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REPORT OF THE DIRECTOR

The importance of the roles placed by culture collections has gained increasing recognition in science and technology. To support a broad range of disciplines in the biotechnological sciences and to keep cultures as living assets for the next generation. We are promoting international and national cooperation among culture collections and other organizations. We are proud of our experience at the Institute, which has been singled out as one of the world's major culture collections.

From 1989 to 1990, the Institute underwent great changes. At the Annual Meeting of Councilor of IFO, May 1989, Councilors Teruhiko Beppu and Yoshiro Okami were nominated as members of the Board of Trustees. At the same time Dr. Shinsaku Hayashida, Kyushu University, was nominated as a new Councilor. Mr. Ryohei Kisaki, auditor of IFO, was appointed by the Cabinet Council as Associate Justice of the Supreme Court on September 4, 1990, and he was therefore replaced as auditor at the Meeting of Councilors on September 7, 1990, by Dr. Takezi Hasegawa, a member of the Board of Trustees. At the same time, Dr. Tōru Hasegawa, was nominated as a new member of the Board of Trustees.

Changes in the research staff as follows: Dr. T. Yokoyama resigned from the Institute in January 1990. His dedication for 22 years in the mycology section in IFO was much appreciated. Dr. T. Iijima retired from the position of director after 14 years of service in April 1990, and was succeeded by Dr. Tōru Hasegawa. Dr. I. Banno was raised to the depty director of IFO in October 1990. Mr. Y. Kaneko received a doctorate from Osaka University in September 1990.

IFO Research Communications No. 14 was published in March 1989. The total number of cultures preserved in the IFO culture collection reached 13,500 at the end of 1989 and 13,656 at the end of 1990. The newly accepted strains during these two years will be listed in IFO Research Communications No. 15, which will be issued in March 1991. The total number of cultures distributed from the IFO culture collection reached 10,788 in 1989 and 9,507 in 1990.

Given the increase in the number of accessions and in distribution

of strains, new data processing facilities were urgently required for rapid and efficient administration of these activities. An IBM business computer system (IBM 36) was introduced in April 1990, and all of the data in IBM 23 were transferred to the new system. From August 1990, all documentation of acceptances and distributions was done using the new computer. Moreover, the microorganism database system, CD-STRAINS, by Hitachi Software Engineering Co., Ltd., and the system has been on the market since August 1990.

In October 1989 and September 1990, the Committee for Confirmation of ISP (International Streptomyces Project) strains in Japan conducted regular confirmatory tests of ISP strains stored at IFO. L-dried specimens, which were prepared for distribution to customers, were tested for viability, taxonomic characteristics and authenticity of the strains. The tested specimens were confirmed to be satisfactory.

Members of the Institute attended several international meetings: Dr. I. Banno attended the Fourth International Symposium of the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Tokyo, February 1989, and presented a paper. Dr. Tōru Hasegawa attended the First International Marine Biotechnology Conference in Tokyo, September 1989. The Fifth International Symposium on Microbial Ecology (ISME 5) was held in Kyoto, August to September 1989. Drs. A. Yokota and A. Nakagiri attended the Symposium and Dr. A. Nakagiri presented a paper. After that, Dr. A. Nakagiri attended the Post-Fifth-ISME Mycology Symposium in Sugadaira, Nagano Prefecture, in September 1989. Dr. Masao Takeuchi attended the Tenth Meeting of the European Society for Animal Cell Technology in Avignon, France, May 1990. After the meeting, he was able to visit the PHLS Center for Applied Microbiology and Research in Salisbury, U.K., see the facilities of the famous animal cell culture collection, and exchange information on matters of mutual interest. Dr. A. Nakagiri attended the Fifth International Symposium of the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Tokyo, March 1990, and presented a paper. In September 1990, the IUMS Congress: Bacteriology and Mycology - Osaka, Japan (IUMS-C - Osaka'90) and the Pre- and Post-congress meetings were held in Osaka. Drs. T. Iijima, T. Hasegawa, I. Banno, K. Imai, A. Yokota, Y. Kaneko, Mr. T. Ito and Ms. Mariko Takeuchi attended the Congress or the meetings. During the IUMS-C - Osaka'90, the WFCC meeting on "100 Years of Culture Collec-

tions" was organized by Drs. T. Iijima and L. Sly (UQM, Australia). Mr. T. Ito also joined the foray of IUMS-C - Osaka'90 in Hachimantai, Iwate Prefecture, in September 1990. Dr. A. Nakagiri received a Grant-in-Aid for Encouragement of Young Scientists for fiscal 1989, and studied marine mastigomycetes.

Lectures and seminars were given by the following guest speakers in the past two years.

Dr. D. Smith, CAB International Mycological Institute, Kew, U.K.: Preservation and services in CMI.

Dr. R. J. Bandoni, University of British Columbia, Vancouver, Canada: Mycoporasitism and mating in Tremellaceous fungi.

Dr. P. Hoffmann, Deutsche Sammulung von Mikroorganismen, Braunschweig, FRG: Methods for long-term storage of fungus cultures.

Dr. G. Okada, Japan Collection of Microorganisms, RIKEN: Recent development of systematics of fungi imperfecti and proposal of Ballistosporomyces gen. nov., a ballistospore-forming yeast genus.

Dr. K. Ando, Kyowa Hakko Kogyo Co., Ltd.: Cultivation of obligate parasitic fungi on artificial media.

Dr. P. Jackman, AFRC Institute of Food Research, Norwich, U.K.: Current developments at the UK National Collection of Yeast Cultures.

As a cooperative activity, Dr. Tōru Hasegawa visited the Food Industry Research and Development Institute (FIRDI) in Hsinchu, Taiwan, December 1989, undertook analyses of chemotaxonomic markers of actinomycetes, and gave lectures about new actinomycete taxa and chemotaxonomic criteria of actinomycetes. He spent 10 days in Taiwan, and visited several universities and organizations. Dr. I. Banno was invited to lecture on yeast taxonomy at the International Postgraduate University Course held annually in Osaka University. Dr. I. Banno visited FIRDI in November 1990, and demonstrated several techniques such as pulsed-field gel electrophoretic analysis and DNA homology on yeast taxonomy, and gave lectures.

IFO received guests researchers during this period: Mr. S. Matsumoto from Hikari Plant, Takeda Chemical Industries Co., Ltd.; Mr. Yong-Kook Shin from the Institute of Applied Microbiology, University of Tokyo; Dr. K. Takeuchi from Ehime College of Health Science; Mr. K. Arai from National Institute of Animal Health, Ministry of Agriculture, Forestry and Fishery; Ms. S. Artjariyasripong from Thailand Institute of Science

and Technological Research; Mr. M. Sakaguchi from Osaka University of Pharmaceutical Sciences; Mr. H. Maruyama from Seikagaku Kogyo Co., Ltd.; and Mr. S. Kayano and Ms. M. Konyo from Miki Trading Co., Ltd.

(Tōru Hasegawa)

Heartfelt condolence is extended to the bereaved of:

Professor Emeritus Hideo Kikkawa, who passed away on 4th October, 1990.

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

IFO Res. Comm. 15,
5-14, 1991 (March)

CHARACTERIZATION OF FASTIDIOUS STRAINS OF MYCOPLASMA HYORHINIS ISOLATED FROM CELL CULTURES

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

Summary

We isolated two strains of mycoplasmas from F111 and Lovo cell cultures. The two strains did not grow in a conventional culture medium for mycoplasmas and cross-reacted to anti-Mycoplasma hyorhinis (M. hyorhinis) serum specifically. From these results, the two strains (NC-1, NC-2) were identified as fastidious M. hyorhinis. They showed the following characteristics. 1) Like M. hyorhinis DBS1050, they required BHK-21 (C13) cell extract for growth. 2) Their total cell protein profiles and antigen profiles of cell membranes, as examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blots, respectively, were identical to those of M. hyorhinis DBS1050. They were also similar to those of M. hyorhinis BTS7, but showed the several differeces. 3) In their electrophoretic mobility, nucleoside phosphorylases of the two strains were similar to that of M. hyorhinis DBS1050, but different from

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that of M. hyorhinis BTS7. These results showed that the two newly isolated strains had the same characteristics as M. hyorhinis DBS1050 and several different biochemical characteristics from M. hyorhinis BTS7.

Keywords: Mycoplasma hyorhinis, cell culture.

Since the first report (8) of contamination of cell cultures by mycoplasmas, microbiological and serological procedures have shown a great number of cell cultures to be contaminated (7). In 1973 Hopps et al. (4) described a fastidious strain that fails to grow on a cell-free, conventional medium for mycoplasmas. This fastidious strain (DBS1050) was identified as Mycoplasma hyorhinis (M. hyorhinis) by serological tests. It was subsequently shown that fastidious strains represent a significant portion of cell culture isolates of M. hyorhinis. DelGiudice and Hopps (2) reported that 244 (61.9%) of 394 strains of M. hyorhinis isolated from cell cultures did not grow on conventional agar or broth media which support the growth of M. hyorhinis BTS7 (the type strain) and a wide variety of mycoplasma species. These fastidious strains of M. hyorhinis required techniques other than microbiological culture for efficient detection, such as DNA staining (1). DelGiudice et al. (3) have shown that inhibitors in yeast extract, a constituent of the conventional medium for mycoplasmas, are responsible for the lack of growth of M. hyorhinis DBS1050. They have also shown that BHK-21 cell extract supports growth of this strain in an appropriate medium. Further characterization of fastidious strains of M. hyorhinis possessing distinct growth characteristics has not, however, been pursued. This report shows the characteristics of fastidious M. hyorhinis isolated from cell cultures.

Materials and Methods

Mycoplasmas. Six strains of M. hyorhinis were examined: M. hyorhinis BTS7 (IFO 14858), M. hyorhinis DBS1050 and four strains isolated from

four cell cultures. These mycoplasmas were cultivated on the following culture media. I) A conventional agar medium for mycoplasmas contains Bacto PPLO agar medium (6 vol.), horse serum (2 vol.), fresh yeast extract (1 vol.) and supplements (1 vol.) (10). II) Macpherson broth medium and Macpherson agar medium were prepared as described by DelGiudice et al. (3). Macpherson broth medium consists of Ham's F12 medium (8 vol.), heat-inactivated fetal calf serum (1 vol.) and Bacto tryptose phosphate broth (1 vol.). Macpherson agar medium consists of twice-concentrated Ham's F12 medium (4 vol.), 2.5% Noble agar (4 vol.), heat-inactivated fetal calf serum (1 vol.) and Bacto tryptose phosphate broth (1 vol.). III) BHK-21 (C13) cell extract-supplemented Macpherson broth medium and Macpherson agar medium (3): these media consist of Macpherson broth medium (9 vol.) or Macpherson agar medium (9 vol.) and BHK-21 (C13) cell extract (1 vol.). BHK-21 (C13) cell extract was prepared as follows. BHK-21 (C13) cells in a flask (75 cm^2) at the confluent stage were harvested with 0.25% trypsin and suspended in 5 ml of Ham's F12 medium containing 5% fetal calf serum. This cell suspension was freeze-dried 7 times, then centrifuged at 1,500 g for 10 min. The supernatant was collected and passed through a 0.22 μm filter. The suspension obtained was used as BHK-21 (C13) cell extract.

For testing mycoplasmal growth, 0.1 ml of each mycoplasma specimen was inoculated onto 5 ml of each agar medium. After 14 days, colonies were counted. Mycoplasma specimens for testing biochemical characterization were cultivated in BHK-21 (C13) cell extract-supplemented Macpherson broth medium. The cell suspension was centrifuged at 1,500 g for 30 min and its supernatant was centrifuged again at 10,000 g for 30 min. The precipitates were washed twice with phosphate-buffered saline and examined for their biochemical characteristics. All cultures were incubated at 37 C in 5% CO_2-95% air.

Detection of mycoplasmal contamination of cell cultures. Mycoplasmal contamination of cell cultures was examined by a culture method and by DNA staining (10). For the culture method, supernatants of cell cultures were inoculated onto the conventional agar medium for mycoplasmas and it was observed whether fried-egg-like colonies formed. For DNA staining, supernatants of cell cultures were inoculated into an indicator cell culture (CKT-1 cells; IFO 50003) and incubated for 5 days. The indicator cells were stained with Hoechst 33258, which specifically binds

DNA, then observed extrachromosomal fluorescence was sought in cytoplasmic regions.

Anti-mycoplasma sera. Antisera against cell membranes of M. hyorhinis BTS7 were prepared by the method of Jeansson et al. (5). After disruption by hypotonic treatment and sonication of M. hyorhinis BTS7, which grew in the conventional broth medium for mycoplasmas, the specimen was centrifuged at 10,000 x g for 60 min. The precipitate was taken as the cell membrane fraction, which was used at a content of 0.6 mg of protein to raise antiserum in rabbit. Antisera against cell membranes of M. fermentans PG18 (IFO 14854), M. orale CH19299 (IFO 14477) and M. hominis PG21 (IFO 14850) were prepared by the same procedure.

Dot-blotting. Each mycoplasma specimen was identified by the dot-blotting method as described previously (11). The antisera used were anti-M. hyorhinis, anti-M. fermentans, anti-M. orale and anti-M. hominis.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Total proteins of each mycoplasma specimen were analyzed by the method of Laemmli et al. (6). SDS-PAGE was carried out using 10% polyacrylamide gel at a constant voltage (100 V) for 90 min. The gel was stained with silver stain kit (Bio-Rad Laboratories Inc., CA).

Western blots. Antigen profiles of cell membranes of mycoplasmas were analyzed by the method of Towbin et al. (9). A nitrocellulose filter was incubated first with the anti-M. hyorhinis serum (1:1,000 diluted), then with peroxidase-conjugated antibody against rabbit IgG (1:1,000 diluted, Cappel Laboratories, PA), and the bound antibody was visualized by use of the enzyme substrate, 4-chloro-1-naphtol.

Electrophoretic mobility of enzymes. The electrophoretic mobility of four enzymes, peptidase B, lactate dehydrogenase, phosphoglucose isomerase and nucleoside phosphorylase, was examined using an Authentikit system (Corning Science Products, MA). The migration distances of the four enzymes in each mycoplasma extract were determined after electrophoresis and appropriate staining.

Results

Isolation of mycoplasmas from cell cultures

Mycoplasmal contamination was detected in four cell cultures, i.e.,

Chimp Liver, Chang conjunctiva, F111 and Lovo, by DNA staining with CKT-1 as indicator cells (Table 1). In the case of Chimp Liver and Chang conjunctiva, the mycoplasmal contamination was also detected by the culture method, but not in the case of F111 and LoVo. The mycolasmas contaminating the four cell cultures were identified using the dot-blotting method. Each mycoplasma specimen cross-reacted with anti-M. hyorhinis serum and did not cross-react with anti-M. fermentans, anti-M. orale or anti-M. hominis. These four M. hyorhinis strains isolated from the cell cultures of Chimp Liver, Chang conjunctiva, F111 and LoVo were designated as CU-1, CU-2, NC-1 and NC-2, respectively.

Table 1. Strains of M. hyorhinis isolated from contaminated cell cultures.

Cell cultures	Detection method			Strains
	Culture	DNA staining	Dot-blotting	
Chimp Liver	+	+	M. hyorhinis	CU-1
Chang conjunctiva	+	+	M. hyorhinis	CU-2
F111	-	+	M. hyorhinis	NC-1
LoVo	-	+	M. hyorhinis	NC-2

+; detected -; not detected

Table 2. Growth of four M. hyorhinis strains on the three media.

Strains	No. colonies/plate (mean±SD)		
	Conventional	Macpherson	Macpherson (+cell extract)
BTS7	153±42	23±7	29±5
DBS1050	0	4±1	23±8
NC-1	0	2±1	18±7
NC-2	0	2±1	12±7

Growth characteristics of M. hyorhinis strains

Growth characteristics of NC-1 and NC-2 were compared with those of BTS7 (the type strain) and DBS1050 (a fastidious strain) using three culture media. The results are shown in Table 2. BTS7 formed colonies on the conventional agar medium. However, NC-1 and NC-2 did not grow on this agar medium as well as DBS1050. On Macpherson agar medium, NC-1,

NC-2 and DBS1050 formed small numbers of colonies, which increased significantly when the medium was supplemented with BHK-21 (C13) cell extract. From these growth characteristics, NC-1 and NC-2 were classified as fastidious strains of M. hyorhinis. These three fastidious strains of M. hyorhinis cultivated on the BHK-21 (C13) cell extract-supplemented Macpherson agar medium formed fried-egg-like colonies which showed similar morphology to the colonies of BTS7 (Fig. 1).

Profiles of total cell proteins and antigens in cell membranes

Total cell protein profiles of the three fastidious strains (NC-1, NC-2, DBS1050) on SDS-PAGE were compared with those of BTS7, CU-1 and CU-2. Although the silver-stained patterns of these six strains specimens were similar, there were three differences (Fig. 2). Two bands (19 kDa, 39 kDa) present in the three fastidious strains were absent in the three other strains; and one band (73 kDa) present in BTS7, CU-1 and CU-2 was absent in the three fastidious strains.

Western blots were performed to analyze the antigen profiles of each strain against anti-M. hyorhinis serum, which was obtained by immunization using the cell membrane fraction of BTS7 specimen. As shown in Fig. 3, the patterns of antigens in cell membrane fractions of the three fastidious strains (DBS1050, NC-1, NC-2) were essentially similar to those of the other three strains (BTS7, CU-1, CU-2). However, several bands (48-55 Kd) present in strains BTS7, CU-1, and CU-2 were absent in the three fastidious strains.

Electrophoretic mobility of enzymes

The migration distances of four enzymes in four strains (NC-1, NC-2, DBS1050, BTS7) after the electrophoresis were measured. Table 3 shows the migration distance of each enzyme relative to that of the respective L929. The mobility of nucleoside phosphorylase in the three fastidious strains (NC-1, NC-2, DBS1050) were significantly different from that of BTS7. The relative migration distances of other three enzymes examined were essentially identical in the four strains.

DISCUSSION

In this report, we isolated two fastidious strains of M. hyorhinis from cell cultures and compared them with M. hyorhinis BTS7 and

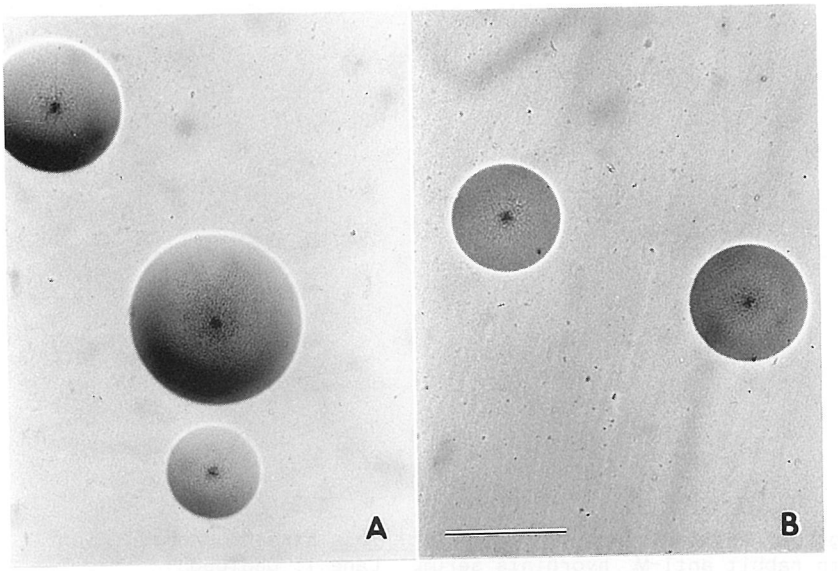

Fig. 1. Colonies of M. hyorhinis BTS7 (A) and NC-1 (B) on the surface of BHK-21 (C13) cell extract-supplemented Macpherson agar medium. The bar: 200 μm.

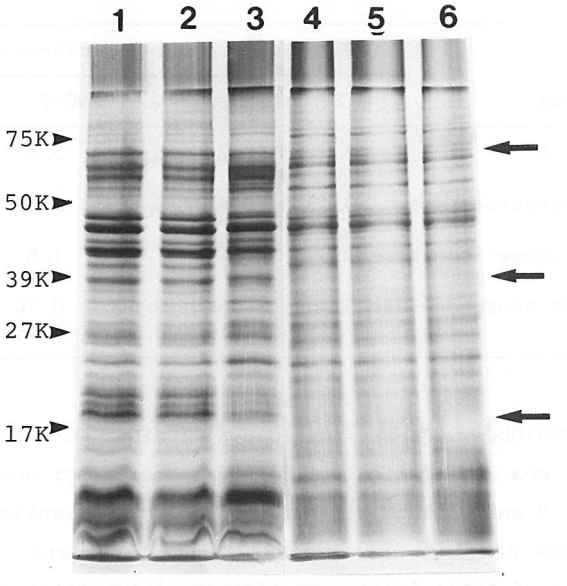

Fig. 2. SDS-PAGE protein profiles of six M. hyorhinis strains. Lane 1: DBS1050 Lane 2: NC-1 Lane 3: NC-2 Lane 4: CU-1 Lane 5: CU-2 Lane 6: BTS7.

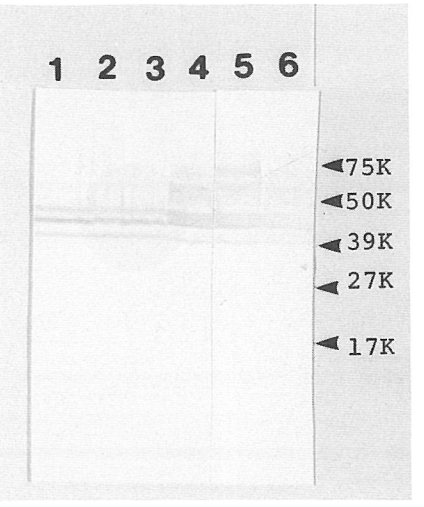

Fig. 3. Western blots of six M. hyorhinis strains probed with rabbit anti-M. hyorhinis serum. Lane 1: DBS1050, Lane 2: NC-1, Lane 3: NC-2, Lane 4: CU-1, Lane 5: CU-2, Lane 6: BTS7.

Table 3. Electrophoretic mobilities of four enzymes in four M. hyorhinis strains.

Enzymes	Relative migration distances				
	BTS7	DBS1050	NC-1	NC-2	L-929
Peptidase B	3.5	3.2	3.2	3.4	1
Lactate dehydrogenase	0.45	0.47	0.42	0.46	1
Phosphoglucose isomerase	1.5	1.7	1.5	1.6	1
Nucleoside phosphorylase	0.45	0.68 0.15	0.60 0.15	0.70 0.15	1 1

M. hyorhinis DBS1050. The two fastidious strains, NC-1 and NC-2, showed similar growth characteristics to DBS1050 and distinct properties from BTS7: NC-1, NC-2 and DBS1050 did not grow on the conventional agar medium and required BHK-21 (C13) cell extract for their growth. The biochemical characteristics of these fastidious strains, i.e., total protein profiles, antigens on cell membranes and electrophoretic mobility of the

enzymes, were essentially similar to those of BTS7, although several differences were found. The relationship between these biochemical differences and growth characteristics was unclear. DelGiudice et al. presumed that the fastidious strains may "turn off" essential metabolic systems which permit these organisms to grow on the cell-free, conventional broth and agar media for mycoplasmas (3). Furthermore, it is unclear whether these fastidious strains are particulary adapted to cell cultures or exist in their natural porcine hosts. Whatever the mechanisms may be, the phenomenon is obviously important as an in vitro model of fastidious mycoplasmas possibly involved in chronic infections of animals, humans and plants. Further characterization of biochemical differences between the fastidious strains and the strains which grow well in the conventional media for mycoplasmas is necessary.

Mycoplasmal contamination of cell cultures by fastidious M. hyorhinis presents serious problems for researchers. To detect efficiently the fastidious strains of M. hyorhinis in cell cultures, it is necessary to employ techniques other than culture on the conventional media for mycoplasmas. We confirmed that these fastidious strains can be detected when BHK-21 (C13) cell extract-supplemented Macpherson agar medium is employed for their cultivation. However, this medium is impractical for routine use, because the efficiency of BHK-21 (C13) cell extract in promoting growth of fastidious strains of M. hyorhinis may vary depending on its preparation. We are now searching for simpler, chemically defined media which are efficient for detecting the fastidious M. hyorhinis.

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IFO Res. Comm. 15,
15-21, 1991 (March)

EFFECT OF NGF-2 ON THE SURVIVAL OF CHICK SENSORY GANGLION NEURONS IN VITRO

MOTONOBU SATOH, YOSHIHIKO KAISHO* and MASAO TAKEUCHI

Summary

The biological activity of NGF-2, the third member of the NGF family, was investigated. Conditioned medium of COS cells transfected with expression plasmid for human NGF-2 cDNA promoted the survival of sensory neurons from dorsal root ganglia (DRG) and nodose ganglia (NG). Supernatant of mock transfected COS cells was less effective for the survival of DRG neurons and ineffective for the survival of NG neurons. These results suggest that NGF-2 is a novel neurotrophic factor whose biological activity is distinct from NGF.

Keywords: NGF-2, Neuronal survival.

In vertebrate neurogenesis, some neuronal populations require trophic factors from their target tissues for survival, and these target-derived neurotrophic factors are therefore thought to play an important role in establishing the correct neuron-target tissue connection (1, 4). Among them, nerve growth factor (NGF) has been most extensively studied and its survival-promoting effects on neurons of sympathetic and several sensory ganglia have been demonstrated (10, 13, 20). Brain-derived neurotrophic factor (BDNF) (3) has also been demonstrated to support the

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survival of several neuronal populations in peripheral nervous system (6, 7, 15). BDNF was cloned and its deduced amino acid sequence was homologous to NGF (12). Recently, Kaisho et al. (11) cloned a cDNA encoding a polypeptide homologous to NGF from a human glioma cDNA library and named this polypeptide NGF-2. NGF-2 mRNA is detected in several peripheral tissues and the central nevous system, and its expression is developmentally regulated (11), suggesting that NGF-2 is a biologically active peptide with a role in neuronal development. In this paper, we investigated the effect of NGF-2 on the survival of chick sensory neurons in vitro.

Materials and Methods

Establishment of NGF-2 producing cells. To obtain native NGF-2 peptide, we introduced expression vector for NGF-2 cDNA into monkey kidney COS-7 cells. A 1.1 kb Eco RI fragment of human NGF-2 cDNA (11) was inserted downstream of the LTR region of Abelson murine leukemia virus and the SV40 promoter in the expression plasmid (16) to give pNTL145. COS-7 cells were transfected with the plasmid pNTL145 using the calcium phosphate coprecipitation technique (8). After 24 hr, the medium was replaced with Dulbecco's modified Eagle's medium (DMEM) containing 0.5 % fetal calf serum (FCS) and was collected at 72 hr.

Biological assays. Cultures of sensory neurons from dissociated 8-day chick dorsal root ganglia (DRG) and nodose ganglia (NG) were prepared as described by Barde et al. (2) with some modifications. Isolated ganglia were dissociated by trypsin treatment and preplated for 2 hr to remove non-neuronal cells. The resultant neuron-enriched cells were plated on poly-L-ornithine (10 μg/ml)-coated or poly-L-ornithine, laminin (10 μg/ml)-coated multiwell plates. Neurons were grown in a medium consisting of a 1:1 mixture of DMEM and Ham's F12 supplemented with 10% FCS. As an antimitotic agent to non-neuronal cells, citosine arabinoside (1 μM) was added to DRG cultures and 5'fluorodeoxyuridine (100 μM) plus uridine (100 μM) to NG cultures. Supernatant of COS-7 cells transfected with expression plasmid for NGF-2 (NGF-2(COS)) or with control plasmid pTB 389 (control(COS)) was added after dialysis of the culture medium. Beta NGF was purchesed from Boehringer Mannheim. Viable neurons were judged

from their round phase-bright soma and the presence of neurites at least twice as long as the cell diameter.

Results

Effect of NGF-2 on the survival of DRG neurons

A preliminary experiment showed that above 95 % of cells were immunoreactive to 70 kD neurofilament protein in NGF-supplemented DRG cultures, thus confirming the purity of neurons. All neurons died within 24 hr in the absence of trophic factors such as NGF. Addition of NGF-2 (COS) or control (COS) resulted in survival of neurons but neurite owtgrowth was poor in the latter case (Fig. 1). The number of viable neurons was counted on day 4. NGF-2 (COS) promoted the survival of DRG neurons in a dose-dependent manner (Fig. 2). Control (COS) also supported their survival, but less effectively (Fig. 2). This seems to be due to a small amount of NGF which is endogenously secreted from COS-7 cells.

Effect of NGF-2 on the survival of NG neurons

NG neurons were plated on poly-L-ornithine-laminin coated substrate and viable neurons were counted after 3 days (Fig. 3). NG neurons cultured with NGF-2 (COS) survived and developed neurites (Fig. 3, 4). Unlike DRG neurons, however, their survival was not promoted by control (COS). NGF also failed to support their survival.

Discussion

We have demonstrated that NGF-2 promotes the survival of embryonic chick DRG neurons and NG neurons *in vitro*. NGF is known to support the survival of a subpopulation of mainly neural crest-derived sensory neurons of young animals, including DRG neurons, *in vitro* and *in vivo* (9, 13). On the other hand, it does not affect the survival of placode-derived neurons such as NG neurons (5, 14, 15). These observations are also supported by the fact that NGF receptor is not detected in placodal neurons (17, 18, 19). Our results show that the biological activity of NGF-2 is distinct from NGF and suggest that the receptor of NGF-2 is different from that of NGF.

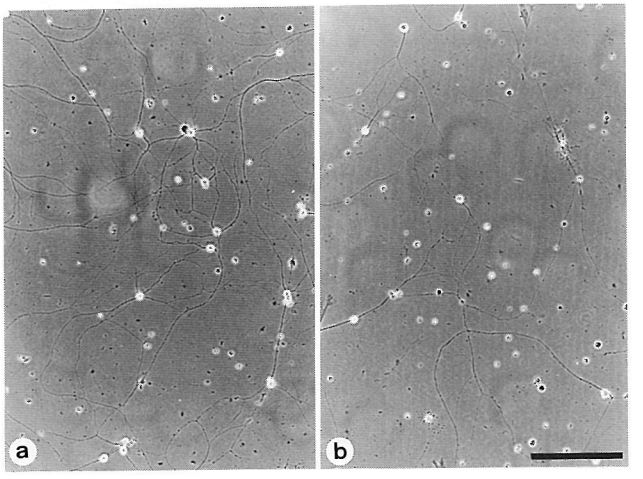

Fig. 1. Photomicrographs of DRG neurons cultured for 5 days in the presence of 5% NGF-2 (COS) (a), or control (COS) (b). Phase contrast. Bar, 250 μm.

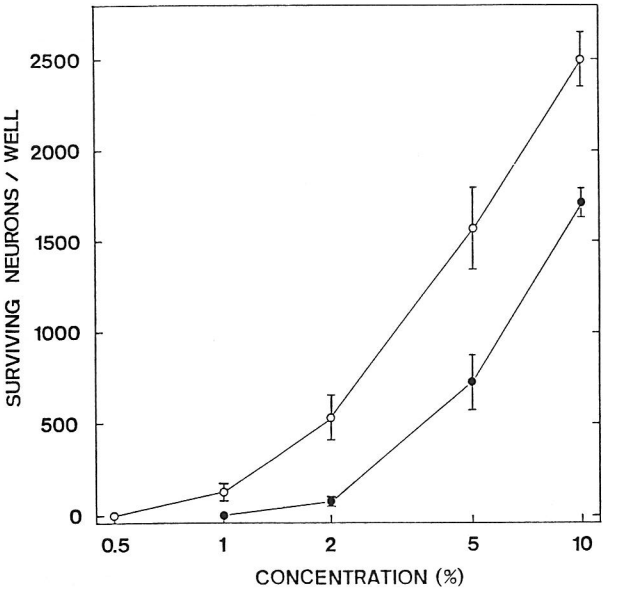

Fig. 2. Survival of DRG neurons in the presence of NGF-2 (COS) (o), or control (COS) (●). Poly-L-ornithine-coated 24-well plates were seeded with 10^4 neurons per well and scored after 4 days. Each point represents the mean number of surviving neurons per well from six determinants. Bar represents standard deviation.

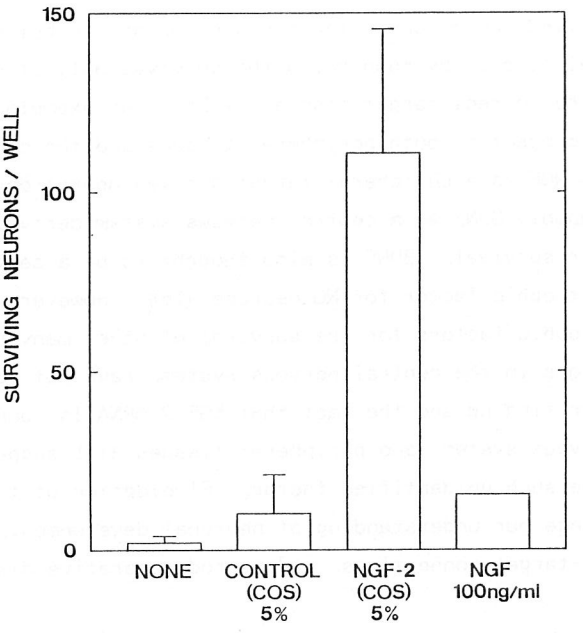

Fig. 3. Survival-promoting effect of NGF-2 on NG neurons. Poly-L-ornithine, laminin-coated 48-well plates were seeded with 10^4 neurons per well and scored after 3 days. Values represent the mean number of surviving neurons per well ± standard deviation.

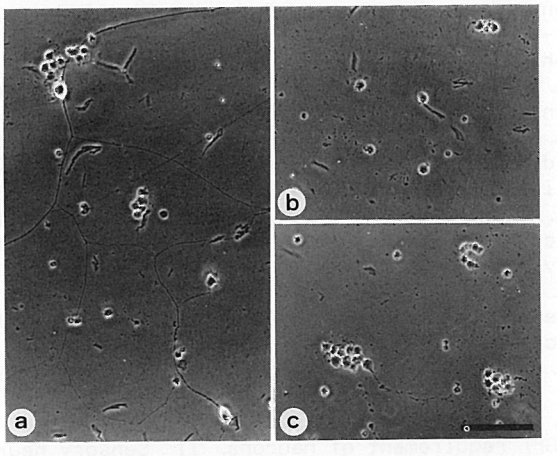

Fig. 4. Photomicrographs of NG neurons cultured for 3 days in the presence of 5% NGF-2 (COS) (a), control (COS) (b), or 100 ng/ml NGF (c). Phase contrast. Bar, 100 μm.

Target-derived neurotrophic factors are thought to build up a correct neuronal circuit by supporting the survival only of neurons connecting to the correct target tissues (1, 5). For example, DRG neurons, which project to both peripheral tissues and the central nervous system, require NGF as a peripheral target-derived neurotrophic factor (4, 10), and probably BDNF as a central nervous system-derived factor (1, 15) for their survival. BDNF is also thought to be a central nervous system-derived trophic factor for NG neurons (15). However, target-derived neurotrophic factors for the survival of other many neurons, especially neurons in the central nervous system, have not yet been identified. Our finding and the fact that NGF-2 mRNA is found in both the central nervous system and peripheral tissues (11) suggest that NGF-2 may be one such unidentified factor. Elucidation of the role of NGF-2 will advance our understanding of neuronal development, the maintenance of neuron-target connections, and neurodegenerative diseases.

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PROPERTIES OF A CELL LINE (MEG-01SSF) OF HUMAN MEGAKARYOBLASTIC LEUKEMIA CELLS

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HIDEHIKO SAITO, ** AND KIKUKO TAKEUCHI***

Summary

MEG-01SSF cells were prepared from MEG-01S by conditioning in a chemically defined, serum-free medium. The cells grew well in the medium and required transferrin for growth. Doubling time was about 20 hours. The cells retained GpIIb/IIIa as a marker of megakaryocytes and transferrin receptors on the cell surface. Some of the cells in the stationary phase of growth produced platelet-like particles, which bore characteristic microtubule rings.

Keywords: Human, Megakaryoblast, Platelet-like particle.

Megakaryoblastic cells differentiate to megakaryocytes in bone marrow and then begin to produce platelets. Many reports have described cell lines with megakaryoblastic characters (4), but few have dealt with the platelet-like particles produced by the cell lines (9, 10). MEG-01 cells have been used for research as a cell line having only megakaryo-

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blastic markers (8). Microtubules localized under the cell membrane of platelets are present in the form of a marginal ring and detection of such marginal rings by an immunological technique showed that MEG-01 cells produced platelet-like particles (13).

Various factors are thought to be required for the differentiation of megakaryoblasts and the production of platelets (3). To analyze the process of differentiation of megakaryoblastic cells, the cells cultivable in seum-free medium are desirable. This paper reports the cell-biological properties of MEG-01SSF cells conditioned for growth in a serum-free medium.

Materials and Methods

Cell culture. MEG-01S cells (9), were cultivated in high-glucose Dulbecco's modified E-MEM supplemented with transferrin, insulin, selenite and biotin (01-medium), as developed by Bottenshtein (7). MEG-01S cells were cultivated in the 01-medium for about 4 weeks, then conditioned with the medium. The cell line was named MEG-01SSF and was used through this experiment. Cell counts were carried out with a Coulter Counter. Human platelets were prepared from blood of a volunteer by centrifugation at 500 xg (8).

Immunofluorescence staining against GpIIb/IIIa or transferrin receptor. After subcultivating MEG-01SSF cells ($1x10^5$ ml^{-1}) for 3 days in a tissue culture multi-well plate, the cells were harvested and fixed in 3.7% paraformaldehyde at room temperature. The cells were put on a coverslip by centrifugation. Monoclonal antibodies against GpIIb/IIIa (clone HPL 1, Kyowa Medex Co., Ltd.) (2) or against transferrin receptor (NU-TfR1, Nichirei) were applied to the cells and the slips incubated for 1-2 hr at 37 C. After washing with phosphate-buffered saline, FITC-conjugated anti-mouse Ig sheep antibody (Cappel) was applied.

Immunofluorescence staining against α-tubulin. On day 7-8 after seeding MEG-01SSF cells ($1x10^5$ ml^{-1}) in a tissue culture multi-well plate, the cells were fixed in 3.7% paraformaldehyde at room temperature, put on a coverslip by cytospin, then treated with methanol at -20 C. Monoclonal antibody against α-tubulin (Amersham) was applied, and the slip was incubated for 1-2 hr at 37 C. After washing with phosphate-

buffered saline, Texas Red-conjugated anti-mouse Ig sheep antibody (Amersham) was applied. Immunofluorescence images were observed under an immnofluorescence microscope (Olympus, type BH2).

Results and Discussion

Growth of cells

MEG-01SSF cells were seeded in tissue culture multi-well plates and cultivated in various media. Their growth curves are shown in Fig. 1A and 1b. The cells grew at similar rates in the 01-medium and RPMI-1640 supplemented with 10% fetal bovine serum, but did not grow in RPMI-1640 without serum. Doubling time of the cell calculated from a growth curve indicated by closed circles in Fig. 1A was about 20 hr. Fig. 1B shows that the cells strongly required transferrin for growth. Transferrin is important for growth of many tumor cell lines (11). No difference was observed morphologically between MEG-01SSF and MEG-01S (data not shown).

Surface antigens

It was found that transferrin was bound specifically to the cell surface of MEG-01SSF, because all cells had trasferrin receptors on the surface as shown in Fig. 2A. MEG-01SSF cells also have been expressing GpIIb/IIIa on the surface (Fig. 2B), as descrived on MEG-01 (13). The intensity of fluorescence on the surface of cells was similar in cells cultivated in 01-medium and RPMI-1640 supplemented with 10% fetal bovine serum.

Immunological observation with anti-α-tubulin antibody

Many particles were observed in culture medium outside of MEG-01SSF cells about five days after subcultivation. To characterize the particles produced by MEG-01SSF, immunofluorescence tests were examined. Platelets have such a characteristic cytoskeleton, which is composed of microtubules forming a circumferential band just inside the cell membranes (1). To detect platelet-like particles, therefore, we used these microtubule bands as a marker of platelets. This structure is detected as a closed brightly fluorescent ring when probed with anti-tubulin antibody (12). We observed brightly stained rings around in culture fluid of MEG-01SSF cells (Fig. 3A-D). The rings were 2-5 μm in diameter and localized in the circumference of particles.

