizophora stylosa*).

32489 ***Massarina ramunculicola*** **24** **16**
IFO (A. Nakagiri; AN-1199; submerged wood of *Rhizophora stylosa*).

32490 ***Massarina ramunculicola*** **24** **16**
IFO (A. Nakagiri; AN-1200; submerged wood of *Rhizophora stylosa*).

32491 ***Quintaria lignatilis*** **24** **16**
IFO (A. Nakagiri; AN-1193; submerged wood of *Rhizophora stylosa*).

32492 ***Quintaria lignatilis*** **24** **16**
IFO (A. Nakagiri; AN-1194; submerged wood of *Rhizophora stylosa*).

32493 ***Savoryella paucispora*** **24** **16**
IFO (A. Nakagiri; AN-1276; submerged wood of *Bruguiera gymnorrhyza*).

32494 ***Savoryella paucispora*** **24** **16**
IFO (A. Nakagiri; AN-1277; submerged wood of *Bruguiera gymnorrhyza*).

32495 ***Swampomyces triseptatus*** **24** **16**
IFO (A. Nakagiri; AN-1184; submerged wood of *Rhizophora stylosa*).

32496 ***Swampomyces triseptatus*** **24** **16**
IFO (A. Nakagiri; AN-1185; submerged wood of *Rhizophora stylosa*).

32497 ***Verruculina enalia*** **24** **16**
IFO (A. Nakagiri; AN-1243; submerged wood of *Rhizophora stylosa*).

32498 ***Verruculina enalia*** **24** **16**
IFO (A. Nakagiri; AN-1244; submerged wood of *Rhizophora stylosa*).

32499 ***Cirrenalia tropicalis*** **24** **16**
IFO (A. Nakagiri; AN-1150; submerged wood of *Rhizophora stylosa*).

32500 ***Cirrenalia tropicalis*** **24** **16**
IFO (A. Nakagiri; AN-1151; submerged wood of *Rhizophora stylosa*).

32501 ***Dictyosporium elegans*** **24** **16**

IFO (A. Nakagiri; AN-1196; submerged wood of *Rhizophora stylosa*).

32502 ***Dictyosporium elegans*** **24** **16**
IFO (A. Nakagiri; AN-1197; submerged wood of *Rhizophora stylosa*).

32503 ***Mycocentrolobium platysporum*** **24** **16**
IFO (A. Nakagiri; AN-1249; submerged wood of *Rhizophora stylosa*).

32504 ***Mycocentrolobium platysporum*** **24** **16**
IFO (A. Nakagiri; AN-1250; submerged wood of *Rhizophora stylosa*).

32505 ***Phragmospathula phoenicis*** **24** **16**
IFO (A. Nakagiri; AN-1241; submerged wood of *Rhizophora stylosa*).

32506 ***Phragmospathula phoenicis*** **24** **16**
IFO (A. Nakagiri; AN-1242; submerged wood of *Rhizophora stylosa*).

32507 ***Stachybotrys mangiferae*** **24** **16**
IFO (A. Nakagiri; AN-1177; submerged wood of *Rhizophora stylosa*).

32508 ***Stachybotrys mangiferae*** **24** **16**
IFO (A. Nakagiri; AN-1178; submerged wood of *Rhizophora stylosa*).

32509 ***Trichocladium achrasporum*** **24** **16**
IFO (A. Nakagiri; AN-1182; submerged wood of *Rhizophora stylosa*).

32510 ***Trichocladium achrasporum*** **24** **16**
IFO (A. Nakagiri; AN-1183; submerged wood of *Rhizophora stylosa*).

32511 ***Cladosporium oxysporum*** **24** **5**
IFO (T. Ito; T. Ito D6-15-6; field soil).

32512 ***Phialophora cyclaminis*** **24** **1**
IFO (T. Ito; T. Ito C-15-13; field soil).

32513 ***Phoma eupyrena*** **24** **1**
IFO (T. Ito; T. Ito C6-5-32; field soil).

50375 ***MSS31*** **37**
The Res. Inst. for Tuberculosis & Cancer, Tohoku Univ. (N. Yanai; mouse; spleen). spleen stromal cell, mouse C57BL/6, spleen, fibroblast-like

50376 ***MSS62*** **37**
The Res. Inst. for Tuberculosis & Cancer, Tohoku Univ. (N. Yanai; mouse; spleen). spleen stromal cell, mouse, C57BL/6, spleen, fibroblast-like

50377 ***FLS3*** **37**
The Res. Inst. for Tuberculosis & Cancer, Tohoku Univ. (N. Yanai; mouse; liver). liver stromal cell, mouse, C57BL/6, liver, epithelial-like

50378 ***FLS5*** **37**
The Res. Inst. for Tuberculosis & Cancer, Tohoku Univ. (N. Yanai; mouse; liver). liver stromal cell, mouse, C57BL/6, liver, fibroblast-like

ABSTRACTS 1991 - 1992

The molecular phylogeny of the Q10-equipped species of the heterobasidiomycetous yeast genus Rhodosporidium Banno based on the partial sequences of 18S and 26S ribosomal ribonucleic acids

Y. Yamada[1], Y. Nakagawa[2], and I. Banno

J. Gen. Appl. Microbiol. 36: 435-444 (1990)

Twelve strains of the Q10-equipped species of the teleomorphic yeast genus Rhodosporidium and the anamorphic yeast genus Rhodotorula were examined for the partial sequences of 18S and 26S rRNAs. In the positions 492 through 625 of 26S rRNA, the strains of R. kratochvilovae, R. paludigenum, R. diobovatum, R. sphaerocarpum, and Rh. graminis were linked at 71% or more maximum homologies to the type species of the genus Rhodosporidium (R. toruloidis). Rhodosporidium dacryoidum constituted its own separate cluster (66% maximum homology). In the positions 1451 through 1618 of 18S rRNA, the strains of the adove-mentioned species were divided into two clusters. The first cluster included R. kratochvilovae, R. paludigenum, R. diobovatum, R. sphaerocarpum, and Rh. graminis (within 0 or 2 base differences), and the second cluster included the only species, R. dacryoidum (11 base differences). These results indicate that R. dacryoidum should be classified in a separate new genus.

1) Department of Agricultural Chemistry, Shizuoka University.
2) Institute of Applied Microbiology, The University of Tokyo.

The phylogenetic relationship of the genera Phaffia Miller, Yoneyama et Soneda and Cryptococcus Kützing emend. Phaff et Spencer (Cryptococcaceae) based on the partial sequences of 18S and 26S ribosomal ribonucleic acids

Y. Yamada[1], T. Nagahama[1], H. Kawasaki[2] and I. Banno

J. Gen. Appl. Microbiol. 36: 403-414 (1990)

The partial sequences of 18S and 26S rRNAs were studied in eighteen strains of Phaffia, Cryptococcus, Candida, and Filobasidiella species.

The position determined were 1451 through 1618(168 bases) of 18S rRNA, and 492 through 625(134 bases), and 1686 through 1835(150 bases) of 26S rRNA. These three determinations showed that the genus Cryptococcus is phylogenetically heterogeneous: the maximum homologies of 26S rRNA were 65-94% in the positions 492 through 625 and 69-93% in the positions 1686 through 1835, and the base differences of 18S rRNA were 0-13 in the positions 1451 through 1618. Filobasidiella neoformans, a teleomorph of the genus Cryptococcus, had partial sequences somewhat different from the species examined of the genus Cryptococcus (maximum homologies, 74-84% and 74-86%; base differences, 4-12, respectively). Candida humicola was included in the cluster of the genus Cryptococcus. However, the base differences of the species were not so small (maximum homologies, 69-77% and 72-84%; base differences, 5-13). the genus Phaffia constituted its own cluster separate from the species of Cryptococcus, Candida, and Filobasidiella (maximum homolpgies, 61-70% and 71-85%; base differences, 6-16, respectively). So the genus Phaffia is retained as an in dependent genus.

1) Department of Agricultural Chemistry, Shizuoka University.
2) Institute of Applied Microbiology, The University of Tokyo.

Studies on motile arthrospore-bearing rare actinomycetes

Toru Hasegawa

Actinomycetol. 5: 64-71 (1991)

In the course of a search program to acquire rare actimomycete species from natural habitats, a remarkable organism was isolated from a grass blade that formed true synnemata and had motile arthrospores and was named as the new genus Actinosynnema. By a selective isolation method based on the characteristics of Actinosynnema, Actinosynnema-like organisms have been effectively isolated from soil habitats, and subsequently, the new genus Actinokineospora was discovered. Furthermore, a number of "Catenuloplanes"-like organisms have been obtained. In this way, Sporichthya, Actinosynnema, Actinokineospora and "Catenuloplanes" have come to be recognized as rare genera having motile arthrospores. No doubt these rare genera await establishment of their status in the rational actinomycete taxonomy.

Mangrovispora pemphii gen. et sp. nov., a new marine fungus from *Pemphis acidula*

K. D. Hyde[1] and A. Nakagiri

Systema Ascomycetum 10: 19-25 (1991)

During a study of the marine fungi of northern Australia, a species new to science was found on *Pemphis acidula* Forster & Forster f. This taxon cannot be accommodated in any previously described genus and therefore a new genus *Mangrovispora* is proposed. The genus is compared with *Belizeana*, *Clypeothecium*, *Exarmidium*, *Pyrenidium*, *Massarina*, and *Swampomyces*, genera with some similar characteristics.

1) Plant Pathology Branch, Queensland Department of Primary Industries, Australia.

Preservation of yeast cultures by freezing at -80 C: viability after 5 years-storage

K. Mikata

Bull. J. F. C. C. 7: 11-19 (1991)

Cultures of 139 strains of yeasts sensitive to the L-drying were suspended in 10% glycerol solution and subjected to long-term storage and preservation by deep-freezing at -80 C. Survival count of one frozen culture of each strains was determined by repeatedly thawing after preservation for 1 m, 6 m, 2 y, and 5 y, while one other frozen cultures was first thawed and examined after 5 years of storage.

Survival curves gradually decreased with the repetition of freezing and thawing in 19 strains, while in 120 other strains it did not decrease with repetition. Calculations based on the survival ratio after 5 years of storage indicated that all the frozen cultures semipermanently retained a survival count of more than 10^5 cells/ml.

Examination revealed that in all yeast strains, freezing at -80 C using an electric-freezer is as effective in maintaining survival during long-term preservation as is freezing in liquid nitrogen (-196 C).

Basidiocarp development of the cyphelloid gasteroid aquatic basidiomycetes Halocyphina villosa and Limnoperdon incarnatum

A. Nakagiri and T. Ito

Can. J. Bot. 69: 2320-2327 (1991)

Basidiocarp structure and development in the aquatic basidiomycetes Halocyphina villosa and Limnoperdon incarnatum were observed under a scanning electron microscope. Halocyphina villosa produces completely closed gasteroid basidiocarps at the early developmental stage while it shows cyphelloid hymenium distribution and ballistosporic basidiospore formation. Alternatively, the basidiocarp of L. incarnatum is cyphelloid at the initial stage but becomes closed at maturity. Basidia and basidiospores are gasteroid and nonballistosporic and are distributed on a loosely formed hymenium. These observations suggest that a cyphelloid hymenomycete and a gasteromycete, terrestrial Basidiomycetes by origin, have evolved into the two aquatic species by now possessing similar closed basidiocarps adapted to aquatic habitats.

Kockovaella, a new ballistospore-forming anamorphic yeast genus

T. Nakase[1], M. Itoh[1], A. Takematsu[1], K. Mikata, I. Banno, and Y. Yamada[2]

J. Gen. Appl. Microbiol. 37: 175-197 (1991)

A new genus Kockovaella Nakase, Banno et Yamada in the Hyphomycetes in described for yeasts that reproduce by non-ballistosporous stalked conidia, ballistospores and budding yeast cells. Strains in this genus have Q-10 as the major isoprenologue of ubiquinones, contain xylose in the cells, and are positive to diazonium blue B reaction. Two new species, Kockovaella thailandica (type species) and K. imperatae, are described in the genus. Kockovaella thailandica has a G+C content of DNA of 49.5 mol% (from Tm) or 47.7-48.4 mol% (by HPLC) and forms asymmetrical kindney-shaped or ellipsoidal ballistospores, whereas K. imperatae has a G+C content of DNA of 52.3 mol% (from Tm) or 49.0 mol% (by HPLC) and forms symmetrical globose to napiform ballistospores. Electrophoretic comparison of eight enzymes and DNA similarity indicated that K. thailandica and K. imperatae are distinct species from each other. The partial sequenc-

ing study on 18S ribosomal RNA suggested a close relationship among species of Kockovaella, Fellomyces and Sterigmatosporidium. The latter two genera resemble Kockovaella in the production of non-ballistosporous stalked conidia and the presence of xylose in the cells but produce neither ballistospores nor budding yeast cells.

1) Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN).
2) Department of Agricultural Chemistry, Shizuoka University.

Liquid nitrogen storage of oomycetous fungi: examination of cooling rates and improvement of the freezing tube case

T. Nishii and A. Nakagiri

Bull. JFCC 7: 90-96 (1991)

To find the best cooling rate for cryopreservation of oomycetous fungi, the survival rates of freeze sensitive strains (three and four strains of Phytophthora and Pythium, respectively) were examined after freezing the tubes with cultures in a programable freezer at the temperature decreasing rate of -0.5, -1, -2 or -5 C/min or by dipping the tubes into liquid nitrogen directly. Cooling at a slower speed (-0.5 or -1 C /min) was found to result in a relatively higher survival rate than cooling at a higher speed. Also, survival rate varied greatly among the strains, but as the cooling rate became faster, the differences were greater. From these results, the cooling rate of -1 C/min was found to be most useful from the viewpoint of practical convenience in storing many and various fungal strains.