It has been reported that no significant difference exists between the particles released from MEG-01 and human blood platelets in the experimental results of double staining with antibodies against tubulin and platelet glycoproteins, GpIIb/IIIa (8). Also no significant difference was observed between the particles derived from MEG-01SSF cells and human blood platelets in their immunofluorescent staining images (Fig. 3E). These results suggest that these particles are platelet-like particles produced by MEG-01SSF cells.

The present experiment showed that MEG-01SSF produced platelet-like particles. This suggests that MEG-01SSF are already committed to differentiation into megakaryocytes (6), and that therefore no additional factors may be required for differentiation, or that MEG-01SSF cells produce differentiation-inducing factor(s) autonomously. CMK cells, which are a megakaryoblastic cell line, produce factors which are related to the differentiation of megakaryocytes, e.g., GM-CSF and IL-6 (5). It seems probable that MEG-01SSF cells also produce factors for differentiation in cell culture under physiological conditions. The MEG-01SSF cells are useful for study of differentiation of megakaryoblasts and the process of megakaryocytopoiesis in a serum-free medium.

We are deeply indebted to Dr. Toru Hasegawa (Institute for Fermentation, Osaka) for his continuing encouragement throughout this work. We also thank Mr. T. Yoshida for useful discussions.

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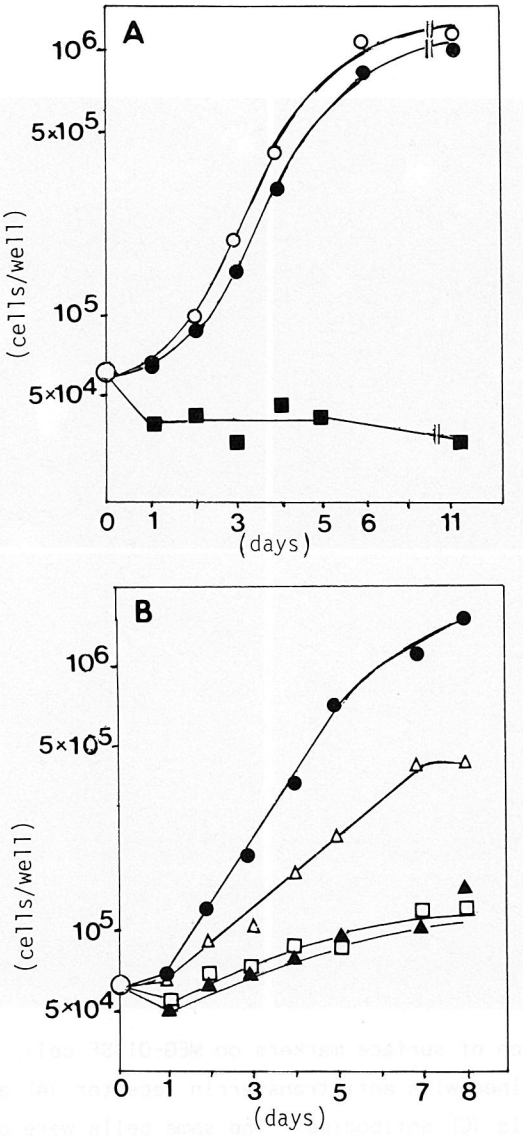

Fig. 1. Growth curve of MEG-01SSF. 6×10^5 cells were seeded in each well and cultivated in each medium. Symbols: ○, RPMI-1640 with 10% fetal bovine serum; ■, RPMI-1640 without serum; ●, 01-medium; △, 01-medium without insulin; □, 01-medium without insulin and transferrin; ▲, 01-medium without transferrin.

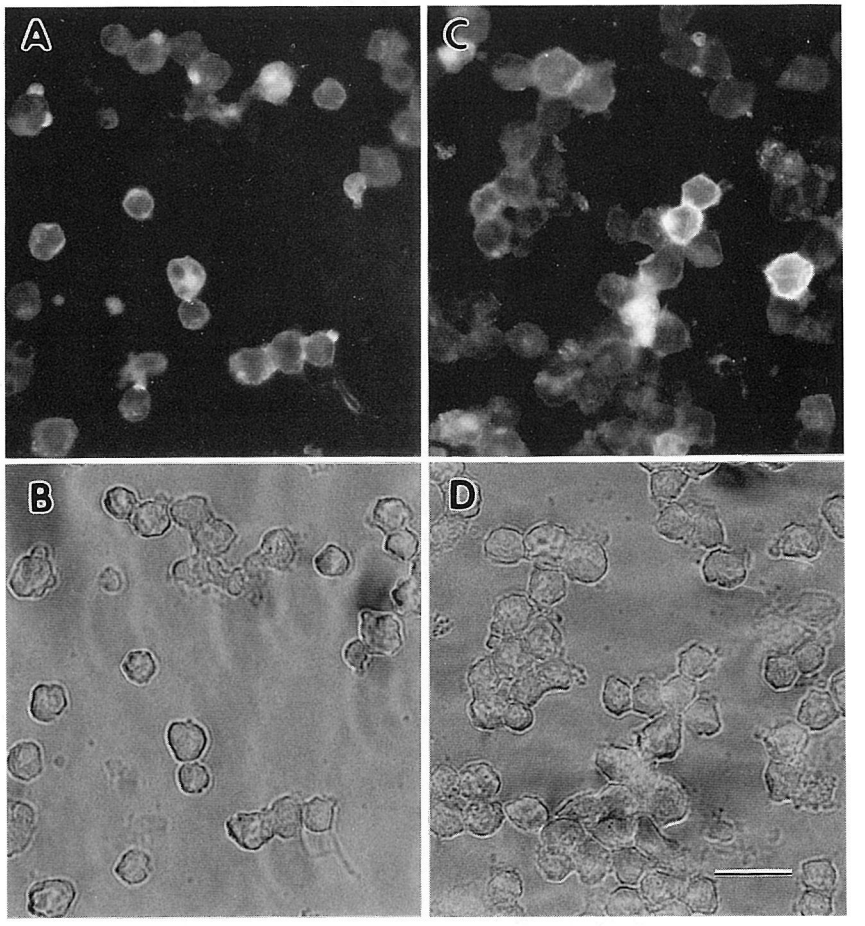

Fig. 2. Expression of surface markers on MEG-01SSF cell. MEG-01SSF cells were stained with anti-transferrin receptor (A) and anti-GpIIb/IIIa (C) antibodies. The same cells were observed by phase-contrast microscopy (B, D). Scale bar, 25 μm.

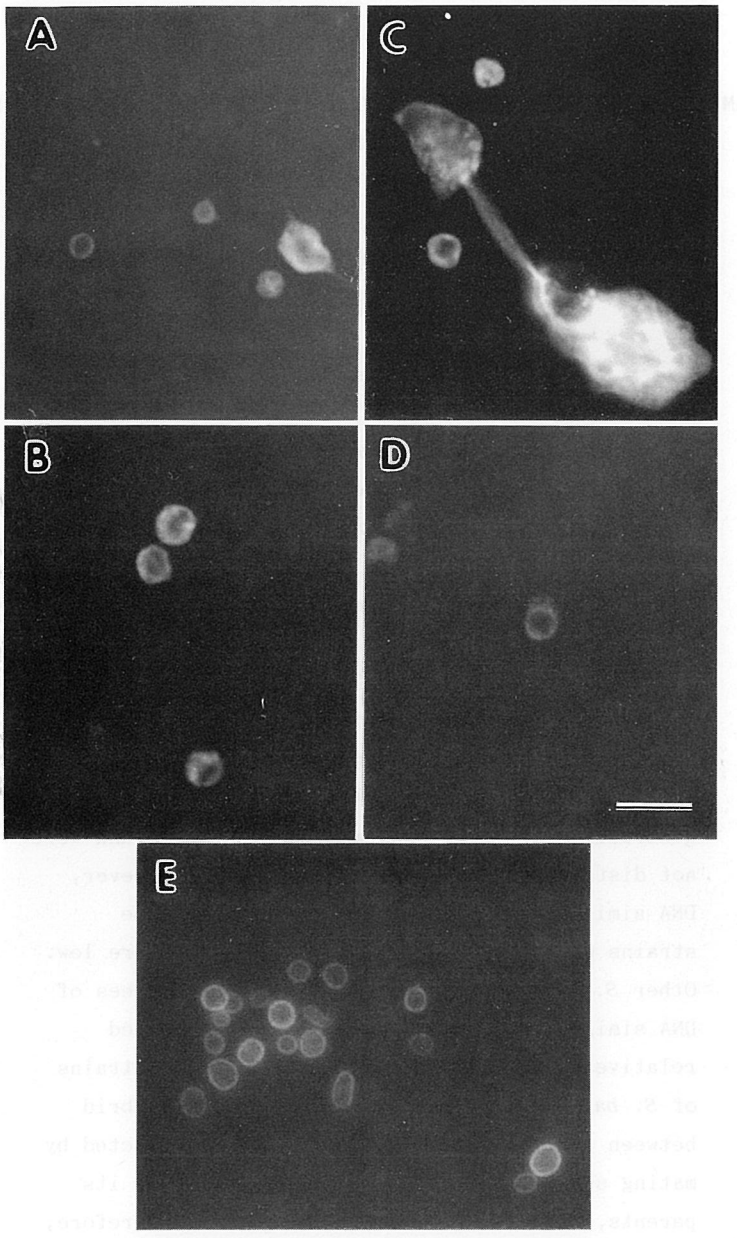

Fig. 3. Localization of microtubules in particles produced by MEG-01SSF. Particles (A-D) and human platelets (E) were stained with anti-α-tubulin antibody. Platelet-like particles can been seen in A-D (arrows). Scale bar, 10 μm.

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REEXAMINATION OF *SACCHAROMYCES BAYANUS* STRAINS BY DNA-DNA HYBRIDIZATION AND ELECTROPHORETIC KARYOTYPING

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Summary

Twelve strains of *Saccharomyces bayanus* preserved in our collection were investigated for their electrophoretic karyotype and their DNA similarity. While the type strain of *S. bayanus* (IFO 1127) gave 17 chromosomal bands in the same size range as *S. cerevisiae* by pulsed-field gel electrophoresis (PFG), its progenies lost 2 or 3 bands in the middle range. The disappearance of up to 3 chromosomal bands in the progenies suggests that IFO 1127 is a 2n+3 aneuploid strain. All *S. bayanus* strains gave generally similar chromosomal band patterns and were not distinguishable from *S. cerevisiae*. However, DNA similarity values of IFO 1127 to the type strains of *S. cerevisiae* and *S. paradoxus* were low. Other *S. bayanus* strains showed various degrees of DNA similarity. One of them, IFO 2027, showed relatively high DNA similarity to the type strains of *S. bayanus* and *S. cerevisiae*. Since a hybrid between *S. bayanus* and *S. cerevisiae* constructed by mating showed about 70% of DNA similarity to its parents, IFO 2027 seems to be a hybrid. Therefore, *S. bayanus* strains identified on the basis of fermentation of sugars are heterogeneous in DNA similarity.

Keywords: *Saccharomyces bayanus*, Electrophoretic karyotyping, DNA-DNA hybridization, DNA similarity.

In 1984, *Saccharomyces bayanus* and other *Saccharomyces* sensu stricto yeasts were taxonomically included in the same species, *Saccharomyces cerevisiae* (22). However, Vaughan Martini and her colleagues (19,20,21) found that at least four species exist in the new *S. cerevisiae* on the basis of DNA-DNA reassociation and proposed that *S. bayanus*, *S. paradoxus* and *S. pastorianus* should be revived. *S. pastorianus* is considered to be a hybrid of *S. bayanus* and *S. cerevisiae* (20). Genetic crosses among *S. bayanus*, *S. cerevisiae* and *S. paradoxus* were performed and it was found that their hybrids produced no viable ascospores (2,14). These findings support the proposal that *S. bayanus* and *S. paradoxus* should be classified separately from *S. cerevisiae*. Subsequently, it has become possible to analyze the chromosomal sets of yeast by pulsed-field gel electrophoresis (PFG) (3). The PFG karyotypes of various synonymous strains of *S. cerevisiae* have been examined by us and others (4,9). These studies indicate that there is no significant difference among synonymous strains of *S. cerevisiae*.

In this paper, we reexamined 12 *S. bayanus* strains preserved in our collection by DNA-DNA hybridization and electrophoretic karyotyping. PFG karyotype analysis of *S. bayanus* type strain (IFO 1127) suggests that IFO 1127 is a 2n+3 aneuploid strain and its chromosomal set is essentially the same as that of *S. cerevisiae*. Other *S. bayanus* strains showed PFG karyotypes which were explainable by chromosome length polymorphism of *S. cerevisiae*. However, DNA similarity study by microplate-hybridization indicated that the *S. bayanus* strains preserved in our collection were heterogeneous.

Materials and Methods

Strains and media. Strains used in this study are shown in Table 1. Nutrient (YPD) and sporulation media were described previously (10). Agar plates were prepared by adding 2% agar. Cultivation was carried out at 28 C.

Table 1. Yeast strains used in this study.

Strain[a]	Synonym	Source or other institute no.[b]
S. bayanus		
IFO 0206		
IFO 0261	*S. odessa*	CBS 422
IFO 0262	*S. oviformis*	CBS 429
IFO 0539	*S. pastorianus*	
IFO 0613	*S. pastorianus*	CBS 1538, ATCC 12752, NRRL Y-1551
IFO 0676	*S. pastorianus*	
IFO 0853	*S. oviformis*	
IFO 1127^T		CBS 380
IFO 1344		CBS 1545
IFO 1802		
IFO 1803		
IFO 2027		
Sb1		spore clone of IFO 1127
Sb3A		spore clone of IFO 1127
Sb5		spore clone of IFO 1127
Sb3A-1C		spore clone of Sb3A (10)
B19-3C		auxotrophic mutant derived from Sb3A-1C (10)
S. cerevisiae		
AX66-10D		(10)
SH964		Satoshi Harashima, Osaka Univ., (11)
IFO 10217^T		CBS 1171
S. paradoxus		
IFO 0259^T		CBS 406, NRRL Y-1548

a) T indicates type strain.
b) CBS: Centraalbureau voor Schimmelcultures, Baarn, Netherlands
ATCC: American Type Culture Collection, Rockville, U.S.A.
NRRL: ARS Culture Collection, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, U.S.A.

Preparation of DNA. Total DNA was prepared according to the method of Holm *et al.* (8) with minor modification. Cells were harvested from 5 ml of YPD culture. After washing with 2 ml of ice-cold 50 mM EDTA (pH 7.5) twice, cells were suspended in 150 μl of SCE (1 M sorbitol, 0.1 M sodium citrate, 0.06 M EDTA, pH 7.0). The cell suspension was transferred to a 1.5-ml Eppendorf microtube and 10 μl of Zymolyase solution [3 mg/ml of Zymolyase 60000 (Seikagaku Cooperation) in SCE containing 10% 2-mercaptoethanol] was added for spheroplasting. Spheroplasts were lysed gently by adding 150 μl of GuHCl (4.5 M guanidine hydrochloride, 0.1 M EDTA, 0.15 M NaCl, 0.05% sodium N-lauroyl sarcosinate, pH 8.0) and incubating for 10 min at 65 C. After addition of 150 μl of ice-cold ethanol, the precipitate was harvested by centrifugation (12000 rpm, 5 min) and resus-

pended completely in 0.3 ml of 10X TE (1X TE is 1 mM EDTA, 10 mM Tris-HCl, pH 8.0). After RNase A (16) and proteinase K (16) treatments, 50 μl of 5 M NaCl and 40 μl of CTAB (10% cetyltrimethyl ammoniumbromide, 0.7 M NaCl) were added and the suspension was kept at 65 C for 10 min. Chloroform-isoamyl alcohol (24:1) extraction followed by phenol extraction (16) was carried out and crude DNA was obtained by isopropanol precipitation. The precipitate was rinsed with 70% ethanol, dried and dissolved in 0.2 ml of 1X TE. RNase A treatment and phenol extraction were again performed, then DNA was precipitated by addition of 100 μl of 7.5 M ammonium acetate and 0.6 ml of ethanol. The cocoon of DNA was rinsed with 70% ethanol, dried and dissolved in 50 μl of distilled water. Concentration of DNA solution was measured by using a photometer (Beckman DU-65). DNA samples having an $A_{260/280}$ ratio of 1.8-1.9 were used in the DNA similarity experiment.

Measurement of DNA similarity. The microplate-hybridization method developed by Ezaki *et al.* (5,6) was applied with minor modification to determine DNA similarity of yeast. A heat-denatured yeast DNA (400 ng) was immobilized in a well of a microplate (Immuno Plate II, Nunc Inc.) at 28 C overnight. After washing with 0.2 ml of PBS (8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl), the microplate was dried at 60 C for 2 hr. Probe DNA was prepared by labeling with photobiotin acetate (Bresatec Ltd.) according to the supplier's instructions. Before hybridization with probe DNA, 0.2 ml of prehybridization solution [50% deionized formamide, 2X SSC (1X SSC is 0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0), 5X Denhardt's solution (1X Denhardt's solution is 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% ficoll 400), 0.2 mg/ml heat-denatured and sonicated salmon testes DNA] was added to the well and the plate was incubated for 30 min at 37 C. The prehybridization solution was discarded and 0.1 ml of a hybridization solution (the prehybridization solution containing 5% dextran sulfate and 0.5 μg/ml heat-denatured probe DNA) was added to the well. Hybridization was performed at 42 C for 24 hr, then wells were washed twice with 0.2 ml of 0.2X SSC. After washing, 0.1 ml of PBS containing 0.5% bovine serum albumin and 0.1% Triton X-100 was added to the well and the plate was kept for 10 min at room temperature. The solution was discarded, and 0.1 ml of the same solution containing 0.1% streptavidin-β-galactosidase conjugate (GIBCO BRL, Cat.No.9536SA) was added. After incubation at 37 C for 30 min, wells were washed twice with 0.2 ml of PBS. For assay of β-galactosidase, 0.2 ml of PBS containing 1

mM $MgCl_2$ and 0.1 mg/ml 4-methylumbelliferyl-β-D-galactopyranoside (Seikagaku Cooperation) was added and plates were incubated for an appropriate time at 37 C or room temperature. Fluorescence intensity in wells was measured with a microplate reader MTP-32 (Corona Electric Co., Ltd.). DNA similarity was calculated by using the following equation:

$$\text{DNA similarity (\%)} = 100 \times (X-N)/(P-N)$$

where X is fluorescence intensity of unknown DNA, P is fluorescence intensity of DNA used in making probe (positive reference), and N is fluorescence intensity of calf thymus DNA (negative reference).

Pulsed-field gel electrophoresis (PFG). Cells were harvested from 5 ml of YPD overnight culture. Agarose blocks containing chromosomal DNA were prepared essentially according to the method of Carle and Olson (3). Electrophoresis was carried out by using the Pulsaphor system with hexagonal array (Pharmacia-LKB Biotechnology) described previously (11). After electrophoresis, the gel was stained in 0.5 μg/ml ethidium bromide solution for 10 min and kept in deionized water more than 1 hr to reduce background staining. The DNA bands were observed on a transilluminator (TL-33, Ultra-Violet Products, Inc.) and photographed.

Results

Electrophoretic karyotype of *S. bayanus* type strain

Chromosomal DNA banding patterns of *S. bayanus* type strain IFO 1127 and its progenies were examined by PFG (Fig. 1). IFO 1127 showed 17 DNA bands as indicated by white arrows (Fig. 1, lane 2). This is consistent with the result of Johnston and Mortimer (9). This pattern was similar to that of *S. cerevisiae* strains. Chromosome size of IFO 1127 was estimated by using SH964 as size standard and is presented in Table 2. Chromosomal bands were numbered 1 to 17 from the bottom band.

One of its spore clones, Sb1, had an identical PFG karyotype to IFO 1127, while Sb3A had lost 2 bands (Nos. 6 and 7) and Sb5 3 bands (Nos. 5, 6 and 7). Sb3A-1C, a spore clone of Sb3A, was identical to Sb3A; and B19-3C, derived from Sb3A-1C, was identical to Sb5. These observations indicate that IFO 1127 is an aneuploid strain containing three chromosomes that are not essential for growth. Judging from its intensity, band 11 of IFO 1127 seems to be a doublet. In addition, the band of chromosome XII

was barely detectable under the conditions of PFG used in this study. This is also mentioned by Carle and Olson (3). Therefore, it is thought that the sufficient genome set of IFO 1127 consists of 16 chromosomes and is the same as that of *S. cerevisiae*.

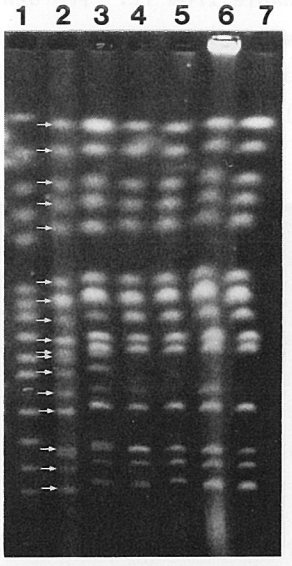

Fig. 1. PFG karyotypes of *S. bayanus* type strain and its progenitors. PFG was performed at 240 V and 60 sec of pulse time for 20 hr. White arrows beside lane 2 indicate the positions of bands detected. Lanes: 1, *S. cerevisiae* SH964; 2, *S. bayanus* type strain IFO 1127; 3, Sb1; 4, Sb3A; 5, Sb5; 6, Sb3A-1C; 7, B19-3C.

Table 2. Chromosome size of *S. bayanus* type strain.

No. of band	Size (kbp)[a]	No. of band	Size (kbp)
1	235	10	740
2	295	11	805
3	335	12	850
4	445	13	960
5	500	14	1015
6	570	15	1075
7	620	16	1165
8	630	17	1550
9	680		

a) kbp is kilobase pairs.

Electrophoretic karyotyping of *S. bayanus* strains preserved in our collection

PFG karyotype analyses of 12 strains preserved as *S. bayanus* in our collection were carried out (Fig.2). All strains except for IFO 0613 and

IFO 0676 have their own PFG karyotypes. Since IFO 0613 and IFO 0676, which had been designated as *S. pastorianus*, showed the same PFG karyotype, they may be identical or have the same pedigree. IFO 0539, which had also been designated as *S. pastorianus*, is similar in PFG karyotype to IFO 0613 but distinguishable from it. IFO 0262 and IFO 0853, previously designated as *S. oviformis*, have distinct PFG karyotypes. IFO 1802 and IFO 1803, which were isolated at different places (Mt. Daisen and Yakushima island, respectively), showed very similar PFG karyotypes.

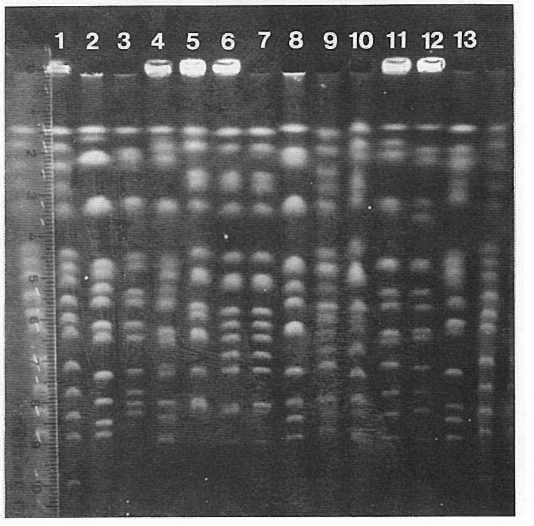

Fig. 2. PFG karyotypes of *S. bayanus* strains preserved in our collection. PFG was performed at 240 V and 60 sec of pulse time for 20 hr. Lanes: 1, SH964 (*S. cerevisiae*); 2, IFO 0206; 3, IFO 0261; 4, IFO 0262; 5, IFO 0539; 6, IFO 0613; 7, IFO 0676; 8, IFO 0853; 9, IFO 1127; 10, IFO 1344; 11, IFO 1802; 12, IFO 1803; 13, IFO 2027.

These *S. bayanus* PFG karyotypes are very similar to that of *S. cerevisiae*, and the minor differences between them are explicable in terms of the chromosome length polymorphism (CLP) observed in *S. cerevisiae* strains (3,4,9). Thus, the 12 strains are indistinguishable from *S. cerevisiae* by PFG karyotype.

DNA similarity of *S. bayanus* strains

As PFG karyotyping is not useful for distinguishing *S. bayanus* from *S. cerevisiae*, we tried to determine the "DNA similarity" of 12 *S. bayanus* strains preserved in our collection. "DNA similarity" refers DNA

Table 3. DNA similarity of *S. bayanus* strains.

Strain	Synonym[a]	DNA similarity (%)[b] IFO 0259	IFO 1127	IFO 10217
IFO 0206		69± 4	35± 4	127± 52
IFO 0261	*S. odessa*^{T}	63± 14	23± 8	92± 35
IFO 0262	*S. oviformis*^{T}	42± 18	30± 5	57± 5
IFO 0539	*S. pastorianus*	29± 9	58± 11	25± 7
IFO 0613	*S. pastorianus*^{T}	44± 10	96± 3	46± 18
IFO 0676	*S. pastorianus*	41± 3	101± 4	45± 18
IFO 0853	*S. oviformis*	46± 5	27± 16	92± 26
IFO 1127[c]		28± 11	100	37± 18
IFO 1344		40± 6	97± 8	33± 7
IFO 1802		51± 13	31± 15	36± 12
IFO 1803		30± 9	26± 10	31± 8
IFO 2027		49± 13	61± 6	79± 4
IFO 0259[c]		100	34± 3	61± 1
IFO 10217[c]		57± 20	30± 4	100

a) T indicates type strain.
b) Mean value and standard deviation were calculated from three independent experiments.
c) IFO 0259, IFO 1127 and IFO 10217 are type strains of *S. paradoxus*, *S. bayanus* and *S. cerevisiae*, respectively.

relatedness as measured by the microplate-hybridization method. The results are shown in Table 3. DNA similarity values of IFO 1127 to the type strains of *S. cerevisiae* (IFO 10217^T) and *S. paradoxus* (IFO 0259) were 37% and 28%, respectively. Since this is consistent with the result of Vaughan Martini (21), it means that the microplate-hybridization method can be used to estimate DNA homology as well as the DNA-DNA reassociation method (19). Out of eleven *S. bayanus* strains, three strains (IFO 0613, IFO 0676 and IFO 1344) showed more than 90% of DNA similarity to IFO 1127, another three (IFO 0206, IFO 0261 and IFO 0853) showed more than 90% to IFO 10217, and two (IFO 1802 and IFO 1803) had low DNA similarity to all three type strains. IFO 0539 showed higher DNA similarity to IFO 1127 than to IFO 10217 and IFO 0259, but only 58%. IFO 0262 showed higher DNA similarity to IFO 10217 than to IFO 1127 and IFO 0259, but only 57%. IFO 2027 had relatively high DNA similarity to the type strains of *S. bayanus* and *S. cerevisiae* (61% and 79%, respectively), but low similarity to IFO 0259. Thus, the 12 *S. bayanus* strains preserved in our collection are heterogeneous on the basis of DNA similarity, and three of them (IFO 0206, IFO 0261 and IFO 0853) should be classified as *S. cerevisiae*.

DNA similarity of a hybrid between *S. bayanus* and *S. cerevisiae*

The relatively high DNA similarity of IFO 2027 to the type strains of both *S. bayanus* and *S. cerevisiae* suggested that it may be a hybrid strain of these species. To confirm this possibility, an artificial hybrid was constructed and its DNA similarity was examined. Spore-to-cell mating with spores of Sb3A-1C and cells of AX66-10D produced the hybrid Sbc1 (2). The DNA similarity values of Sbc1 to Sb3A-1C and AX66-10D were measured by the microplate-hybridization method (Table 4), and in both cases a relatively high similarity value (70%) was obtained. This result supports the possibility that IFO 2027 is a hybrid strain *S. bayanus* and *S. cerevisiae*.

Table 4. DNA similarity of a hybrid between *S. bayanus* and *S. cerevisiae*.

Strain	taxonomic name	DNA similarity (%) Sb3A-1C	AX66-10D
Sb3A-1C	*S. bayanus*	100	40
AX66-10D	*S. cerevisiae*	35	100
Sbc1	hybrid	71	70

Discussion

The genome of *S. cerevisiae* consists of 16 chromosomes (12). PFG karyotype analysis of *S. bayanus* type strain indicated that the *S. bayanus* genome also essentially consists of 16 chromosomes, and that the type strain is a 2n+3 aneuploid. Three extra chromosomes were estimated to be 500 kilobase pairs (kbp), 570 kbp and 620 kbp in size (Table 2, band Nos. 5, 6 and 7). In *S. cerevisiae*, physical size is an important factor in the mitotic stability of a chromosome, the minimal size for stable maintenance being about 100 kbp (13,18,23). The three extra chromosomes of the type strain of *S. bayanus* are of sufficient size to be mitotically stable. Although aneuploidy is generally thought to be unstable, these extra chromosomes seem to have been stably maintained during preservation by serial transfer. A study of single ascospore cultures from triploids indicated that there is a high degree of tolerance of aneuploid chromosome numbers in *S. cerevisiae* (15). Furthermore, it was found that aneuploidy

is a common phenomenon among industrial strains of *S. cerevisiae* (1). Goto *et al.* (7) reported that the type strain of *S. bayanus* is an aneuploid strain on the basis of its DNA content. This is consistent with our estimation of ploidy. Therefore, the electrophoretic karyotyping is useful for confirming aneuploidy. Furthermore, as the PFG karyotype is stable during vegetative growth, minor differences in PFG karyotype can be used for identification of strains and estimation of pedigree relationships. For a culture collection, electrophoretic karyotyping is therefore very useful for quality control of preserved strains.

Since chromosome length polymorphism (CLP) has been reported not only in *S. cerevisiae* strains (3,4,9) but also in *Kluyveromyces* yeasts (17), it seems that minor differences of PFG karyotype observed in *S. bayanus* strains are explained in terms of the CLP of *S. cerevisiae*. Therefore, *S. bayanus* is not distinguishable from *S. cerevisiae* by PFG karyotype. On the other hand, the DNA simiarity experiment indicated that the type strain and some strains of *S. bayanus* are distinguishable from *S. cerevisiae*. We do not know why their DNA relatedness to *S. cerevisiae* is low in spite of the PFG karyotype being the same. Since the formation of DNA hybrids is affected by hybridization conditions, a low DNA similarity value may not reflect real DNA homology. Future investigation, such as DNA sequence analysis, may lead to a better understanding of the genetic relationship between *S. bayanus* and *S. cerevisiae*.

Vaughan Martini *et al.* (20,21) reported that the *S. pastorianus* type strain (CBS 1538, DBVPG 6047) showed intermediate DNA relatedness with type strains of *S. bayanus* and *S. cerevisiae*. IFO 0613 preserved in our collection as *S. pastorianus* type strain, however, showed a very high DNA similarity with *S. bayanus* type strain. Therefore, IFO 0613 belongs to *S. bayanus*. This inconsistency may arise from different methods of measuring DNA relatedness or the use of different strains. If our result is correct, the species *S. pastorianus* should not be reinstated.

IFO 1802 and IFO 1803 isolated from a partially decayed leaf showed low DNA similarity to the three type strains but very high DNA similarity to each other (data not shown). Therefore, they appear to belong to a new species which is closely related to *S. cerevisiae*, and this should be confirmed by genetic crossing study.

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RAPID METHOD FOR LPS ANALYSIS WITH DOC-PAGE AND PAS STAIN

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Summary

A rapid procedure for resolving lipopolysaccharides (LPSs) of gram-negative bacteria in crude samples is described. The method is based on the high resolvability of sodium deoxycholate (DOC) and the selective staining of LPS by periodic acid-Schiff (PAS) reagent. Crude LPS samples were prepared by boiling whole cells with 1% DOC. Adequate profiles of LPS in crude samples were obtained by using DOC-polyacrylamide gel electrophoresis (DOC-PAGE) followed by visualization with PAS stain, and the profiles agreed with those of purified LPS samples.

Keywords: Analysis of lipopolysaccharide, DOC-PAGE, PAS stain.

The analysis of LPS by polyacrylamide gel electrophoresis has been the subject of several studies. Tsai and Frash (10) employing sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver stain, Diedrich and his coworkers (2) employing SDS-PAGE and PAS stain, and Komuro and Galanos (7) employing DOC-PAGE and silver stain have demonstrated high resolution of purified LPS. However, procedures for preparation of purified LPS require careful handling and are time-consuming. Simple methods for analysis of salmonella LPS in crude samples by SDS-PAGE were reported by Hitchcock and Brown (4) and by Kido et al. (6).

In studying the damage of cell membranes of aquaspirilla cells by

drying (9), we attempted to analyze the LPS in whole cells by the method of Hitchcock and Brown but could not obtain satisfactory results. Therefore, we sought an alternative method for the analysis and finally got good resolution with a combination of DOC-PAGE and PAS staining.

This communication presents a simple method for the resolution of LPS extracted from whole cells of Aquaspirillum, Agrobacterium, Rhizobium and Bradyrhizobium strains without purification.

Materials and Methods

Microorganisms. The microorganisms used are listed in Table 1. Type strains are indicated by the letter T after the strain number. Aquaspirillum were grown on PYS medium (5 g of Polypepton, 2 g of Bacto-yeast extract, 1 g of magnesium sulfate, 1 g of sodium succinate, 1 liter of distilled water, pH 7.0) at 28 C, except for Aquaspirillum psychrophilum IFO 13611, which was grown at 20 C. Agrobacterium, Rhizobium and Bradyrhizobium were grown on YM medium (0.4 g of Bacto-yeast extract, 10 g of mannitol, 0.2 g of magnesium sulfate, 0.5 g of potassium dihydrogen-phosphate, 0.1 g of sodium chloride, 1 liter of distilled water, pH 7.2) at 28 C.

Preparation of LPS. Crude LPS samples were prepared by the procedure described below.

(1) Cells were streaked on PYS-agar or YM-agar plates and grown for 2 days.
(2) A loopful of cells was harvested, suspended in 1 ml of distilled water in a 1.5 ml-Eppendorf microcentrifuge tube, and then centrifuged for 5 min at 8,000 rpm.
(3) The cells were resuspended in 200 ul of sample buffer (100 mg of DOC, 2 ml of glycerol, 8 mg of bromophenol blue, 8 ml of 0.01M Tris-glycine buffer, pH 6.8), and heated at 100 C for 5 min.
(4) After centrifugation in a microcentrifuge (Kubota, Tokyo, Japan) for 5 min at 12,000 rpm, 10 ul of the supernatant was subjected to electrophoresis.

Purification of LPS. Purified LPSs were isolated and purified from 15 g to 18 g of wet cells grown for 2 days by the method described by Westphal and Jann (11). Authentic LPSs from the wild type and Re mutant of Salmonella minnesota were purchased from Sigma Chemical Company (St. Louis,

Table 1. Bacterial strains studied

Species	Strain	Other strain designations*
Aquaspirillum aquaticum	IFO 14918 T	ATCC 11330
Aquaspirillum delicatum	IFO 14919 T	ATCC 14667
Aquaspirillum giesbergeri	IFO 13959 T	ATCC 11334
Aquaspirillum gracile	IFO 14920 T	ATCC 19624
Aquaspirillum itersonii	IFO 14921	ATCC 11331
Aquaspirillum itersonii subsp. nipponicum	IFO 13615 T	ATCC 33333
Aquaspirillum metamorphum	IFO 13960 T	ATCC 15280
Aquaspirillum metamorphum	IFO 12012	
Aquaspirillum peregrinum	IFO 14922 T	NCIB 9435
Aquaspirillum peregrinum subsp. integrum	IFO 13617 T	ATCC 33334
Aquaspirillum polymorphum	IFO 13961 T	ATCC 11332
Aquaspirillum psychrophilum	IFO 13611 T	ATCC 33335
Aquaspirillum putridiconchylium	IFO 13962 T	ATCC 15279
Aquaspirillum serpens	IFO 14923 T	ATCC 11335
Aquaspirillum serpens	IFO 14924	ATCC 12638
Aquaspirillum sinuosum	IFO 14925 T	ATCC 9786
Spirillum lunatum	IFO 13958 T	ATCC 11337
Aeromonas hydrophila	IFO 3820	NRRL B-909
Aeromonas hydrophila	IFO 13286	ATCC 14715
Rhizobium leguminosarum biovar viceae	IFO 14778 T	ATCC 10004
Rhizobium leguminosarum biovar phaseoli	IFO 14785 T	ATCC 14482
Rhizobium leguminosarum biovar trifolii	IFO 14784 T	ATCC 14480
Rhizobium fredii	IFO 14780 T	ATCC 35423
Rhizobium loti	IFO 14779 T	ATCC 33669
Rhizobium galegae	IFO 14965 T	ATCC 43677
Rhizobium leguminosarum	IFO 13337	
Rhizobium leguminosarum	IFO 13338	
Rhizobium leguiminosarum	IFO 14168	
Bradyrhizobium japonicum	IFO 14792	USDA 110
Bradyrhizobium japonicum	IFO 14791	USDA 76
Bradyrhizobium japonicum	IFO 15001	
Bradyrhizobium japonicum	IFO 15002	
Bradyrhizobium sp.	IFO 15004	
Bradyrhizobium sp.	IFO 15006	
Bradyrhizobium sp.	IFO 15007	
Agrobacterium tumefaciens biovar 1	IFO 13264	ATCC 11157
Agrobacterium tumefaciens biovar 1	IFO 13265	ATCC 11158
Agrobacterium tumefaciens biovar 1		SAES CH-2
Agrobacterium tumefaciens biovar 1		SAES CH-2B
Agrobacterium tumefaciens biobar 1		SAES CH-3A
Agrobacterium tumefaciens biovar 1		SAES CH-5
Agrobacterium tumefaciens biovar 2		SAES R-6
Agrobacterium tumefaciens biovar 2		SAES R-184
Agrobacterium tumefaciens biovar 2		SAES R-243
Agrobacterium tumefaciens biovar 2		SAES R-257
Agrobacterium tumefaciens biovar 2		SAES R-288
Agrobacterium tumefaciens biovar 2		SAES R-294
Agrobacterium tumefaciens biovar 2		SAES R-300
Agrobacterium tumefaciens biovar 3		MAFF 63001
Agrobacterium rubi	IFO 13261 T	ATCC 13335
Agrobacterium rubi	IFO 13260	ATCC 13334

* ATCC, American Type Culture Collection, Rockville, USA; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; NRRL, ARS Culture Collection, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, USA; USDA, United States Department of Agriculture, Maryland, USA; SAES, Shizuoka Agricultural Experiment Station, Shizuoka, Japan; and MAFF, Ministry of Agriculture, Foresty and Fisheries, Tsukuba, Japan.

Missouri, U. S. A.).

Polyacrylamide gel electrophoresis. DOC-PAGE was performed according to the procedure described by Komuro and Galanos (7). The separating gels contained 13% acrylamide and 0.9% DOC with a 4% acrylamide stacking gel containing 0.5% DOC. Electrophoresis was performed using 26 mM Tris-192 mM glycin buffer (pH 8.4) containing 0.25% DOC. After pre-electrophoresis at 25 mA without substance, 10 μl portions of the samples were applied to sample wells and subjected to electrophoresis at a constant current of 20 mA until the bromophenol blue migrated to the bottom of gel. SDS-PAGE was performed as described by Laemmli (9), using 12.5% polyacrylamide gel slabs. Purified and authentic LPSs were dissolved in sample buffer at a concentration of approximately 5 mg/ml, heated at 100 C for 5 min, then subjected to electrophoresis. The size of the gels used was 100 x 70 x 1 mm.

Staining procedures. After electrophoresis, the LPSs in gels were stained with PAS reagent. This is a modification of the procedure of Dubray and Bezard (3).

(1) The gel slab was soaked in 40% methanol-10% acetic acid solution overnight at room temperature with mild agitation using an orbital shaker. It was then soaked in 7.5% acetic acid for 30 min.

(2) The gel slab was put in 0.35% aqueous periodic acid solution and stored for 3 hr at room temperature with mild agitation.

(3) The gel slab was transferred into cold Schiff's reagent (5 C) without rinsing, and stored overnight in a refrigerator.

(4) The gel slab was destained by several changes of 8% acetic acid at room temperature. The destaining process is essential to avoid coloring the gel background.

The LPSs in gels were also detected by silver staining with a Bio-Rad silver stain Kit (Bio-Rad Laboratories, Richmond, California) according to the manufacturer's instructions.