Stainless cases for freezing tubes were lined inside with thick paper and compared with cases without lining for the survival of two Phytophthora species in frozen tubes. Phytophthora capsici IFO 9752 showed a higher survival rate (91.3 %) after cooling at -1 C/min in a lined case than in an ordinary case (67.5 %), while P. vesicula IFO 32216 survived equally well in both cases. Since the lined freezing case was more efficient for freezing one sensitive strain and no adverse effect was found on the other strain, we decided to use a lined freezing case for routine liquid nitrogen storage of fungi.

A constitutive thiamine metabolism mutation, thi80, causing reduced thiamine pyrophosphokinase activity in Saccharomyces cerevisae

H. Nishimura[1], Y. Kawasaki[1], K. Nosaka[1], Y. Kaneko and A. Iwashima[1]

J. Bacteriol. 173: 2716-2719 (1991)

We identified a strain carrying a recessive constitutive mutation (thi80-1) with an altered thiamine transport system, thiamine-repressible acid phosphatase, and several enzymes of thiamine synthesis from 2-methyl-4-amino-5-hydroxymethylpyrimidine and 4-methyl-5-β-hydroxyethylthiazole. The mutant shows markedly reduced activity of thiamine pyrophosphokinase (EC 2.7.6.2) and high resistance to oxythiamine, a thiamine antagonist whose potency depends on thiamine pyrophosphokinase activity. The intracellular thiamine pyrophosphate content of the mutant cells grown with exogenous thiamine ($2X10^{-7}$ M) was found to be about half that of the wild-type strain under the same conditions. These results suggest that the utilization and synthesis of thiamine in Saccharomyces cerevisiae is controlled negatively by the intracellular thiamine pyrophosphate level.

1) Department of Biochemistry, Kyoto Prefectural University of Medicine.

Occurrence of Lipid A Variants with 27-Hydroxyoctacosanoic acid in Lipoplysaccharides from the Members of the Family Rhizobiaceae

U. Ramadas Bhat[1], H. Mayer[2], A. Yokota, R. I. Hollingthworth[3], and R. W. Carlson[1]

J. Bacteriol. 173: 2155-2159 (1991)

Lipoplysaccharides (LPSs) isolated from several strains of Rhizobium, Bradyrhizobium, Agrobacterium, and Azorhizobium were screened for the presence of 27-hydroxyoctacosanoic acid. The LPSs from all strains, with the exception of Azorhizobium caulinodans, contained various amounts of this long-chain hydroxy fatty acid in the lipid A fractions. Analysis of the lipid A sugars revealed three types of backbones: those containing glucosamine (as found in Rhizobium meliloti and Rhizobium fredii), those containing glucosamine and galacturonic acid a (as found in Rhizobium leguminosarum bv. phaseoli, trifolii, and visiae), and those containing

2, 3-diamino-2, 3-dideoxyglucose either alone or in combination with glucosamine (as found in Bradyrhizobium japonicum and Bradyrhizobium sp. [Lupinus] strain DSM 30140). The distribution of 27-hydroxyoctacosanoic acid as well as analysis of lipid A backbone sugars revealed the taxonomic relatedness of various strains of the Rhizobiaceae.

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2) Max-Planck-Institut für Immunbiologie, D-7800 Freiburg-Zähringen, Federal Republic of Germany.
3) Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824-1319, U. S. A.

Production of platelet-like particles by a human megakaryoblastic leukemia cell line (MEG-01)

K. Takeuchi[1], M. Ogura[2], H. Saito[3], M. Satoh and Masao Takeuchi

Exp. Cell Res. 193: 223-226 (1991)

The distribution of microtubules and platelet-specific glycoproteins (GPIIb/IIIa) in particles was probed by an immunofluorescence method using anti-tubulin and anti-GPIIb/IIIa antibodies to identify whether particles released from a human megakaryoblastic cell line (MEG-01) are platelets. The fluorescence image showing anti-tubulin staining of the particles revealed a characteristic ring structure observed in platelets. Anti-platelet GPIIb/IIIa antibody staining showed an image in which small patches or spots were seen throughout the particle with brighter staining at the periphery. No significant difference was observed between these particles and human blood platelets under immunofluorescent staining. These results show that MEG-01 cells released platelet-like particles.

1) Ehime College of Health Science.
2) Aichi Cancer Hospital.
3) Nagoya University School of Medicine.

Amino acid compositions and partial sequences of xylanases from a new subspecies, Nocardiopsis dassonvillei subsp. alba OPC-18

H. Tsujibo[1], T. Sakamoto[1], K. Miyamoto[1], Toru Hasegawa, M. Fujimoto[1] and Y. Inamori[1]

Agric. Biol. Chem. 55: 2173-2174 (1991)

We have already reported the purification and properties of the three xylanases, as well as the taxonomy of alkalophilic actinomycete OPC-18. This paper deals with amino acid compositions and partial amino acid sequences of X-I (Mr 23,000), X-II (Mr 23,000), and X-III (Mr 37,000) isolated from the culture filtrate of N. dassonvillei subsp. alba OPC-18. X-I and X-II had very similar amino acid compositions like isozymes. On the other hand, the amino acid composition of X-III was different from those of X-I and X-II, with much asparatic acid, glutamic acid, prolin, alanine, and leucine. The amino-terminal 40 amino acid residues of X-II were sequenced, and compared with those of other microbial xylanases. Particularly, X-II showed high sequence homology with the xylanase from B. pumilus (51% homology). The sequence experiment on X-I was unsuccessful. The amino-terminal amino acid seems to be blocked. X-III showed no apparent sequence homology with R. pumilus-like xylanases. However, this enzyme showed high sequence homology with the xylanase from Bacillus sp. C-125 (33% homology).

1) Osaka University of Pharmaceutical Sciences.

The molecular phylogeny of the Q9-equipped ascomycetous teleomorphic yeast genus Debaryomyces Lodder et Kreger-van Rij based on the partial sequences of 18S and 26S ribosomal ribonucleic acids

Y. Yamada[1], T. Nagahama[1], and I. Banno

J. Gen. Appl. Microbiol. 37: 277-288 (1991)

The partial base sequences of 18S and 26S rRNAs were examined in eighteen strains of Debaryomyces, Torulaspora, and Yamadazyma species including two strains of D. udenii. All of the strains of Debaryomyces species constituted a single group(cluster) phylogenetically. In the partial base sequence (positions 493 through 622, 130 bases) of 26S rRNA, the maximum homologies were 79-99% among Debaryomyces species. T. globosa and Y. philogaea had 71-78% and 81-87% maximum homologies, respectively, with Debaryomyces species. In the partial base sequence (positions

1611 through 1835, 225 bases) of 26S rRNA, the base differences numbered 5-0 among Debaryomyces species. T. globosa and Y. philogaea had 15-13 and 10-8 base differences, respectively, with Debaryomyces species. In the partial base sequence (Positions 1451 through 1618, 168 bases) of 18S rRNA, Debaryomyces species were divided into two subgroups (subclusters). The first subgroup was comprised of D. hansenii, D. melissophilus, D. udenii, and so on, and the second subgroup comprised of D. castellii, D. polymorphus, D. yamadae, and so on. The base difference numbered 1 between the two subgroups. T. globosa and Y. philogaea had 5-4 and 1-0 base differences, respectively, with Debaryomyces species. Between T. globosa and S. cerevisiae, there was 1 base difference. D. tamarii occupied a distant position (maximum homologies, 63-71%; base differences, 50-48 and 20-19, respectively).