Analysis of chemical composition of LPS. Neutral sugars and fatty acids in purified LPS were analyzed by the method of Yokota et al. (12). Amino sugars were determined with an amino acid analyzer. Total phosphorus in LPS was analyzed by the method of Ames (1). 2-Keto-3-deoxyoctonate (KDO) was analyzed by the method of Karkhanis et al. (5).

Results and discussion

Comparison of methods for electrophoresis and staining

Electrophoretic patterns of LPS preparations were examined. The resolution by SDS-PAGE and DOC-PAGE were compared by using the purified LPSs of Aquaspirillum. The chemical composition of these LPSs is shown in Table 2. The LPSs in each gel were visualized by silver staining or PAS staining. The profiles obtained are shown in Figs. 1a-d. The migration patterns of aquaspirilla LPSs in SDS-PAGE were apparently different from those in DOC-PAGE. In SDS-PAGE, the LPS of Aquaspirillum itersonii subsp. nipponicum IFO 13615 gave a ladder-like banding profile, which suggests an S-chemotype LPS with a heterotrophic O-side chain; and the LPS of Aquaspirillum metamorphum IFO 13960 gave a diffuse profile (Figs. 1a and 1b). In contrast, the LPSs of IFO 13615 and IFO 13960 were both resolved in one compact dense band by the DOC-PAGE (Figs. 1c and 1d), which suggests that these LPSs have homologous O-side chains. An LPS of Salmonella minnesota wild-type strain used as an authentic standard gave a similar profile in both SDS-PAGE and DOC-PAGE, where typical ladder-like bands can be seen, which was essentially similar to that already reported (4). These results suggest that the SDS-PAGE procedure employed in this study led to

Table 2. Chemical composition of LPSs of A. itersonii subsp. nipponicum IFO 13615, A. metamorphum IFO 13960 and A. aquaticum IFO 14918

Component		μg per mg of LPS		
		IFO 13615	IFO 13960	IFO 14918
Glucosamine		88	59	116
2-Keto-3-deoxyoctonate		19	9	20
Phosphorus		4	12	8
Fatty acid:	3-OH-12:0	-	27	61
	3-OH-16:0	35	-	-
	3-OH-18:0	42	-	-
	12:0	3	2	3
Neutral sugar:	Rhamnose	17	22	ND [b]
	Galactose	17	-	ND
	Glucose	12	13	ND
	Mannose	20	-	ND
	Arabinose	-	17	ND
	D, D-Heptose [a]	322	-	-
	L, D-Heptose [a]	-	16	+
	Unknowns	-	405	ND

a: D, D-Heptose, D-glycero-D-manno-heptose; and L, D-Heptose, L-glycero-D-manno-heptose. Tentatively identified from their retention times on GLC.
b: ND, not determined.

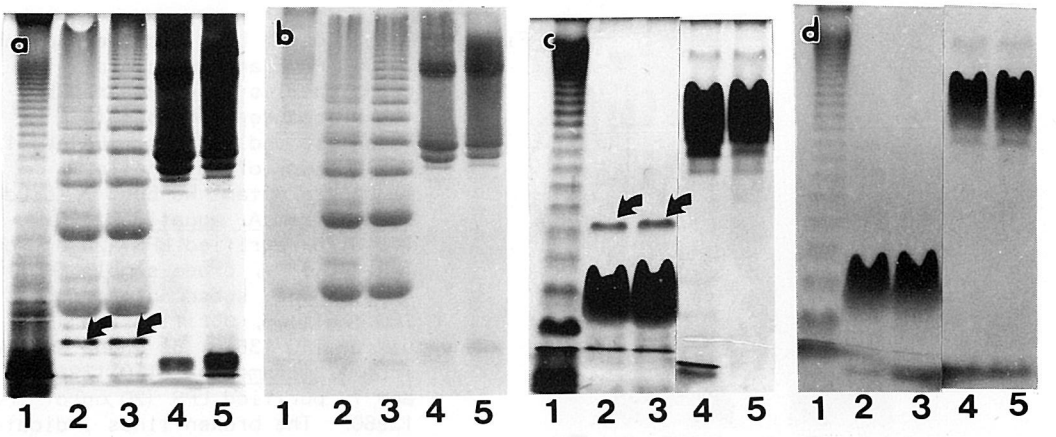

Fig. 1. Migration patterns of purified LPSs resolved by SDS-PAGE (a and b) and by DOC-PAGE (c and d). The LPSs were detected by silver stain (a and c) and by PAS stain (b and d). Lanes: 1, LPS of Salmonella minnesota wild type; 2, LPS of A. itersonii subsp. nipponicum IFO 13615; 3, LPS of IFO 13615 treated with Proteinase K; 4, LPS of A. metamorphum IFO 13960; and 5, LPS of IFO 13960 treated with Proteinase K. Each lane contains 50 ug of LPS. The arrows indicate bands of impurity.

formation of multimers of the aquaspirilla LPS molecules.

The silver staining method is known to be useful for the detection of LPSs, but it requires many steps. In contrast, although PAS staining showed lower sensitivity than silver staining, the method is simple and selective for LPSs (Figs. 1b and 1d). For example, the bands indicated by arrows in Figs. 1a and 1c, which are due to a contaminant in the sample, were not stained by PAS reagent. Thus, DOC-PAGE and PAS staining were found to be effective for the analysis of Aquaspirillum LPSs.

Application of DOC-PAGE and PAS stain to whole-cell LPSs

We also evaluated the resolution by DOC-PAGE and PAS staining for the crude LPS samples. Figure 2 shows the DOC-PAGE profiles of purified LPSs and LPSs in crude samples prepared from three different Aquaspirillum species. Similar migration patterns of LPSs were obtained for the purified LPS and crude sample of each strain. The LPSs were detected as dense red bands. A sharp red band was also detected near the top of gel in the crude sample from A. metamorphum IFO 13960. Since this band is sensitive to Coomassie blue stain and is lost after digestion with Proteinase K (Boehringer Mannheim GmbH, West Germany), it probably represents

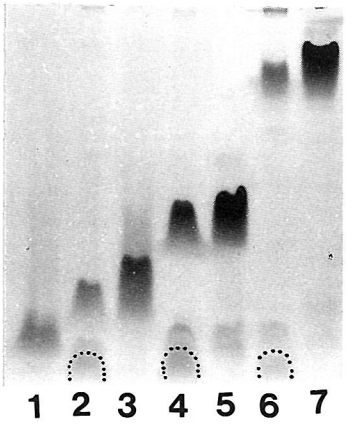

Fig. 2. Detection of LPS in crude samples by DOC-PAGE and PAS stain. The crude samples were prepared by the procedure described in Materials and Methods. Lanes: 1, LPS (20 μg) of Salmonella minnesota mutant Re 595; 2, crude sample from A. aquaticum IFO 14918; 3, purified LPS (20 μg) of IFO 14918; 4, crude sample from A. itersonii subsp. nipponicum IFO 13615; 5, purified LPS (20 μg) of IFO 13615; 6, crude sample from A. metamorphum IFO 13960; and 7, purified LPS (20 μg) of IFO 13960. The broken lines indicate the band stained blue-violet.

glycoproteins composing the surface layer of the cells (data not shown). Another band which migrated to the bottom of gel and was stained blue-violet was detected from each crude sample. These appeared to be a non-LPS component since the band is missing in the purified LPS samples.

Crude LPS samples from sixteen strains of Aquaspirillum, one strain of Spirillum, two strains of Aeromonas, sixteen strains of Agrobacterium, nine strains of Rhizobium and seven strains of Bradyrhizobium were also prepared and subjected to DOC-PAGE. Figure 3 shows the profiles of LPSs of Aquaspirillum visualized by PAS staining. The LPSs of Aquaspirillum aquaticum IFO 14918, Aquaspirillum serpens IFO 14923 and IFO 14924, Aquaspirillum putridiconchylium IFO 13962, Aquaspirillum gracile IFO 14920, and Aquaspirillum peregrinum subsp. integrum IFO 13617 showed the migration pattern of R-chemotype LPSs. Those of Aquaspirillum delicatum IFO 14919, A. itersonii subsp. nipponicum IFO 13615 and Aquaspirillum peregrinum IFO 14922 migrated to middle of the gel, and these seemed to be SR-chemotype LPSs. The LPSs of Aquaspirillum polymorphum IFO 13961, A. itersonii IFO 14921, A. metamorphum IFO 12012 and IFO 13960, Aquaspirillum psychrophilum IFO 13611, Aquaspirillum giesbergeri IFO 13959 and Aquaspirillum sinuosum IFO 14925 were S-chemotype LPSs, however, the DOC-PAGE profiles of these LPSs were not a ladder-like pattern, but showed only one to three bands. The LPSs of Spirillum lunatum IFO 13958 and Aeromonas hydrophila IFO 3820 and IFO 13286 were S-chemotype LPSs showing the ladder-like migration pattern. Figures 4 and 5 show the profiles of LPSs of Rhizobiaceae. The results indicate that the LPSs of Agrobacterium tumefaciens biovar 1, A.

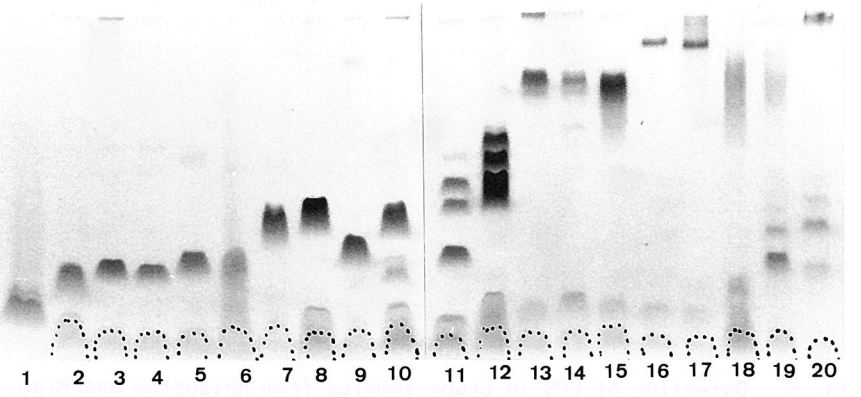

Fig. 3. Detection of LPS in crude samples from Aquaspirillum, Spirillum and Aeromonas by DOC-PAGE and PAS stain. Lanes: 1, LPS (20 ug) of Salmonella minnesota mutant Re 595; 2, A. aquaticum IFO 14918; 3, A. serpens IFO 14924; 4, A. serpens IFO 14923; 5, A. putridiconchylium IFO 13962; 6, A. gracile IFO 14920; 7, A. delicatum IFO 14919; 8, A. itersonii subsp. nipponicum IFO 13615; 9, A. peregrinum subsp. integrum IFO 13617; 10, A. peregrinum IFO 14922; 11, A. polymorphum IFO 13961; 12, A. itersonii IFO 14921; 13, A. metamorphum IFO 13960; 14, A. metamorphum IFO 12012; 15, A. psychrophilum IFO 13611; 16, A. giesbergeri IFO 13959; 17, A. sinuosum IFO 14925; 18, Spirillum lunatum IFO 13958; 19, Aeromonas hydrophila IFO 3820; and 20, Aeromonas hydrophila IFO 13286. The broken lines indicate the band stained blue-violet.

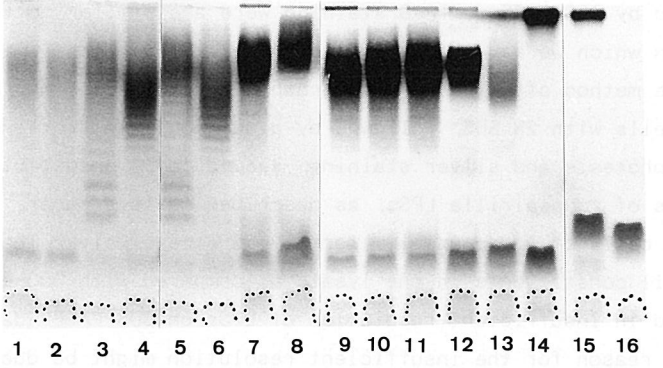

Fig. 4. Detection of LPS in crude samples from Agrobacterium by DOC-PAGE and PAS stain. Lanes: 1 to 14 are LPSs of A. tumefaciens; 1, IFO 13264; 2, IFO 13265; 3, SAES CH-2; 4, SAES CH-2B; 5, SAES CH-3A; 6, SAES CH-5; 7, SAES R-6; 8, SAES R-184; 9, SAES R-243; 10, SAES R-257; 11, SAES R-288; 12, SAES R-294; 13, SAES R-300; 14, MAFF 63001; 15, A. rubi IFO 13261; and 16, A. rubi IFO 13260.

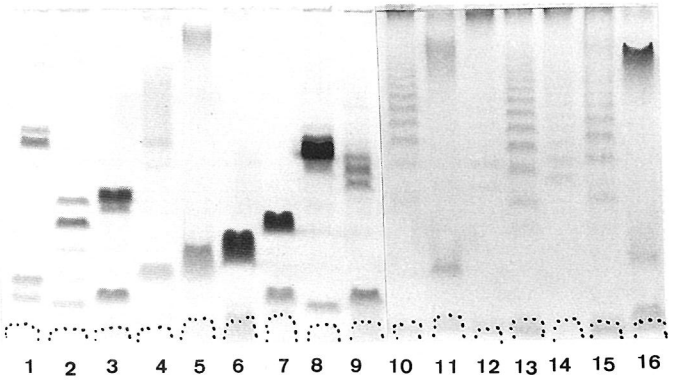

Fig. 5. Detection of LPS in crude samples from Rhizobium and Bradyrhizobium by DOC-PAGE and PAS stain. Lanes: 1, R. leguminosarum biovar viceae IFO 14778; 2, R. leguminosarum biovar phaseoli IFO 14785; 3, R. leguminosarum biovar trifolii IFO 14784; 4, R. fredii IFO 14780; 5, R. loti IFO 14779; 6, R. galegae IFO 14965; 7, R. leguminosarum IFO 14168; 8, R. leguminosarum IFO 13338; 9, R. leguminosarum IFO 13337; 10, B. japonicum IFO 14792; 11, B. japonicum IFO 14791; 12, B. japonicum IFO 15001, 13, B. japonicum IFO 15002; 14, B. sp. IFO 15004; 15, B. sp. IFO 15006; and 16, B. sp. IFO 15007.

tumefaciens biovar 2 and Bradyrhizobium are S-chemotype LPSs; A. tumefaciens biovar 3 and Agrobacterium rubi have R-chemotype LPSs; and Rhizobium has SR-chemotype LPSs. These profiles of LPSs in crude samples resolved by DOC-PAGE and PAS staining were and similar to those of purified LPSs, on which we have reported elsewhere (13).

The method of Hitchcock and Brown (4), extraction of LPS by boiling whole cells with 2% SDS, followed by digestion with Proteinase K, SDS-PAGE electrophoresis and silver staining, seemed to be unsuitable for the analysis of aquaspirilla LPSs, as described in this paper. By boiling with SDS the cells of Aquaspirillum were lysed excessively. The presence of more cell constituents in the lysate as compared with DOC-extraction resulted in insufficient resolution of LPSs on SDS-PAGE (data not shown). Another reason for the insufficient resolution might be due to their characteristic sugar compositions (Table 2). Thus, the procedure of the extraction of LPS by boiling with 1% DOC, and DOC-PAGE followed by visualization by PAS staining appears to be effective for the resolution of LPS in gram-negative bacteria.

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52-56, 1991 (March)

ACTINOMYCETE STRAINS ISOLATED FROM FALLEN LEAVES IN MANGROVE AREAS

YUMIKO NAKAGAITO and TŌRU HASEGAWA

Summary

From fallen leaves collected in mangrove areas in Iriomote Island and Ishigaki Island, Okinawa Prefecture, 13 strains of actinomycetes were isolated. Based on their morphological, physiological and chemotaxonomic characteristics, the one strain was placed in the genus Promicromonospora and the others in the genus Streptomyces.

Keywords: Actinomycetes on mangrove leaves, Streptomyces, Promicromonospora, Taxonomy of actinomycetes.

Mangrove forests are located in tropical and subtropical regions, for example, the Ryukyu Islands and Amami Island in Japan. In those area, high temperature and high humidity, tidal ebb and flow and inflow of fresh water from rivers give rise to complex environmental conditions. The mycological ecology of such unique environments has been studied extensively, but ecological studies of actinomycete in such locations are very few (1). We attempted to isolate actinomycetes from fallen leaves collected in mangrove areas to study the actinomycetes community of these areas.

Here, we describe the identification of 13 strains of actinomycetes isolated from fallen leaves of mangroves.

Materials and Methods

Isolation of actinomycete strains. Actinomycete strains were isolated from fallen leaves which were collected in mangrove areas of Iriomote Island and Ishigaki Island. The leaves were placed on yeast extract agar (0.02% yeast extract, 1.5% agar, pH 7.0), and incubated at 28 C. Aerial mycelium of actinomycetes formed on the leaves were isolated under stereoscopic microscope.

Morphology. For morphological observation by optical microscope and scanning electron microscope (SEM, Model JSM-T20, JEOL Ltd.), strains were grown on media described by Shirling and Gottlieb (5). The preparations for SEM were peformed by dehydration of agar blocks containing cells through graded ethanol series, followed by treatment in a critical point drying apparatus (Hitachi HCP-2).

Chemotaxonomic analysis. Isomers of diaminopimelic acid (A2pm) were analyzed by the TLC method of Hasegawa et al. (3). Whole-cell sugar compositions were determined by HPLC (6). Amino acid compositions in cell walls and cellular fatty acid compositions were determined by GLC, menaquinones and the DNA base compositions were analyzed by HPLC, and mycolic acids and phospholipids were analyzed by TLC, as described by Goodfellow and Minnikin (2).

Results

Isolation of actinomycete strains

Forty fallen leaves of mangrove (Bruguiera gymnorrhiza Lamk.) were incubated on yeast extract agar at 28 C for 14 days. Colonies obtained from different leaves were purified on yeast extract-malt extract agar (ISP Medium 2) plates, and a total of 13 strains (NAK-2 - NAK-14) of actinomycetes were isolated.

Taxonomic characterization of isolates

Of the isolates, 12 strains contained LL-A2pm, and one strain (NAK-14) contained meso-A2pm in their cell walls. The former formed green, gray, red or white aerial mycelia on agar media, and their hyphae were not broken up into fragments. SEM revealed spore chains with smooth surfaces, and neither sporangia or verticils (Figs. 1 and 2). Based on

these data, 12 strains were considered to belong to the genus Streptomyces and 6 of these strains were identified as Streptomyces griseus on the basis of taxonomic characteristics (Table 1). The other strains have not been identified. Strain NAK-14, which contains meso-A2pm in its cell wall, was identified as Promicromonospora citrea based on the following characteristics: it shows branched hyphae broken up into fragments in broth; it forms short aerial mycelia on an agar medium (Fig. 3); growth is pasty to leathery; cell wall type is VI; mycolic acids are lacking; it contains menaquinone MK-9 (H_4); it contains phospholipid of type PV; cellular fatty acids are branched; the guanine plus cytosine content of the DNA is 72.7 mol%; and it is susceptible to Promicromonospora-specific phages (4) (Table 1).

Table 1. Actinomycete strains isolated from mangrove leaves

Strain	Spore surface	AM	AM's color	A2pm	Genus or species name identified	Collection site
NAK-2	s	RF	r	LL	Streptomyces sp.	Iriomote Is.
NAK-3	s	RF	r	LL	Streptomyces sp.	Iriomote Is.
NAK-4	s	RF	g-gr	LL	Streptomyces griseus	Iriomote Is.
NAK-5	s	RF	r	LL	Streptomyces sp.	Iriomote Is.
NAK-6	s	RF	r	LL	Streptomyces sp.	Iriomote Is.
NAK-7	s	RF	g-gr	LL	Streptomyces griseus	Iriomote Is.
NAK-8	s	RF	g-gr	LL	Streptomyces griseus	Iriomote Is.
NAK-9	s	RF	g-gr	LL	Streptomyces griseus	Iriomote Is.
NAK-10	s	RF-RA	w	LL	Streptomyces sp.	Iriomote Is.
NAK-11	s	RF	g-gr	LL	Streptomyces griseus	Iriomote Is.
NAK-12	s	RA-S	g-gr	LL	Streptomyces griseus	Iriomote Is.
NAK-13	s	RF	w	LL	Streptomyces sp.	Iriomote Is.
NAK-14	s	RF	w	meso	Promicromonospora citrea	Iriomote Is.

AM; aerial mycelium; s, smooth; RF, Rectiflexibilis; RA, Retinaculiaperti; S, Spira; r, red; g, green; gr, gray; w, white.

The taxonomic position of the isolate NAK-14 will be published elsewhere (Nakagaito and Hasegawa, in preparation).

Discussion

Actinomycete strains hitherto reported were mostly isolated from soil, aquatic environments such as fresh water of a river and sea water, plants, animals or air. Streptomyces are known to exist in various

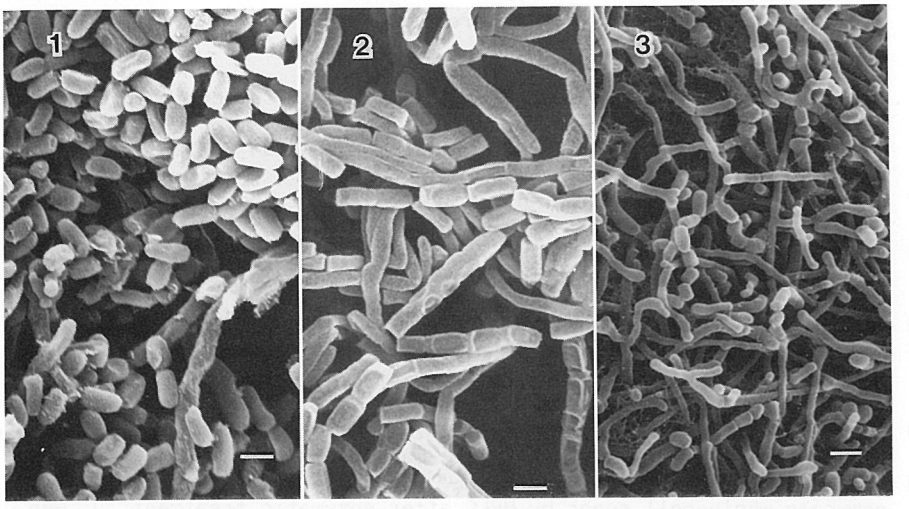

Fig. 1. Cells of the isolate NAK-5 (Streptomyces sp.) grown on yeast extract-malt extract agar. (Bar = 1 μm)
Fig. 2. Cells of the isolate NAK-8 (Streptomyces sp.) grown on yeast extract-malt extract agar. (Bar = 1 μm)
Fig. 3. Cells of the isolate NAK-14 (Promicromonospora citrea) grown on Czapeks' agar. (Bar = 1 μm)

environments. Weyland and Helmke (7) reported varieties of actinomycetes in segments of mangrove, and suggested that a striking variety of actinomycetes could be found in such circumstances. It is not surprising, therefore, that we could isolate many Streptomyces strains from fallen leaves in mangrove areas that are regularly inundated.

Concerning Promicromonospora, P. citrea and P. sukumoe have been isolated from soil, and P. enterophila from millipede feces, but the isolation from leaves has not been reported. From fallen leaves in mangrove areas, we isolated 1 strain of Promicromonospora citrea in addition of 12 strains of Streptomyces.

The isolates of Streptomyces were divided into 3 groups on the basis of their taxonomic characteristics: the one consists of NAK-2, 3, 5, and 6; the second, NAK-10, and 13; and the third, NAK-4, 8, 9, 11, and 12.

It is not known whether the existence of mangrove leaves was indispensable for thier isolation.

This is our first investigation into the distribution of actinomycetes in mangrove areas, and it will be necessary to isolate more strains from these areas for the full exploration to the distribution of actinomycetes and the composition of genera in mangrove area.

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TAXONOMIC SIGNIFICANCE OF FATTY ACID COMPOSITIONS IN WHOLE CELLS AND LIPOPOLYSACCHARIDES IN RHIZOBIACEAE

AKIRA YOKOTA and TAKESHI SAKANE

Summary

The cellular fatty acid compositions of 71 strains from 13 species of the genera Rhizobium, Bradyrhizobium and Agrobacterium, including strains isolated from plants, were re-investigated by gas-liquid chromatography. Almost all the species and biovar groups of Rhizobiaceae showed different 2- and 3-hydroxy fatty acid profile. Thus, species of Rhizobiaceae could be easily distinguished on the basis of cellular fatty acid profiles, which were therefore proven to be useful for the identification of these bacteria.

From 9 strains of the genera Rhizobium and Agrobacterium lipopolysaccharides (LPSs) were isolated and their hydroxy fatty acids were examined. 3-Hydroxy fatty acid profiles of whole cells and LPSs correlated well in Rhizobiaceae. Thus, the cellular hydroxy fatty acid profile mainly reflected that of LPSs which are essential for the growth of Gram-negative bacteria. Therefore, the hydroxy fatty acid profile was shown to be valuable as a chemotaxonomic marker for the systematics of Rhizobiaceae.

Keywords: Taxonomy of Rhizobiaceae, cellular fatty acid, 2-hydroxy fatty acid, 3-hydroxy fatty acid, Rhizobium, Bradyrhizobium, Agrobacterium.

Gas chromatographic analysis of whole cell fatty acid methyl esters is a well-known tool in the identification of various bacteria. In Gram-negative bacteria both 2- and 3-hydroxy fatty acids are cell constituents and their distribution in cells varies with the type of bacteria. Therefore, their profile has been shown to be valuable for differentiation and identification of these bacteria (25). The fatty acid profile of Rhizobiaceae strains was described in the previous paper (26). Recently two new species of Rhizobiaceae, Rhizobium galegae (14) and Agrobacterium vitis (18), have been proposed. To obtain more information on these fatty acid profiles in order to establish criteria for distinguishing between species of Rhizobium, Bradyrhizobium and Agrobacterium, we re-examined the cellular fatty acids of 71 strains of Bradyrhizobium, Rhizobium and Agrobacterium, including 35 strains isolated from plants.

In Gram-negative bacteria, 3-hydroxy fatty acids are known to be distributed mostly in LPSs (25). Although studies on the fatty acid composition of LPSs in Rhizobiaceae have been reported by many workers (1-6,9,13, 17,20,22,28), only a few species have been studied.

Here we describe the results of re-examination of cellular fatty acid compositions of type strains, reference strains and many isolates of Rhizobiaceae, and also the hydroxy fatty acid profiles of LPSs of Rhizobiaceae strains. The significance of these hydroxy fatty acid profiles is discussed with regard to the classification and identification of these bacteria.

Materials and Methods

Microorganisms. The bacterial strains used in this study are listed in Table 1. Nine strains of Bradyrhizobium species, two strains of Rhizobium leguminosarum and seven strains of R. loti were isolated from root nodules of legume plants and identified according to Bergey's Manual of Systematic Bacteriology (11). Strains of Agrobacterium rhizogenes and A. tumefaciens were isolated by Makino and Morita (15), and were kindly supplied by Dr. T. Makino (Shizuoka Agricultural Experiment Station, Shizuoka, Japan).

Cultivation of Microorganisms. Composition of the medium (YEM) and conditions used for the cultivation of strains were described in the previous paper (27). Type strains are indicated by the letter "T" after the

Table 1. Bacterial strains studied.

Taxon	Strain	Source and Comments
Genus *Bradyrhizobium*		
B. japonicum	IFO 14783 T	DNA homology group I (10)
B. sp. (*Lupinus*)	IFO 14781	DNA homology group I (10)
B. japonicum	IFO 14792	DNA homology group Ia (10)
B. japonicum	IFO 14791	DNA homology group II (10)
B. japonicum	Isolate Rz-5	*Glycine max*
B. japonicum	Isolate Rz-6	*Glycine max*
B. japonicum	Isolate Rz-7	*Glycine max*
Bradyrhizobium sp.	Isolate Rz-8	*Phaseolus angularis*
Bradyrhizobium sp.	Isolate Rz-10	*Vicia hirsuta*
Bradyrhizobium sp.	Isolate Rz-11	*Pueraria thunbergiana*
Bradyrhizobium sp.	Isolate Rz-12	*Kummerowia striata*
Bradyrhizobium sp.	Isolate Rz-13	*Kummerowia striata*
Bradyrhizobium sp.	Isolate Rz-14	*Kummerowia stipulacea*
Genus *Rhizobium*		
R. leguminosarum biovar *viceae*	IFO 14778 T	
R. leguminosarum biovar *viceae*	IFO 14168	
R. leguminosarum biovar *phaseoli*	IFO 14785 T	
R. leguminosarum biovar *phaseoli*	IFO 13338	
R. leguminosarum biovar *trifolii*	IFO 14784 T	
R. leguminosarum biovar *trifolii*	IFO 13337	
R. leguminosarum	Isolate Rz-9	*Phaseolus vulgaris*
R. leguminosarum	Isolate Rz-20	*Medicago cupulina*
R. melilot	IFO 14782 T	
R. fredii	IFO 14780 T	
R. galegae	IFO 14965 T	*Galega orientalis* (14)
R. loti	IFO 14779 T	
R. loti	IFO 13336	
R. loti	Isolate Rz-15	
R. loti	Isolate Rz-16	
R. loti	Isolate Rz-18	*Lotus corniculata*
R. loti	Isolate Rz-19	var. *japonicus*
R. loti	Isolate Rz-21	
R. loti	Isolate Rz-24	
R. loti	Isolate Rz-26	

Table 1. (Continued)

Taxon	Strain	Source and Comments
Genus Agrobacterium		
A. radiobacter biovar 1	IFO 12607	
A. radiobacter biovar 1	IFO 12664	
A. radiobacter biovar 1	IFO 12665	
A. radiobacter biovar 1	IFO 13258	
A. radiobacter biovar 1	IFO 13259	
A. radiobacter biovar 1	IFO 13532 T	
A. radiobacter biovar 1	IFO 13533	
A. tumefaciens biovar 1	IFO 12667	
A. tumefaciens biovar 1	IFO 13264	
A. tumefaciens biovar 1	IFO 13265	
A. tumefaciens biovar 1	CH-2	Makino
A. tumefaciens biovar 1	CH-2B	Makino
A. tumefaciens biovar 1	CH-3	Makino
A. tumefaciens biovar 1	CH-5	Makino
A. rhizogenes biovar 1	IFO 14554	
A. rhizogenes biovar 1	IFO 14555	
A. rhizogenes biovar 1	MR-1	Makino
A. rhizogenes biovar 1	MR-2	Makino
A. rhizogenes biovar 1	MR-4	Makino
A. rhizogenes biovar 1	MR-7	Makino
A. rhizogenes biovar 1	MR-8	Makino
A. sp. biovar 1	IFO 12470	
Alcaligenes faecalis var. myxogenes biovar 1	IFO 13714	Harada (8)
A. tumefaciens biovar 2	IFO 14793	
A. tumefaciens biovar 2	R-6	Rose, Makino (15)
A. tumefaciens biovar 2	R-184	Rose, Makino (15)
A. tumefaciens biovar 2	R-243	Rose, Makino (15)
A. tumefaciens biovar 2	R-257	Rose, Makino (15)
A. tumefaciens biovar 2	R-288	Rose, Makino (15)
A. tumefaciens biovar 2	R-294	Rose, Makino (15)
A. tumefaciens biovar 2	R-300	Rose, Makino (15)
A. rhizogenes biovar 2	IFO 13257 T	
A. tumefaciens biovar 3	MAFF 06-63001	Grapevine
A. rubi	IFO 13260	
A. rubi	IFO 13261 T	
Others		
Azorhizobium caulinodans	IFO 14845 T	(7)
Xanthobacter autotrophicus	IFO 14758 T	
Xanthobacter flavus	IFO 14759 T	
Mycoplana bullata	IFO 13290 T	
Mycoplana dimorpha	IFO 13291 T	

T, type strain; MAFF, Ministry of Agriculture, Foresty and Fisheries, Kannondai, Tsukuba, Japan.

strain numbers.

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 as described previously (26). The fatty acid composition of LPSs was analyzed as described previously (27).

Gas-liquid chromatography (GLC) and gas-liquid chromatography-mass spectrometry (GLC-MS). Fatty acid methyl esters were analyzed with a Shimadzu GC-9A chromatograph. The column employed was a glass column (5 m x 0.28 cm) packed with 10% diethyleneglycol succinate (DEGS) on Chromosorb W at 180 C, 195 C or 210 C. 2-Hydroxy fatty acid methyl esters were identified by GLC-MS using Hewlett Packard 5890A mass spectrometer equipped with a capillary OV-101 fused silica column (25 m x 0.25 mm i.d.).

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

Preparation of lipopolysaccharides (LPSs). LPSs were isolated from the lyophilized bacterial cells (ca. 5 g of dry weight) by the hot phenol-water extraction procedure of Westphal and Jann (24), followed by repeated ultracentrifugation. The purification was modified by additional digestions with ribonuclease A and proteinase K (27).

Deoxycholate-polyacrylamide gel electrophoresis (DOC-PAGE). DOC-PAGE was carried out according to Komuro and Galanos (12), with separating gels formed of 13% acrylamide and 0.5% DOC with a 4% acrylamide stacking gel containing 0.5% DOC. Electrophoresis was performed with Tris-glycine buffer containing 0.25% DOC, with a constant current of 18 mA until the tracking dye entered the separating gel, and at 25 mA thereafter. Pre-electrophoresis was not carried out. LPS bands were visualized by silver staining as described by Tsai and Frasch (22).

Results

Fatty acid profiles of whole cells of Bradyrhizobium strains

The distribution of fatty acids in whole cells of 13 strains of Bradyrhizobium is shown in Table 2. All the strains studied had similar

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

Strain	Non-hydroxylated fatty acid (%)[a]							3-Hydroxy fatty acid (%)[b]								2-Hydroxy fatty acid[c]
	16:0	16:1	17cyc	18:0	18:1	19cyc	21:1	12:0	i-13:0	14:0	i-15:0	ai-15:0	16:0	16:1	18:0	18:1	
Bradyrhizobium																	
B. japonicum IFO 14783 T	17	7	-	2	74	-	-	36	-	64	-	-	-	-	-	-	-
B. sp. (Lupinus) IFO 14781	18	5	-	2	74	-	-	24	-	76	-	-	-	-	-	-	-
B. japonicum IFO 14792	14	1	-	1	75	6	3	45	-	55	-	-	-	-	-	-	-
B. japonicum IFO 14791	13	1	-	1	71	10	5	38	-	62	-	-	-	-	-	-	-
B. japonicum Rz-5	13	1	-	2	79	4	2	48	-	52	-	-	-	-	-	-	-
B. japonicum Rz-6	15	1	-	4	71	3	2	46	-	54	-	-	-	-	-	-	-
B. japonicum Rz-7	16	2	-	1	74	4	4	43	-	57	-	-	-	-	-	-	-
B. sp. (Phaseolus) Rz- 8	14	6	-	3	63	14	-	40	-	60	-	-	-	-	-	-	-
B. sp. (Vicia) Rz-10	13	5	-	4	58	20	-	28	-	72	-	-	-	-	-	-	-
B. sp. (Paeraria) Rz-11	12	7	-	7	60	16	-	38	-	62	-	-	-	-	-	-	-
B. sp. (Kummerowia) Rz-12	14	2	-	5	68	11	-	41	-	59	-	-	-	-	-	-	-
B. sp. (Kummerowia) Rz-13	14	2	-	1	81	1	1	44	-	56	-	-	-	-	-	-	-
B. sp. (Kummerowia) Rz-14	14	2	-	6	72	5	2	40	-	60	-	-	-	-	-	-	-

Table 2. (Continued)

Strain	Non-hydroxylated fatty acid (%)[a]							3-Hydroxy fatty acid (%)[b]									2-Hydroxy fatty acid[c]
	16:0	16:1	17cyc	18:0	18:1	19cyc	21:1	12:0	i-13:0	14:0	i-15:0	ai-15:0	16:0	16:1	18:0	18:1	
Rhizobium																	
R. leguminosarum IFO 14778 T	10	-	-	16	42	17	15	-	-	39	-	17	14	-	30	-	-
R. leguminosarum IFO 14168	7	-	-	13	69	6	5	-	-	48	-	10	10	-	32	-	-
R. leguminosarum IFO 14785 T	9	-	-	22	36	23	11	-	-	58	-	3	8	-	32	-	-
R. leguminosarum IFO 13338	17	3	-	10	51	8	12	-	-	46	-	14	22	-	18	-	-
R. leguminosarum IFO 14784 T	8	-	-	15	55	14	9	-	-	50	-	7	11	-	32	-	-
R. leguminosarum IFO 13337	7	1	-	16	71	2	4	-	-	43	-	11	11	-	35	-	-
R. leguminosarum Rz-9	7	1	-	13	51	11	16	-	-	39	-	13	12	-	36	-	-
R. leguminosarum Rz-20	8	-	-	22	54	11	7	-	-	40	-	6	14	-	40	-	-
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 Rz-15	13	3	3	3	76	1	2	11	37	3	-	-	24	8	18	-	-
R. loti Rz-16	15	-	6	10	57	7	6	16	49	8	-	-	6	10	10	-	-
R. loti Rz-18	11	-	5	8	70	2	3	17	45	8	-	-	8	6	17	-	-
R. loti Rz-19	13	-	5	10	58	4	10	19	46	4	-	-	8	8	15	-	-
R. loti Rz-21	13	-	7	8	57	8	7	13	50	2	-	-	9	11	13	-	-
R. loti Rz-24	13	-	5	10	58	6	7	22	48	9	-	-	6	4	12	-	-
R. loti Rz-26	12	-	5	7	65	6	6	19	48	10	-	-	6	6	12	-	-

[a] Percentages of the total non-hydroxylated acids.
[b] Percentages of the total 3-hydroxy acids.
[c] -, absent.