1) Department of Agricultural Chemistry, Shizuoka University.

The phylogenetic relationships of the Q6-equipped genera Torulaspora Lindner and Zygosaccharomyces Barker (Saccharomycetaceae) based on the partial sequences of 18S and 26S ribosomal ribonucleic acids

Y. Yamada[1], K. Maeda[1], T. Nagahama[1], and I. Banno

J. Gen. Appl. Microbiol. 37: 503-513 (1991)

Eleven strains of Torulaspora and Zygosaccharomyces species were examined for the partial base sequences of 18S and 26S rRNAs. In the partial base sequences in positions 493-622 (130 bases) of 26S rRNA, there were 88-91% and 70-92% maximum homologies within the genera Torulaspora and Zygosaccharomyces, respectively. The maximum homologies were 70-90% between the two genera. The species of the two genera had 73-88% maximum homologies with Saccharomyces cerevisiae. In the partial base sequences in positions 1611-1835 (225 bases) of 26S rRNA, there were 3-0 and 16-1 base differences within the two genera, respectively. The base differences were 15-0 between the two genera. The two genera had 17-8 base differences with S. cerevisiae. In the partial base sequences in positions 1451-1618 (168 bases) of 18S rRNA, there were 0 and 3-0 base differences within the two genera, respectively. The base differences were 2-0 between the two genera. The two genera had 3-1 base differences with S. cerevisiae. Zygosaccharomyces cidri and Z. fermentati occupied a

unique situation (base differences, 3-2). The data obtained were discussed with regard to the phylogenetic relationships and the taxonomic positions of Torulaspora, Zygosaccharomyces, and Saccharomyces.

1) Department of Agricultural Chemistry, Shizuoka University.

The phylogenetic relationship of Kurtzmanomyces tardus Gimenez-jurado et van Uden (Cryptococcaceae) based on the partial sequences of 18S and 26S ribosomal RNAs

Y. Yamada[1], T. Nagahama[1], I. Banno, G. Gimenez-jurado[2], and N. van Uden[2]

J. Gen. Appl. Microbiol. 37: 321-324 (1991)

Sequences of 157 bases of 26S rRNA and 168 bases of 18S rRNA of the second species of genus Kurtzmanomyces, K. tardus was determined and compared with those of K. nectairei, Sterigmatomyces halophilus, S. elviae, Tsuchiyaea wingfieldii, and Fellomyces polybolus. In a dendrogram based on the calculated number of base difference in the partial sequence of 18S rRNA. K. tardus and K. nectairei constituted separate clusters (7 base difference). S. halophilus and F. polyborus were linked to K. tardus at 5 and 13 base differences, respectively. K. tardus is considered to be phylogenetically separable at the generic level from any other stalked conidium-forming yeast species and to be accommodated in its own genus.

1) Department of Agricultural Chemistry, Shizuoka University.
2) The Portuguese Yeast Culture Collection, Laboratory of Microbiology, Gulbenkian Institute of Science.

Primary culture and cryopreservation of mouse astrocytes under serum-free conditions

T. Yoshida and Masao Takeuchi

Cytotechnology 5: 99-106 (1991)

The methods of primary culture and cryopreservation of mouse astrocytes under serum-free conditions were examined. Cerebra from newborn C3H/He mice were employed as the source of astrocytes. The cultured

cells were able to grow in a serum-free, chemically defined medium containing transferrin, hydrocortisone, biotin, sodium selenite, insulin, fibroblast growth factor and epidermal growth factor. After the culture was maintained in the medium for 3 weeks, purity was assessed using immunofluorescence staining. The great majority of the cells (>98%) contained glial fibrillary acidic protein and S-100 protein which are cell markers of astrocytes. To cryopreserve the enriched astrocytes under serum-free conditions, various cryoprotectants were examined. The combination of 10% dimethylsulfoxide and 0.1% methylcellulose gave the highest survival rate. These methods of primary culture and cryopreservation will be useful in physiological and biochemical studies which require mouse astrocytes.

Expression of fibronectin and laminin by different types of mouse glial cells cultured in a serum-free medium

T. Yoshida and Masao Takeuchi

Cytotechnology 7:187-196 (1991)

The expression of fibronectin and laminin by cultured glial cells was studied. The glial culture from neonatal mouse cerebra maintained in a chemically defined, serum-free medium consisted of type-1 astrocytes, oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells, oligodendrocytes and type-2 astrocytes. Double-labelling immunofluorescent experiments performed using the mixed glial culture indicated that fibronectin and laminin are expressed in different patterns among glial subtypes. The staining intensities with anti-fibronectin or anti-laminin antibodies decreased in the order: type-1 astrocytes, O-2A progenitor cells and type-2 astrocytes. Both molecules were deposited in a fibrillar matrix underneath type-1 astrocytes, whereas only intracytoplasmic localization of these molecules was observed with O-2A progenitor cells and type-2 astrocytes. Western blot analysis showed that glial fibronectin has a slightly higher molecular weight than mouse plasma fibronectin (230 kDa) and that glial laminin is a varient with a 220 kDa B chain present and the 400 kDa A chain missing. Using enzyme-linked immunosorbent assays (ELISA), these molecules were detected in the glial extracellular matrix at the concentration of 4 ng/10^6 cells. A large amount of fibronectin

(82 ng/10^6 cells) was secreted into the culture medium, while secretion of laminin was not detected.

Selective isolation of Actinoplanes and Dactylosporangium from soil by using γ-collidine as the chemoattractant

M. Hayakawa[1], T. Tamura and H. Nonomura[1]

J. Ferment. Bioeng. 72: 426-432 (1992)

Zoospores of the Actinoplanes missouriensis and Dactylosporangium thailandense were attracted to a number of amino acids, aromatic compounds and sugars. Among these compounds, D-xylose and γ-collidine served as universal chemoattractants for most or all of other 9 species belonging to the genera, and moreover, γ-collidine always elicited an extremely strong response. In contrast, potassium chloride was a weak to moderate attractant only for a half of the strains tested. Palleroni's chemotactic method (Palleroni, N. J., Arch. Microbiol., 128, 53-55, 1980) for the isolation of motile actinoplanetes was complemented by using γ-collidine as a substitute for the originally used chemoattractant, potassium chloride, and by using humic acid-vitamin (HV) agar in place of the isolation medium, starch-casein agar. The improved method not only enabled the increased recovery of Actinoplanes spp. but also the consistent recovery of Dactylosporangium spp., which have only incidentally been isolated by the original method and by conventional dilution plating techniques, from various field soils (16 samples). The recovery of motile actinoplanetes was more successfully achieved by using HV agar supplemented with nalidixic acid to reduce associated bacterial contaminants.

1) Faculty of Engineering, Yamanashi University

Pollen-baiting and drying method for the highly selective isolation of Actinoplanes spp. from soil

M. Hayakawa[1], T. Tamura, H. Iino[1] and H. Nonomura[1]

J. Ferment. Bioeng. 72: 426-432 (1992)

A simplified enrichment method for the highly selective isolation of the zoosporic actinomycetes Actinoplanes spp. from soil is described.

The method consists of baiting the species with Pinus pollen grains, desiccating (30 C, 2 h) the baits bearing sporangia in dried soil particles with the aid of silica gel and following the spore liberation upon immersion in water. Portions of the liquid enriched with zoospores are plated out on humic acid-vitamin (HV) agar supplemented with nalidixic acid at a concentration of 10 $\mu g\ ml^{-1}$. The desiccation stage has enable the almost complete elimination of associated bacteria from colonized baits while allowing the Actinoplanes sporangia to survive and still possess the ability to release many spores. A total of four different soil samples from fields of corn, peach, vegetable and paddy rice were examined. The pollen-baiting and drying method consistently resulted in the highly selective isolation of Actinoplanes spp. which accounted for over 83% of the total number of micro-organisms recovered on HV agar containing nalidixic acid.

1) Faculty of Engineering, Yamanashi University

A new species of Halophytophthora from Atlantic and Pacific subtropical islands

H. H. Ho[1], A. Nakagiri, and S. Y. Newell[2]

Mycologia 84: 548-554 (1992)

Halophytophtora exoprolifera is described from decaying mangrove leaves in coastal marine environments. It was discovered wholly independently and nearly simultaneously in Atlantic and Pacific mangrove systems, both near latitude 26° N. Key characteristics that distinguish H. exoprolifera from other species of Halophytophthora are: a) sporangial dehiscence tubes containing clear closure material that rapidly expands outward and immediately dissolves at zoospore release; b) external proliferation of the sporangia; and c) readily produced sexual structures.

1) Department of Biology, State University of New York, USA.
2) University of Georgia Marine Institute, Sapelo Island, USA.

A new DNA profiling system for cell line identification for use in cell banks in Japan

M. Honma[1], E. Kataoka[1], K. Ohnishi[1], T. Ohno[2], Masao Takeuchi,

N. Nomura[3] and H. Mizusawa[1]
In Vitro Cell. Dev. Biol. 28A: 24-28 (1992)

Using the polymorphic DNA probes, ChdTC-15, ChdTC-114, pYNH24, and λTM-18, a DNA profiling system was developed that verified identities of individual cultured cell lines collected in the Japanese cell banks, JCRB, RCB, and IFO. These highly polymorphic DNA probes include both VNTR (Variable Number of Tandem Repeats) sequences and substantial lengths of unique regions. In the mixed probe system, several distinct bands from four to eight can be used for cell line identification. These bands were widely spread in a range of molecular sizes, and were stable and reproducible under stringent conditions of Southern blot hybridization. Because the DNA profile was specific for each individual human cell line, it is useful not only to authenticate many existing cultured cell lines but also to monitor their identity during propagation in a laboratory, and to confirm newly established lines as unique.

1) Division of Genetics and Mutagenesis, Cell Bank (JCRB), National Institute of Hygienic Sciences.
2) Cell Bank, Riken (RCB, The institute of Physical and Chemical Research).
3) Molecular Oncology Laboratory, Nippon Veterinary and Zootechnology Collage.

Intertidal fungi from Australia. The genus Swampomyces including S. triseptatus sp. nov.

K. D. Hyde[1] and A. Nakagiri
Sydowia 44: 122-130 (1992)

The genus Swampomyces is reported from Australia and Papua New Guinea, with Swampomyces triseptatus sp. nov. described and illustrated. S. triseptatus differs from S. armeniacus in having 3-septate ascospores and ascomata developing under a pseudostroma. The fungus is compared with Marinosphaera mangrovei and Mangrovispora pemphii, marine taxa with similar ascospores.

1) Plant Pathology Branch, Queensland Department of Primary Industries, Australia.

Three new species of the genus Streptomyces: Streptomyces cochleatus sp. nov., Streptomyces paracochleatus sp. nov., and Streptomyces azaticus sp. nov.

Y. Nakagaito, A. Yokota and Toru Hasegawa

J. Gen. Appl. Microbiol. 38: 105-120 (1992)

Three actinomycete strains isolated from soil, 'Streptomyces candidus subsp. azaticus' IFO 13803^T (T=type strain) and 'Nocardiopsis streptosporus' IFO 14362^T were found to contain both LL- and meso-2, 6-diaminopimelic acid (A_2pm) in their cell wall peptidoglycans and supposed to belong to new members of Streptomyces, yielded by unification of the genus Kitasatosporia with the genus Streptomyces. Based on the phenetic and DNA-DNA hybridization studies, these strains were considered to belong to new species of the genus Streptomyces. We propose the names Streptomyces cochleatus sp. nov. for the species represented by the soil isolate M-5^T (=IFO 14768^T) and S. paracochleatus sp. nov. for the soil isolate M-13^T (=IFO 14769^T), and S. azaticus sp. nov. for strain IFO 13803^T. 'Nocardiopsis streptosporus' IFO 14362^T and 'Kitasatosporia brunnea' IFO 14627^T were included in S. phosalacinea. 'K. melanogena' IFO 14327^T, 'K. cystarginea' IFO 14836^T and 'K. kifunense' IFO 15206^T were new species belonging to the genus Streptomyces. 'K. clausa' IFO 15240^T was supposed to belong to a maduromycete. 'K. papulosa' and 'K. grisea' are synonymous, and should be reclassified as a species belonging to the cluster consisting of original Streptomyces species.

Cloning and characteristics of a positive regulatory gene, THI2 (PHO6), of thiamin biosynthesis in Saccharomyces cerevisiae

H. Nishimura[1)], Y. Kawasaki[1)], Y. Kaneko, K. Nosaka[1)] and Akio Iwashima[1)]

FEBS Letters 297: 155-158 (1992)

A thi2 (pho6) mutant of Saccharomyces cerevisiae, defective in the expression of structural genes of thiamin-repressible acid phosphatase and enzymes involved in thiamin biosysnthesis, was found to retain sufficient thiamin transport activity. The transport activity was repressed by thiamin in growth medium. We isolated from an S. cerevisiae genomic library two hybrid plasmids, pTSR1 and pTSR2, containing 10.2- and 12.0-

kilobase (kb) DNA fragments, respectively, which complement the thi2(pho6) mutation of S. cerevisiae. This gene was localized on a 6.0-kb ClaI-ClaI fragment in the subclone pTSR3. Complementation of the enzyme activities for thiamin metabolism in the thi2(pho6) mutant transformed by some plasmids with the THI2(PHO6) gene was also examined.

1) Department of Biochemistry, Kyoto Prefectural University of Medicine.