Table 3. Cellular fatty acid composition of *Agrobacteium* strains

Strain	Non-hydroxylated fatty acid (%)[a]									3-Hydroxy fatty acid (%)[b]								2-Hydroxy fatty acid[c]
	16:0	16:1	18:0	18:1	19:0	19:1	19cyc	21:1	22:1	12:0	i-13:0	14:0	i-15:0	ai-15:0	16:0	16:1	18:0	18:1	
Genus *Agrobacterium* [Biovar 1]																			
A. radiobacter IFO 12607	12	3	tr	74	1	1	3	5	1	-	-	54	-	-	42	-	4	-	-
A. radiobacter IFO 12664	12	2	tr	63	2	1	5	11	3	-	-	58	-	-	39	-	3	-	-
A. radiobacter IFO 12665	11	3	tr	80	-	-	2	3	1	-	-	61	-	-	35	-	3	-	-
A. radiobacter IFO 13258	11	2	tr	67	2	1	5	9	2	-	-	60	-	-	38	-	2	-	-
A. radiobacter IFO 13259	9	2	1	76	1	1	3	5	1	-	-	63	-	-	34	-	3	-	-
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. tumefaciens IFO 13265	11	2	1	70	2	1	3	8	1	-	-	57	-	-	40	-	2	-	-
A. tumefaciens CH-2	14	2	2	34	4	3	11	24	5	-	-	55	-	-	44	-	1	-	-
A. tumefaciens CH-2B	10	2	1	42	5	3	10	22	5	-	-	58	-	-	40	-	2	-	-
A. tumefaciens CH-3	13	3	1	55	2	2	8	14	3	-	-	58	-	-	40	-	2	-	-
A. tumefaciens CH-5	10	2	3	42	3	2	13	21	4	-	-	63	-	-	35	-	2	-	-
A. rhizogenes IFO 14554	20	-	-	25	-	-	40	5	-	-	-	65	-	-	29	-	2	4	-
A. rhizogenes IFO 14555	20	-	-	29	-	-	38	14	-	-	-	65	-	-	29	-	2	4	-
A. rhizogenes MR-1	17	2	1	41	2	2	11	20	3	-	-	57	-	-	40	-	3	-	-
A. rhizogenes MR-2	19	2	1	58	tr	1	6	11	2	-	-	56	-	-	41	-	3	-	-
A. rhizogenes MR-4	18	2	1	47	2	2	8	17	3	-	-	55	-	-	43	-	2	-	-
A. rhizogenes MR-7	14	1	4	31	1	2	13	27	6	-	-	63	-	-	36	-	1	-	-
A. rhizogenes MR-8	16	2	1	49	2	2	9	15	4	-	-	59	-	-	40	-	1	-	-
A. sp. IFO 12470	12	3	tr	75	tr	-	4	5	1	-	-	61	-	-	36	-	3	-	-
Alc. faecalis var. *myxogenes* IFO 13714	9	3	1	62	1	2	6	13	2	-	-	61	-	-	37	-	2	-	-

Table 3. (Continued)

Strain		Non-hydroxylated fatty acid (%)[a]									3-Hydroxy fatty acid (%)[b]									2-Hydroxy fatty acid[c]
		16:0	16:1	18:0	18:1	19:0	19:1	19cyc	21:1	22:1	12:0	i-13:0	14:0	i-15:0	ai-15:0	16:0	16:1	18:0	18:1	
[Biovar 2]																				
A. tumefaciens	IFO 14793	15	3[d]	4	19	-	-	35	25	-	-	-	22	32	-	28	-	18	-	+
A. tumefaciens	R-6	13	2[d]	2	28	2	3	17	27	5	-	-	13	32	-	30	-	25	-	+
A. tumefaciens	R-184	22	3[d]	4	23	3	3	13	23	5	-	-	15	30	-	36	-	18	-	+
A. tumefaciens	R-243	13	2[d]	5	27	3	3	12	28	7	-	-	14	34	-	32	-	20	-	+
A. tumefaciens	R-257	12	2[d]	3	27	4	5	11	29	6	-	-	13	28	-	34	-	25	-	+
A. tumefaciens	R-288	11	2[d]	4	29	6	3	17	22	5	-	-	17	31	-	29	-	23	-	+
A. tumefaciens	R-294	12	2[d]	2	26	3	4	11	31	7	-	-	12	27	-	35	-	26	-	+
A. tumefaciens	R-300	17	3[d]	5	21	3	2	16	27	7	-	-	19	37	-	27	-	16	-	+
A. rhizogenes	IFO 13257 T (L)[e]	21	4[d]	8	22	2	3	14	23	4	-	-	10	36	-	28	-	25	-	+
A. rhizogenes	IFO 13257 T (S)[e]	21	4[d]	7	24	6	3	10	21	4	-	-	10	36	-	31	-	24	-	+
[Biovar 3]																				
A. tumefaciens	MAFF 06-63001	6	7	-	83	-	-	1	1	-	-	-	51	-	-	16	-	26	7	+
[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	-	-
Others																				
A. caulinodans	IFO 14845 T	7	-	13	73	-	-	7	-	-	-	-	50	-	-	-	-	50	-	-
X. autotrophicus	IFO 14758 T	5	4	5	87	-	-	-	-	-	-	-	-	-	-	43	-	30	27	-
X. flavus	IFO 14759 T	3	-	6	88	-	-	-	-	-	-	-	-	-	-	35	-	38	15	-
M. bullata	IFO 13290 T	35	1	-	62	-	-	-	-	-	76	-	24	-	-	-	-	-	-	-
M. dimorpha	IFO 13291 T	22	-	12	65	-	-	2	-	-	-	-	67	-	-	-	-	-	33	-

[a,b,c] See Table 2.

[d] Tentatively identified as straight chain acid 16:1 5 for these strains, in contrast to 16:1 7 in the other strains.

[e] Segregants on YEM agar plate which formed a large colony (L) and a small colony (S), respectively.

Table 4. 2-Hydroxy fatty acid compositions of biovar 2 and biovar 3 strains of Agrobacterium species.

Strain	2-Hydroxy fatty acid		
	18:1	i-19:0	19:0
A. tumefaciens IFO 14793	30	65	4
A. tumefaciens R-6	62	17	21
A. tumefaciens R-184	57	28	15
A. tumefaciens R-243	71	20	9
A. tumefaciens R-257	74	17	9
A. tumefaciens R-288	56	30	14
A. tumefaciens R-294	58	32	10
A. tumefaciens R-300	44	31	25
A. rhizogenes IFO 13257 T	59	24	16
A. tumefaciens MAFF 06-63001	89	11	0

Table 5. Distribution of 3-hydroxy fatty acids in whole cells and LPSs of Rhizobiaceae

Strain	3-Hydroxy fatty acid composition of		References for LPSs
	whole cells	LPS (Lipid A)	
Bradyrhizobium	3-OH 12:0, 3-OH 14:0	3-OH 12:0, 3-OH 14:0 30-OH 31:0[a]	(1,16,17)
Rhizobium			
R. leguminosarum	3-OH 14:0, 3-OH ai-15:0, 3-OH 16:0, 3-OH 18:0	3-OH 14:0, 3-OH ai-15:0, 3-OH 16:0, 3-OH 18:0 27-OH 28:0[a]	(1-3,19,28)
R. meliloti	3-OH 14:0, 3-OH 18:0	3-OH 14:0, 3-OH 18:0 27-OH 28:0[a]	This work
R. fredii			(1,5)
R. galegae	3-OH 14:0, 3-OH 16:0 3-OH 18:0	3-OH 14:0, 3-OH 16:0 3-OH 18:0 27-OH 28:0[a]	This work
R. loti	3-OH 12:0, 3-OH i-13:0 3-OH 14:0, 3-OH 18:0	3-OH 12:0, 3-OH i-13:0 3-OH 14:0, 3-OH 18:0 27-OH 28:0[a]	This work
Agrobacterium			
[Biovar 1]	3-OH 14:0, 3-OH 16:0	3-OH 14:0, 3-OH 16:0 27-OH 28:0[a]	This work (17)
[Biovar 2]	3-OH 14:0, 3-OH i-15:0 3-OH 16:0, 3-OH 18:0	3-OH 14:0, 3-OH i-15:0 3-OH 16:0 27-OH 28:0[a]	This work
[Biovar 3]	3-OH 14:0, 3-OH 16:0 3-OH 18:0, 3-OH 18:1	Not determined	
A. rubi	3-OH 14:0, 3-OH 16:0	3-OH 14:0, 3-OH 16:0 27-OH 28:0[a]	This work

[a] Long chain fatty acids (data from Bhat et al. (1)).

profiles, with 18:1 and 19cyc as the major cellular fatty acids. The hydroxy acids 3-OH 12:0 and 3-OH 14:0 were present, but 2-hydroxy acids were absent. Thus, the fatty acid profile is homogeneous in this genus.

Fatty acid profile of whole cells of Rhizobium strains

The non-hydroxylated fatty acid composition of Rhizobium strains was similar to that of Bradyrhizobium strains (Table 2), but the 3-hydroxy acid profiles differed significantly. Furthermore, distribution of hydroxy acids differed among the species. Strains of Rhizobium meliloti, Rhizobium fredii and Rhizobium galegae differed from Rhizobium leguminosarum in lacking 3-OH ai-15:0, and R. meliloti and R. fredii differed from R. galegae in having low contents of 3-OH 16:0. The 3-hydroxy acid profiles of Rhizobium loti differed significantly from those of the other four species. It contained 3-OH 12:0, 3-OH i-13:0, 3-OH 14:0, 3-OH 16:0, 3-OH 16:1 and 3-OH 18:0. Three of them, 3-OH 14:0, 3-OH 16:0 and 3-OH 18:0 also present in others, but R. loti contains 3-OH 12:0, 3-OH i-13:0 and 3-OH 16:1, which the others do not.

Therefore, species of Rhizobium could be allocated to four groups on the basis of their 3-hydroxy fatty acids in whole cells: first, three biovars of R. leguminosarum; second, R. meliloti and R. fredii; third, R. galegae; and fourth, R. loti (Table 6).

Fatty acid profile in whole cells of Agrobacterium strains

Cellular fatty acid profiles of 35 strains of Agrobacterium are shown in Table 3. Strains of Agrobacterium biovar 2 and biovar 3 significantly differed from strains of Agrobacterium biovar 1 and Agrobacterium rubi, both in 3-hydroxy acid composition and in the presence of 2-hydroxy acids. Fatty acid profiles of the biovar 2 strains were similar to that of the biovar 3 strain, but were distinguished by the presence of 3-OH i-15:0. Fatty acid profiles of biovar 1 strains were basically the same as those of A. rubi strains.

2-Hydroxy fatty acid compositions in the strains of biovar 2 and biovar 3 are summarized in Table 4. These three fatty acids were assigned from their electron impact (EI)- and chemical ionization (CI)-mass spectra and ECL values on GLC by a similar method described previously (26). EI-MS of the fatty acids showed intensive fragments m/z 90 and M-59 (m/z 253 or 267). The molecular weight deduced from CI-MS were 312, 326 and 326, respectively (data not shown). Based on these results, the 2-hydroxy fatty acids were assigned as 2-OH 18:1, 2-OH i-19:0, and 2-OH 19:0, respective-

ly. The 2-hydroxy fatty acid profile of the biovar 3 strain was the same as those of biovar 2.

Species (biovars) of Agrobacterium could be divided into three groups on the basis of their 3-hydroxy fatty acids in whole cells: first, biovar 1 and A. rubi; second, biovar 2 strains; and third, biovar 3 strain. The fatty acid analysis thus indicates that the three biovars have different 3-hydroxy fatty acid compositions, and can be distinguished on this basis.

The strain IFO 13714 formerly designated Alcaligenes faecalis var. myxogenes (8), which show taxonomic characteristics of Agrobacterium biovar 1, were also examined. From the physiological data and the fatty acid compositions obtained here, the strain was re-identified as Agrobacterium sp. biovar 1.

Fatty acid profile in whole cells of related bacterial strains

To compare strains of Rhizobiaceae and their taxonomic neighbors, the fatty acid profiles of strains of phylogenetically related species were also examined (Table 3). The fatty acid profiles of Mycoplana dimorpha (23) and the stem nodulating bacterium Azorhizobium caulinodans (7) were found to be similar to those of R. fredii and R. meliloti, and that of Mycoplana bullata (23) to be similar to that of Bradyrhizobium.

LPSs from Rhizobium and Agrobacterium strains

LPSs were isolated from R. meliloti IFO 14782 T, R. fredii IFO 14780 T, R. loti IFO 14779 T, R. galegae IFO 14965 T, A. tumefaciens IFO 12667 (biovar 1), A. tumefaciens IFO 14793 (biovar 2), A. rhizogenes IFO 14554 (biovar 1), A. rhizogenes IFO 13257 T (biovar 2), and R. rubi IFO 13261 T. Electrophoretic patterns of these LPSs are shown in Figs. 1 and 2, which reveal that all the LPSs from Rhizobium species are of the rough type, and those from Agrobacterium species are of the smooth type.

3-Hydroxy fatty acid compositions of these LPS preparations were compared with those of whole cells. As summarized in Table 5, the two sets of 3-hydroxy fatty acid profiles correlated well. Typical elution patterns of 3-hydroxy acids on GLC are shown in Figs. 3-6. Other chemical components in these LPS preparations are presently being analyzed and the results will be published elsewhere.

Hollingsworth and Carlson (8) and Bhat et al. (1) have recently found the long chain hydroxy fatty acids 27-hydroxy-octacosanoic acid and 30-hydroxyhentriacontanoic acid to be major structural fatty acid components of LPSs in Agrobacterium and Bradyrhizobium species. As the determinations

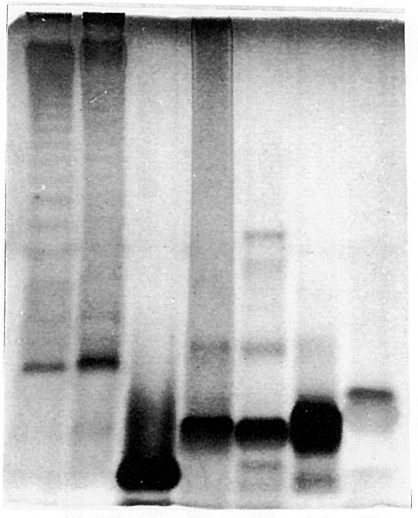

Fig. 1. Deoxycholate-polyacrylamide gel electrophoresis and silver staining of LPSs from Rhizobium species.
Lanes: 1, Salmonella minnesota S type; 2, Salmonella minnesota Rd mutant; 3, Rhizobium meliloti IFO 14782 T; 4, Rhizobium fredii IFO 14780 T; 5, Rhizobium loti IFO 14779; 6, Rhizobium galegae IFO 14965 T.

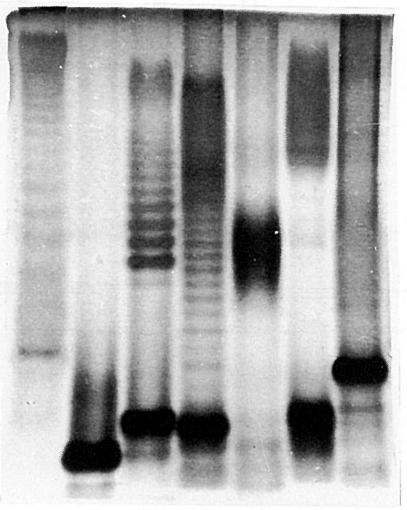

Fig. 2. Deoxycholate-polyacrylamide gel electrophoresis and silver staining of LPSs from Agrobacterium species.
Lanes: 1, Salmonella minnesota S type; 2, Salmonella minnesota Rd mutant; 3, Agrobacterium tumefaciens IFO 12667 (biovar 1); 4, Agrobacterium tumefaciens IFO 14793 (biovar 2); 5, Agrobacterium rhizogenes IFO 14554 (biovar 1); 6, Agrobacterium rhizogenes IFO 13257 T (biovar 2); 7, Agrobacterium rubi IFO 13261 T.

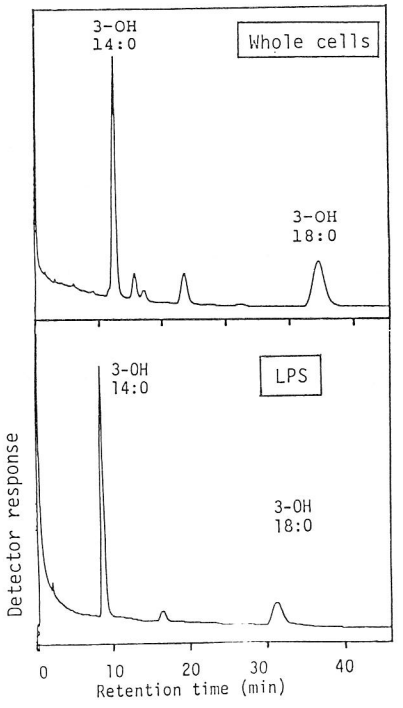

Fig. 3. 3-Hydroxy fatty acid profiles of whole cells and LPS of Rhizobium meliloti IFO 14782 T

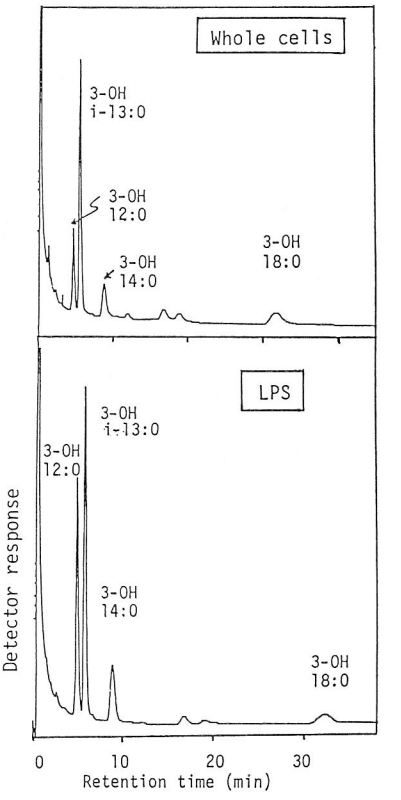

Fig. 4 3-Hydroxy fatty acid profiles of whole cells and LPS of Rhizobium loti IFO 14779 T

of long chain acids by Bhat et al. (1) were carried out on the same LPS preparations as described here, these results are included in Table 5. It can be seen that 27-OH 28:0 is distributed throughout the Rhizobium and Agrobacterium species and that 30-OH 31:0 is present in Bradyrhizobium species; and thus the long chain fatty acids can be used to differentiate between Bradyrhizobium and Rhizobium species.

Discussion

The chemotaxonomic value of hydroxy acid profiles has been illustrated for various Gram-negative bacteria (23) and is apparent from numerous studies at both the generic level and the species level. The present study

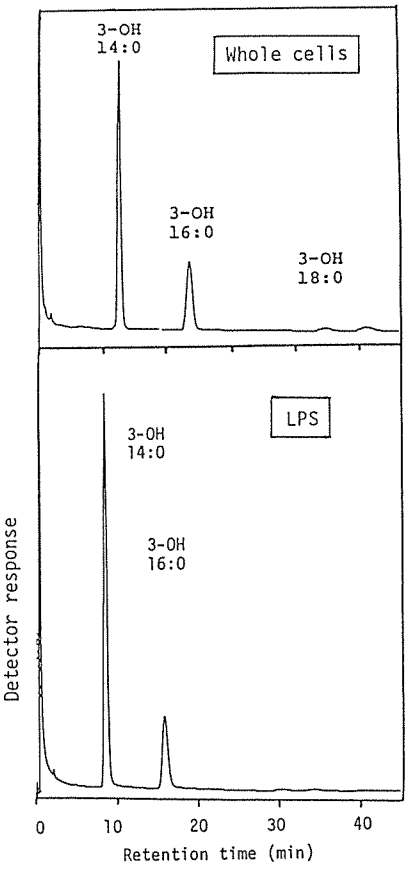

Fig. 5 3-Hydroxy fatty acid profiles of whole cells and LPS of Agrobacterium rhizogenes IFO 14554 (biovar 1)

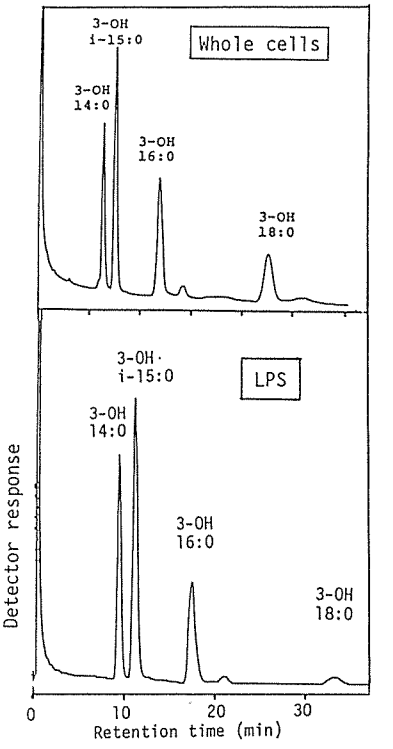

Fig. 6. 3-Hydroxy fatty acid profiles of whole cells and LPS of Agrobacterium rhizogenes IFO 13257 (biovar 2)

revealed that hydroxy acids in Rhizobiaceae, as in most other Gram-negative bacteria, are derived mainly from LPSs (25), and that they are therefore valuable for the chemotaxonomy of this group of bacteria.

The cellular fatty acid profiles of the species belonging to Bradyrhizobium, Rhizobium and Agrobacterium and five related species obtained in this study are summarized in Table 6. This study shows that for identifying new isolates of Rhizobiaceae, analysis of cellular fatty acids may be a favorable method. Especially in the three biovar groups of Agrobacterium, where the capacity of carbohydrate utilization cannot reliably be used as a criterion to differentiate among the groups, data on the fatty acid composition can be of great help in classification. As only one

Table 6. Summary of the cellular fatty acid profiles in Rhizobiaceae and related species

Genera and species	Major fatty acid	2-Hydroxy fatty acid	3-Hydroxy fatty acid						
			12:0	i-13:0	14:0	i-15:0	ai-15:0	16:0	18:0
Bradyrhizobium	18:1 19cyc	-	+	-	+	-	-	-	-
Rhizobium									
R. leguminosarum	18:1 19cyc	-	-	-	+	-	+	+	+
R. meliloti R. fredii	18:1 19cyc	-	-	-	+	-	-	±	+
R. galegae	16:0 19cyc	-	-	-	+	-	-	+	+
R. loti	18:1	-	+	+	+	-	-	+	+
[Agrobacterium biovar 1] A. rubi	18:1 19cyc 21:1	-	-	-	+	-	-	+	-
[Agrobacterium biovar 2]	18:1 19cyc 21:1	+	-	-	+	+	-	+	+
[Agrobacterium biovar 3]	18:1 19cyc 21:1	+	-	-	+	-	-	+	+
Azorhizobium	18:1	-	-	-	+	-	-	-	+
Xanthobacter	18:1	-	-	-	-	-	-	+	+
Mycoplana bullata	18:1+16:0	-	+	-	+	-	-	-	-
Mycoplana dimorpha	18:1	-	-	-	+	-	-	-	+

Table 7. Differential characteristics of the species in Rhizobiaceae

Group	Genus or species	3-Ketolactose formation	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	-	-	+	+	-	-	+	+
6	Agrobacterium biovar 1	+	-	-	-	-	-	+	-
7	Agrobacterium biovar 2	-	+	-	-	+	-	+	+
8	Agrobacterium biovar 3	-	+	-	-	-	-	+	+
9	A. rubi	-	-	-	-	-	-	+	-

biovar 3 strain was examined, the hydroxy fatty acid composition of more biovar 3 strains needs to be examined.

Table 7 shows the differential characteristics of the species in Rhizobiaceae based on the combination of a biochemical tests and their hydroxy fatty acid compositions. On this basis, the species in Rhizobiaceae can be divided into 9 groups. Agrobacterium biovar 1 has the same hydroxy acid profile as A. rubi, but these groups can be clearly differentiated by the biochemical test of 3-ketolactose formation. The only species difficult to differentiate are R. meliloti and R. fredii, which have the same cellular fatty acid profiles. Thus, combination of these chemotaxonomic and biochemical tests can be used as criteria to differentiate among genera and species of Rhizobiaceae.

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76-82, 1991 (March)

RE-IDENTIFICATION OF ACTINOMYCETE STRAINS IN THE IFO CULTURE COLLECTION

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Summary

Chemotaxonomic analysis of 4 species of the genus Streptomyces in the IFO culture collection revealed that these strains should be excluded from the genus Streptomyces, as 3 strains of them contained meso-diaminopimelic acid (A2pm) in their cell walls and 1 strain contained both meso- and LL-A2pm. Based on chemotaxonomic and morphological characteristics, Streptomyces galtieri IFO 13366 and Streptomyces hikiziensis IFO 13785 should be transferred to the genera Nocardia and Saccharothrix, respectively. Streptomyces hofunensis IFO 13810 was regarded as a member of the genus Microtetraspora. Streptomyces candidus subsp. azaticus IFO 13803 was expected to belong to the genus Kitasatosporia.

Keywords: Taxonomy of actinomycetes, Streptomyces galtieri, Streptomyces hikiziensis, Streptomyces hofunensis, Streptomyces candidus subsp. azaticus.

Strains of the genus Streptomyces generally form extensively branched mycelium and spore chains, and contain LL-diaminopimelic acid (A2pm) in their cell walls. These characteristics allow them to be distinguished roughly from strains of other genera.

A2pm isomer analysis by TLC of the strains of the genus Streptomyces

in the IFO culture collection revealed that 3 of these strains, S. galtieri IFO 13366, S. hikiziensis IFO 13785 and S. hofunensis IFO 13810 don't contain LL-A2pm but meso-A2pm in their cell walls and S. candidus subsp. azaticus contains both meso- and LL-A2pm. This indicates that they belong to genera other than Streptomyces. All these strains had been identified mainly on the basis of morphological characteristics, and the taxonomic data in the literature were not enough to allow identification. To determine the correct taxonomic placement of these strains, we analyzed their chemotaxonomic properties, such as cell wall amino acids, whole-cell sugars, mycolic acids, phospholipids, menaquinones, fatty acids and the DNA base composition, together with morphological and physiological characteristics. Based on these chemotaxonomic, morphological and physiological features, we tried to identify them correctly.

Materials and Methods

Actinomycete strains. The strains used in this study were Streptomyces galtieri IFO 13366 (=ISP 5350) (4), S. hikiziensis IFO 13785 (22), S. candidus subsp. azaticus IFO 13803 (7), and S. hofunensis IFO 13810 (14), which are preserved in the IFO culture collection.

Chemotaxonomic analyses. Cells used for chemotaxonomic analyses were obtained from cultures incubated in yeast extract-glucose broth (pH 7.1) containing (per liter) 10 g of yeast extract and 10 g of glucose at 28 C for 4 days, except for S. hofunensis, which was cultured at 28 C for 5 days in Bennett's broth containing (per liter) 1 g of yeast extract, 1 g of beef extract, 2 g of NZ-amine type A and 10 g of glucose (pH 7.1). Isomers of A2pm were analyzed by the TLC method of Hasegawa et al. (6) and the HPLC method of Takahashi et al. (20). Amino acid compositions of cell wall were analyzed by GLC (15). Whole-cell sugars were analyzed by HPLC (23). Phospholipid patterns were determined by the method of Minnikin et al. (13). Mycolic acids were analyzed by the method of Minnikin et al. (12). Menaquinones were extracted, purified by the method of Collins et al. (1), and analyzed by HPLC (2). Cellular fatty acids were analyzed by GLC (19). The DNA base compositions were analyzed by HPLC (21).

Morphological observation. For morphological observation by

optical microscope and scanning electron microscope (SEM, Model JSM-T20, JEOL Ltd.), the strains were grown on media suggested by Shirling and Gottlieb (18). The samples for SEM were prepared by dehydrating agar blocks of culture through a graded ethanol series, and then in a critical point drying apparatus (Hitachi HCP-2).

Results and Discussion

Streptomyces galtieri IFO 13366

Streptomyces galtieri was proposed as a new species in the genus Streptomyces by Goret and Joubert (4) in 1951 with some doubts about the classification, such as the lack or poverty of sporulation on aerial mycelium. In this study, we confirmed the presence of meso-A_2pm, arabinose and galactose in the whole-cell hydrolysate of strain IFO 13366. This is the typical pattern of cell wall type IV/A after Lechevalier's (11). The fatty acid composition was n-C16:0, n-C18:0, n-C16:1, n-C18:1 and 10-methyl-C19:0, belonging to the Kroppenstedt's

Table 1. Chemotaxonomic characteristics of 4 strains of the genus Streptomyces.

Characteristics	S. galtieri IFO 13366	S. hikiziensis IFO 13785	S. candidus subsp. azaticus IFO 13803	S. hofunensis IFO 13810
cell wall amino acid	ala glu meso-	ala glu meso-	ala glu meso-, LL-	ala glu meso-
Whole-cell sugar pattern	A gal, ara glc, man	C rha, rib man, gal	man, gal	B man, gal glc, mad
cell wall type	IV	III		III
Major menaquinone	MK-8 (H_4)	MK-9 (H_4)	MK-9 (H_6, H_8)	MK-9 (H_4, H_6)
Phospholipid type	PII	PII	PII	PIV
Fatty acid composition (%)				
saturated	35	12	43	44
unsaturated	54	12	15	12
iso-14/16/18	0	35	9	14
iso-15/17	0	14	5	0
anteiso-15/17	0	20	26	0
10Me-17	0	0	0	2
10Me-18	0	4	0	23
10Me-19	11	0	0	0
2OH acid	-	+	-	+
Fatty acid pattern	1b	3f	2a or 2b	3c
G+C content (mol %)	68.1	70.7	72.1	70.5

Abbreviations: ala, alanine; glu, glutamic acid; meso-, meso-A_2pm; LL-, LL-A_2pm; ara, arabinose; rib, ribose; gal, galactose; man, mannose; glc, glucose; rha, rhamnose; mad, madurose.

pattern 1b (9). The presence of mycolic acid was also confirmed. The phospholipid composition placed this strain in phospholipid pattern group PII: phosphatidyl ethanolamine was present, while phosphatidyl choline, phosphatidyl glycerol and an unknown glucosamine-containing phospholipid were absent. The predominant menaquinone was MK-8(H4), and the DNA base composition was 68.1 mol% of guanine plus cytosine. Based on these chemotaxonomic data together with phenotypic characteristics shown in Table 1, we propose that S. galtieri should be transferred to the genus Nocardia.

Streptomyces hikiziensis IFO 13785

Streptomyces hikiziensis, which forms long, straight aerial mycelium without verticils, loops or spirals, and forms spores with smooth surface was proposed as a new species of the genus Streptomyces by Uchida et al. (22) in 1971. However, as shown in Table 1, on chemical analyses indicate that this placement was incorrect, because the strain contains meso-A2pm in its cell walls. Whole-cell hydrolysate of the strain contained rhamnose, galactose, ribose and mannose, but lacked madurose. These data showed that cell wall type of the strain is III/C of Lechevalier's (11). The phospholipid composition was in phospholipid pattern group PII: phosphatidyl ethanolamine was present, while phosphatidyl choline, phosphatidyl glycerol and an unknown glucosamine-containing phospholipid were absent. The predominant menaquinone was MK-9(H4). The cellular fatty acids present were iso- and anteiso- branced chain fatty acids and 2-hydroxylated fatty acids. This fatty acid profile placed the strain in pattern 3f of the classification after Kroppenstedt (9). The DNA base composition was 70.7 mol% of guanine plus cytosine. These characteristics are within the range specified for the genus Saccharothrix (5). Accordingly, we propose that this strain should be transferred to the genus Saccharothrix.

Streptomyces hofunensis IFO 13810

Streptomyces hofunensis, described by Nara et al. (14) in 1977, forms long spore chains and spores with warty surface, and was proposed as a new species of the genus Streptomyces. However, as shown in Table 1, the presence of meso-A2pm indicates that strain IFO 13810 does not belong to the genus Streptomyces and that further taxonomic study is necessary.

Whole-cell hydrolysate of the strain contained mannnose, galactose,

glucose, and madurose. Diamino acid in cell walls was meso-A2pm. These data showed that cell wall type was III/B of Lechevalier s (11). Phosphatidyl ethanolamine was present as a component of phospholipids, but phosphatidyl choline, phosphatidyl glycerol, and an unknown glucosamine-containing phospholipid were absent. The predominant menaquinones were MK-9 (H_4, H_6). The fatty acids consisted of iso-C16:0, 10-methyl-C18:0, saturated, unsaturated and 2-hydroxylated acids, which placed the strain in pattern group 3a (9). The DNA base composition of this strain was 70.5 mol% of guanine plus cytosine. Based on the morphological and physiological characteristics and chemotaxonomical data described here, it is clear that strain IFO 13810 belongs to the group maduromycetes (3), which includes the Actinomadura pussila group, Microbispora, the Microtetraspora glauca group, Planobispora, Planomonospora and Streptosporangium. By comparing taxonomic characteristics, the possibilities that strain IFO 13810 might belong to Microbispora, Planobispora, Planomonospora and Streptosporangium were eliminated, leaving the Actinomadura pussila group and the Microtetraspora glauca group as possibilities. As shown in Table 1, the properties of strain IFO 13810 matched those of the Actinomadura pussila group. Recently it has been proposed that the Actinomadura pussila group and the Microtetraspora glauca group will be combined as Microtetraspora (10). Therefore, we propose that S. hofunensis should be transferred to the genus Microtetraspora.

Streptomyces candidus subsp. azaticus IFO 13803

Streptomyces candidus subsp. azaticus was described by Hata et al. (6) and was represented by a strain which forms branched aerial mycelium, straight sporophore and oval spores with smooth surface. However, the presence of meso- and LL-A2pm was confirmed in this strain, and mannose and galactose in whole-cell hydrolysate were detected. These data showed the strain does not belong to the genus Streptomyces. The phospholipid composition, particularly the presence of phosphatidyl ethanolamine as the characteristic phospholipid, placed the strain in phospholipid pattern group PII. The predominant menaquinone was MK-9 (H_6, H_8). The cellular fatty acids consisted of iso- and anteiso- branched chain and saturated and unsaturated straight chain acids, and presumably, the fatty acid pattern is 2a or 2b (9). The DNA base composition was 72.1 mol% of guanine plus cytosine.

Among actinomycetes, the genera containing both meso- and LL-A2pm in

the cell wall are Kineosporia (8) and Kitasatosporia (16). Concerning the genus Kineosporia, it is characterized by lacking of aerial mycelium and by formation of the motile spore. The strain IFO 13803 formed aerial mycelium and its spore were unmotile. Therefore, it became clearly that this strain did not belong to the genus Kineopsporia. Concerning the genus Kitasatosporia, cell wall type of this genus is X, containing meso- and LL-A2pm, galactose and glycine as minor component. Although glycine were not detected in the strain IFO 13803, many chemotaxonomic characteristics of the strain were similar to those of the genus Kitasatosporia. Further taxonomic studies on strain IFO 13803, therefore, are needed.

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RECLASSIFICATION OF STRAINS OF *FLAVOBACTERIUM* - *CYTOPHAGA* GROUP IN IFO CULTURE COLLECTION

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Summary

The taxonomic positions of 14 strains of *Flavobacterium* or *Cytophaga* species in the IFO culture collection were investigated by chemotaxonomic and phenotypic characterization. "*Flavobacterium autothermophilum*" IFO 14593 was reclassified as "*Pseudomonas hydrogenothermophila*". "*Cytophaga heparina*" IFO 12017 was a new species in the genus *Sphingobacterium*, for which we propose the new combination *Sphingobacterium heparinum*. Other strains were reclassifed at the the genus level; "*Flavobacterium lutescens*" was a species of the genus *Xanthomonas*, *Flavobacterium esteraromaticum* and "*Flavobacterium suaveolens*" were species of the genus *Aureobacterium*, and "*Flavobacterium gasogenes*" and *Flavobacterium* sp. IFO 14590 were species of the genus *Arthrobacter*. *Flavobacterium* sp. IFO 14594 and IFO 14592 were species of the genus *Xanthomonas* and *Microbacterium*. The remaining two strains, "*Flavobacterium dormitator* subsp. *glucanolytica*" IFO 14591 and "*Flavobacterium okeanokoites*" IFO 12536 should be excluded from *Flavobacterium*, but their taxonomic positions are uncertain.

Keywords: Taxonomy of *Flavobacterium*-*Cytophaga* group, *Sphingobacterium*, chemotaxonomy, "*Flavobacterium autothermophilum*", "*Flavobacterium dormitator* subsp. *glucanolytica*", "*Flavobacterium lutescens*", *Flavobacterium*

esteraromaticum, "Flavobacterium suaveolens", "Flavobacterium gasogenes", "Flavobacterium okeanokoites", "Cytophaga heparina", "Cytophaga keratolytica"

The main criteria for identifying the genus Flavobacterium or Cytophaga were the formation of yellow or orange-pigmented colonies on culture media and the ability to produce acid weakly from carbohydrates. From the start, this genus was taxonomically heterogenous, and historically it contained motile or non-motile Gram-negative and Gram-positive strains (9). But, Gram-positive species were excluded from the seventh edition of the Manual (3). In the eighth edition of the Manual, Flavobacterium was further restricted to species not showing gliding or spreading, and was divided into two sections, non-motile low G+C (30-42 mol%) strains and non-motile or motile high G+C (63-70 mol%) strains. Bergey's Manual of Systematic Bacteriology recognizes seven species of Flavobacterium and 20 species of Cytophaga (9, 17). Six additional species of Flavobacterium have subsequently been proposed and officially recognized in the genus Flavobacterium: Flavobacterium branchiophila, Flavobacterium gleum, Flavobacterium indologenes, Flavobacterium mizutae, Flavobacterium thalpophilum and Flavobacterium yabuuchiae (6, 10, 11, 26, 27); but the new genus Sphingobacterium has been proposed by Yabuuchi et al.(27) for the four species, Flavobacterium spiritivorum, Flavobacterium multivorum, Flavobacterium mizutae and Flavobacterium thalpophilum. Chemotaxonomic data derived from G+C contents of DNA, respiratory quinones and cellular fatty acid composition indicated homogeneity in this group. The quinone system and G+C contents of DNA of Flavobacterium and Cytophaga are MK-6 or MK-7 and 31-45 mol%, and cellular fatty acids consist of branched-chain non-polar and hydroxy acids.

The IFO culture collection contains 15 strains named as Flavobacterium or Cytophaga species (IFO List of Cultures, 8th edition, 1988). Recently, Yabuuchi et al.(28) proposed transfering one of them, Flavobacterium capsulatum IFO 12533^{T}, to the new genus Sphingomonas, as Sphingomonas capsulata comb. nov.; but the taxonomic position of the other 14 strains remained uncertain. Therefore, it is necessary to determine the correct taxonomic placement of these strains.

This paper deals with the characterization of 14 strains named as

Flavobacterium or Cytophaga species (12 strains of Flavobacterium and 2 strains of Cytophaga species) in IFO, and discusses their taxonomic position on the basis of such chemotaxonomic characteristics as their quinone system, DNA base composition, and cellular fatty acid composition, together with phenotypic characteristics.

Materials and Methods

Bacterial strains. The strains used are shown in Table 1. For the comparison of taxonomic characteristics, "Pseudomonas hydrogenothermophila" IFO 14978^T (8), and 5 strains belonging to the genus Sphingobacterium (6, 11, 27), Sphingobacterium spiritivorum IFO 14948^T, Sphingobacterium multivorum IFO 14947^T, Sphingobacterium mizutae IFO 14946^T, Sphingobacterium thalpophilum IFO 14963^T, and "Sphingobacterium yabuuchiae" IFO 14975^T were also used.

Determination of phenotypic characters. "Flavobacterium autothermophilum" IFO 14593 and "Pseudomonas hydrogenothermophila" IFO 14978^T were cultured aerobically at 50 C, and the other 14 strains were incubated at 28 C. The PY medium used for cultivation contained 1% Polypepton, 0.2% yeast extract, 0.2% NaCl and 0.5% Difco brain-heart infusion (pH 7.0). DNase activity was tested on DNase test agar (Catalog No. 0632; Difco Lab. U.S.A.). API50 CH carbohydrate substrate strips, API ZYM chromogenic enzyme substrate strips and API 20NE were used to determine the assimilation pattern, the enzymatic activities and phenotypic characteristics, respectively.

Cell wall analysis. Cell walls were prepared by disrupting the cells by ultrasonic oscillator, followed by treatment with SDS, RNase A and pronase E. For determination of sugar composition, purified cell wall was hydrolyzed with 2N HCl at 100 C for 3 hr and analyzed by high performance liquid chromatography (HPLC) as described previously (24). After acid hydrolysis with 6N HCl at 100 C overnight, amino acids were analyzed by HPLC using Wakopak WS-PTC column (Wako Pure Chemical Ltd., Japan).

Cellular lipids and fatty acid analysis. Wet cells harvested from 24-hr culture in PY medium were mixed with 2 ml of a mixture of 12N HCl/methanol (1:5, v/v) and heated at 100 C for 3 hr. Fatty acid methyl esters extracted with n-hexane/diethyl ether (1:1, v/v) were separated by

Table 1. Bacterial strains studied.

Species name	IFO	Other strain designation	Source and comments	Reference
Gram-negative strains				
"F. autothermophilum"	14593^{T}		TH-4; T. Kodama (Univ. Tokyo)	(8)
"F. dormitator subsp. glucanolytica"	14591		FA-5; S. Yamamoto (Kochi Univ.)	(29)
"F. lutescens"	3084	ATCC 25311	Received as "Achromobacter liquidum"	(2)
"F. lutescens"	3085		Received as "Flavobacterium flavescens"	(2)
"F. lutescens"	12997		Received as "Flavobacterium fuscum"	(2)
Flavobacterium sp.	14594		SH-548; K. Aoki (Kobe Univ.)	(1)
"C. heparina"	12017	ATCC 13125,	Received as Flavobacterium heparinum	(22)
"C. keratolytica"	14087		F-179, M. Kitamikado (Kyushu Univ.) Received as "Flavobacterium keratolyticus"	(15)
Gram-positive strains				
F. esteraromaticum	3751^{T}	ATCC 8091		(20)
"F. suaveolens"	3752	ATCC 958		(3)
"F. gasogenes"	12065$_{T}$	OUT 8077		
"F. okeanokoites"	12536^{T}	AJ 2511, CCM 320		(31)
Flavobacterium sp.	14590		KI-72; H. Okada (Osaka Univ.)	(14)
Flavobacterium sp.	14592		M-73; M. Kobayashi (Tohoku Univ.)	(19)

T: type strain.
Abbreviations for culture collections: ATCC, American Type Culture Collection, Rockvile, Maryland, U.S.A.; OUT, Department of Fermentation Technology, Osaka University, Suita, Japan; AJ, Central Research Laboratories, Ajinomoto, Co., Kawasaki, Japan; CCM, Czechoslovak Collection of Microorganisms, J.E. Purkyne University, Brno, CSSR.

thin layer chromatography (TLC) using a solvent system of n-hexane/diethyl ether (1:1, v/v). Non-polar, 2-hydroxy (2-OH) and 3-OH fatty acids were visualized by spraying (dichlorofluorescein in 0.02% ethanol), extracted with diethyl ether, and analyzed by gas-liquid chromatography (GLC). The long-chain base components of the cellular sphingolipids were obtained from acid hydrolysates of dried cells as described by Yano et al.(30). The long-chain base was developed by TLC with a solvent system of chloroform/methanol/water (65:25:4, v/v), derivatized into trimethylsilyl ether, and analyzed by GLC.

Gas-liquid chromatography (GLC). GLC analyses were carried out using a Shimadzu GC-9A gas chromatograph. Columns employed were 10% DEGS (diethyleneglycol succinate)(2 m and 5 m) at 180 C and 3% OV-1 (2 m) at 165 C for fatty acid analysis, and OV-1 (2 m) at 190 C for long-chain base analysis.

DNA base composition. DNAs of test strains were isolated by the method of Saito and Miura (23). G+C contents of DNA were determined by reversed phase HPLC (18, 25) after nuclease P1 and alkaline phosphatase treatment.

DNA/DNA hybridization. DNA/DNA hybridization was carried out by fluorometric hybridization in microdilution wells (7) using biotinylated DNA.

Quinone system. Isoprenoid quinone was extracted twice with chloroform/methanol (2:1, v/v) for 4 hr, and analyzed by HPLC.

Electrophoretic whole-cell protein pattern (13). Cells (0.1 g wet weight) were suspended in 1.0 ml buffer (0.0625 M Tris-HCl, pH 6.8; 2% SDS, 5% 2-mercaptoethanol, 10% glycerol), heated at 100 C for 10 min, then centrifuged at 13,000 x g for 10 min. Supernatant obtained was subjected to electrophoresis. SDS-PAGE was performed in SDS-containing alkaline gel (10%) with a buffer system (3.1 g of Tris, 145.4 g of glycine, 1 g of sodiumdodecyl-sulfate, total to 1000 ml distilled water) at a constant current of 25 mA. After electrophoresis, gels were stained overnight in a stirred solution of Coomassie Blue.

RESULTS AND DISCUSSION

Among the 14 strains named as Flavobacterium or Cytophaga species, 5

Table 2. Chemotaxonomic characteristics of Gram-negative strains.

Species name	IFO	Quinone	G+C (mol%)	Fatty acid[a] non-polar	2-OH	3-OH	Name re-identified
Flavobacterium							
"F. autothermophilum"	14593	Q-8	62.2	16:0, 18:0	-	10:0	"P. hydrogenothermophila"
"F. dormitator subsp. glucanolytica"	14591	Q-8	55.6	i15:0,i16:0	-	i11:0	Unknown
"F. lutescens"	3084	Q-8	63.5	i15:0	-	i11:0,i12:0,i13:0	Xanthomonas sp.
"F. lutescens"	3085	Q-8	65.1	i15:0	-	i11:0,i12:0,i13:0	Xanthomonas sp.
"F. lutescens"	12997	Q-8	65.7	i15:0	-	i11:0,i12:0,i13:0	Xanthomonas sp.
Flavobacterium sp.	14594	Q-8	70.0	ai15:0	-	i11:0,	Xanthomonas sp.
Cytophaga							
"C. heparina"	12017	MK-7	42.3	i15:0,i17:0	i15:0	i15:0, 16:1	S. heparinum
"C. keratolytica"	14087	MK-7	40.8	i15:0,i17:0	i15:0	i15:0, 16:1	Sphingobacterium sp.

a: For fatty acids, the number before the colon indicates the carbon chain length, and the number after the colon indicates the number of double bonds in the chain; ai and i designate anteiso or iso branchings, respectively, at the end of the chain.

Table 3. Chemotaxonomic characteristics of Gram-positive strains.

Species name	IFO	Quinone	G+C (mol%)	Fatty acid[a] (non-polar)	Cell-wall amino acid[b] (molar ratio)	Name re-identified
Flavobacterium						
F. esteraromaticum	3751	MK-12,13	68.8	ai15:0	Gly:Hyg:Hsr:Ala:Orn (2:1:1:1:1)	Aureobacterium sp.
"F. suaveolens"	3752	MK-12,13	67.7	ai15:0	Gly:Hyg:Hsr:Ala:Orn (2:1:1:1:1)	Aureobacterium sp.
"F. gasogenes"	12065	MK-9(H_2)	67.3	ai15:0	Glu:Lys:Ala (1:1:6)	Arthrobacter sp.
"F. okeanokoites"	12536	MK-7,8^2	45.3	i16:0	Glu:Lys:Ala:Asp (1:1:2:1)	Unknown
Flavobacterium sp.	14590	MK-9(H_2)	62.1	ai15:0	Glu:Lys:Ala (1:1:3)	Arthrobacter sp.
Flavobacterium sp.	14592	MK-11,12	72.5	ai15:0	Gly:Hyg:Ala:Lys (2:1:1:2)	Microbacterium sp.

a: See Table 1.
b: Gly, glycine; Hyg, threo-3-hydroxy-glutamic acid; Hsr, homoserine; Ala, alanine; Orn, ornithine; Lys, lysine; Glu, glutamic acid; Asp, aspartic acid.

strains of 3 species of Flabobacterium, 1 strain of Flavobacterium sp. and 2 strains of Cytophaga species were Gram-negative, but the other 6 strains of Flavobacterium were Gram-positive (Table 1). Chemotaxonomic data of these strains are summarized in Tables 2 and 3.

I. Gram-negative strains

"Flavobacterium autothermophilum" IFO 14593

"Flavobacterium autothermophilum" is a thermophilic hydrogen bacterium which was proposed as a new species of Flavobacterium by Goto et al in 1978 (8). They pointed out that "F. autothermophilum" resembles "Pseudomonas hydrogenothermophila" in many characteristics except for motility and the formation of spherical bodies like "microcysts". But we could not detect "microcysts". Concerning its chemotaxonomic features, isoprenoid quinone was Q-8, G+C content of DNA was 62.2 mol%, and major cellular fatty acids were non-polar 16:0 and 18:0 and 3-OH 10:0. 2-OH Fatty acids were not detected. These chemotaxonomic data indicate that strain IFO 14593 cannot be retained in the genus Flavobacterium and should be reclassified in the genus Pseudomonas, where quinone system is Q-8,9 or 10 and G+C content of DNA is 58-70 mol%. "P. hydrogenothermophila" IFO 14978 showed the same chemotaxonomic features as "F. autothermophilum" IFO 14593, the only difference between the two strains being that IFO 14593 is non-motile.

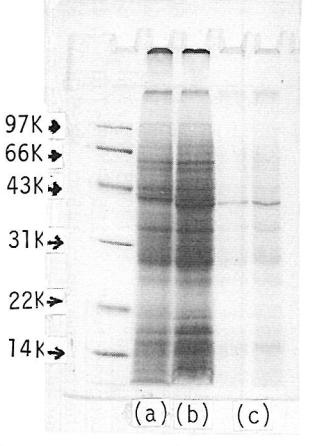

Fig. 1 SDS-polyacrylamide gel electrophoresis of whole-cell protein.
(a) F. autothermophilum IFO 14593
(b) P. hydrogenothermopila IFO 14978
(c) flagella from P. hydrogenothermophila IFO 14978

Comparison of SDS-PAGE patterns of whole cell protein from "F. autothermophilum" IFO 14593 and "P. hydrogenothermophila" IFO 14978 revealed that both strains have the same banding pattern except for the presence of a band corresponding to flagella protein in the strain IFO 14978 (Fig. 1c), which suggested that a lack of flagella protein resulted in strain IFO 14593 being non-motile. As already described by Goto et al. (8), both strains have the same physiological characteristics.

On the basis of these data, together with the description by Goto et al (8), we propose that "F. autothermophilum" IFO 14593 should be reclassified in the genus Pseudomonas as "P. hydrogenothermophila".

"Flavobacterium dormitator subsp. glucanolytica" IFO 14591, "Flavobacterium lutescens" IFO 3084, IFO 3085 and IFO 12997 and Flavobacterium sp. IFO 14594

The quinone systems of these five strains were Q-8, and G+C contents of DNAs were 55.6 mol% for "Flavobacterium dormitator subsp. glucanolytica" IFO 14591, 63.5-65.7 mol% for "Flavobacterium lutescens" IFO 3084, 3085 and 12997, and 70.0 mol% for IFO 14594. The major cellular fatty acids in all strains were iso-branched chain non-polar and 3-hydroxylated acids. Non-polar fatty acids detected were i-15:0 and/or ai-15:0 and i-16:0; and the 3-OH fatty acids detected were 3-OH i-11:0 and/or 3-OH i-12:0, and 3-OH i-13:0. These fatty acid profiles are characteristic of strains of the genera Xanthomonas, Alteromonas and Schwanella among Gram-negative, aerobic rods (16). The quinone system and G+C contents of DNA are known to be Q-8 and 63-71 mol% in Xanthomonas species, Q-8 with MK-8 and methylmenaquinone (MMK)-8, and 38-50 mol% in Alteromonas species, and Q-8 with MK-8 and MMK-8, and 44.7-54.7 mol% in Schwanella species, respectively.

These data indicate that "F. lutescens" IFO 3084, 3085 and 12997, and Flavobacterium sp. IFO 14594 might belong to the genus Xanthomonas. Physiological characteristics of Flavobacterium sp. IFO 14594 tested by the rapid method, namely, the API 20NE system, coincide with those of Xanthomonas maltophilia (21).

The physiological characteristics of "F. dormitator subsp. glucanolytica" IFO 14591 by API 20NE did not coincide with those of Xanthomonas maltophilia or other species of this genus, and its DNA contains G+C 55.6 mol% is below the range of the genus Xanthomonas. Therefore, the taxonomic position of this strain remains uncertain.

"Cytophaga heparina" IFO 12017 and "Cytophaga keratolytica" IFO 14087

"Cytophaga heparina" is an organism described in 1956 by Payza and Korn (22) as Flavobacterium heparinum and then reclassified as "Cytophaga heparina" by Christensen (4) for the reason that it had been shown to glide. On the other hand, "Cytophaga keratolytica" IFO 14087 was first deposited under the name of "Flavobacterium keratolyticus" by Kitamikado and Ito (15), then reclassified as a species of the genus Cytophaga by Imai (12), despite of the absence of obvious gliding movement. In Bergey's Manual of Systematic Bacteriology Vol. 3 (17), these two strains are now excluded from the genus Cytophaga by reason of their non-gliding, and their taxonomic positions are uncertain.

G+C content of DNA was 42.3 mol% for "C. heparina" IFO 12017 and 40.8% for "C. keratolytica" IFO 14087, and both species contained MK-7 as the major isoprenoid quinone. Branched chain non-polar acid i-15:0, and the hydroxy acids 2-OH i-15:0, 3-OH i-15:0, and 3-OH i-17:0, were detected as cellular fatty acids in these strains. Both strains contained relatively large amounts of long-chain base, which was identified as dihydrosphingosin (d17:0br)(28) using gas-liquid chromatography/mass spectrometry (GLC/MS), and is characteristic of the genus Sphingobacterium. On the basis of these data, these two strains should be reclassified as species of Sphingobacterium. S. multivorum, S. spiritivorum, S. mizutae, S. thalpophilum and "S. yabuuchiae" are recognized in the genus Sphingobacterium. Comparison of physiological, biochemical and chemotaxonomic characteristics and DNA/DNA homology tests of these two strains revealed that "C. heparina" should be reclassified in the genus Sphingobacterium as S. heparinum. Precise data will be published elsewhere (Takeuchi and Yokota, in preparation). "C. keratolytica" IFO 14087 was suggested to be close to S. multivorum, but further work is necessary to determine its exact taxonomic position .

II. Gram-positive strains

The following Flavobacterium strains are Gram-positive, aerobic, motile or non-motile rods, and therefore, they could be excluded from the genus Flavobacterium.

Flavobacterium esteraromaticum IFO 3751 and "Flavobacterium suaveolens" IFO 3752

Diamino acid in the cell wall peptidoglycan of Flavobacterium

esteraromaticum IFO 3751 and "Flavobacterium suaveolens" IFO 3752 was ornithine, and the peptidoglycans consisted of glycine, threo-3-hydroxy-glutamic acid, homoserine, alanine, and ornithine (2:1:1:1:1). Galactose was the cell wall sugar. Neither strain contained mycolic acids. The major fatty acids were non-polar ai-15:0 and ai-17:0. Major menaquinones were MK-12,13. G+C content of DNA was 67.7-68.8 mol%. These chemotaxonomic data indicate that IFO 3751 and IFO 3752 might belong to the genus Aureobacterium (5, 16). Studies on their proper taxonomic position are now in progress.

"Flavobacterium gasogenes" IFO 12065 and Flavobacterium sp. IFO 14590

Cell wall peptidoglycan of "Flavobacterium gasogenes" IFO 12065 contained lysine as diamino acid, together with glutamic acid and alanine in the molar ratio of 1:1:6. Galactose was the cell wall sugar. Major fatty acids were non-polar ai-15:0, i-15:0 and ai-17:0. Major menaquinone was MK-9(H_2). G+C content of DNA was 67.3 mol%. These chemotaxonomic results agree with that of the genus Arthrobacter (16). The molar ratio of alanine to lysine was similar to that of Arthrobacter ramosus (16). Further investigation is necessary to clarify the taxonomic position of "F. gasogenes" IFO 12065.

Diamino acid in the cell-wall peptidoglycan of Flavobacterium sp. IFO 14590 was lysine, and the molar ratio of the amino acids in cell walls, glutamic acid, lysine and alanine, was 1:1:3. The major fatty acids were non-polar ai-15:0 and a small amount of ai-17:0. The major menaquinone was MK-9(H_2), and the G+C content of DNA was 62.1 mol%. These chemotaxonomic data indicate that Flavobacterium sp. IFO 14590 is a species of the genus Arthrobacter (16). The molar ratio of alanine to lysine was similar to that of Arthrobacter pascens, but further investigation is necessary to clarify the taxonomic position.

"Flavobacterium okeanokoites" IFO 12536

Diamino acid in the cell-wall peptidoglycan of "Flavobacterium okeanokoites" IFO 12536 was lysine, and the molar ratio of the amino acids in cell walls, lysine, glutamic acid, alanine and aspartic acid, was 1:1:2:1. The major fatty acids were non-polar i-16:0 and a small amount of i-14:0 and i-15:0. Equal amounts of MK-7 and 8 were contained as major menaquinone. G+C content of DNA was 45.3 mol%. Although these chemo-taxonomic characteristics of the strain IFO 12536 are within the range specified for the genus Bacillus, formation of spores by the strain was

not confirmed in the present study. Further studies are necessary to determine its proper taxonomic position.

Flavobacterium sp. IFO 14592

Diamino acid in the cell-wall peptidoglycan of IFO 14592 was lysine, and the molar ratio of the amino acids in cell walls, glycine, threo-3-hydroxy-glutamic acid, alanine and lysine, was 2:1:1:2. The major fatty acids consisted of non-polar ai-15:0 and a small amount of i-16:0. The major menaquinones were MK-11 and 12, and G+C content of DNA was 72.5 mol%. These chemotaxonomic data characterize the genus Microbacterium (16), and therefore Flavobacterium sp. IFO 14592 (M-73) should be transferred to the genus Microbacterium (16).

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COENZYME Q SYSTEMS IN THE GENUS COROLLOSPORA AND ALLIED MARINE FUNGI

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Summary

The ubiquinone systems of 13 species of the genus Corollospora and 3 species of allied marine fungi were determined by high performance liquid chromatography and found to contain ubiquinone Q-10, Q-10(H_2), and Q-10(H_4). Eleven species of Corollospora have Q-10(H_2) as the major ubiquinone, whereas two species of the genus have mainly Q-10(H_4). By considering not only the major ubiquinone but also the minor ones, ubiquinone systems were found to be useful for the taxonomy of the genus, because the ubiquinone data supported well the species classifcation proposed on the basis of morphology.

Keywords: ubiquinone, Corollospora, marine fungi.

The isoprene side chains of ubiquinone molecules have been investigated as a chemotaxonomic characteristic that has been considered useful for the taxonomy of bacteria (13), yeasts (12, 14, 15, 16, 17), and fungi (4, 9). The distribution of ubiquinone systems in certain fungal groups has been investigated and applied to the taxonomy of smut and rust fungi (10), Aspergillus and its teleomorphs (3), and Penicillium and related genera (2). Takachi et al. (11) examined the ubiquinone systems in marine fungi including ascomycetes, basidiomycetes, and deuteromycetes and found that the types of isoprene side chain of ubiquinones of marine

fungi corresponded with those of the terrestrial fungi that are classified into the same taxonomic group as the marine species.

In the genus Corollospora (Halosphaeriaceae, Sphaeriales, Pyrenomycetes, Ascomycotina), 14 species have so far been described from marine environments, especially from sandy beach (1, 5). Some of them are known to have anamorph states. Ascospore morphology is mainly used for classification at the species level, but some species whose ascospores are similar in shape may be difficult to identify. For precise identification of these species, it is necessary to compare other characteristics such as the peridial wall structure of the ascocarp (8), anamorphs, or chemotaxonomical characteristics. In this study, I investigated a distribution of ubiquinones in the genus Corollospora and allied fungi. The possibility of using the data as an aid to identification or classification of the species will be discussed.

Materials and Methods

Strains. The strains used here are listed in Table 1. The ubiquinone systems of 25 strains in 13 species of the genus Corollospora, and 2 strains in 1 anamorphic species and 4 strains in 2 species of allied fungi were investigated.

Cultivation. All strains were cultured on seawater starch agar medium (SWSA: 1% soluble starch, 0.1% soytone, 1.5% agar, in 20% salinity artificial seawater, pH 8.2) in tubes for two weeks. Agar medium containing mycelium was broken into small pieces with a sterilized stick. The agar pieces were inoculated in 5 ml of a liquid medium (SWS: as SWSA but without agar) in a tube. Four tubes for each strain were incubated on a shaker for one week at 28 C. The grown mycelium was used as a "seed" for the cultivation in 1-liter Erlenmeyer flasks containing 250 ml of SWS. Four flasks for each strain were incubated on a rotary shaker for one to three weeks at 28 C. The grown mycelial balls were washed in distilled water and freeze-dried for preservation before extracting ubiquinones.

Extraction and purification of ubiquinones. Dried mycelia were pow-

dered in a mortar. About 1 g of the mycelium powder was suspended in 240 ml of chloroforme/methanol (2:1, v/v). Ubiquinones were extracted for 8 hr under continuous stirring. After separating the mycelial powder by filtration, secondary extraction were done with fresh solvent for 8 hr. After the mycelial powder had been removed by filtration, the two extracts were combined and evaporated to dryness in a rotary evaporater. The dried extract was dissolved in ca. 1 ml of acetone and the solution was used for purifying ubiquinones under thin-layer chromatography (TLC). TLC was performed using 0.5 mm layers of Merck Kiesel-gel HF254 and benzene as a developing solvent. Vitamin-K_1 (SIGMA) and ubiqinone-10 (SIGMA) were used as standards. Ubiquinones were detected under ultraviolet light. The silica gel containing ubiquinones was scratched from the plates and the ubiquinones were eluted with acetone. The acetone was evaporated off, and the dried materials were dissolved in ca. 0.5 ml of ethanol. The ethanol solution was preserved at -20 C before analysis by high performance liquid chromatography (HPLC).

Analysis of ubiquinones. HPLC was carried out on a Shimadzu Liquid Chromatograph LC-6AD with a ZORBAX (4.6 mm x 15 cm) column. Ubiquinones were detectecd by their absorbance at 275 nm by means of a Shimadzu Spectrophotometric Detector SPD-6A. Samples were eluted with methanol/isopropyl ether (4:1, v/v) at 1 ml/min at 30 C. The elution time and area of each peak of ubiquinone were calculated with Shimadzu Chromatopac C-R3A. Authentic samples of ubiquinones Q-6, 7, 8, 9, 10, 10 (H_2), 10 (H_4) were used as standards.

Results and Discussion

The distribution of ubiquinones in Corollospora and allied species is shown in Table 1, where all ubiquinones that constitute more than 1% of a total amount of ubiquinones are listed.

Ubiquinone Q-10 and its hydrogenated derivatives Q-10 (H_2) and Q-10 (H_4) were found in these fungi. This pattern corresponds to those of other pyrenomycetous terrestrial fungi (4, 9). Of 13 species of Corollospora, the following 11 species have Q-10 (H_2) as the major ubiquinone (74-100%): C. angusta, C. cinnamomea, C. colossa, C. filiformis, C. fusca, C.

Table 1. Distribution of ubiquinones in Corollospora and allied species.

Species	IFO No.	Ubiquinones*		
		Q-10	Q-10 (H_2)	Q-10 (H_4)
C. angusta Nakagiri & Tokura	32100		100	
	32101		100	
C. cinnamomea Koch	32125		96	4
	32126		97	3
C. colossa Nakagiri & Tokura	32103		100	
	32105		100	
C. filiformis Nakagiri	32106	12	86	2
C. fusca Nakagiri & Tokura	32107	2	98	
	32108	1	97	2
C. gracilis Nakagiri & Tokura	32110	1	99	
	32111		100	
C. intermedia I. Schmidt	32119	1	99	
	32120	1	99	
C. lacera (Linder) Kohlm.	32121	2	40	58
	32122	1	28	71
C. luteola Nakagiri & Tubaki	31315	4	95	1
	31316	5	95	
C. maritima Werdermann	32117	19	74	7
	32118	24	76	
C. pseudopulchella Nakagiri & Tokura	32112	1	95	4
	32113	2	80	18
C. pulchella Kohlm., Schmidt, & Nair	32123	1	7	92
	32124	1	6	93
C. quinqueseptata Nakagiri	32114	4	96	
	32116	1	96	3
Clavatospora bulbosa (Anastasiou) Nakagiri & Tubaki	(AN-847)		5	95
	(ATCC 14677)		3	97
Sigmoidea marina Haythorn & Jones	32159	1	97	2
	32160		100	
Varicosporina ramulosa Meyers & Kohlm.	32163		100	
	(AN-1017)		100	

* Numbers indicate percentages of total ubiquinones.

gracilis, C. intermedia, C. luteola, C. maritima, C. pseudopulchella, and C. quinqueseptata. Q-10(H_4) is the main ubiquinone in C. lacera (58-71%) and C. pulchella (92-93%). Q-10 is a minor ubiquinone in the genus, whereas relatively higher contents were observed in C. maritima (19-24%) and C. filiformis (12%).

Classification of these species based on morphology presents the following problems. The two distinct species C. maritima and C. gracilis have been often confused because their ascospores are similar in shape and size, though they are distinguishable by ascospore diameter, colony colour, presence or absence of chlamydospores, etc. (5). The data of ubiquinones showed a clear distinction between the two species (Table 2, A). The three species C. colossa, C. lacera, and C. quinqueseptata are distinguished by ascospore size and septation, peridial wall structure of ascocarps, etc. (5), but they have been misidentified by many authors. The distinction of these three species was suported by the ubiquinone data, which were distinctive for C. lacera against C. colossa or C. quinqueseptata (Table 2, B). Another example is that of C. pulchella and C. pseudopulchella, which are very similar in ascospore morphology, but distinguishable by the surface structure of the ascocarp wall and anamor-

Table 2. Comparison of ubiquinones in morphologically similar species of Corollospora.

(A)

Ubiquinones	C. maritima	C. gracilis
Q-10	19-24*	0-1
Q-10(H_2)	74-76	99-100
Q-10(H_4)	0-7	

(B)

Ubiquinones	C. colossa	C. lacera	C. quinqueseptata
Q-10		1-2	1-4
Q-10(H_2)	100	28-40	96
Q-10(H_4)		58-71	0-3

(C)

Ubiquinones	C. pulchella	C. pseudopulchella
Q-10	1	1-2
Q-10(H_2)	6-7	96
Q-10(H_4)	92-93	0-3

* Numbers indicate percentages of total ubiquinones.

phic state (5). The ubiquinone systems of these species are completely different, as C. pulchella has Q-10 (H_4) as the major ubiquinone, while C. pseudopulchella mainly possesses Q-10 (H_2) (Table. 2, C).

From the viewpoint of teleomorph-anamorph relationships, the ubiquinone systems of teleomorphs, anamorphs, and related species are summarized in Table 3, A-C. Corollospora pulchella is known to have an anamorphic state in Clavatospora bulbosa (7). The strains of C. pulchella, IFO 32123, 32124, are known to have both morphs in the life cycle (holomorphic), since they were isolated as an ascospore and produced conidia of Clav. bulbosa in culture. On the other hand, two strains of Clav. bulbosa, AN-847 and ATCC 14677, were isolated in the anamorphic state, and it has not been confirmed whether they have lost the ability of sexual reproduction (anamorphic). The ubiquinone systems of these four strains are similar, but a slight difference is seen between the holomorphic strains and anamorphic (?) strains (Table. 3, A). However, it is uncertain whether this difference corresponds to the type of life cycle of the strains. Two strains of C. luteola, IFO 31315, 31316, are holomorphic and have Sigmoidea luteola as their anamorph. Sigmoidea marina is a distinct species, but similar to S. luteola in conidium morphology (6).

Table 3. Comparison of ubiquinones in holomorphic, anamorphic, and allied species of Corollospora.

(A) Ubiquinones	C. pulchella - Clav. bulbosa	Clav. bulbosa
Q-10	1*	
Q-10 (H_2)	6-7	3-5
Q-10 (H_4)	92-93	95-97

(B) Ubiquinones	C. luteola - S. luteola	S. marina
Q-10	4-5	0-1
Q-10 (H_2)	95	97-100
Q-10 (H_4)	0-1	0-2

(C) Ubiquinones	C. intermedia - V. prolifera	V. ramulosa
Q-10	1	
Q-10 (H_2)	99	100
Q-10 (H_4)		

* Numbers indicate percentages of total ubiquinones.

No teleomorph is known so far. The same situation was observed in the relation between C. intermedia-Varicosporina prolifera and an allied species, V. ramulosa. The ubiquinone data show that there is a slight difference between holomorphic strains and allied species in the former case (Table. 3, B), and that similar patterns are found in both species in the latter case (Table. 3C).

From these observations, the ubiquinone system was found to be a useful character supporting morphology-based classification at species level in the genus Corollospora. In this study, I observed the minor as well as the major ubiquinones and found that they are more informative for species classification within a genus than the major ubiquinone alone, which has been treated as a sole character in many previous studies. Further research into the relationships between teleomorph-anamorph fungi and their allied species may reveal that the ubiquinone data are informative in investigating their phylogenetical relationship.

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CRYOPRESERVATION OF OOMYCETOUS FUNGI IN LIQUID NITROGEN

TADASHI NISHII and AKIRA NAKAGIRI

Summary

Liquid nitrogen storage was examined as a means of safe, long-term preservation of oomycetous fungi in the Institute for Fermentation, Osaka (IFO). Agar discs with mycelium of the fungal culture were frozen at a constant cooling rate in a programmable freezer. In a preliminary study, eight strains of Phytophthora and two strains of Pythium were successfully preserved for two years by using 10% glycerol or 10% dimethylsulfoxide (DMSO) as a cryoprotectant. The viability of 171 strains of oomycetous fungi preserved in liquid nitrogen was examined. Immediately after freezing, 165 strains were recovered. After six months storage, 164 strains survived. The eight strains that were lost or partly survived in the first recovery test (four strains each of Phytophthora and Pythium) were used to examine the methods for agar disc preparation and thawing. It was found that the agar discs derived from the central part of a precultured fungal colony were stored more successfully than those from edge of the colony. Thawing the frozen agar discs at 30 C for 5 min yielded better results than thawing at 40 C for 3 min.

Keywords: cryopreservation, liquid nitrogen, Oomycetes.

Oomycetes (Mastigomycotina) have been preserved by subculturing (1, 6, 12), storing in water (6) or in liquid paraffin (1), or by freezing in an ultra-low freezer at -80 C (11, 13) or in liquid nitrogen at -196 C (1, 2, 3, 4, 5, 8, 10, 11). The Institute for Fermentation, Osaka (IFO) maintains oomycetous cultures mainly by subculturing, but also by storage in liquid paraffin and freezing at -80 C. However, about 10% of strains are not successfully recovered at each routine subculturing at three-month intervals. Freezing storage at -80 C has not given satisfactory results (13). Storage in water or liquid paraffin involves higher risk of contamination by other organisms or changes in fungal properties during storage. For safe, long-term preservation, the cryopreservation of filamentous fungi in liquid nitrogen has been widely employed. We examined the application of this method to the oomycetous strains deposited in IFO.

Materials and Methods

Three experiments were carried out.

Experiment 1. Eight strains of Phytophthora and two of Pythium (Table 1) were cultured on medium no. 1 (see below) at 24 C for 4 to 12 days. Agar discs containing mycelium were removed from the edge of the fungal colony with plastic tubes 8 mm in diameter. Two agar discs were soaked in 1 ml of each of the following three cryoprotectants in a cryotube (Nunc, 3-66656): 10% glycerol, 10% dimethylsulfoxide (DMSO), and 10% polyethylene glycol (PEG). Eighteen tubes were prepared for each strain (six for each cryoprotectant). After precooling at 5 C for 30 min, the tubes were frozen in a programmable freezer (Taiyo Sanso Ltd., CM-2) at the cooling rate 1 C/min until -40 C and at 2 C/min from -40 to -80 C (Fig. 1). This programmable freezer monitors temperature both in one freezing tube and the freezing chamber, and controls the temperature according to the cooling program by automatically supplying liquid nitrogen to the freezing chamber. This system is possibly able to reduce the injurious effect on frozen cultures caused by latent heat generated at the freezing point. Frozen tubes were submerged in liquid nitrogen and stored for up to 2 years. Recovery tests were carried out at immediately

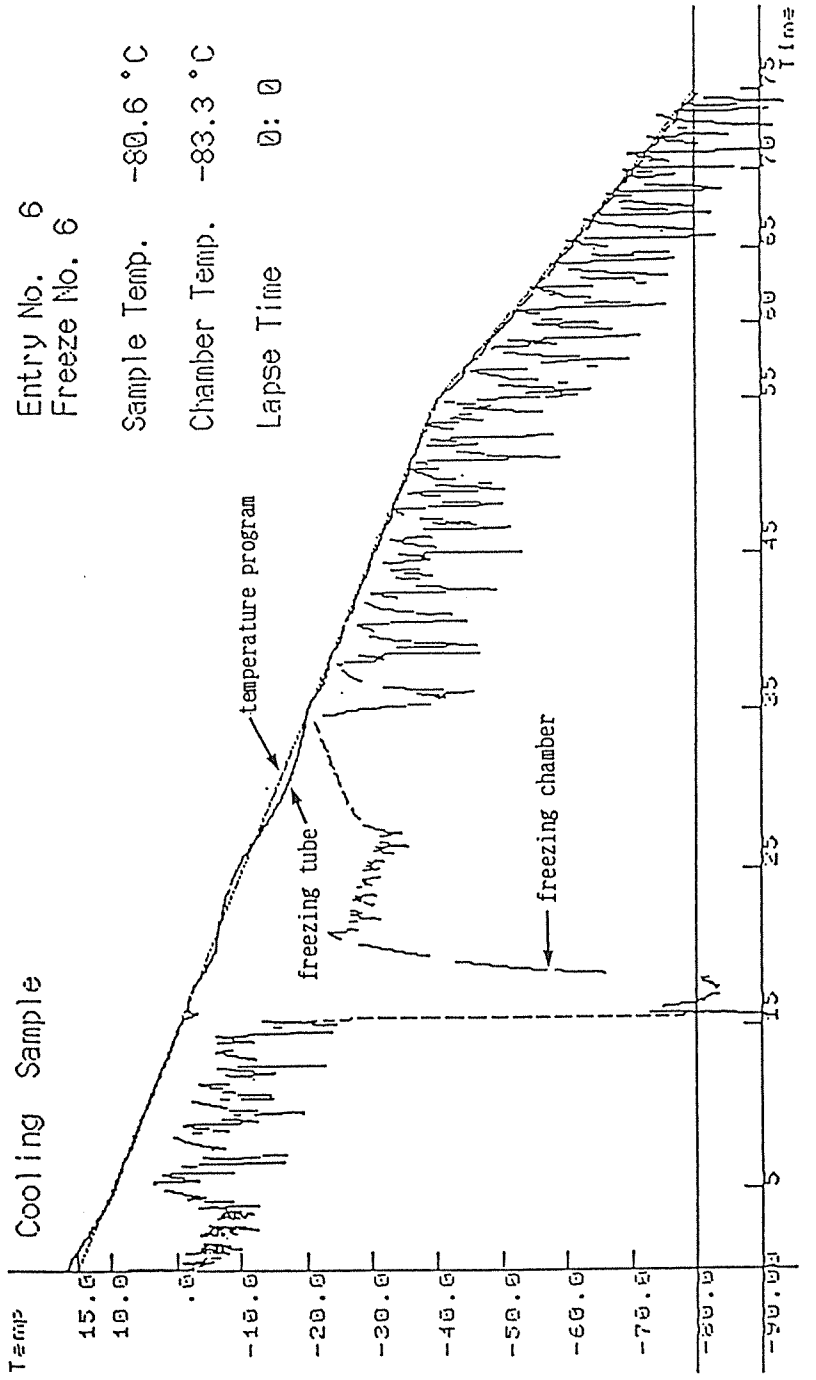

Fig. 1. Temperature change during freezing with programing freezer.

after freezing, and 1 month, 6 months, 12 months, and 24 months after freezing. For thawing the cultures, the lower half of the cryotubes were immersed in water at 40 C and agitated for 3 min. The thawed agar disks were incubated on agar plates of medium no. 1 at 24 C for one week.

Experiment 2. One hundred and seventy-one strains of oomycetous fungi preserved in IFO (24 species, 88 strains of Phytophthora; 26 species, 79 strains of Pythium; 1 species, 1 strain of Saprolegnia; 1 species, 3 strains of Aphanomyces) (Table 2) were examined for their suitability for cryopreservation in liquid nitrogen. All strains were precultured on the appropriate agar media at a suitable temperature (see Table 2 and below for the medium contents). Agar discs for storage were prepared in the same manner as in Exp. 1. Two discs were put in a cryotube containing 0.7 ml of 10% glycerol. Four tubes were prepared for each strain. After freezing with the programmable freezer, the tubes were stored in liquid nitrogen. Survival was examined immediately after freezing and at six months after freezing.

Experiment 3. The eight strains (four strains of each Phytophthora and Pythium) which had been lost completely or partly survived in the first recovery test at Exp. 2 and three strains of Aphanomyces iridis were used for the following examination. From the precultured colony, ten agar discs each were removed from the edge and the central part of the colony and used to prepare five tubes for each strain. Freezing was carried out in the same manner as in Exp. 2. Frozen discs were recovered three days after freezing. In the recovery test, the tubes were thawed either at 40 C for 3 min or at 30 C for 5 min.

The agar media used for preculturing fungal strains and for recovery tests were as follows:

Medium No. 1. Potato sucrose agar

200 g potato, 20 g sucrose, 20 g agar, 1000 ml distilled water, pH 5.6.

Medium No. 8. Oatmeal agar

50 g oatmeal, 20 g agar, 1000 ml distilled water

Medium No. 12. Semi-solid CMSA

20 g cornmeal, 3 g agar, 1000 ml half-strength seawater, pH 7.0-

7.5.

Medium No. 14. Corn fishmeal extract agar

20 g corn fishmeal mixture (feed for birds), 20 g agar, 1000 ml distilled water, pH 6.5.

Preparation of these media is detailed in the IFO List of Cultures, Appendix 2.

Results

In Exp. 1, all ten strains survived two years of storage in liquid nitrogen when 10% glycerol or 10% DMSO was used as cryoprotectant, whereas when 10% PEG was used, six of ten strains were not recovered even immediately after freezing (Table 1). Accordingly, Exp. 2 and 3 were carried out with 10% glycerol as cryoprotectant. In Exp. 2, the viability of 171 strains of the oomycetous fungi was examined immediately after freezing and after six months' storage (Table 2). In the first recovery test, six strains failed to survive, namely, Phy. capsici IFO 9752, Phy. palmivora IFO 30812, Phy. vesicula IFO 32216, Py. zingiberum IFO 30818, Py. myriotylum IFO 31022, and Py. periplocum IFO 31933; and only one each of the two agar discs of Phytophthora sp. IFO 30635 and Py. iwayamai IFO 31991 survived. After six months' storage, four strains were completely dead, namely, Phytophthora sp. IFO 30635, Py. porphyrae IFO 30347, 30801, and Py. iwayamai IFO 31991; and only one of the two agar discs each of Phy. vignae IFO 30473, Py. porphyrae IFO 30800, Py. graminicola IFO 31997, and 31998 survived (Table 2). In these tests, Phytophthora sp. IFO 30635 and Py. iwayamai IFO 31991 showed comparatively low viability in liquid nitrogen storage, while there was no strain that was completely dead in the both recovery tests. From the results of Exp. 3 (Table 3), it was found that thawing at 30 C gave higher recovery in most strains than thawing at 40 C. In addition, discs derived from the central part of the colony showed higher survival rate than those from the edge of the colony in most cases. The differences in survival rate were more apparent in the cases of Phy. palmivora IFO 30812 thawed at 40 C, Phytophthora sp. IFO 30635 thawed at 30 C, and the three strains Pythium IFO 31022, 31933, and 30818 thawed at 30 C (Table 3). In contrast, for three strains of A. iridis, IFO 31934, 31935, and 31936, the agar discs from

Table 1. Viability of *Phytophthora* and *Pythium* strains stored in liquid nitrogen for two years using different cryoprotectants.

Species	IFO No.	10% Glycerol						10% Dimethylsulfoxide (DMSO)						10% Polyethylene Glycol (PEG)					
		0M*	1M	3M	6M	12M	24M	0M*	1M	3M	6M	12M	24M	0M*	1M	3M	6M	12M	24M
Phytophthora cactorum	30474	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phy. capsici	30697	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Phy. combivora	30472	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Phy. nicotianae var. *nicotianae*	4873	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phy. nicotianae var. *parasitica*	30595	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phy. palmivora	9755	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
Phy. porri	30417	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Phy. vignae	30613	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Pythium butleri	31214	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Py. zingiberum	30817	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+

+ : viable. - : non-viable. 0M* : immediately after freezing.

Table 2. Viability of oomycetous fungi immediately after freezing and after six months' storange in liquid nitrogen.

Species	IFO No.	0 M*	6 M**	Medium No.	Temp.(C)
Phytophthora cactorum (Lebert & Cohn) Schroter	30474	+ +	+ +	1	24
Phy. cactorum	31084	+ +	+ +	1	24
Phy. cactorum	31151	+ +	+ +	1	24
Phy. cactorum	32191	+ +	+ +	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	+ +	+ +	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	+ +	+ +	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	+ +	+ +	1	24
Phy. megasperma	32174	+ +	+ +	1	24
Phy. megasperma	32175	+ +	+ +	1	24
Phy. megasperma	32176	+ +	+ +	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 2. (continued)

Species	IFO No.	0 M*	6 M**	Medium 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	- -	+ +	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. vesicura Anastasiou & Churchland	32216	- -	+ +	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 2. (continued)

Species	IFO No.	0 M*	6 M**	Medium No.	Temp.(C)
Pythium afertile Kanouse & Humphrey	32195	+ +	+ +	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	+ +	+ +	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	+ -	- -	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	- -	+ +	1	24
Py. porphyrae Takahashi & Sasaki apud Takahashi et al.	30347	+ +	- -	13	24
Py. porphyrae	30800	+ +	+ -	13	24
Py. porphyrae	30801	+ +	- -	13	24
Py. sp.	32197	+ +	+ +	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 2. (continued)

Species	IFO No.	0 M*	6 M**	Medium 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	+ +	+ +	8	24
Py. spinosum	32213	+ +	+ +	8	24
Py. spinosum	32214	+ +	+ +	8	24
Py. sylvaticum Campbell & Hendrix	31942	+ +	+ +	1	24
Py. sylvaticum	31943	+ +	+ +	1	24
Py. sylvaticum	32198	+ +	+ +	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	+ +	+ +	8	24
Py. ultimum	7216	+ +	+ +	8	24
Py. ultimum	7217	+ +	+ +	8	24
Py. ultimum Trow var. ultimum	32210	+ +	+ +	8	24
Py. ultimum	32211	+ +	+ +	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	- -	+ +	1	34
Saprolegnia parasitica Coker	8978	+ +	+ +	1	24
Aphanomyces iridis Ichitani & Kodama	31934	+ +		14	24
Ap. iridis	31935	+ +		14	24
Ap. iridis	31936	+ +		14	24

+ : viable
- : non-viable (Each sign indicates the viability of the each one of two agar discs)
* : immediately after freezing
** : six months after freezing

the edge of the colony recovered well, while all of those from the central part of the colony did not.