A positive regulatory gene, THI3, is required for thiamine metabolism in Sccharomyces cerevisiae

H. Nishimura[1], Y. Kawasaki[1], Y. Kaneko, K. Nosaka[1] and A. Iwashima[1]

J. Bacteriol. 174: 4701-4706 (1992)

We have isolated a thiamine auxotrophic mutant carrying a recessive mutation which lacks the positive regulatory gene, THI3, which differs in the regulation of thiamine transport from the THI2(PHO6) gene described previously (Y. kawasaki, K. Nosaka, Y. Kaneko, H. Nishimura, and A. Iwashima, J. Bacteriol. 172:6145-6147, 1990) for expression of thiamine metabolism in Saccharomyces cerevisiae. The mutant (thi3) had a markedly reduced thiamine transport system as well as reduced activity of thiamine-repressible acid phosphatase and of several enzymes for thiamine synthesis from 2-methyl-4-amino-5-hydroxymethylpyrimidine and 4-methyl-5-β-hydroxyethylthiazole. These results suggest that thiamine metabolism in S. cerevisiae is subject to two positive regulatory genes, THI2(PHO6) and THI3. We have also isolated a hybrid plasmid, pTTR1, containing a 6.2-kb DNA fragment from an S. cerevisiae genomic library which complements thiamine auxotrophy in the thi3 mutant. This gene was localized on a 3.0-kb ClaI-BglII fragment in the subclone pTTR5. Complementation of the activities for thiamine metabolism in the thi3 mutant transformed by some plasmids with the THI3 gene was also examined.

1) Department of Biochemistry, Kyoto Prefectural University of Medicine.

Role of ethylenediamine dihydrochloride in the protection of cell membrane of Aquaspirillum metamorphum subjected to L-drying

T. Sakane and A. Yokota

Japan. J. Freez. Dry. 38: 21-26 (1992)

The protective effect of ethylenediamine dihydrochloride (ED) in preventing damage of the bacterial cell membrane by L-drying was examined. The bacterium examined was Aquaspirillum metamorphum, which might be susceptible to damage of the cell membrane during desiccation. Addition of 30 mM ED to the basal suspending medium (BSM) was found to provide effective protection of both the wild-type strain and a surface layer protein-less mutant after drying. Vegetative cells of A. metamorphum IFO 13960 (wild type) were insensitive to 1% Triton X-100, but the cells became sensitive to the detergent after drying, and this change was prevented by addition of ED to the BSM. Analysis of lipopolysaccharide (LPS) and surface layer protein by deoxycholate-polyacrylamide gel electrophoresis (DOC-PAGE) revealed an apparent decrease in the amount of LPS in the cells dried without ED, whereas those dried with ED showed little decrease. The protective action of ED was reduced by addition of 100-300 mM NaCl to the suspending medium containing 30 mM ED and abolished completely by addition of 1 M NaCl. These results suggest that ED binds to the outer membrane and stabilizes the cell membrane, in the same way that magnesium ions have bridging and stabilizing effects on the cell membrane. The stabilized membrane can prevent damage from occurring during desiccation.

Long-term preservation of halophilic archaebacteria and thermoacidophilic archaebacteria by liquid drying

T. Sakane, I. Fukuda[1)], T. Itoh[1)] and A. Yokota

J. Microbiol. Methods 16: 281-287 (1992)

A liquid drying (L-drying) method was applied satisfactorily to the long-term preservation of extremely halophilic archaebacteria and thermoacidophilic archaebacteria that are sensitive to freezing and freeze-drying. Survival values of Halobacterium, Haloferax, Haloarcula, Halococcus, Natronobacterium, Thermoplasma, Sulfolobus and Acidianus were

more than 5% after drying and more than 0.04% after being stored at 37 C for 2 weeks. The survival values of these dried specimens after long-term preservation at 5 C corresponded to those estimated from the results of the accelerated storage test. Therefore, it is considered that the accelerated storage test is useful for estimating the stability of the dried specimens of archaebacteria during preservation and that the dried specimens of even the most fragile bacterium, Thermoplasma, will remain viable for more than 15 years when they are kept at 5 C.

1) College of Liberal Arts and Science, Kitasato University

Taxonomic significance of arabinose in the family Pseudonocardiaceae

Mariko Takeuchi, T. Nishii and A. Yokota

Actinomycetol. 6: 79-90 (1992)

Sugar composition of whole cells and cell wall preparations of the family Pseudonocardiaceae, mycolate-less wall chemotype IV actinomycetes, was examined to clarify the taxonomic significance of cell-wall sugars. Among the strains of the genera examined, cell wall types of Actinopolyspora, Saccharopolyspora and Amycolata were confirmed to be chemotype IV/A, however, those of Pseudonocardia, Amycolatopsis, Saccharomonospora, Psedoamycolata, Actinokineospora and Kibdelosporangium were found to be III/A, and that of Saccharothrix to be III/A or III/C. Thus, the family Pseudonocardiaceae, which was proposed for the closely related genera on the basis of 16S rRNA sequencing studies, was indicated to include organisms with wall chemotypes III and IV, and the description of the family should therefore be emended.

Purification, properties, and partial amino acid sequences of thermostable xylanases from Streptomyces thermoviolaceus OPC-520

H. Tsujibo[1], K. Miyamoto[1], T. Kuda[1], K. Minami[1], T. Sakamoto[1], Toru Hasegawa and Y. Inamori[1]

Appl. Environ. Microbiol. 58: 371-375 (1992)

Two types of xylanases (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8)

were isolated from the culture filtrate of a thermophilic actinomycete, Streptomyces thermoviolaceus OPC-520. The enzymes (STX-I and STX-II) were purified by chromatography with DEAE-Toyopearl 650M, CM-Toyopearl 650M, Sephadex G-75, Phenyl-Toyopearl 650M, and Mono Q HR. The purified enzymes showed single bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The molecular weights of STX-I and STX-II were 54,000 and 33,000, respectively. The pIs were 4.2 (STX-I) and 8.0 (STX-II). The optimum pH levels for the activity of STX-I and STX-II were pH 7.0. The optimum temperature for the activity of STX-I was 70 C, and that for the activity of STX-II was 60 C. The enzymes were completely inhibited by N-bromosuccinimide. The enzymes degraded xylan, producing xylose and xylobiose as the predominant products, indicating that they were endoxylanases. STX-I showed high sequence homology with the exoglucanase from Cellulomonas fimi (47% homology), and STX-II showed high sequence homology with the xylanase from Bacillus pumilus (46% homology).

1) Osaka University of Pharmaceutical Sciences.

Purification and properties of two types of chitinases produced by an alkalophilic actinomycetes

H. Tsujibo[1], Y. Yoshida[1], K. Miyamoto[1], Toru Hasegawa and Y. Inamori[1]

Biosci. Biotech. Biochem. 56: 1304-1305 (1992)

Two types of chitinases (EC 3.2.1.14) were isolated from the culture filtrate of an alkalophilic actinomycete, Nocardiopsis dassonvillei subsp. prasina OPC-131. The enzymes (chi-A and chi-B) were purified by the successive chromatographies of DEAE-Sepharose CL-6B, Sephadex G-75 and FPLC Q Sepharose. The purified enzymes showed a single band on SDS-PAGE and the molecular weights of both enzymes were estimated to be 35,200. The pIs were 4.6 (chi-A) and 4.7 (chi-B). The optimum pH of chi-A was pH 5.0, and that of chi-B was pH 7.0. The optimum temperature of chi-A and -B was 60 C. The most characteristic properties of the enzymes indicated that the enzyme activities remained considerably in the alkaline pH range. In particular, even at pH 10 chi-B showed 50% of the activity at optimum pH. Chi-A and -B were stable in the range of pH 6-10 up to 50 C for 30 min.

1) Osaka University of Pharmaceutical Sciences.

The phylogenetic relationships of the Q9-equipped, spheroidal ascospore-forming Pichia species based on the partial sequences of 18S and 26S ribosomal RNAs

Y. Yamada[1], K. Maeda[1] and I. Banno

J. Gen. Appl. Microbiol. 38: 247-252 (1992)

Seven strains of Pichia abadieae, P. carsonii, P. etchellsii, P. humboldtii, and Candida ingens were examined for partial base sequences in positions 493-622 (130 bases) and positions 1611-1835 (225 bases) of 26S rRNA and in position 1451-1618 (168 bases) of 18S rRNA. These three partial base sequencings indicated that P. abadieae and P. humboldtii (and C. ingens) are phylogenetically distant from P. carsonii and P. etchellsii. In contrast, the latter two species, P. carsonii and P. etchellsii had very close relationships to Debaryomyces species. Some discussions were made, especially on transferring the two Pichia species to the genus Debaryomyces.

1) Department of Agricultural Chemistry, Shizuoka University.

The phylogenetic relationships of the genus Sporopschydermia Rodrigues de Miranda (Sacharomycetaceae) based on the partial sequences of 18S and 26S ribosomal RNAs

Y. Yamada[1], K. Maeda[1], T. Nagahama[1], I. Banno, and M.-A. Lachance[2]

J. Gen. Appl. Microbiol. 38: 179-183 (1992)

The primary partial base sequences of 18S and 26S rRNAs of Sporopachydermia cereana, S. lactativora and S. quercuum were determined. A dendrogram was drawn by the simple linkage method based on base differences of partial base sequences of 18S rRNA among the three species of Sporopachydermia along with those of the Co-Q9 equipped species of ascomycetous yeast genera. The three species of Sporopachydermia constituted their own cluster with 2 base difference and linked to species of the other genera at 7 base difference.

1) Department of Agricultural Chemistry, Shizuoka University.
2) Department of Plant Sciences, University of Western Ontario.

PRESENTATION OF PAPERS AT SCIENTIFIC MEETINGS 1991 - 1992

The Japanese Tissue Culture Association (March, 1991, Sagamihara)

H. Mizusawa[1], Masao Takeuchi, T. Kudo[2] and T. Ohno[3]
Construction of data base for cultured cell lines available in Japanese cell banks.
1) National Institute of Hygienic Sciences.
2) Tohoku University.
3) Riken Gene Bank.

K. Takeuchi[1], M. Satoh, M. Ogura[2], H. Saito[3] and Masao Takeuchi
Induction of platelet-like particles by a human megakaryoblastic leukemia cell line (MEG-01).
1) Ehime College of Health Science.
2) Aichi Cancer Hospital.
3) Nagoya University School of Medicine.

Japanese Society for Bacteriology (March, 1991, Osaka)

Y. Kaneko
Application of chromosome-banding pattern by pulsed-field gel electrophoresis to yeast taxonomy and identification.

Japan Society for Bioscience, Biotechnology, and Agrochemistry (March-April, 1991, Kyoto)

T. Sakane and A. Yokota
Rapid method for lipopolysaccharide analysis with deoxycholate-polyacrylamide gel electrophoresis and PAS stain.

Mariko Takeuchi, F. Kawai[1], Y. Shimada[1] and A. Yokota
Taxonomic study of polyethylene glycol-utilizing bacteria.
1) Department of Biology, Kobe University of Commerce.

VAAM-DGHM Spring Meeting (April, 1991, Freiburg, Germany)

S. A. Campos-Portugeuz[1], A. Yokota and H. Mayer[1]
Composition of the O-chain of lipopolysaccharide of Thiobacillus ferrooxidans.

1) Max-Planck-Institut für Immunbiologie, Freiburg, Germany.
VAAM: Vereinigung für Allgemeine und Angewandte Mikrobiologie
DGHM: Deutche Gesellschaft für Hygiene und Mikrobiologie

Mycological Society of Japan (May, 1991, Chiba)

T. Ito
Change of fungal flora in field soils after bonfire (I); Total number of fungi and thermophilic/thermotolerant fungi.

K. Mikata, Y. Yamada and I. Banno
A new ascosporogenous yeast species: Saccharomyces yakushimaensis sp. nov.

A. Nakagiri
A new Trinacrium-like aquatic hyphomycete with predacity for nematodes.

World Congress on Cell and Tissue Culture (June, 1991, Anaheim, USA)

Masao Takeuchi, M. Satoh, M. Ogura[1], H. Saito[2] and K. Takeuchi[3]
Production of platelet-like particles by human megakaryoblastic leukemia cell line (MEG-01).
1) Aichi Cancer Hospital.
2) Nagoya University School of Medicine.
3) Ehime College of Health Science.

Annual Meeting of the Society for Actinomycetes Japan (July, 1991, Tokyo)

N. Nakagaito, A. Yokota and Toru Hasegawa
Two new species of the genus Kitasatosporia, Kitasatosporia paracochleata sp. nov. and Kitasatosporia azatica sp. nov.

Yeast Genetics Society of Japan (August, 1991, Hachioji)

Y. Kaneko and I. Banno
Cloning of actin gene in Saccharomyces bayanus.

Japan Federation for Culture Collections (July, 1991, Chiba)

T. Nishii and A. Nakagiri

Liquid nitrogen storage of oomycetous fungi: examination of cooling rates and improvement of the freezing tube case.

T. Sakane, I. Fukuda[1)], T. Ito[1)] and A. Yokota

Preservation of thermoacidophilic archaebacteria by L-drying.

1) College of Liberal Arts and Science, Kitasato University.

Masao Takeuchi, T. Yoshida and M. Satoh

Activities of IFO animal cell bank.

Annual Meeting on Microbial Chemotaxonomy (November, 1991, Atami)

Mariko Takeuchi and A. Yokota

Taxonomic significance of cell-wall sugar composition in coryneform bacteria.

Japan Society for Cell Biology (November, 1991, Fukuoka)

T. Yoshida and Masao Takeuchi

Expression of fibronectin and laminin by different types of mouse glial cells cultured in a serum-free medium.

Society of Fermentation Technology, Japan (November, 1991, Hiroshima)

A. Yokota and Mariko Takeuchi

Proposals of two new species of the genus Microbacterium: Microbacterium dextranolyticum sp. nov. and Microbacterium aurum. sp. nov.

Regular Colloquia of the Society for Actinomycetes Japan (December, 1991, Osaka)

A. Yokota

Significance of sugars in chemotaxonomy of actinomycetes.