Discussion

More than 97% of 168 stains of oomycetous fungi were successful after six months storage in liquid nitrogen (Exp. 2). This suggests that cryopreservation in liquid nitrogen is equally as effective for oomycetous fungi as for other fungal groups. Of the unsuccessful strains, some survived freezing but not six months' storage, while others that had shown no survival immediately after freezing recovered well after six months' storage. The former finding suggests that the storage period may affect the viability of frozen cultures; and this possibility will be examined through recovery tests at 12 months and 24 months after freezing. The latter phenomenon suggests that the conditions of the agar discs for freezing, such as the age of the mycelium in the agar disc, the extent of hyphal septation, the presence or absence of oospores or other resting spores, etc., may affect their survival rate. From this point of view, we examined the survival of the agar discs of diffent ages in Exp. 3. In the strains of Phytophthora and Pythium, the agar discs from the central part of the colony (older mycelium) achieved higher survival than those from the edge of the colony (younger mycelium). This phenomenon may correspond to the fact that bacterial cells in the stationary phase are generally more tolerant to freezing or freeze-drying than those in the logarithmic growth phase (9). Aphanomyces iridis, however, showed the opposite result. We supposed more oospores or chlamydospores were produced in older mycelia of Phytophthora and Pythium and in younger mycelia of Aphanomyces than younger and older ones, respectively, but microscopically no clear difference was observed between them. The viability of the preserved cultures seems to depend significantly on the conditions of the agar disc, and requirements may differ from species to species. When preserving a new strain in liquid nitrogen, it will be necessary to examine beforehand the viability of agar discs derived from different parts of the precultured fungal colony, or to neutralize the agar disc effect by, for example, mixing agar discs from different parts of the colony for preservation.

Table 3. Viability of young and old agar discs of some Oomycetes after thawing at different temperaturs.

Species	IFO No.	Thawing at 40 C for 3 min, Agar discs derived from Colony edge					Thawing at 40 C for 3 min, Agar discs derived from Colony center					Thawing at 30 C for 5 min, Agar discs derived from Colony edge					Thawing at 30 C for 5 min, Agar discs derived from Colony center				
Phytophthora capsici	9752	--	--	--	++	+-	--	--	+-	+-	--	+-	+-	++	++	++	++	+-	++	++	++
Phy. palmivora	30812	--	--	--	--	--	++	+-	+-	+-	+-	++	++	++	++	++	++	++	++	++	++
Phy. sp.	30635	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+-	--	+-	+-	+-
Phy. vesicula	32216	++	--	++	++	--	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Pythium iwayamai	31991	++	+-	++	+-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Py. myriotylum	31022	--	--	+-	+-	--	--	--	--	--	+-	++	++	--	+-	--	++	++	++	++	++
Py. periplocum	31933	--	--	--	--	--	++	--	+-	+-	--	--	--	--	--	--	++	++	++	++	++
Py. zingiberum	30818	+-	+-	--	--	--	--	+-	++	--	--	--	--	--	--	--	++	++	++	++	++
Ahanomyces iridis	31934											++	++	++	++	++	--	--	--	--	--
Ap. iridis	31935											++	++	++	++	++	--	--	--	--	--
Ap. iridis	31936											++	++	++	++	++	--	--	--	--	--

+ : viable. - : non-viable.

The cooling rate for freezing of cultures was set at 1 C/min until -40 C and 2 C/min from -40 to -80 by using the programmable freezer. Optimal cooling rates for the various fungal groups are known to have a wide range. Morris et al. (7) reported the highest recovery after cooling at 5 to 10 C/min with several oomycete strains. However, their study was not conducted using a programmable freezer as was used in this study, so reexamination is necessary. Practically, the cooling rate permitting all strains concerned to survive should be determined before storing. As a cryoprotectant, we found 10% glycerol and 10% DMSO to be effective for liquid nitorgen storage. Further investigation of their optimal concentrations is necessary. As to the thawing procedure, immersing a frozen tube in water at 35 to 40 C has been employed widely (7, 10, 11). However, this study revealed that a temperature of 30 C for 5 min gave better survival than 40 C. Although this phenomenon may depend on the fungus concerned, thawing at the lower temperature could be used for the oomycetous fungi. Further improvement on various points is necessary for the safe, long-term preservation of a wide range of oomycetous cultures.

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FROZEN STORAGE OF FUNGAL CULTURES DEPOSITED IN THE IFO CULTURE COLLECTION

TADAYOSHI ITO

Summary

Of a total of 313 strains of fungal cultures deposited in the Institute for Fermentation, Osaka (IFO) between 1985 and 1986 tested, 311 strains survived after 4 or 5 years of storage at -80 C and only 2 strains of species of Ascomycotina did not survive. The percentage of viability in this experiment was 99.4%. It was confirmed that the use of an electric refrigerator at -80 C was effective for the long-term preservation of fungal cultures in Ascomycotina and Deuteromycotina.

Keywords: deposited strains in IFO, storage at -80 C, effective preservation method.

Many methods have been developed and recommended for the stabe, long-term preservation of fungal cultures. In paticlar, as shown by Carmichael (1, 2), Hamilton and Weaver (3), Ito and Yokoyama (5, 6), Kramer and Mix (7), and Yokoyama and Ito (8, 9), the preservation of fungi by freezing in an electric refrigerator is simple, practical and economical.

Based on the results of effectiveness of fungal preservation in our preliminary test and previous papers (5, 6, 8, 9), this freezing method was adapted for the preservation of all of the fungal cultures in IFO. In particular, cultures including non-sporulating strains of Ascomycotina and Deuteromycotina deposited in IFO since 1982 have been preserved by

this method.

The purpose of the present study is to confirm the viability of strains frozen between 1985 and 1986.

Materials and Methods

A total of 313 strains of Ascomycotina and Deuteromycotina were tested. These strains were stored at -80 C as soon as possible after being deposited in the IFO Culture Collection of fungi between 1985 and 1986. The procedures for the growth of cultures, thawing and incubation of frozen mycelial disks were exactly as described in our previous reports (5, 8, 9).

Results and Discussion

Table 1 shows the number of strains used, the number of viable strains and the proportion of survivors (%) of these strains, which are grouped the taxonomic rank by Ainsworth & Bisby's Dictionary of Fungi (4). Table 2 shows the number of strains tested and the number of viable strains for each species.

Of a total of 313 strains used in this experiment belong to 92 species including varieties, in 51 genera of Ascomycotina, and 132 species in 54 genera of Deuteromycotina. Among these strains, 184 strains of Ascomycotina including 7 strains of Dothideales, 2 of Endomycetales, 85 of Eurotiales, 48 of Gymnoascales, 9 of Helotiales, 4 of Microascales and 3 of Pezizales, and 127 of Deuteromycotina including 127 of Hyphomycetes and 2 of Coelomycetes survived. Only 2 strains, one each of Hypocreales and Sordariales in Ascomycotina, did not survive. The percentage of viability was 99.4%. Except for one strain each of Chaetomidium fimeti (Fuckel) Saccardo in Hypocreales and Roumegueriella rufula (Berkeley & Broome) Malloch & Cain in Sordariales, the various species belonging to Ascomycotina and Deuteromycotina all survived after preservation for 4 to 5 years at -80 C. The species of Grovesinia Cline et al. and Sclerotinia Fuckel considered to be non-sporulating strains as well as the well sporulated cultures of Emericella Berkeley & Broome, Eupenicil-

Table 1. Viability of strains of Ascomycotina and Deuteromycotina fungi deposited in IFO between 1985 and 1986.

Group	Number of strains	Number of viable strains	Proportion of survivors (%)
ASCOMYCOTINA			
Dothideales*	7	7	100
Endomycetales	2	2	100
Eurotiales	85	85	100
Gymnoascales	48	48	100
Helotiales	9	9	100
Hypocreales	3	2	66.7
Microascales	4	4	100
Pezizales	3	3	100
Sordariales	23	22	95.7
DEUTEROMYCOTINA			
Hyphomycetes	127	127	100
Coelomycetes	2	2	100
Total	313	311	99.4

* Classification based on Ainsworth & Bisby s Dictionary of Fungi (7th ed., 1983).

lium Ludwig, Talaromyces C. R. Benjamin, Aspergillus Micheli: Link, and Penicillium Link survived. As shown in our previous papers (5, 6, 8, 9) and the present experiment, the results obtained indicated that the effectiveness of long-term preservation of fungal cultures in Ascomycotina and Deuteromycotina was as high as for Basidiomycotina. Even the two strains that did not survive might be preservable by this method if detailed investigations were conducted into thawing rate and cultural growth prior to freezing. Furthermore, it is predicted that the frozen strains deposited between 1987 and the present time, and the fungi other than the present groups in this experiment will also survive by this method. This method can be applied by cutting mycelial disks from a colony grown vigorously on agar in test tube and putting the disks directly into cryotubes.

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Table 2. Fungi of Ascomycotina and Deuteromyctina preserved by freezing at -80 C.

IFO Name	Number of strains tested	Number of viable strains
ASCOMYCOTINA		
Amorphotheca resinae Parbery	4	4
Amylocarpus encephaloides Currey	2	2
Anixiella reticulata (Booth & Ebben) Cain	1	1
Anixiopsis fulvescens (Cooke) de Vries var. fulvescens	1	1
A. fulvescens (Cooke) de Vries var. stercoraria (Hansen) de Vries	2	2
Arachniotus aurantiacus (Kamyschko) von Arx	1	1
A. aureus (Eidam) Schroeter	1	1
A. ruber (van Tieghem) Schroeter	5	5
Arachnomyces nitidus Massee & Salmon	1	1
Arthroderma curreyi Berkeley	2	2
A. uncinatum Dawson & Gentles	1	1
Ascodesmis sphaerospora Obrist	1	1
Auxarthron pseudauxarthron Orr & Kuehn	1	1
A. reticulatum (Zukal) Orr & Plunkett	1	1
A. thaxteri (Kuehn) Orr & Kuehn	1	1
A. umbrinum (Boudier) Orr & Plunkett apud Orr et al.	2	2
Byssoascus striatisporus (Barron & Booth) von Arx	1	1
Byssochlamys fulva Olliver & G. Smith	4	4
B. nivea Westling	1	1
Chaetomidium fimeti (Fuckel) Saccardo	3	2
Chaetomium aureum Chivers	1	1
C. bostrychodes Zopf	1	1
C. elatum Kunze: Fries	1	1
C. funicola Cooke	1	1
C. fusiforme Chivers	1	1
C. globosum Kunze: Fries	1	1
C. murorum Corda	1	1
C. olivaceum Cooke & Ellis	1	1
C. seminudum Ames	1	1
C. thermophilum La Touche var. dissitum Cooney & Emerson	1	1
Ctenomyces serratus Eidam emend. R. K. Benjamin	3	3
Dichotomomyces cejpii (Milko) Scott var. cejpii	2	2
D. cejpii (Milko) Scott var. spinosus (Udagawa) Malloch & Cain	1	1
Diplogelasinospora grovesii Udagawa & Horie	1	1
Emericella nidulans (Eidam) Vuillemin var. acristata (Fennell & Raper) Subramanian	1	1
E. nidulans (Eidam) Vuillemin var. nidulans	1	1
E. variecolor Berkeley & Broome	2	2
Eupenicillium alutaceum Scott	1	1
E. anatolicum Stolk	1	1

Table 2. (continued)

IFO Name	Number of strains tested	Number of viable strains
Eupenicillium brefeldianum (Dodge) Stolk & Scott	2	2
E. catenatum Scott	1	1
E. cinnamopurpureum Scott & Stolk	1	1
E. crustaceum Ludwig	1	1
E. erubescens Scott	1	1
E. javanicum (van Beyma) Stolk & Scott	1	1
E. lassenii Paden	1	1
E. meridianum Scott	1	1
E. molle Malloch & Cain	1	1
E. ornatum Udagawa	2	2
E. parvum (Raper & Fennell) Stolk & Scott	1	1
E. pinetorum Stolk	1	1
E. shearii Stolk & Scott	1	1
E. tularense Paden	1	1
E. zonatum Hodges & Perry	1	1
Eurotium chevalieri Mangin	1	1
E. repens de Bery	2	2
Gelasinospora calospora (Mouton) C. Moreau & M. Moreau	1	1
G. reticulospora (Gries & Greis-Dengler) C. Moreau & M. Moreau	2	2
Grovesinia pruni Harada & Noro	1	1
G. pyramidalis M. Cline et al.	1	1
Gymnoascus demonbreunii Ajello & Cheng	2	2
G. dugwayensis Orr & Kuehn	1	1
G. uncinatus Eidam	1	1
Hamigera avellanea Stolk & Samson	2	2
H. avellanea Stolk & Samson var. alba Morinaga et al.	1	1
H. striata Stolk & Samson	1	1
Hapsidospora irregularis Malloch & Cain	1	1
Keratinophyton terreum Randhava & R. S. Sandhu	1	1
Melanodothis caricis Arnold	1	1
Melanospora zamiae Corda	1	1
Microascus cinereus (Emilé-Weil & Gaudin) Curzi	1	1
Monascus ruber van Tieghem	1	1
Myxotrichum chartarum Kunze	1	1
M. deflexum Berkeley	2	2
M. stipitatum (Lindfors) Orr & Kuehn	2	2
Nectria gliocladioides Smalley & Hansen	1	1
N. inventa Pethybridge	1	1
Neosartorya fischeri (Wehmer) Malloch & Cain var. fischeri	2	2
N. fischeri (Wehmer) Malloch & Cain var. glabra (Fennell & Raper) Malloch & Cain	1	1
N. fischeri (Wehmer) Malloch & Cain var. spinosa (Fennell & Raper) Malloch & Cain	1	1

Table 2. (continued)

IFO Name	Number of strains tested	Number of viable strains
Neosartorya quadricincta (Yuill) Malloch & Cain	1	1
Neurospora tetrasperma Shear & B. Dodge	1	1
Nigrosabulum globosum Malloch & Cain	2	2
Onygena corvina Albertini & Schweinitz: Fries	1	1
O. equina (Willdenow: Fries) Persoon	1	1
O. piligena Fries: Fries	1	1
Petalosporus afilamentosus Orr & Kuhen	1	1
P. anodosus Kuehn et al.	1	1
P. nodosus Ghosh et al.	1	1
Petriella setifera (Schmidt) Curzi	2	2
Petriellidium boydii (Shear) Malloch	1	1
Pleospora herbarum (Persoon: Fries) Rabenhorst	1	1
Podospora carbonaria (Phillips & Plowright) Niessl	1	1
Pseudeurotium ovale Stolk	2	2
P. zonatum van Beyma	2	2
Pseudogymnoascus roseus Raillo	5	5
P. vinaceus Raillo	1	1
Roumegueriella rufula (Berkeley & Broome) Malloch & Cain	1	0
Sclerotinia minor Jagger	3	3
Shanorella spirotricha R. K. Benjamin	2	2
Spiromastix warcupii Kuehn & Orr	1	1
Talaromyces byssochlamydoides Stolk & Samson	1	1
T. emersonii Stolk	2	2
T. flavus (Klöcker) Stolk & Samson var. flavus	2	2
T. flavus (Klöcker) Stolk & Samson var. macrosporus Stolk & Samson	1	1
T. galapagensis Samson & Mahoney	1	1
T. gossypii Pitt	1	1
T. helicus C. R. Benjamin apud Stolk & Samson var. helicus	3	3
T. intermedius (Apinis) Stolk & Samson	1	1
T. leycettanus Evans & Stolk	1	1
T. luteus (Saccardo) Stolk & Samson	3	3
T. mimosinus Hocking	1	1
T. ohiensis Pitt	1	1
T. purpureus (E. Müller & Pacha-Aue) Stolk & Samson	1	1
T. rotundus C. R. Benjamin apud Stolk & Samson	1	1
T. stipitatus C. R. Benjamin apud Stolk & Samson	1	1
T. thermophilus Stolk	1	1
T. trachyspermus (Shear) Stolk & Samson	2	2
T. ucrainicus Udagawa apud Stolk & Samson	3	3
T. udagawae Stolk & Samson	1	1

Table 2. (continued)

IFO Name	Number of strains tested	Number of viable strains
Talaromyces wortmannii C. R. Benjamin apud Stolk & Samson	2	2
Thermoascus aurantiacus Miehe	2	2
T. crustaceus (Apinis & Chesters) Stolk	1	1
Thielavia arenaria Mouchacca	1	1
Trichophaea abundans (Karsten) Boudier	1	1
Warcupiella spinulosa (Warcup) Subramanian	1	1
Westerdykella dispersa (Clum) Cejp & Milko	2	2
W. multispora (Saito & Minoura) Cejp & Milko	1	1
W. nigra (Routien) von Arx	1	1
W. purpurea (Cain) von Arx	1	1
Xylogone sphaerospora von Arx & Nilsson	1	1
Zopfiella leucotricha (Spegazzini) Malloch & Cain	1	1
DEUTEROMYCOTINA		
Acremonium fusidioides (Nicot) W. Gams	1	1
A. persicinum (Nicot) W. Gams	1	1
A. strictum W. Gams	1	1
A. terricola (Miller et al.) W. Gams	1	1
Alatosessilispora bibrachiata Ando & Tubaki	1	1
Alternaria alternata (Fries) Keissler	1	1
Amblyosporium botrytis Fresenius	1	1
Arthrinium phaeospermum (Corda) M. B. Ellis	1	1
Arthrobotrys ellipsospora Tubaki & Yamanaka	1	1
Aspergillus clavatus Desmazieres	1	1
A. deflectus Fennell & Raper	1	1
A. fumigatus Fresenius	1	1
A. melleus Yukawa	1	1
A. terreus Thom	1	1
A. versicolor (Vuillemin) Tiraboschi	1	1
Beauveria bassiana (Balsamo) Vuillemin	2	2
Botrytis cinerea Persoon: Fries	1	1
Chrysosporium merdarium (Link: Greville) Carmichael	1	1
Cladosporium cladosporioides (Fresenius) de Vries	1	1
C. resinae (Lindau) de Vries f. albidum de Vries	1	1
C. resinae (Lindau) de Vries f. avellaneum de Vries	4	4
C. resinae (Lindau) de Vries f. resinae	5	5
C. sphaerospermum Penzig	2	2
C. staurophorum (Kendrick) M. B. Ellis	1	1
Cristulariella moricola (Hino) Redhead	1	1
Curucispora ombrogena Ando & Tubaki	1	1
Cylindrocarpon destructans (Zinssmeister) Scholten f. sp. panacis Matuo & Miyazawa	2	2
C. sclerotigenum N. Matsumoto & Tajimi	1	1

Table 2. (continued)

IFO Name	Number of strains tested	Number of viable strains
Dicranidion fissile Ando & Tubaki	1	1
Dicyma olivacea (Emoto & Tubaki) von Arx	1	1
Discosia artocreas (Tode) Fries	1	1
Doratomyces microsporus (Saccardo) Morton & Smith	1	1
D. nanus (Ehrenberg: Link) Morton & Smith	1	1
D. stemonitis (Persoon: Fries) Morton & Smith	1	1
Fusarium oxysporum Schlechtendahl emend. Snyder & Hansen f. sp. fragariae Winks & Williams	5	5
F. oxysporum Schlechtendahl emend. Snyder & Hansen f. sp. raphani Kendrick & Snyder	1	1
Geomyces asperulatus Sigler & Carmichael	2	2
G. pannorum (Link) Sigler & Carmichael var. pannorum	3	3
Geotrichum candidum Link: Persoon emend. Carmichael	1	1
Gilmaniella humicola Barron	1	1
Gliocladium catenulatum Gilman & Abbott	1	1
G. virens Miller et al.	1	1
Harposporium helicoides Drechsler	1	1
Humicola fuscoatra Traaen	1	1
Idriella lunata Nelson & Wilhelm	1	1
Malbranchea pulchella Saccardo & Penzig var. sulfurea (Miehe) Cooney & Emerson	1	1
Metarhizium anisopliae (Metschnikoff) Sorokin	2	2
Microstella pluvioriens Ando & Tubaki	1	1
Myceliophthora thermophila (Apinis) van Oorschot	2	2
Oidiodendron echinulatum Barron	1	1
O. truncatum Barron	1	1
Paecilomyces carneus (Duché & Heim) Brown & G. Smith	1	1
P. inflatus (Burnside) Carmichael	1	1
P. marquandii (Massee) Hughes	2	2
P. variotii Bainier	2	2
Penicillium argillaceum Stolk et al.	1	1
P. chermesinum Biourge	2	2
P. crustosum Thom	1	1
P. donkii Stolk	1	1
P. funiculosum Thom	1	1
P. herquei Bainier & Sartory	1	1
P. hispanicum Ramirez et al.	1	1
P. janthinellum Biourge	2	2
P. lilacinum Thom	3	3
P. nigricans (Bainier) Thom	1	1
P. novae-zeelandiae van Beyma	1	1
P. oxalicum Currie & Thom	1	1
P. piceum Raper & Fennell	1	1
P. quercetorum Baghdadi	1	1
P. rubrum Stoll	1	1

Table 2. (continued)

IFO Name	Number of strains tested	Number of viable strains
Pestalotiopsis acaciae (Thümen) Yokoyama & S. Kaneko	1	1
Phialocephala humicola Jong & Davis	1	1
Phialophora alba van Beyma	1	1
Pithomyces chartarum (Berkeley & Curtis) M.B. Ellis	1	1
Scolecobasidium humicola Barron & Busch	2	2
Scopulariopsis brevicaulis (Saccardo) Bainier	2	2
Stachybotrys bisbyi (Srinivasan) Barron	1	1
Staphylotrichum coccosporum Meyer & Nicot	1	1
Thermomyces lanuginosus Tsiklinsky	1	1
Tolypocladium niveum (Rostrup) Bissett	5	5
Trichoderma aureoviride Rifai	3	3
T. harzianum Rifai	1	1
T. longibrachiatum Rifai	2	2
T. pseudokoningii Rifai	2	2
Tricladiella pluvialis Ando & Tubaki	1	1
Tripospermum infalcatum Ando & Tubaki	1	1
Ustilaginoidea virens (Cooke) Takahashi	2	2
Verticillium nigrescens Pethybridge	1	1
V. psalliotae Treschow	1	1
Warcupia terrestris Paden & Cameron	1	1
Wardomyces anomalus Brooks & Hansford	1	1
W. inflatus (Marchal) Hennebert	1	1
Zygosporium mycophilum (Vuillemin) Saccardo	1	1

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DEOXYRIBONUCLEIC ACID HOMOLOGY AMONG LIPOPOLYSACCHARIDE-SPECIFIC BACTERIOPHAGES OF PSEUDOMONAS AERUGINOSA

KO IMAI and YORIKO YAMADA

Summary

Six of fourteen bacteriophages collected for the typing of Pseudomonas aeruginosa, namely, phages 44 (IFO 20038), 1214 (IFO 20039), F8 (IFO 20040), E79 (IFO 20041), 16 (IFO 20049), and 109 (IFO 20053), were inactivated by the lipopolysaccharide isolated from the host bacteria and exhibited more than 85% relative binding values with photobiotinylated DNAs of E79 and 44 in DNA/DNA hybridization experiments.

Keywords: LPS, P. aeruginosa phage, DNA homology.

Typing phages for Pseudomonas aeruginosa have been collected and divided into twelve groups by their lytic spectra on 707 strains of P. aeruginosa (9). Reports indicate that some phages of P. aeruginosa are inactivated by the lipopolysaccharide (LPS) and use it as a receptor (2, 3, 4, 6, 7, 10). Except for phages 352 and 2, these LPS-specific phages fall in two groups of lytic spectra reported by Sakamoto et al. (9). However, there are no reports on the phylogenetic relationships among P. aeruginosa phages. The purposes of this communication are (i) to identify LPS-specific phages among the fourteen P. aeruginosa phages in the IFO collection and (ii) to quantify the phylogenetic relatedness among the LPS-specific phages by DNA/DNA hybridization experiments.

Materials and Methods

Microorganisms used. Bacteriophages and their propagating hosts used here are shown in Table 1.

Media. The PY broth used for liquid bacterial cultures and for dilution of phage suspensions for assay contained 10 g of Polypepton (Nippon Seiyaku, Co.), 2 g of yeast extract (Difco), 2 g of NaCl, and 1,000 ml of distilled water and adjusted to pH 7.2 with NaOH. Solid and soft agar media consisted of PY plus 1.5% and 0.8% agar, respectively.

Table 1. Bacteriophages and their host bacterial strains.

Phage		Propagating host
Name	IFO No.	Strain No.
7	20036	IFO 13739
352	20037	IFO 13740
44	20038	IFO 13741
1214	20039	IFO 13742
F8	20040	IFO 13743
E79	20041	IFO 13744
M4	20042	IFO 13745
C11	20043	IFO 13746
C188	20045	IFO 13748
2	20048	Ps2
16	20049	Ps16
31	20051	Ps31
109	20053	Ps109
G101	20058	PAO1

Phage assay. Infective phage particles (plaque-forming units, PFUs) were determined by the agar layer technique (1) using the propagating host as the indicator.

Preparation of LPS. LPSs used in phage inactivation tests were ex-

tracted from lyophilized cells of host bacteria by the method of Westphal and Jann (11), and phenol was removed by dialysis against tap water. After treatment with RNase, LPSs were purified by repeating ultracentrifugation at 35,000 rpm for 60 min twice or more, then lyophilized. Protein contents of the purified LPSs were less than 4% by the determination of Lowry et al. (8).

Inactivation of phages by LPS. Reaction mixtures for the phage inactivation test consisted of 1 ml of PY broth, ca. 10^6 to 10^9 PFUs of phage particles, and 100 μg of LPS isolated from their respective hosts. These were incubated at 37 C for 1 hr, then the phage titer was determined by the agar layer technique. The phage titer of reaction mixtures without LPS was determined as the control.

Preparation of bacteriophage DNA. Phage particles in 1 L of lysate were precipitated by addition of 35 g of NaCl and 80 g of polyethylene glycol (PEG 6000) and collected by centrifugation. The pellets were washed with and resuspended in 30 ml of saline-EDTA (0.15 M NaCl, 0.1 M EDTA, pH 8.0). An equal volume of saturated phenol was added to phage suspensions and the mixtures were vigorously shaken for 10 min. After centrifugation at 8,000 rpm for 15 min, the water layer was collected, and DNAs were precipitated with two volumes of cold ethanol. The precipitated DNAs were dissolved in saline-citrate (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and further purified by repeating the phenol treatment.

DNA/DNA hybridization. The DNA homology among phages was determined by the photobiotin microplate assay method described by Ezaki et al. (5).

Results and Discussion

Inactivation of bacteriophages by LPS

Results of the phage inactivation test are shown in Table 2. The PFUs of phages 44, 1214, F8, E79, 16, and 109 in the reaction mixtures with LPS were reduced to less than one-hundredth of those in the mixtures without LPS, and these six phages were judged to be LPS-specific. The remaining eight phages were not inactivated by LPS.

DNA homology among phages

To examine the phylogenetic relationships among these phages, DNA/DNA hybridization experiments were done. The results are shown in Table 3.

Table 2. The phage inactivation test by LPS.

Phage	PFUs* after incubation for 1 hr in the reaction mixture		Inactivation
	LPS not added	LPS added	
7	$(2.44\pm0.08)\times10^{6}$	$(2.21\pm0.13)\times10^{6}$	-
352	$(1.11\pm0.05)\times10^{7}$	$(1.28\pm0.02)\times10^{7}$	-
44	$(1.20\pm0.20)\times10^{8}$	$(2.20\pm0.35)\times10^{6}$	+
1214	$(7.60\pm1.30)\times10^{7}$	$(2.30\pm0.40)\times10^{5}$	+
F8	$(5.10\pm0.35)\times10^{7}$	$(4.90\pm0.69)\times10^{5}$	+
E79	$(1.20\pm0.12)\times10^{8}$	$(2.30\pm0.75)\times10^{6}$	+
M4	$(7.88\pm0.31)\times10^{6}$	$(7.56\pm1.30)\times10^{6}$	-
C11	$(1.68\pm0.46)\times10^{7}$	$(1.64\pm0.22)\times10^{7}$	-
C188	$(6.67\pm1.72)\times10^{7}$	$(6.83\pm1.56)\times10^{7}$	-
2	$(5.02\pm0.38)\times10^{7}$	$(4.68\pm0.32)\times10^{7}$	-
16	$(2.24\pm0.78)\times10^{7}$	$(5.76\pm1.80)\times10^{5}$	+
31	$(8.30\pm0.53)\times10^{6}$	$(7.83\pm0.83)\times10^{6}$	-
109	$(1.12\pm0.02)\times10^{8}$	$(8.50\pm3.95)\times10^{4}$	+
G101	$(1.93\pm0.03)\times10^{8}$	$(1.78\pm0.21)\times10^{8}$	-

* Values represent the average of triplicate assays and their standard deviations.

The DNAs prepared from the six LPS-specific phages exhibited relative binding values of more than 85% with photobiotinylated DNAs of LPS-specific phages E79 and 44, but less than 20% with the labeled DNAs of phages 7 and 352, which were not inactivated with LPS. The remaining eight phages had DNA relatedness values of less than 21% with labeled DNAs of E79 and 44. These results indicate that LPS-specific phages have close phylogenetic relationships with each other, but low DNA relatedness with phages that were not inactivated by LPS.

The DNA relatedness values among phages 7, 2, 352, and M4 were more than 85%, which indicated that these phages are closely related. The propagating strain for phage 7 (IFO 13739) was also sensitive to phages 2, 352, and M4. A resistant mutant to phage 7, which was derived from IFO

13739, was insensitive to the phages 2, 352 and M4 and did not adsorb these phages. This finding suggests that phages 2, 7, 352, and M4 use a common receptor site.

Table 3. DNA relatedness among P. aeruginosa phages.

Unlabeled	% Homology to labeled DNA from			
DNA from	E79	44	7	352
E79	100	87	13	19
44	87	100	7	18
1214	87	92	NT	NT
F8	98	86	6	17
16	96	88	NT	NT
109	85	94	NT	NT
7	20	19	100	96
2	13	NT	85	89
352	21	19	90	100
M4	18	NT	92	89
C11	16	22	14	15
31	18	17	5	16
C188	16	NT	4	16
G101	14	17	14	19

NT, not tested.

Temple et al. (10) reported that phage 352, obtained from Colindale typing set, and phage 2, provided by Bartell, were neutralized by LPS isolated from their propagating strains. However, our results indicated that phages 352 and 2 preserved in the IFO are not LPS-specific and have extremely low DNA relatedness with LPS-specific phages and suggested that both phages are not identical to those in the Colindale typing set.

We thank Ihomi Nishiura for excellent technical assistance.

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IFO Res. Comm. 15,
135-144, 1991 (March)

DESCRIPTIVE CATALOGUE OF IFO FUNGUS COLLECTION XII.

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 fungi have been found to be taxa either new to Japan or obscurely or insufficiently described. In some cases, the first record of a fungus in Japan gives only the name of a taxon, without an adequate description of the species concerned. The object of this series is to provide descriptions of the fungi preserved or newly deposited in the IFO fungus collection and/or in the IFO herbarium and to contribute to the knowledge of the fungal flora of Japan.

The authors of the descriptions of these fungal taxa are shown in parentheses.

88. Hemicarpenteles acanthosporus Udagawa & Takada (Figs. 1-4) Eurotiales

Bull. Nat. Sci. Mus. Tokyo 14: 503 (1971); Samson, Studies in Mycology 18: 6 (1979).

Colonies on malt extract agar grow rapidly, floccose, white to pale tan, later becoming grayish brown; reverse uncolored. Colonies on oatmeal agar and potato carrot agar are almost the same as malt extract agar.

Cleistothecia formed in abundance, superficial, solitary to gregarious, spherical, at first pale yellow-brown, later becoming gray brown, covered with flexuous pale tan mycelium, 350-600 μm in diam; peridium membranaceus, consisting of three to five layers with angular cells, grayish brown, 45-75 μm thick. Asci globose to ovoid, 8-spored, evanescent at maturity, 10-12 x 9-10 μm. Ascospores lenticular, hyaline, showing two distinct, parallel, equatorial crests, with conspicuously

echinulate surfaces, sporebodies 3.5-4.5 x 3.0-4.0 μm. Conidial heads white to gray green, at first radiate, later becoming loosely columnar. Conidiophores single, short, straight to flexuous, smooth, hyaline, 120-400 x 6-10 μm; vescicles pyriform to clavate, concolore with conidiophores, having uniseriate phialides over the upper half to two-thirds, 10-25 μm wide. Phialides hyaline to pale green, flask-shaped, 10-12 x 4-5 μm. Conidia pale green, globose, conspicuously echinulate, 5.5-6.5 x 6.0 μm.

Growth is nil at 37 C.

Hab. field soil, Kabira, Ishigaki, Okinawa Pref., Sept., 19, 1989 (T. Ito S64-8E-3 = IFO 32304); soil, Komi, Taketomi-cho, Yaeyama-gun, Okinawa Pref., Sept., 20, 1989 (T. Ito S64-12E-3 = IFO 32305); pasture soil, Mihara, Taketomi-cho, Yaeyama-gun, Okinawa Pref., Sept., 21, 1989 (T. Ito S64-17E-1).

This fungus was originally described by Udagawa and Takada based on a culture from soil at Bougainville Island, Solomon Islands. This species is distinguished from other species by its conspicuously echinulate ascospores.

(T. Ito & A. Nakagiri)

89. Didymostilbe matsushimae Seifert (Figs. 5-7) Hyphomycetes

Studies in Mycology 27: 138 (1985).

Syn. Stilbum macrosporum Matsushima, Microfungi of the Solomon Islands and Papua-New Guinea, p. 63 (1971).

Colonies on oatmeal agar grow moderately, velvety, white to pale brown, later becoming yellow-orange, partly appearing glandular; reverse brown at the center, white to pale yellow-orange at the margin. Colonies on malt extract agar and potato carrot agar grow restrictedly, velvety, white to pale brown, partly immersed; reverse white to pale brown.

Synnemata solitary to gregarious, cylindrical to clavate, sometimes proliferating apically to form over two new synnemata, later becoming

brown to black at the base, white at the upper parts, up to 1.5 mm tall, 60-100 μm wide at the base, 30-120 μm wide at the conidial mass below, bearing brown oil droplets in synnemata. Conidiophores long, straight, branched, verrucose, hyaline; metulae smooth-walled, hyaline, 12-28 x 3.0-3.5 μm; phialides cylindrical, hyaline, smooth-walled, 20-50 x 3.0-3.5 μm, with conspicuously collarettes. Conidial mass orange, later becoming dark brown, globose, 100-250 μm diam. Conidia ellipsoidal with nipple-like base, round at the apex, pale orange with conspicuously mucoid, 23-34 x 7.0-9.0 μm, with wall 0.5-2.0 μm thick.

Growth is nil at 37 C.

Hab. rotten stem of Arenga englerri. Komi, Taketomi-cho, Yaeyama-gun, Okinawa Pref., Sept., 21, 1989 (T. Ito S64-21-2 = IFO 32328).

This fungus was originally described by Matsushima as Stilbum macrosporum. Seifert accommodated S. macrosporum to D. matsushimae by having dark brown to black synnemata and by its larger conidia and verrucose subapical marginal hyphae. According to Dr. Okada (Japan Collection of Microorganisms, RIKEN), this fungus is often found on dead stems in the southern islands of Okinawa Pref., and this strain was identified by him.

(T. Ito & A. Nakagiri)

90. Gyrothrix circinata (Berkeley & Curtis) Hughes

(Figs. 8-14) Hyphomycetes

Can. J. Bot. 36: 771 (1958); Pirozynski, Mycol. Pap. No. 84: 12 (1962); Ellis, Dematiaceaus Hyphomycetes, CMI, p. 360 (1971).

Syn. Campsotrichum circinatum Berkeley & Curtis, apud Berkeley, Grevillea 36: 771 (1874).

Colonies on natural substrates velvety, dark brown to black, amphigenous, scattered. Superficial mycelium composed of a network of branched and anastomosing, smooth-walled, subhyaline hyphae bearing conidiogenous cells. Setae erect, thick walled, septate, dark brown, rough with ruptured tubercles, circinate, 2-4 times branched, 80-120 μm

high, 3.5-4 μm in diam at the base, branches perpendicular to the main axis, sometimes with 2nd and 3rd branches, pale colored, rough, circinate. Conidiogenous cells phialidic, obclavate to lageniform, subhyaline, 6-8 μm high, 3-4 μm wide at the base, with mucilaginous material surrounding the apex of phialides. Conidia cylindrical to fusoid, straight or slightly curved, one-celled, hyaline, 12-14 X 1-1.5 μm, adherent with mucilage, aggregated into a whitish layer at the bases of setae.

Growth on PSA moderate, velvety to somewhat cottony, white, with sparse tufts of brown hyphae; reverse reddish orange to brown. Growth on OA moderate, cottony, white. No conidium formation on agar media.

Hab. on the fallen leaves and petioles of Tachycarpus excelsa Wendl. Shimoda, Shizuoka Pref., 14 March 1990 (A. Nakagiri AN-1111, 1112 = IFO 32309, 32310; IFO H 12126).

This fungus has been reported from fallen leaves of Artocarpus, Carapa, Cocos, Crataegus, and Magnolia in subtropical to tropical regions of North America, Asia, and Africa.

Conidiogenesis of this fungus has not been determined clearly. Pirozynski (1962) mentioned that it formed conidia in the same way as Circinotrichum Nees ex Persoon, which was described as producing up to 10 conidia simultaneously in a ring just below the apex of a sporogenous cell; the apex then elongated vertically to produce second ring of conidia, and this cycle was repeated several times. Ellis (1971) described a polyblastic, percurrent conidiogenesis in the species of Gyrothrix (Corda) Corda, Circinotrichum, and Ceratocladium Corda, which are all setigerous fungi that produce unicellular, cylindrical, hyaline conidia on the obclavate conidiogenous cells. Our SEM observation on the conidiogenesis of G. circinata revealed that the conidiogenous cell is a type of phialide and that mucilaginous material surrounds and holds the conidia in mass at the apex of phialide. The deposited mucilage at the fertile apex might be seen as conidiogenous cell elongated percurrently under a light microscope. Conidia are formed successively, not simultaneously, from alternate sides of the conidiogenous cell apex. Similar conidium formation was observed in Chloridium Link ex Fr., Cacumisporium Preuss, Codinaea Maire. This peculiar type of conidiogenesis and muci-

laginous material at the fertile apex may result in the ring-like arrangement of conidia which was described by the previous authors. On the other hand, conidiogenesis of G. pediculata Cunningham was revealed to be normal phialidic by time-lapse observation of sporulating culture (J. L. Cunningham, Mycologia 66: 122-129 (1974)). Further research on conidiogenesis of the species of Gyrothrix, Circinotrichum, and Ceratocladium under the electron microscope is necessary to clarify and emend the generic definition.

(A. Nakagiri & T. Ito)

91. Trichobotrys effusa (Berkeley & Broome) Petch

(Figs. 15-17) Hyphomycetes

Ann. R. bot. Gdns Peradeniya 9: 169 (1924); M. B. Ellis, Dematiaceous Hyphomycetes, CMI, p. 340 (1971).

Syn. Trichobotrys pannosa Penzig & Saccardo, Malpighia 15: 245 (1902).

Colonies on oatmeal agar grow moderately, floccose to funiculose, at first white, later becoming pale yellow-orange; reverse yellow-orange, yellow at the margin. Colonies on malt extract agar grow restrictedly, floccose, partly fasciculate, white to pale yellow-orange, raised at the center, rugged at the margin; reverse yellow-orange at the center, uncolored at the margin, partly dark brown. Colonies on potato carrot agar grow moderately, floccose at the center, velvety at the margin, white; reverse uncolored to pale yellow.