Japan Society for Bioscience, Biotechnology, and Agrochemistry (March-April, 1992, Tokyo)

Y. Murooka[1], Y. Xin[1], T. Morinaga[1] and A. Yokota
Characterization and identification of root nodule-forming bacteria of Astragalus sinicus.
1) Department of Fermentation Technology, Hiroshima University

Mariko Takeuchi, T. Nishii and A. Yokota
Taxonomic significance of arabinose in the family Pseudonocardiaceae.

T. Tamura, T. Nishii, A. Yokota and Toru Hasegawa
A new aerobic, Gram-positive, motile coccus. I.

Y. Yamada[1], K. Maeda[1], H. Nagahama[1], and I. Banno
The molecular phylogeny of genera Torulaspora and Zygosaccharomyces based on the partial base-sequence of 18S and 26S ribosomal RNAs.
1) Department of Agricultural Chemistry, Shizuoka University.

A. Yokota, Mariko Takeuchi, T. Sakane and K. Komagata[1]
Taxonomic studies of strains of the genus Aureobacterium: Proposals of 4 new species and a new combination.
1) Tokyo University of Agriculture

Japanese Society for Research of Freezing and Drying (April, 1992, Tokyo)

T. Sakane and A. Yokota
Role of ethylendiamine dihydrochloride in the protection of cell membrane of Aquaspirillum metamorphum subjected to L-drying.

Phytopathological Society of Japan (May, 1992, Morioka)

H. Nasu[1], A. Nakagiri and M. Hatamoto[1]
Pathogen of fruit stain of pear in Okayama Prefecture.
1) Okayama Prefectural Agricultural Experiment Station.

H. Sawada[1], H. Oyaizu[2], Mariko Takeuchi, A. Yokota and S. Matsumoto[1]

Phylogenetic relationships of the genera Agrobacterium and Rhizobium.

1) Akitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries.

2) Faculty of Agriculture, The University of Tokyo.

The 10th Yeast Symposia Japan (May, 1992, Kyoto)

I. Banno

Thinking on Phylogeny of Yeasts.

Japan Federation for Culture Collections (June, 1992, Wako)

T. Ito

Cryopreservation (-80 C) of filamentous fungi and their transport after defrosting.

K. Mikata, Y, Yamada and I. Banno[1]

Electrophoretic karyotypes of Torulaspora and Zygosaccharomyces yeasts.

1) Settsu Oil Mill, Ltd.

T. Sakane and A. Yokota

Preservation of cyanobactria by L-drying.

Y. Yamada, I. Banno[1] and K. Mikata

Re-identification of strains of genus Saccharomyces maintained in IFO.

1) Settsu Oil Mill, Ltd.

Conference on Taxonomy and Automated Identification of Bacteria (July, 1992, Prague, Czechoslovakia)

A. Yokota, Mariko Takeuchi and T. Sakane

New species of the genus Aureobacterium with murein types B2α and B2α'.

A. Yokota, T. Tamura, T. Nishii and Toru Hasegawa
Kineococcus aurantiacus gen. nov., sp. nov., a new Gram-positive, aerobic, motile coccus with meso-diaminopimelic acid and arabinogalactan in the cell wall.

Annual Meeting of the Society for Actinomycetes Japan (July, 1992, Kurashiki)

Y. Nakagaito, A. Yokota and Toru Hasegawa
Reidentification of Micropolyspora and Kitasatosporia strains in the IFO culture collection.

E. Higashide[1)], M. Kanamori[1)], H. Tsuji[1)], S. Nihongi[1)], T. Oomi[1)], Y. Kimura[1)] and A. Yokota
Microbial properties on actinomycetes from sea weeds.
1) Department of Agriculture, Okayama University.

Summer Meeting of the Phytopathological Society of Japan (July, 1992, Chiba)

H. Sawada[1)], H. Oyaizu[2)], Mariko Takeuchi, A. Yokota and H. Ieki[1)]
Taxonomic study of crown-gall bacteria isolated from kiwifruit and cherry.
1) Akitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries.
2) Faculty of Agriculture, The University of Tokyo.

UNESCO Regional Training Workshop (August, 1992, Taejon, Korea)

Toru Hasegawa
Source of bioactive secondary metabolites other than Streptmyces.

Yeast Genetics Society of Japan (August, 1992, Kyoto)

Y. Kaneko, I. Banno and Masao Takeuchi
Microevolution of Saccharomyces bayanus: Comparison of actin gene.

Kobokenkyukai (August, 1992, Nagaokakyo)

Y. Kaneko
A specific relationship among Saccharomyces cerevisiae complex.

Mycological Society of Japan (September, 1992, Beppu)

T. Ito

Change of fungal flora in field soils after bonfire (II); Fungal species detected by three isolation methods.

Y. Yamada[1], K. Maeda[1], I. Banno and J. P. van der Walt[2]

Molecular phylogeny of Pichia equipped with Q-9 and round ascospores based on partial sequences of 18S and 26S rRNAs.

1) Department of Agricultural Chemistry, Shizuoka University.
2) University of the Orange Free State, Bloemfontein, South Africa.

A. Nakagiri

Growth and reproduction of Halophytophthora species.

Annual Meeting on Plant Microbiology (September, 1992, Tokyo)

H. Sawada[1], H. Oyaizu[2], Mariko Takeuchi, A. Yokota and H. Ieki[1]

Taxonomic study of genus Agrobacterium.

1) Akitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries.
2) Faculty of Agriculture, The University of Tokyo.

Symposium "Microbial Diversity" (October, 1992, Tokyo)

Y. Kaneko

Species in fungi: Microevolution of Saccharomyces cerevisiae complex.

The 7th International Congress of Culture Collections (October, 1992, Beijing, China)

S. Arai[1], R. Harasawa[2], T. Ohno[3], Masao Takeuchi, K. Hikizi[4], S. Hanai[5], N. Kobayashi[6], T. Uemori[7], K. Takagi[8], I.-E. Lee[9], K. Kato[10] and Japan Bioindustry Association

Japan Bioindustry Association (JBA) is validating standard methods for the detection of mycoplasmas.

1) Kurume University
2) The University of Tokyo
3) Riken Cell Bank
4) SRL Inc.

5) Kyowa Medex Co. Ltd.
6) Dainippon Pharmaceutical Co. Ltd.
7) Takara Shuzo Co. Ltd.
8) Chugai Pharmaceutical Co. Ltd.
9) Nihon Pall Ltd.
10) Wakunaga Pharmaceutical Co. Ltd.

Toru Hasegawa
The culture collection at the Institute for Fermentation, Osaka (IFO).

T. Ito
Cryopreservation (-80 C) of fungal cultures and their transport after defrosting.

K. Mikata and I. Banno
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CORRECTIONS

In the issue of IFO Research Communications No. 15, the following corrections should be made.

Page	Line	Type	Should read
101	Table 2 (C)	C. pseudopulchella	C. pseudopulchella
		1-2	1-2
		96	80-95
		0-3	4-18
113	34-36	Medium No. 13	Medium No. 12

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