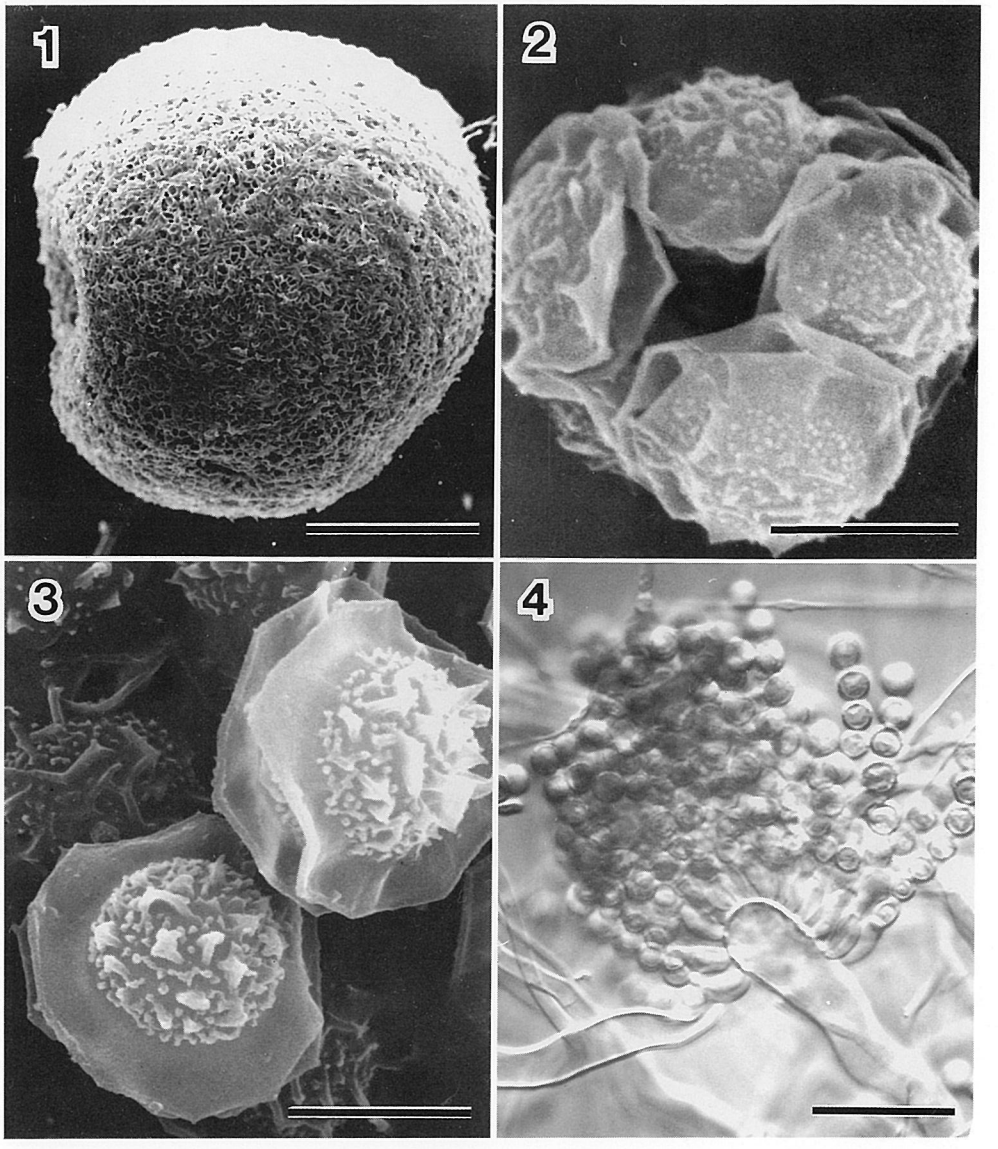
Conidiophores macronematous, mononematous, laterally branched, straight to flexuous, rigid, echinulate, long, with sterile apex, up to 1-1.2 mm high; lateral branch up to 400 μm, straight, dark brown at the base, paler towards the apex, verrucose to echinulate; short lateral branch, smooth, pale brown at the base, dark brown at the apex. Conidiogenous cells polyblastic, determinate, apical or lateral on the conidiophores, branches up to 25 μm long, 3-5 μm thick. Conidia pale brown to dark brown, globose, smooth to finely echinulate, 4.5-6.0 μm including spines.

Growth is nil at 37 C.

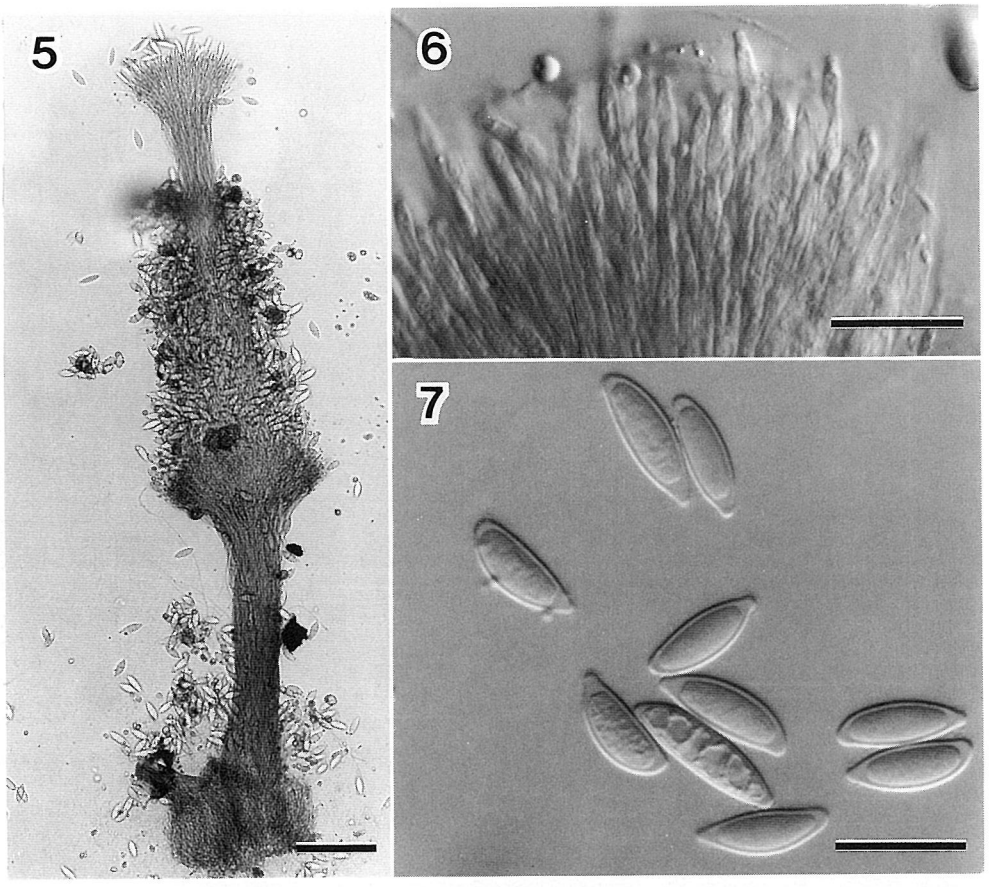
Hab. soil in front of a charcoal kiln, Tateiwa-mura, Minamiaizu-gun, Fukushima Pref., Sept., 30, 1988 (T. Ito S63-19-4 = IFO 32329).

This fungus was described by Petch (1924) as having short unciform lateral branches, walls verruculose or echinulate, resembling genus Periconia Tode ex Schw. According to M. B. Ellis (1971), this fungus is known to occur on pineapple, bamboo, palm etc. in tropical countries.

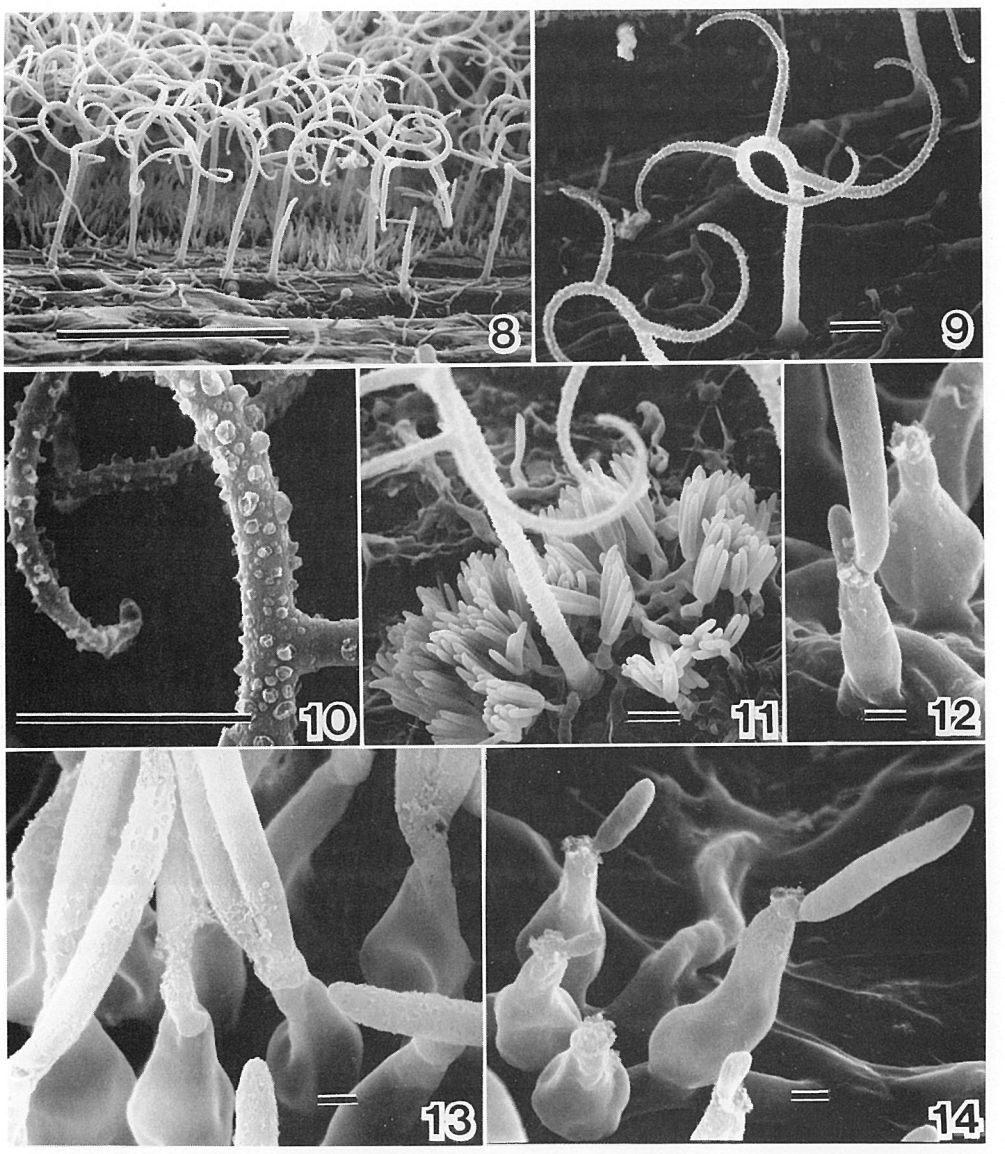
(T. Ito)



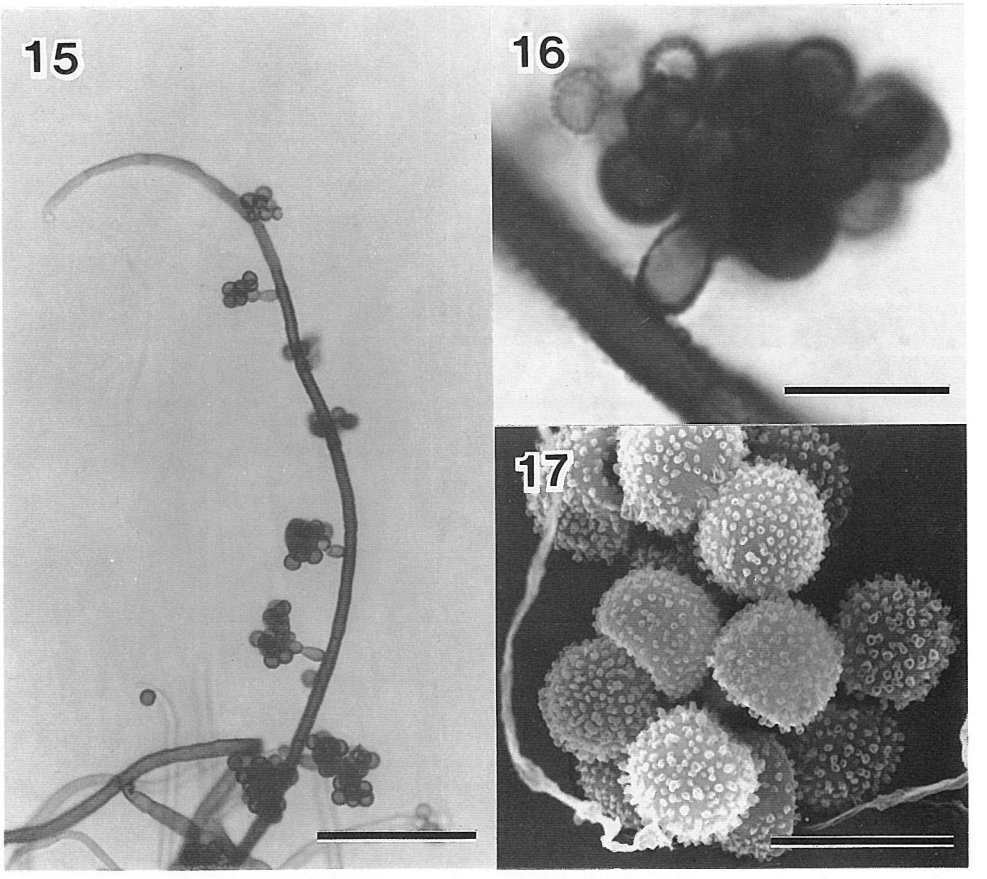
Figs. 1-4. Hemicarpenteles acanthosporus. 1. Cleistothecium. 2. Ascus. 3. Ascospores. 4. Conidial structure. (Bars: Fig. 1 = 250 μm; Figs. 2 & 3 = 3 μm; Fig. 4 = 20 μm)



Figs. 5-7. Didymostilbe matsushimae. 5. Mature synnema. 6. Conidiophores. 7. Conidia.
(Bars: Fig. 5 = 100 μm; Figs. 6 & 7 = 20 μm)



Figs. 8-14. Gyrothrix circinata. 8. Habit on leaf of Tachycarpus excelsa. 9. Circinate seta. 10. Rough surface of seta with ruptured tubercles. 11. Conidia produced at the base of seta. 12, 14. Phialidic conidiogenous cells producing conidia successively from alternate sides of the apex. 13. Conidial mass surrounded with mucilaginous material. (Bars: Fig. 8 = 100 μm; Figs. 9-11 = 10 μm; Figs. 12-14 = 1 μm)



Figs. 15-17. Trichobotrys effusa. 15. Conidiophore. 16. Conidiogeneous cells and conidia. 17. Conidia.
(Bars: Fig. 15 = 50 μm; Fig. 16 = 20 μm; Fig. 17 = 3 μm)

IFO Res. Comm. 15,
145-148, 1991 (March)

DESCRIPTIVE CATALOGUE OF IFO BACTERIAL COLLECTION IX.

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

74. **Comamonas acidovorans** Tamaoka, Ha and Komagata

Tamaoka, J., D.-M. Ha, and K. Komagata. 1987. Int. J. Syst. Bacteriol. 37: 52-59.

IFO 13582

75. **Comamonas testosteroni** Tamaoka, Ha and Komagata

Tamaoka, J., D.-M. Ha, and K. Komagata. 1987. Int. J. Syst. Bacteriol. 37: 52-59.

IFO 12048

The strains IFO 13582 and IFO 12048 were obtained under the name of Pseudomonas acidovorans den Dooren de Jong and Pseudomonas dacunhae Gray and Thornton, respectively. These have been reidentified as Comamonas acidovorans and Comamonas testosteroni, respectively, according to the description of species given by Tamaoka et al. in 1987.

Characteristic	IFO 13582	IFO 12048
Cell morphology	Rod	Rod
Gram-staining	Negative	Negative
Motility	+	+
Flagellation	Polar	Polar
Oxidase	+	+
Catalase	+	+
Growth factor requirement	-	-

Assimilation of:		
Glucose	+	+
Glutamate	+	-
Fructose	+	-
Mannitol	+	-
Ethylene glycol	+	-
Testosterone	-	+
β-Hydroxybutylate	+	+
Gluconate	+	+
L-Alanine	+	-
β-Alanine	+	-
Cellular fatty acid compostion		
Non-polar	16:0, 16:1 18:1	16:0, 16:1 18:1
2-Hydroxylated	None	16:0, 14:0
3-Hydroxylated	10:0	10:0
Mol% G+C of DNA	66.8	60.9
Isoprenoid quinone	Q-8	Q-8

(A. Yokota)

76 and 77. Haloarcula vallismortis (Gonzalez et al.) Torreblanca, Rodriguez-Valera, Juez, Ventosa, Kamekura and Kates
IFO 14954 and IFO 14955

These two strains were isolated from salted, boiled Wakame and labeled as Halobacterium sp., by K. Fukuta, Tokushima prefectural Food Research Institute, Tokushima, Japan. They were identified as Haloarcula vallismortis from the properties in the following Table:

	IFO 14954	IFO 14955
Cells:		
Pleomorphic rods	+	+
Presence of gas vacuoles	-	-
Motility	+	+
Lysis when shifted to distilled water	+	+
Colonies are pink to red	+	+
Catalase test	+	+
Growth in broth with:		
NaCl 10%	-	-
NaCl 15%	+	+
NaCl 25%	+	+
Magnesium ions requirement for growth, mM	10	10
Mol% G+C of DNA (HPLC method)	61.0	61.2
Major menaquinones	MK-8, MK-8 (H2)	MK-8
Minor menaquinones	MK-6	MK-6, MK-8 (H2)
Polar lipid compositions of cells:		

Phosphatidylglycerol	+	+
Phosphatidylglycerophosphate	+	+
Phosphatidylglycerosulfate	+	+
Triglycosyl diether	+	+
Sulfated diglycosyl diether	-	-
Sulfated triglycosyl diether	-	-
Sulfated tetraglycosyl diether	-	-
Hydrolysis of:		
Gelatin	-	-
Tween 80	-	-
Urea	+	+
Reduction of nitrate to nitrite	+	+
Production of gas from nitrate	+	+
Production of hydrogen sulfide	-	-
Production of acid from glucose	+	+
Sensitive to Bacitracin	+	+
Optimum temperature for growth, C	40	40
Optimum pH for growth	7.5-8.0	7.5-8.0

(T. Sakane)

78. Pseudomonas hydrogenothermophila Goto, Komagata and Minoda
Goto, E., T. Kodama, and Y. Minoda. 1978. Agric. Biol. Chem. 42: 1305-1308; Takeuchi, M., and A. Yokota. 1991. IFO Res. Comm. 15:
IFO 14593

The strain was deposited under the name of Flavobacterium autothermophilum Goto et al., but was reidentified as Pseudomonas hydrogenothermophila from the characteristics described by Goto et al. and from data which we have reported elsewhere.

(M. Takeuchi)

79 to 81. Rhizobium leguminosarum (Frank) Frank
IFO 13338, IFO 14994 and **IFO 14995**

82 to 85. Rhizobium loti Jarvis, Pankhurst and Patel
IFO 13336, IFO 14997, IFO 14998 and **IFO 14999**

Strain IFO 13338 was isolated from root nodules of Medicago denticulata in 1972 and entered in the IFO List of Cultures under the name of Rhizobium japonicum (Kirchner) Buchanan; and strain IFO 13336 was isolated from root nodules of Lotus corniculatus var japonicas and listed

under the name of Rhizobium meliloti Dangeard. The taxonomic properties of these two strains were reexamined. Five other strains were newly isolated from root nodules of leguminous plants in 1989, and their taxonomic characteristics were examined. From the findings listed in the Table, strains IFO 13338, 14994 and 14995 were identified as Rhizobium leguminosarum (Frank) Frank; and IFO 13336, 14997, 14998 and 14999 as Rhizobium loti Jarvis et al.

	IFO 13338	IFO 14994	IFO 14995	IFO 13336	IFO 14997	IFO 14998	IFO 14999
Flagella arrangement:							
Lateral, 1	+	+	+	+	+	+	+
Polar, 1	-	-	-	-	-	-	-
Rapid growth on YM agar	+	+	+	+	+	+	+
Alkaline reaction on YM agar	-	-	-	-	-	-	-
Production of hydrogen sulfide from cysteine	+	+	-	-	w	+	w
Alkaline phosphatase	+	-	-	+	-	+	+
Urease	+	+	+	-	-	-	-
Growth at 37 C	-	-	-	-	-	-	-
Growth at pH 9.5	-	+	+	+	+	-	+
Growth on agar with:							
0.5% NaCl	-	-	-	+	+	+	+
0.5% Arginine	-	-	-	+	+	+	+
0.8% Citrate	-	-	-	+	+	+	+
Resistant to:							
Ampicillin, 50 μg/ml	+	+	+	-	-	-	-
Penicillin G, 25 μg/ml	-	-	+	-	-	-	-
Streptomycin, 20 μg/ml	-	-	-	-	-	-	-
Chloramphenicol, 25 μg/ml	-	-	+	-	-	w	-
Tetracycline, 10 μg/ml	-	-	-	-	-	-	-
Noboviocin, 10 μg/ml	-	+	+	-	w	-	-
Utilization as carbon sources:							
Glucose	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+
Erythritol	+	-	+	+	+	+	+
Citrate	w	-	-	+	+	+	+
Malonate	w	-	-	-	-	-	-
Tartrate	w	-	-	-	-	-	-
Arginine	-	w	-	+	+	+	+
Ornithine	-	+	w	-	-	-	-
Host plants	Md	Pv	Ml	Lc	Lc	Lc	Lc

w, weak; Md, Medicago denticulata; Pv, Phaseolus vulgaris; Ml, Medicago lupulina; Lc, Lotus corniculatus var japonicus.

(T. Sakane)

CATALOGUE OF NEWLY ACCEPTED STRAINS NOVEMBER 1988 - OCTOBER 1990

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

IFO	NAME	TEMP	MED
10466	**Trichosporon cutaneum**	24	101
	AJ 4816 (T. Nakase; cabbage). Special Use: Estimation of BOD.		
10478	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 287; recombinant between genetic stock mutants). Lineage of H42. Genotype: MATa arg6		
10479	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 288; recombinant between genetic stock mutants). Lineage of H42. Genotype: MATα arg6		
10480	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 289; recombinant between genetic stock mutants). Lineage of H42. Genotype: MATa trp1		
10481	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 290; recombinant between genetic stock mutants). Lineage of H42. Genotype: MATα his6		
10482	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 291; recombinant between genetic stock mutants). Lineage of S288C. Genotype: MATa his4-519 gal2.		
10483	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 292; recombinant between genetic stock mutants). Lineage of S288C. Genotype: MATα leu2-3,112 gal2.		
10485	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 293; recombinant between genetic stock mutants). Genotype: MATa pho6-7 arg6		
10486	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 294; recombinant between genetic stock mutants). Genotype: MATα pho6-7 arg6		
10487	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 301; recombinant between genetic stock mutants). Genotype: MATa pho2-8 his3 gal2		
10488	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 302; recombinant between genetic stock mutants). Genotype: MATα pho2-8 his3 gal2		
10489	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 303; recombinant between genetic stock mutants). Genotype: MATa pho4-1 trp1 gal2		
10490	**Saccharomyces cerevisiae**	28	112
	History: IFO (Y. Kaneko; KYC 304; recombinant between genetic stock mutants). Genotype: MATα pho4-1 trp1 gal2		
10493	**Rhodosporidium kratochvilovae**	24	101
	History: IAM 13072 (M. Hamamoto; YK 218) -- CCY 62-3-1 -- Biol. Inst. Czechoslovak Acad. Sci. 812/2. self-sporulating		
10494	**Rhodosporidium kratochvilovae**	24	101
	History: IAM 13073 (M. Hamamoto; YK 219) -- CCY 62-3-2. self-sporulating		
10502	***Saccharomyces cerevisiae***	28	109
	History: IFO (Y. Kaneko; YPH250) -- FST (Y. Ohya) -- P. Hieter, YPH250. Genotype: MATa ura3-52 lys2-801amber ade2-101ochre trp1-Δ1 his3-Δ200 leu2-Δ1		
10503	***Saccharomyces cerevisiae***	28	109
	History: IFO (Y. Kaneko; YPH252) -- FST (Y. Ohya) -- P. Hieter, YPH252. Genotype: MATα ura3-52 lys2-801amber ade2-101ochre trp1-Δ1 his3-Δ200 leu2-Δ1		

IFO	NAME	TEMP	MED
10504	**Saccharomyces cerevisiae** History: IFO (Y. Kaneko; YPH274) -- FST (Y. Ohya) -- P. Hieter, YPH274. Genotype: MATa/MATα ura3-52/ura3-52 lys2-801amber/lys2-801amber ade2-101ochre/ade2-101ochre trp1-Δ1/trp1-Δ1 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1	28	109
10505	**Saccharomyces cerevisiae** History: IFO (Y. Kaneko; YPH499) -- FST (Y. Ohya) -- P. Hieter, YPH499. Genotype: MATa ura3-52 lys2-801amber ade2-101ochre trp1-Δ63 his3-Δ200 leu2-Δ1	28	109
10506	**Saccharomyces cerevisiae** History: IFO (Y. Kaneko; YPH500) -- FST (Y. Ohya) -- P. Hieter, YPH500. Genotype: MATα ura3-52 lys2-801amber ade2-101ochre trp1-Δ63 his3-Δ200 leu2-Δ1	28	109
10507	**Saccharomyces cerevisiae** History: IFO (Y. Kaneko; YPH501) -- FST (Y. Ohya) -- P. Hieter, YPH501. Genotype: MATa/MATα ura3-52/ura3-52 lys2-801amber/lys2-801amber ade2-101ochre/ade2-101ochre trp1-Δ1/trp1-Δ1 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1	28	109
10508	**Saccharomyces cerevisiae** History: K. Matsubara -- A. Tohe, AH22 pho80. Genotype: MATa leu2 his4 can1 pho80 cir+ R- rho+	28	112
10512	**Rhodosporidium toruloides** History: IFO (I. Banno; M-919; a mutant of IFO 0559). Methionine- & pantothenate-deficient	24	101
10513	**Rhodosporidium toruloides** History: IFO (I. Banno; M-1057; a mutant of IFO 0880). Yellow & PABA-deficient	24	101
10514	**Saccharomyces cerevisiae** History: IFO (I. Banno; K-2) -- K. Kodama, Ksc 2, tree exudate.	28	101
10515	**Saccharomyces cerevisiae** History: IFO (I. Banno; K-40) -- K. Kodama, Ksc 40, tree exudate.	28	101
10516	**Saccharomyces cerevisiae** History: IFO (I. Banno; K-73) -- K. Kodama, Ksc 73, tree exudate.	28	101
14791	**Bradyrhizobium japonicum** History: USDA 76 (R. Griffin).	28	218
14792	**Bradyrhizobium japonicum** History: USDA 110 (R. Griffin).	28	218
14793	**Agrobacterium tumefaciens** History: Shizuoka Agr. Exp. St. (K. Ohta; AtR 11; crown gall of rose).	28	201
14796	**Pseudomonas putida** History: Univ. of The Ryukyus (K. Yonaha; F-126; soil).	30	201
14797	**Mycobacterium diernhoferi** History: ATCC 19341 -- R. Bonicke, SN 1419 -- K. Diernhofer, drinking trough for cows.	37	201
14798	**Mycobacterium diernhoferi** History: ATCC 19344 -- R. Bonicke, SN 1425 -- K. Diernhofer, drinking trough for cows.	37	201
14799	**Mycoplasma neurolyticum** History: ATCC 19988 -- Walter Reed Army Institute -- NIHA -- Children's Hospital, Cincinnati (A.B. Sabin; mouse brain).	37	246
14800	**Mycoplasma neurolyticum** History: ATCC 15049 -- Walter Reed Army Institute -- Lister Institute (R.M. Lemcke; mouse brain).	37	246
14802	**Streptomyces murinus** History: ATCC 19788 -- ISP 5091 -- NRRL B-2286 -- Frommer. Type strain	28	227
14808	**Pseudomonas fluorescens** History: Nara Women's Univ. (R. Kurushima).	30	201
14811	**Brevibacterium epidermidis** History: JCM 2593 -- NCDO 2286 -- D.G. Pitcher, P159, Human skin.	30	201
14812	**Brevibacterium casei**	30	201

History: JCM 2594 -- NCDO 2048 -- M.E. Sharpe, CMD1, Cheddar cheese.

14814 **Acetobacter pasteurianus** 30 202
History: Shizuoka Univ. (Y. Yamada) -- NCIB 7029 -- NCTC 7029 -- T.K. Walker, vinegar brew.

14815 **Acetobacter hansenii** 30 202
History: Shizuoka Univ. (Y. Yamada) -- NCIB 8752 -- T.K. Walker, Malt vinegar brewery acetifiers.

14816 **Acetobacter hansenii** 30 202
History: Shizuoka Univ. (Y. Yamada) -- NCIB 8246 -- J. Senez -- M. Aschner, local vinegar, Israel.

14817 **Acetobacter hansenii** 30 202
History: Shizuoka Univ. (Y. Yamada) -- NCIB 8747 -- J.L. Shimwell -- M. Schramm, local vinegar brew, Israel.

14818 **Acetobacter aceti** 30 202
History: Shizuoka Univ. (Y. Yamada) -- NCIB 8621 -- W. Verhoeven -- J. Frateur, alcohol turned to vinegar.

14819 **Gluconobacter oxydans** 30 202
History: Shizuoka Univ. (Y. Yamada) -- NCIB 9013 -- J.G. Carr, beer.

14820 **Acetobacter hansenii** 30 202
History: Shizuoka Univ. (Y. Yamada) -- NCIB 8746 -- J.L. Shimwell -- M. Schramm, local vinegar brew, Israel.

14821 **Acetobacter aceti** 30 202
History: Shizuoka Univ. (Y. Yamada) -- NCIB 6656 -- R.M. Nattrass, East African vinegar brew.

14822 **Acetobacter hansenii** 30 202
History: Shizuoka Univ. (Y. Yamada) -- NCIB 4940 -- NCTC 4940 -- A.C. Thaysen.

14823 **Streptomyces vellosus** 28 266
History: JCM 5023 -- KCC S-1023 -- NRRL 8037 -- Upjohn Co. 5656; soil.

14824 **Streptomyces aculeolatus** 28 243
History: JCM 6055 -- Cent. Res. Lab., Meiji Seika Kaisha, Ltd. (T. Shomura; SF-2415; soil). Type strain

14831 **Saccharomonospora glauca** 45 229
History: Inst. for Microb., Tech. Hochschul. Darmstadt (H.J. Kutzner; K 62; carbage compost) -- DSM 43769. Type strain

14834 **Kitasatosporia papulosa** 28 268
JCM 7250 -- Y. Nakamura, AB-110.

14835 **Kitasatosporia grisea** 28 2[illegible]
History: JCM 7249 -- Y. Nakamura, AA-107.

14836 **Kitasatosporia cystarginea** 28 266
History: JCM 7356 -- H. Kusakabe, RK-419.

14837 **Actinoplanes arizonaensis** 28 268
History: NRRL B-16399 -- Abbott Labs. (J. Karwowski; AB660D-122; soil). Type strain

14838 **Kitasatosporia mediocidica** 28 227
History: NRRL B-16110 -- M.P. Lechevalier, LL-81 -- Waksman Inst., L. McDaniels.

14840 **Microbispora griseoalba** 28 265
History: Sichuan Ind. Inst. Antibiot., Chengdu (R.-M. Hu; NA-2468; soil).

14841 **Saccharomonospora cyanea** 28 245
History: Sichuan Ind. Inst. Antibiot., Chengdu (R.-M. Hu; 88134; soil). Type strain

14842 **Streptomyces fragmentosporus** 45 269
History: ATCC 25210 -- A. Henssen, N13, stable manure. Type strain

14843 **Amycolatopsis mediterranei** 28 227
History: RTCI -- ATCC 21789.

14845 **Azorhizobium caulinodans** 30 218
History: Lab. Microbiol. & Microbial Genet., Belgium -- B. Dreyfus, ORS 571, stem nodules of *Sesbania rostrata*.

14846 **Mycoplasma canis** 37 267

History: Nat. Inst. Animal Health (K. Yamamoto; PG 14) -- NIHA, dog throat.

14847 **Acholeplasma granularum** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; BTS 39) -- NIHA, nasal cavity of pig.

14848 **Mycoplasma maculosum** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; PG 15) -- NIHA, throat of dog.

14849 **Mycoplasma spumans** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; PG 13) -- NIHA, vagina of dog.

14850 **Mycoplasma hominis** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; PG 21) -- NIHA, rectal swab.

14851 **Mycoplasma buccale** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; CH 20247) -- NIHA, oropharynx of child.

14852 **Mycoplasma meleagridis** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; strain 17529) -- NIHA, turkey sinus.

14853 **Mycoplasma iners** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; PG 30) -- NIHA, respiratory tract of chicken with chronic respiratory disease.

14854 **Mycoplasma fermentans** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; PG 18) -- NIHA, ulcerative balanitis.

14855 **Mycoplasma gallisepticum** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; PG 31) -- NIHA, airsac tissues of chicken with chronic respiratory disease.

14856 **Mycoplasma salivarium** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; PG 20) -- NIHA, saliva.

14857 **Mycoplasma bovirhinis** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; PG 43) -- NIHA, bovine respiratory tract.

14858 **Mycoplasma hyorhinis** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; BTS 7) -- NIHA, nasal cavity of pig.

14859 **Mycoplasma gallinarum** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; PG 16) -- NIHA, respiratory tract of fowl with coryza.

14860 **Mycoplasma arthritidis** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; PG 6) -- NIHA, knee joint of female rat.

14861 **Mycoplasma anatis** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; strain 1340) -- NIHA, duck sinusitis.

14862 **Mycoplasma bovigenitalium** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; PG 11) -- NIHA, bovine genital tract.

14863 **Micromonospora rhodorangea** 28 231
History: JCM 3189 -- KCC A-0189 -- NRRL 5326 -- Schering Corp. Type strain

14875 **Microbispora karnatakensis** 28 266
History: Meiji Seika Kaisha, Ltd., S. Miyadoh -- ATCC 35927. Type strain

14876 **Microbispora chromogenes** 28 266
History: Meiji Seika Kaisha, Ltd., S. Miyadoh -- JCM 3022. Type strain

14877 **Streptomyces paulus** 28 270
History: The Upjohn Co., Alma Dietz, UC 8560.

14878 **Microtetraspora caesia** 28 227
History: Meiji Seika Kaisha, Ltd., S. Miyadoh, -- ATCC 31295. Type strain

14879 **Microbispora indica** 28 266
History: Meiji Seika Kaisha, Ltd. S. Miyadoh, -- ATCC 35926. Type strain
14880 **Microbispora bispora** 45 227
History: Meiji Seika Kaisha, Ltd., S. Miyadoh -- ATCC 19993. Type strain
14886 **Streptomyces griseus subsp. formicus** 28 271
History: JCM 3140 -- KCC A-0140 -- NRRL 2470 -- Merck Sharp & Dohme Res. Labs., MA-143 (55R2670).
14887 **Streptomyces paradoxus** 28 266
History: JCM 3052 -- KCC A-0052 -- RIA 655 -- INMI 3180. Holotype strain
14888 **Actinomyces fulvoviridis** 28 266
History: JCM 4374 -- KCC S-0374 -- IFO 12863 -- SAJ -- ISP 5210 -- INMI 16-3.
14889 **Actinomyces cyanocolor** 28 266
History: JCM 4469 -- KCC S-0469 -- IFO 13034 -- SAJ -- ISP 5425 -- INMI 31-23.
14890 **Streptomyces armentosus** 28 266
History: JCM 4684 -- KCC S-0684 -- NRRL 3176 -- Upjohn Co.
14891 **Streptomyces laeteviolaceus** 28 227
History: JCM 4875 -- KCC S-0875 -- N. Muto -- R. Shinobu, 760.
14892 **Streptomyces anthocyanicus** 28 227
History: JCM 5058 -- KCC S-1058 -- MS 1456 -- ATCC 19821 -- N.A. Krassilnikov, 69. Holotype strain
14893 **Streptomyces avermitilis** 28 268
History: JCM 5070 -- KCC S-1070 -- NRRL 8165 -- Merck Sharp & Dohme Res. Labs., MA-4680. Holotype strain
14894 **Rhodococcus rhodochrous** 28 231
History: Nippon Medical School (S. Takaichi; RNMS1) -- IAM.
14895 **Mycoplasma lipophilum** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; strain MaBy) -- NIHA, sputum from pneumonia patient.
14896 **Mycoplasma pulmonis** 37 267
History: Nat. Inst. Animal Health (K. Yamamoto; strain Ash) -- NIHA, lung lesion of rat.
14905 **Cytophaga succinicans** 24 272
History: DSM 4002 -- H. Reichenbach, Cy su3 -- J.T. Staley -- R.L. Anderson & E.J. Ordal, 8.
14906 **Paracoccus denitrificans** 30 203
History: DSM 1403 -- T.H. Nokhal, N-1, sewage.
14907 **Paracoccus denitrificans** 30 203
History: DSM 1404 -- T.H. Nokhal, N4, sludge.
14908 **Paracoccus denitrificans** 30 203
History: DSM 1405 -- T.H. Nokhal, N5, meadow soil.
14909 **Paracoccus denitrificans** 30 203
History: DSM 1406 -- T.H. Nokhal, N7, horse manure.
14910 **Paracoccus denitrificans** 30 203
History: DSM 1407 -- T.H. Nokhal, N10, cow dung.
14911 **Paracoccus denitrificans** 30 203
History: DSM 1408 -- T.H. Nokhal, N11, field soil.
14912 **Paracoccus halodenitrificans** 30 273
History: DSM 735 -- CCM 286 -- N.E. Gibbons, wiltshire bacon curing brine.
14913 **Actinoplanes consettensis** 28 227
History: Univ. Newcastle (M. Goodfellow; LA 97; river derwent). Type strain
14914 **Actinoplanes durhamensis** 28 227
History: Univ. Newcastle (M. Goodfellow; LA 139; river derwent). Type strain
14915 **Actinoplanes humidus** 28 227
History: Univ. Newcastle (M. Goodfellow; LA 6; river derwent). Type strain
14916 **Actinoplanes palleronii** 28 227

History: Univ. Newcastle (M. Goodfellow; LA 83; river derwent). Type strain

14917 **Aquaspirillum anulus** 30 203
History: Suzugamine Women's Coll. (Y. Terasaki) -- M.A. Williams, pond water.

14918 **Aquaspirillum aquaticum** 30 203
History: Suzugamine Women's Coll. (Y. Terasaki) -- ATCC 11330, fresh water.

14919 **Aquaspirillum delicatum** 30 203
History: Suzugamine Women's Coll. (Y. Terasaki) -- ATCC 14667, distilled water.

14920 **Aquaspirillum gracile** 30 203
History: Suzugamine Women's Coll. (Y. Terasaki) -- ATCC 19624, pond water.

14921 **Aquaspirillum itersonii** 30 203
History: Suzugamine Women's Coll. (Y. Terasaki) -- ATCC 11331.

14922 **Aquaspirillum peregrinum** 30 203
History: Suzugamine Women's Coll. (Y. Terasaki) -- NCIB 9435, primary oxidation pond.

14923 **Aquaspirillum serpens** 30 203
History: Suzugamine Women's Coll. (Y. Terasaki) -- M.A. Williams, pond water.

14924 **Aquaspirillum serpens** 30 203
History: Suzugamine Women's Coll. (Y. Terasaki) -- M.A. Williams, fresh water.

14925 **Aquaspirillum sinuosum** 30 203
History: Suzugamine Women's Coll. (Y. Terasaki) -- ATCC 9786, fresh water.

14935 **Actinoplanes derwentensis** 25 228
History: Univ. Newcastle (M. Goodfellow; LA107; river sediment). Type strain

14939 **Klebsiella planticola** 37 201
History: RTCI (Y. Sugiyama) -- ATCC 33531.

14940 **Klebsiella pneumoniae** 37 201
History: IFO (K. Imai) -- GIFU 2924 -- KM 2924 -- NCTC 9633.

14941 **Klebsiella terrigena** 37 201
History: RTCI (Y. Sugiyama) -- ATCC 33257.

14942 **Cytophaga johnsonae** 30 201
History: IFO (A. Yokota) -- Gifu Univ. School of Medicine (E. Yabuuchi; GIFU 2500) -- ATCC 17061.

14943 **Flavobacterium breve** 30 201
History: IFO (A. Yokota) -- Gifu Univ. School of Medicine (E. Yabuuchi; GIFU 3159) -- JCM 1276.

14944 **Flavobacterium indologenes** 30 201
History: IFO (A. Yokota) -- Gifu Univ. School of Medicine (E. Yabuuchi; GIFU 1347) -- George Washington Univ. Medical Center (R. Hugh; RH 542; trachea at autopsy).

14945 **Flavobacterium odoratum** 30 201
History: IFO (A. Yokota) -- Gifu Univ. School of Medicine (E. Yabuuchi; GIFU 1357) -- RIMD.

14946 **Sphingobacterium mizutae** 30 201
History: IFO (A. Yokota) -- Gifu Univ. School of Medicine (E. Yabuuchi; GIFU 1203) -- S. Mizuta, ventricular fluid.

14947 **Sphingobacterium multivorum** 30 201
History: IFO (A. Yokota) -- Gifu Univ. School of Medicine (E. Yabuuchi; GIFU 2812) -- NCTC 11343.

14948 **Sphingobacterium spiritivorum** 30 201
History: IFO (A. Yokota) -- Gifu Univ. School of Medicine (E. Yabuuchi; GIFU 3101) -- CDC E7288, intrauterine specimen.

14950 **Comamonas acidovorans** 30 201
History: JCM 5833 (Y. Kosako; 85004) -- Nissui Pharm. Co. Ltd. -- ATCC 15668 -- R. Hugh, 2167 -- Technische Hogeschool, Delft, soil enriched with

acetamide.

14951 **Comamonas testosteroni** 30 201
History: JCM 5832 (Y. Kaneko; 85013) -- Nissui Pharm. Co. Ltd. -- ATCC 11996 -- P. Talalay, soil.

14954 **Haloarcula vallismortis** 37 255
History: Tokushima Pref. Food Res. Inst. (K. Fukuta; 0-1; salted boiled-Wakame).

14955 **Haloarcula vallismortis** 37 255
History: Tokushima Pref. Food Res. Inst. (K. Fukuta; 0-5; salted boiled-Wakame).

14956 **Rhodococcus equi** 30 201
History: JCM 1311 (K. Suzuki; CNF 002) -- AJ 1402 -- ATCC 6939 -- CTC 1621 -- H. Magnusson, lung abscess of foal.

14957 **Cytophaga agarovorans** 30 204
History: ATCC 19043 -- H. Veldkamp, marine mud.

14958 **Cytophaga aquatilis** 24 203
History: ATCC 29551 -- W. Strohl, strain N, gills of diseased salmon.

14960 **Cytophaga flevensis** 24 203
History: ATCC 27944 -- H.J. van der Meulen, A-34, lake water.

14961 **Cytophaga lytica** 30 204
History: ATCC 23178 -- R.A. Lewin, LIM-21, beach mud.

14962 **Flavobacterium uliginosum** 24 204
History: ATCC 14397 -- C.E. ZoBell, 533.

14963 **Flavobacterium thalpophilum** 30 203
History: ATCC 43320 -- NCTC 11429.

14965 **Rhizobium galegae** 30 218
History: Univ. of Helsinki (K. Lindström; HAMBI 540; root nodule of *Galega orientalis*).

14970 **Saccharothrix waywayandensis** 28 231
History: NRRL B-16159 -- IMRU (M.P. Lechevalier; LLR-37Z-15; soil). Type strain

14971 **Saccharothrix texasensis** 28 231
History: NRRL B-16134 -- IMRU (M.P. Lechevalier; LLR-37U-77; soil). Type strain

14972 **Cytophaga xantha** 15 203
History: IAM 12026 -- Univ. Tokyo (K. Inoue; 5-0-c; soil).

14973 **Cytophaga arvensicola** 30 203
History: IAM 12650 -- Univ. Tokyo (H. Oyaizu) -- Osaka Univ. (T. Harada; M64; soil).

14975 **Flavobacterium yabuuchiae** 30 203
History: NCTC 12113 -- CDC D7529.

14978 **Pseudomonas hydrogenothermophila** 50 203
History: ATU (T. Kodama; TH-1; soil).

14983 **Flavobacterium ferrugineum** 30 203
History: IAM 1493 -- IAM (H. Iizuka & K. Komagata; Kp-8).

14984 **Flavobacterium ferrugineum** 30 201
History: JCM 7453 -- H. Oyaizu, KS 0419 -- AJ 2501 -- K. Komagata & H. Ogawa, P-15, Frozen food.

14985 **Flavobacterium ferrugineum** 30 201
History: JCM 7454 -- H. Oyaizu, KS 0420 -- AJ 2502 -- K. Komagata & H. Ogawa, P-48, Frozen rainbow traut.

14986 **Flavobacterium sewanense** 30 201
History: JCM 7461 -- H. Oyaizu, KS 0433 -- AJ 2449 -- IAM 1014.

14987 **Flavobacterium sp.** 30 201
History: JCM 7463 -- H. Oyaizu, KS 0435 -- AJ 2457 -- H. Iizuka & K. Komagata, Kp-16.

14988 **Actinomadura coeruleoviolacea** 28 231
History: VKM Ac-1083 -- INA (L.P. Terekhova; INA 3564; soil). Type strain

14989 **Actinomadura luzonensis** 28 227

History: VKM Ac 873 -- K. Tomita, G455-101, soil. Type strain

14990 **Actinoplanes cyaneus** 28 231
History: VKM Ac-1095 -- INA 1569, soil. Type strain

14991 **Amycolata alni** 28 228
History: VKM Ac-901 -- L.S. Sharaya, 3LS, root nodules of *Alnus incana*.

14992 **Flavobacterium ferrugineum** 30 201
History: ATCC 13524 -- J.M. Coffey.

14993 **Thiobacillus novellus** 30 201
History: ATCC 8093 -- R.L. Starkey.

14994 **Rhizobium leguminosarum** 30 218
History: IFO (T. Sakane; Rz-9; root nodules of *Phaseolus vulgaris*).

14995 **Rhizobium leguminosarum** 30 218
History: IFO (T. Sakane; Rz-20; root nodules of *Medicago lupulina*).

14996 **Rhizobium loti** 30 218
History: IFO (T. Sakane; Rz-15; root nodules of *Lotus corniculatus* var. *japonicus*).

14997 **Rhizobium loti** 30 218
History: IFO (T. Sakane; Rz-18; root nodules of *Lotus corniculatus* var. *japonicus*).

14998 **Rhizobium loti** 30 218
History: IFO (T. Sakane; Rz-19; root nodules of *Lotus corniculatus* var. *japonicus*).

14999 **Rhizobium loti** 30 218
History: IFO (T. Sakane; Rz-24; root nodules of *Lotus corniculatus* var. *japonicus*).

15000 **Rhizobium loti** 30 218
History: IFO (T. Sakane; Rz-29; root nodules of *Lotus corniculatus* var. *japonicus*).

15001 **Bradyrhizobium japonicum** 30 218
History: IFO (T. Sakane; Rz-5; root nodules of *Glycin max*).

15002 **Bradyrhizobium japonicum** 30 218
History: IFO (T. Sakane; Rz-6; root nodules of *Glycin max*).

15003 **Bradyrhizobium sp.** 30 218
History: IFO (T. Sakane; Rz-8; root nodules of *Phaseolus angularis*).

15004 **Bradyrhizobium sp.** 30 218
History: IFO (T. Sakane; Rz-10; root nodules of *Vicia hirsuta*).

15005 **Bradyrhizobium sp.** 30 218
History: IFO (T. Sakane; Rz-11; root nodules of *Pueraria thunbergiana*).

15006 **Bradyrhizobium sp.** 30 218
History: IFO (T. Sakane; Rz-12; root nodules of *Kummerowia striata*).

15007 **Bradyrhizobium sp.** 30 218
History: IFO (T. Sakane; Rz-14; root nodules of *Kummerowia stipulacea*).

15008 **Nocardia brasiliensis** 28 227
History: Pharm. Res. Cent. Meiji Seika Kaisha (S. Miyadoh; SF2457; soil).

15012 **Oerskovia turbata** 28 230
History: IMET 7006 -- H. Prauser, 377-78, soil.

15013 **Oerskovia xanthineolytica** 28 230
History: IMET 7075 -- H. Prauser, 468-29, compost soil.

15014 **Oerskovia turbata** 28 230
History: IMET 7153 -- J. Fabian -- R.E. Gordon, IMRU 761, soil.

15015 **Oerskovia turbata** 28 230
History: IMET 7405 -- NCIB 10587, soil. Type strain

15016 **Promicromonospora citrea** 28 230
History: IMET 7260 -- H. Prauser, 604-56, soil.

15017 **Promicromonospora citrea** 28 230
History: IMET 7261 -- H. Prauser, 623-3, meadow.

15018 **Promicromonospora citrea** 28 230
History: IMET 7001 -- H. Prauser, 393-9, soil.

15019 **Lactobacillus helveticus** 37 205
History: JCM 1120 -- ATCC 15009 -- P.A. Hansen, Lh12 -- S. Orla-Jensen 12,

Thermobacterium helveticum, Emmental (Swiss) cheese.

15030 **Flavobacterium branchiophila** 20 274
History: Dept. of Fisheries, Univ. Tokyo (H. Wakabayashi; BGD-7721; *Onchorhyncus masou*).

15033 **Flavobacterium sp.** 30 201
History: Kobe Univ. of Commerce (F. Kawai; No. 203; soil).

15034 **Escherichia coli** 37 201
History: JCM 5491 -- ATCC 25922.

15035 **Staphylococcus aureus** 37 201
History: JCM 2874 -- NIHJ 85047 -- ATCC 29213.

15036 **Aureobacterium barkeri** 30 201
History: JCM 1343 -- CNF 095 -- NCIB 9658 -- J. Bhat, 7, raw domestic sewage.

15037 **Aureobacterium liquefaciens** 30 201
History: JCM 3879 -- DSM 20638 -- NCIB 11509 -- K. Robinson Mbm 15, milk.

15038 **Aureobacterium saperdae** 30 201
History: JCM 1352 (K. Suzuki; CNF 058) -- AJ 3126 -- CCEB 366 -- O. Lysenko, 48-1-4, insect (*Saperda carcharias*).

15039 **Aureobacterium flavescens** 28 201
History: DSM 20643 -- NCIB 9221 -- A.G. Lochhead, 401, soil.

15041 **Bacillus macerans** 30 203
History: Inst. of Microbiol., Acad. Sinica, China (Z-Q, Lee; AS 1.65) -- GSRICI 1203.

15044 **Escherichia coli** 37 275
History: Osaka Univ. (K. Matsubara; JM109/pAM82).
Genotype: recA1 endA1 gyrA96 thy1 hsdR17 relA1 (lac proAB) supE44 /pAM82

15045 **Saccharopolyspora gregorii** 24 228
History: NCIMB 12823 -- J. Lacey, A333, hay. Type strain

15046 **Saccharopolyspora hordei** 37 228
History: NCIB 12824 -- J. Lacey, Lacey A735, hay. Type strain

15047 **Pseudoamycolata halophobica** 28 227
History: DSM 43092 -- R.E. Gordon, IMRU -- M.P. Lechevalier. Chemolithotrophic growth with hydrogen.

15048 **Pseudoamycolata halophobica** 28 227
History: DSM 43089 -- R.E. Gordon -- M.P. Lechevalier, ss1/1, soil. Type strain

15051 **Cytophaga hutchinsonii** 30 276
History: DSM 1761 -- H. Reichenbach, strain Cyh1 -- NCIB -- P.H.A. Sneath, strain D465.

15052 **Flavobacterium aquatile** 24 277
History: ATCC 11947 -- O.B. Weeks -- E. Taylor, strain F36, deep well.

15053 **Flavobacterium balustinum** 30 203
History: ATCC 33487 -- NCTC 11212 -- V. bonifas -- C. Tysset, heart blood of fish.

15054 **Flavobacterium gleum** 30 203
History: ATCC 35910 -- R.J. Owen, vaginal swab.

15055 **Flexibacter elegans** 30 204
History: ATCC 23112 -- R.A. Lewin, strain NZ-1, hot spring.

15056 **Flexibacter filiformis** 30 277
History: DSM 527 -- H. Reichenbach, strain Fxe1 (*Flexibacter elegans*).

15057 **Flexibacter sancti** 30 272
History: DSM 784 -- H. Reichenbach -- ATCC 23092 -- R.A. Lewin, strain BA-3 -- A. Cataldi.

15059 **Nocardia pinensis** 28 279
History: UQM 3063 -- L.L. Blackall, surface foam, sewage treatment plant. Type strain

15060 **Flexibacter flexilis** 30 278
History: ATCC 23079 -- R.A. Lewin, strain CR-63, Lilly pond.

15061 **Saccharopolyspora taberi** 28 228
History: NRRL B-16173 -- M.P. Lechevalier -- W.A. Tuber, soil sample from

Texas. Type strain

15062 **Promicromonospora citrea** 28
History: IFO (A. Yokota; NAK-14; mangrove leaf). Fragmentation of vegetative mycelia. Cell wall type: VI.

15065 **Amycolatopsis methanolica** 28 227
History: NCIB (T. Dando; Nocardia sp. strain 239 ; LMD 8032; soil). Type strain

15074 **Aureobacteirum sp.** 30 203
History: DSM 20143 -- NCIB 9568 -- T. Gibson -- L.E. Topping, No.69 (Group 2a), soil.

15075 **Aureobacterium sp.** 30 203
History: DSM 20489 -- H. Seiler, S110, activated sludge in dairy factory.

15076 **Aureobacterium sp.** 30 203
History: DSM 20606 -- K.H. Schleifer-- H. Seiler, Co112, activated sludge in dairy factory.

15077 **Aureobacterium sp..** 30 203
History: JCM 1358 (K. Nakayama; 114-2; soil).

15078 **Curtobacteirum albidum** 30 203
History: JCM 1344 (K. Suzuki; CNF 028) -- AJ 1472 -- IAM 1631 -- K. Komagata, Y-3-2, Chinese paddy.

15094 **Bacillus sphaericus** 30 203
History: NCTC 7585.

15095 **Bacillus sphaericus** 30 203
History: NCTC 10338.

15097 **Nocardiopsis albus subsp. albus** 28 227
History: DSM 43377 -- M. Goodfellow, A92 -- W.A. Causey, CDC W2536, drainage from hip.

15099 **Sphingomonas adhaesiva** 30 203
History: JCM 7370 -- GIFU 11458 (E. Yabuuchi; Op-55).

15100 **Sphingomonas parapaucimobilis** 30 203
History: JCM 7510 -- GIFU 11387 --Ohtemae Hospital, Osaka, 3807, urine.

15101 **Sphingomonas parapaucimobilis** 30 203
History: JCM 7512 -- GIFU 2135.

15102 **Sphingomonas yanoikuyae** 30 203
History: JCM 7371 -- GIFU 9882, clinical specimen.

15103 **Sphingomonas sp.** 30 203
History: JCM 7513 -- GIFU 11456 (E. Yabuuchi; Op-1).

20065 **Escherichia coli phage ØX174** 37
History: Milipore Corp. -- Univ.of Massachusettes (C.E. Dowel, Jr. ; ØX174 cs70 am3).

31400 **Phytophthora capsici** 24 1
History: PPCT P 3 (C.-W. Kao & L.-S. Leu; bulb of *Allium cepa*). PP 56-K-2393 Mating type: A2

31402 **Phytophthora capsici** 24 1
History: PPCT P 45 (C.-W. Kao & L.-S. Leu; stem of *Lycopersicum esculentum*). PP 56-K-2393 Mating type: A1

31408 **Phytophthora citrophthora** 24 1
History: PPCT P 50 (C.-W. Kao & L.-S. Leu; basal stem of *Dianthus bartatus*). PP 56-K-2393 Mating type: A1

31410 **Phytophthora citrophthora** 24 1
History: PPCT P 232 (C.-W. Kao & L.-S. Leu; fruit of *Citrus limon*). PP 56-K-2393 Mating type: A1

31411 **Phytophthora cryptogea** 24 1
History: PPCT P 85 (C.-W. Kao & L.-S. Leu; basal stem of *Aster* sp.). PP 56-K-2393

31412 **Phytophthora cryptogea** 24 1
History: PPCT P 90 (C.-W. Kao & L.-S. Leu; root of *Arachis hypogaea*). PP 56-K-2393

31413 **Phytophthora melonis** 24 1
History: PPCT P 40 (C.-W. Kao & L.-S. Leu; fruit of *Benincasa cerifera*).

PP 56-K-2393 Mating type: A2

31414 **Phytophthora melonis** 24 1
History: PPCT P 41 (C.-W. Kao & L.-S. Leu; basal stem of *Cucumis sativus*). PP 56-K-2393 Mating type: A2

31415 **Phytophthora melonis** 24 1
History: PPCT P 169 (C.-W. Kao & L.-S. Leu; fruit of *Momordica charantia*). PP 56-K-2393 Mating type: A2

31416 **Phytophthora nicotianae var. parasitica** 24 1
History: PPCT P 2 (C.-W. Kao & L.-S. Leu; fruit of *Lycopersicum esculentum*). PP 56-K-2393 Mating type: A2

31419 **Phytophthora nicotianae var. parasitica** 24 1
History: PPCT P 24 (C.-W. Kao & L.-S. Leu; fruit of *Carica papaya*). PP 56-K-2393 Mating type: A2

31423 **Phytophthora nicotianae var. parasitica** 24 1
History: PPCT P 48 (C.-W. Kao & L.-S. Leu; leaf of *Ananas comosus*). PP 56-K-2393 Mating type: A1

31425 **Phytophthora nicotianae var. parasitica** 24 1
History: PPCT P 71 (C.-W. Kao & L.-S. Leu; bulb of *Allium cepa*). PP 56-K-2393 Mating type: A1

31428 **Phytophthora palmivora** 24 1
History: PPCT P 54 (C.-W. Kao & L.-S. Leu; fruit of *Carica papaya*). PP 56-K-2393 Mating type: A1

31430 **Colletotrichum gloeosporioides** 24 1
History: PPCT A 1 (H.-C. Yang & L.-S. Leu; fruit of *Mangifera indica*). PP 56-K-2393

31432 **Colletotrichum musae** 24 1
History: PPCT A 7 (H.-C. Yang & L.-S. Leu; fruit of *Musa sapientum*). PP 56-K-2393

31434 **Colletotrichum acutatum** 24 1
History: PPCT A 9-1 (H.-C. Yang & L.-S. Leu; fruit of *Eugenia javanica*). PP 56-K-2393

31436 **Colletotrichum gloeosporioides** 24 1
History: PPCT A 10-1 (H.-C. Yang & L.-S. Leu; fruit of *Vitis vinifera*). PP 56-K-2393

31438 **Colletotrichum lagenarium** 24 1
History: PPCT A 11 (H.-C. Yang & L.-S. Leu; fruit of *Cucumis sativus*). PP 56-K-2393

31439 **Glomerella cingulata** 24 1
History: PPCT A 12 (H.-C. Yang & L.-S. Leu; fruit of *Cucumis melo*). PP 56-K-2393

31440 **Colletotrichum falcatum** 24 1
History: PPCT A 13 (H.-C. Yang & L.-S. Leu; leaf of *Saccharum officinarum*). PP 56-K-2393

31442 **Colletotrichum acutatum** 24 1
History: PPCT A 14-2 (H.-C. Yang & L.-S. Leu; fruit of *Malus pumila* var. *domestica*). PP 56-K-2393

31444 **Glomerella cingulata** 24 1
History: PPCT A 15-1 (H.-C. Yang & L.-S. Leu; fruit of *Carica papaya*). PP 56-K-2393

31459 **Colletotrichum gloeosporioides** 24 1
History: PPCT A 25 (H.-C. Yang & L.-S. Leu; leaf of *Nicotiana tabacum*). PP 56-K-2393

31462 **Colletotrichum lagenarium** 24 1
History: PPCT A 28 (H.-C. Yang & L.-S. Leu; leaf of *Luffa cylindrica*). PP 56-K-2393

31463 **Glomerella cingulata** 24 1
Histroy: PPCT A 29 (H.-C. Yang & L.-S. Leu; leaf of *Hibiscus sabdariffa*). PP 56-K-2393

31481 **Colletotrichum dematium** 24 1
History: PPCT A 44 (H.-C. Yang & L.-S. Leu; pod of *Glycine max*).

PP 56-K-2393

31483 **Colletotrichum acutatum** 24 1
History: PPCT A 46 (H.-C. Yang & L.-S. Leu; fruit of *Pyrus pyrrifolia* var. *culta*). PP 56-K-2393

31484 **Colletotrichum lagenarium** 24 1
Histroy: PPCT A 47-1 (H.-C. Yang & L.-S. Leu; fruit of *Citrullus vulgaris*). PP 56-K-2393

31544 **Colletotrichum lindemuthianum** 24 1
History: PPCT A 95 (H.-C. Yang & L.-S. Leu; leaf of *Pongamia pinnata*). PP 56-K-2393

31545 **Colletotrichum lindemuthianum** 24 1
History: PPCT A 96 (H.-C. Yang & L.-S. Leu; leaf of *Dalbergia latifolia*). PP 56-K-2393

31508 **Colletotrichum truncatum** 24 1
Histroy: PPCT A 135-2 (H.-C. Yang & L.-S. Leu; leaf of *Syngonium auritum*). PP 56-K-2393

31598 **Colletotrichum lindemuthianum** 24 1
History: PPCT A 143 (H.-C. Yang & L.-S. Leu; leaf of *Arachis hypogaea*). PP 56-K-2393

32173 **Shearia formosa** 20 1
History: IFO (A. Nakagiri; TKB-C-1046) -- TKB-C-1046 (A. Nakagiri) -- S. Murata, rotten branches of *Magnolia kobus*.

32174 **Phytophthora megasperma** 24 1
History: Yokohama Plant Protection St. (E. Kimishima; EL-8701; white trumpet lily). Mating type: homothalic

32175 **Phytophthora megasperma** 24 1
History: Yokohama Plant Protection St. (E. Kimishima; EL-8702; white trumpet lily). Mating type: homothalic

32176 **Phytophthora megasperma** 24 1
History: Yokohama Plant Protection St. (E. Kimishima; EL-8703; white trumpet lily). Mating type: homothalic

32179 **Cladosporium carpophilum** 24 1
History: Fukui Agric. Exp. Sta. (N. Honda; Cladosporium 88468; fruit of *Prunus mume*).

32180 **Neosartorya spathulata** 28 8
History: M. Takada, NHL 2947, cultivated soil.

32181 **Talaromyces derxii** 24 8
History: M. Takada, NHL 2980, cultivated soil.

32182 **Talaromyces derxii** 24 8
History: M. Takada, NHL 2981, cultivated soil. Mating type A

32183 **Talaromyces derxii** 24 8
History: M. Takada, NHL 2982, cultivated soil. Mating type α

32184 **Lyophyllum decastes** 24 7
History: Univ. of Lausanne (H. Clémençon; 88/179J).

32185 **Lyophyllum decastes** 24 7
History: Univ. of Lausanne (H. Clémençon; 88/155J).

32186 **Lyophyllum decastes** 24 7
History: Univ. of Lausanne (H. Clémençon; 88/176J).

32187 **Lyophyllum shimeji** 24 7
History: Univ. of Lausanne (H. Clémençon; 88/187J).

32188 **Lyophyllum shimeji** 24 7
History: Univ. of Lausanne (H. Clémençon; 88/178J).

32189 **Lyophyllum shimeji** 24 7
History: Univ. of Lausanne (H. Clémençon; 88/177J).

32190 **Agaricus arvensis** 24 1
History: IFO (T. Ito; T. Ito S64-1; humus).

32191 **Phytophthora cactorum** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 82GM-4; Stem of tulip cv. Golden Melody).

32192 **Phytophthora cactorum** 24 1

History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 83A1-1; Leaf of tulip cv. Allegretto).

32193 **Phytophthora cactorum** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 83GM-4; Stem of tulip cv. Golden Melody).

32194 **Phytophthora cactorum** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 85MC-1; Stem of tulip cv. Monte Carlo).

32195 **Pythium afertile** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 83P-10; Leaf of tulip cv. Parade).

32196 **Pythium dissotocum** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 85QN-1; Leaf of tulip cv. Queen of Night).

32197 **Pythium sp.** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 83Y-1; Leaf of tulip cv. Yokohama).

32198 **Pythium sylvaticum** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 83RM-9; Leaf of tulip cv. Red Matador).

32199 **Coniochaeta cymbiformispora** 24 8
History: IFO (T. Ito; T. Ito S5870-29-2; swamp soil).

32200 **Coniochaeta cymbiformispora** 24 8
History: IFO (T. Ito; T. Ito S6170-32-1; burned soil).

32201 **Pithomyces graminicola** 24 1
History: IFO (T. Ito; Shigemitsu A) -- H. Shigemitsu, mushroom bed.

32202 **Chlorociboria aeruginascens** 24 1
History: IFO (T. Ito; T. Ito S63-61; dead wood).

32203 **Fusarium oxysporum f. sp. tulipae** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 80RB-7; bulb of tulip).

32204 **Fusarium oxysporum f. sp. tulipae** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 80RB-32; bulb of tulip).

32205 **Fusarium oxysporum f. sp. tulipae** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 80WP-104; bulb of tulip).

32206 **Fusarium oxysporum f. sp. tulipae** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 80Ma-105; bulb of tulip).

32207 **Fusarium oxysporum f. sp. tulipae** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 80 Ox-113; bulb of tulip).

32208 **Fusarium oxysporum f. sp. tulipae** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 80RM-116; bulb of tulip).

32209 **Fusarium roseum f. sp. cerealis 'Avenaceum'** 24 1
History: Toyama Agric. Res. Cent. (H. Mukobata; TAC 82RB-1; stem of tulip).

32210 **Pythium ultimum var. ultimum** 24 1
History: Coll. Agric., Univ. Osaka Pref. (T. Ichitani; UOP 360; Root of *Tulipa gesneriana*).

32211 **Pythium ultimum var. ultimum** 24 1
History: Coll. Agric., Univ. Osaka Pref. (T. Ichitani; UOP 376; Root of *Tulipa gesneriana*).

32212 **Pythium spinosum** 24 1
History: Coll. Agric., Univ. Osaka Pref. (T. Ichitani; UOP 371; Root of *Tulipa gesneriana*).

32213 **Pythium spinosum** 24 1
History: Coll. Agric., Univ. Osaka Pref. (T. Ichitani; UOP 372; Root of

Tulipa gesneriana).

32214 **Pythium spinosum** 24 1
History: Coll. Agric., Univ. Osaka Pref. (T. Ichitani; UOP 374; Rhizosphere soil of tulip).

32215 **Penicillium camembertii** 24 1
History: IFO (T. Ito; T. Ito S64-2; commercial cheese).

32216 **Phytophthora vesicula** 24 1
History: CBS 393.81 -- C.J. Anastasiou, *Prunus laurocerasus* leaves submerged in seawater.

32217 **Grovesinia pruni** 20 1
History: Fac. Agr., Hirosaki Univ. (Y. Harada; 839) -- Aomori Field Crops and Horticult. Exp. Stat. (S. Noro; Noro 82 Aa-3; apricot leaf).

32218 **Oxydothis nypae** 24 13
History: IFO (A. Nakagiri; AN-992) -- Inst. Biol. Sci., Univ. Tsukuba (A. Nakagiri; AN-992; decaying fronds of *Nypa fruticans*).

32219 **Pythium aristosporum** 24 1
History: Coll. Agric., Univ. Osaka Pref. (T. Ichitani; UOP 379; Rhizosphere soil of zoysia green).

32220 **Chaetomium bostrychodes** 24 2
History: IFO (T. Ito; 1701-9; soil).

32221 **Chaetomium brasiliense** 24 2
History: IFO (T. Ito; 1701-E5; soil). PP 61-K-1699

32222 **Dichotomomyces cejpii var. cejpii** 24 5
History: IFO (T. Ito; 1739-701; soil). PP 61-K-1699

32223 **Emericella quadrilineata** 24 5
History: IFO (T. Ito; 1761-453; soil).

32224 **Emericella rugulosa** 24 5
History: IFO (T. Ito; 1775-451; soil).

32225 **Gymnoascus reessii** 24 2
History: IFO (T. Ito; 1755-11; soil).

32226 **Microascus cinereus** 24 2
History: IFO (T. Ito; 1725-6; soil). PP 61-K-1699

32227 **Microascus trigonosporus** 24 2
History: IFO (T. Ito; 1774-9; soil).

32228 **Monascus anka** 24 4
History: IFO (T. Ito; 1852-451; red rice).

32229 **Petriellidium boydii** 24 2
History: IFO (T. Ito; 1724-E2; soil).

32230 **Petromyces alliaceus** 24 1
History: IFO (T. Ito; 1811-1; soil). PP 61-K-1699

32231 **Petromyces alliaceus** 24 1
History: IFO (T. Ito; 1784-3; soil). PP 61-K-1699

32232 **Pithoascus intermedius** 24 2
History: IFO (T. Ito; 1724-E3; soil). PP 61-K-1699

32233 **Pseudeurotium ovale** 24 5
History: IFO (T. Ito; 1701-E1; soil).

32234 **Pseudeurotium zonatum** 24 5
History: IFO (T. Ito; 1701-E3; soil).

32235 **Sordaria humana** 24 8
History: IFO (T. Ito; 1797-451; soil).

32236 **Talaromyces byssochlamydoides** 37 5
History: IFO (T. Ito; 1713-453; soil).

32237 **Talaromyces helicus var. helicus** 24 5
History: IFO (T. Ito; 1713-E2; soil).

32238 **Talaromyces trachyspermus** 30 5
History: IFO (T. Ito; 1740-453; soil).

32239 **Thermoascus crustaceus** 37 5
History: IFO (T. Ito; 1760-453; soil).

32240 **Thielavia arenaria** 28 2
History: IFO (T. Ito; 1718-452; soil).

32241	**Acremonium alabamense**	37	5
	History: IFO (T. Ito; 1716-451; soil).		
32242	**Acremonium curvulum**	24	8
	History: IFO (T. Ito; 1725-2; soil).		
32243	**Acremonium potronii**	24	1
	History: IFO (T. Ito; 1806-9; soil).		
32244	**Acremonium strictum**	24	1
	History: IFO (T. Ito; 1704-702; soil). PP 61-K-1699		
32245	**Acrodontium crateriforme**	24	1
	History: IFO (T. Ito; 1823-2; soil).		
32246	**Acrophialophora levis**	28	5
	History: IFO (T. Ito; 1756-453: soil).		
32247	**Arthrobotrys oligospora**	24	1
	History: IFO (T. Ito; 1726-702; soil).		
32248	**Aspergillus candidus**	24	1
	History: IFO (T. Ito; 1801-E1; sheep dung). PP 61-K-1699		
32249	**Aspergillus carneus**	24	5
	History: IFO (T. Ito; 1723-451; soil).		
32250	**Cladosporium herbarum**	24	1
	History: IFO (T. Ito; 1820-8; soil). PP 61-K-1699		
32251	**Doratomyces microsporus**	24	8
	History: IFO (T. Ito; 1705-702; soil).		
32252	**Doratomyces nanus**	24	8
	History: IFO (T. Ito; 1761-3; soil).		
32253	**Geomyces pannorum var. pannorum**	24	1
	History: IFO (T. Ito; 1705-705; soil).		
32254	**Gliocladium catenulatum**	24	1
	History: IFO (T. Ito; 1775-702; soil).		
32255	**Gliomastix cerealis**	24	8
	History: IFO (T. Ito; 1708-704; soil).		
32256	**Gliomastix murorum var. felina**	24	8
	History: IFO (T. Ito; 1708-2; soil).		
32257	**Malbranchea pulchella var. sulfurea**	37	1
	History: IFO (T. Ito; 1701-451; soil).		
32258	**Metarhizium anisopliae**	24	2
	History: IFO (T. Ito; 1705-703; soil).		
32259	**Monocilliun indicum**	24	8
	History: IFO (T. Ito; 1749-8; soil).		
32260	**Monocillium mucidum**	24	8
	History: IFO (T. Ito; 1822-8; soil). PP 61-K-1699		
32261	**Monocillium tenue**	24	8
	History: IFO (T. Ito; 1787-7; soil). PP 61-K-1699		
32262	**Myrothecium cinctum**	24	1
	History: IFO (T. Ito; 1711-1; soil). PP 61-K-1699		
32263	**Myrothecium roridum**	24	1
	History: IFO (T. Ito; 1756-7; soil). PP 61-K-1699		
32264	**Myrothecium verrucaria**	24	1
	History: IFO (T. Ito; 1711-703; soil). PP 61-K-1699		
32265	**Oidiodendron griseum**	24	1
	History: IFO (T. Ito; 1754-8; soil).		
32266	**Paecilomyces variotii**	24	1
	History: IFO (T. Ito; 1848-451; seeds of star anise).		
32267	**Phialocephala humicola**	24	1
	History: IFO (T. Ito; 1778-4; soil).		
32268	**Scolecobasidium variabile**	24	1
	History: IFO (T. Ito; 1717-704; soil).		
32269	**Scopulariopsis brumptii**	24	1
	History: IFO (T. Ito; 1707-7; soil).		
32270	**Stachybotrys microspora**	24	8
	History: IFO (T. Ito; 1701-1; soil).		

32271 **Torulomyces lagena** 24 5
History: IFO (T. Ito; 1806-8; soil).
32272 **Trichurus spiralis** 24 1
History: IFO (T. Ito; 1726-7; soil). PP 61-K-1699
32273 **Ulocladium botrytis** 24 1
History: IFO (T. Ito; 1837-4; soil).
32274 **Verticillium leptobactrum** 24 8
History: IFO (T. Ito; 1739-3; soil).
32275 **Verticillium psalliotae** 24 1
History: IFO (T. Ito; 1793-9; soil).
32276 **Verticillium suchlasporium var. catenatum** 24 8
History: IFO (T. Ito; 1797-4; soil).
32277 **Wallemia sebi** 24 3
History: IFO (T. Ito; 1851-17; soil). PP 61-K-1699
32278 **Wardomyces inflatus** 24 8
History: IFO (T. Ito; 1845-704; soil).
32279 **Absidia corymbifera** 24 1
History: IFO (T. Ito; 1726-451; soil).
32280 **Absidia cylindrospora** 24 1
History: IFO (T. Ito; 1797-1; soil).
32281 **Mortierella alpina** 24 1
History: IFO (T. Ito; 1707-703; soil).
32282 **Mortierella globalpina** 24 1
History: IFO (T. Ito; 1733-1; soil).
32283 **Mortierella minutissima** 24 1
History: IFO (T. Ito; 1716-2; soil).
32284 **Choanephora infundibulifera** 24 1
History: IFO (A. Nakagiri; AN-1097; flower of *Hibiscus mutabilis*).
32285 **Choanephora infundibulifera** 24 1
History: IFO (A. Nakagiri; AN-1098; flower of *Hibiscus* sp.).
32286 **Choanephora infundibulifera** 24 1
History: NRRL-2560 -- IFO 5987.
32287 **Choanephora infundibulifera** 24 1
History: NRRL-2561 -- IFO 5988.
32289 **Lyophyllum sykosporum** 24 7
History: IFO (T. Ito; T. Ito S64-5; ground in forest).
32290 **Gymnopilus spectabilis** 24 7
History: IFO (T. Ito; T. Ito S64-4; trunk of *Quercus* sp.).
32293 **Mortierella longicollis** 24 1
History: CBS 209.32 (E. McLennan; sandy loam).
32294 **Choanephora cucurbitara** 24 1
History: IFO (T. Ito; T. Ito S64-16-1; paddy field soil).
32295 **Choanephora trispora** 24 1
History: IFO (T. Ito; T. Ito S64-17-1; pasture soil).
32296 **Digitodochium rhodoleucum** 20 7
History: Lab. Biol., Coll. Pharmacy, Nihon Univ., Tokyo (K. Tubaki; NUP-98; fallen twing of *Fagus crenata*).
32297 **Coniochaeta angustispora** 24 8
History: IMI 254103 -- H.Y. Yip, *Gahnia radula* roots.
32298 **Dicyma olivacea** 24 1
History: CBS 226.76 -- M. de Bertoldi, 555, sandy soil of river bank.
32299 **Emericella variecolor var. variecolor** 24 1
History: CBS 119.37 -- O. Verona, olives.
32300 **Emericella variecolor var. astellata** 24 1
History: CBS 134.55.
32301 **Emericella variecolor var. astellata** 24 1
History: CBS 135.55 -- NRRL.
32302 **Emericella variecolor var. variecolor** 24 1
History: CBS 136.55 -- A.C. Batista, Laboratory contaminant.
32303 **Emericella variecolor var. variecolor** 24 1

History: CBS 597.65.

32304 **Hemicarpenteles acanthosporus** 24 5
History: IFO (T. Ito; T. Ito S64-8E-3; field soil).

32305 **Hemicarpenteles acnathosporus** 24 5
History: IFO (T. Ito; T. Ito S64-12E-3; soil).

32306 **Melanographium selenioides** 24 1
History: IFO (T. Ito; T. Ito S64-21-1; twig of *Didymosperma engleri*).

32307 **Neurospora crassa** 24 1
History: IFO (T. Ito; T. Ito S64-870-1, No.3; field soil).

32308 **Neurospora crassa** 24 1
History: IFO (T. Ito; T. Ito S64-870-1, No.5; field soil).

32309 **Gyrothrix circinata** 24 1
History: IFO (A. Nakagiri; AN-1111, dead leaf of *Trachycarpus exelsa*).

32310 **Gyrothrix circinata** 24 1
History: IFO (A. Nakagiri; AN-1112; dead leaf of *Trachycarpus exelsa*).

32311 **Verticillium lecanii** 24 1
History: Plant Path. Lab., Hokkaido Nat. Agric. Exp. Sta., AG-Y-1, *Aphis gossypii*.

32312 **Verticillium lecanii** 24 1
History: Plant Path. Lab., Hokkaido Nat. Agric. Exp. Sta., BB-W-1, *Brevicoryne brassicae*.

32313 **Verticillium lecanii** 24 1
History: Plant Path. Lab., Hokkaido Nat. Agric. Exp. Sta., MP-Y-6, *Myzus persicae*.

32314 **Verticillium lacanii** 24 1
History: Plant Path. Lab., Hokkaido Nat. Agric. Exp. Sta., IV-I-1, *Trialeurodes vaporariorum*.

32315 **Verticillium lecanii** 24 1
History: Plant Path. Lab., Hokkaido Nat. Agric. Exp. Sta., V237, *Tetramychus urticae*.

32316 **Monascus anka** 24 1
History: AS 3.554 -- Golden Sea Res. Inst. Chem. Ind. (GSRICI 807; red rice).

32317 **Monascus fuliginosus** 24 1
History: AS 3.2091 (M27; glutinous moromi for Maotai fermentation).

32318 **Monascus ruber** 24 4
History: AS 3.549 -- Golden Sea Res. Inst. Chem. Ind. (GSRICI 801; red rice).

32319 **Fibroporia gossypina** 24 1
History: Forestry & Forest Prod. Res. Inst., (Y. Abe; WD 94; decayed wood of *Larix kaempferi*).

32320 **Fibroporia gossypina** 24 1
History: Forestry & Forest Prod. Res. Inst., (Y. Abe; WD 160b; decayed wood (stump) of *Larix kaempferi*).

32321 **Capnobotryella renispora** 24 5
History: IAM (J. Sugiyama; JS#82-144(b); leaves of *Abies veitchii*). A culture derived from holotype TNS F-198506

32322 **Capnobotryella renispora** 24 5
History: IAM (J. Sugiyama; JS#83-147(b)) -- S. Tokumaru, leaves of *Abies veitchii*. A culture derived from paratype TNS F-197957

32325 **Phytophthora cryptogea** 24 1
HISTORY : Yokohama Plant Protection Station (E. Kimishima; SG-1; root of gerbera).

32326 **Phytophthora cryptogea** 24 1
HISTORY : Yokohama Plant Protection Station (E. Kimishima; SG-4; root of gerbera).

32328 **Didymostilbe matsushimae** 24 8
History: IFO (T. Ito; T. Ito S64-21-2; rotten stem of Arenga englerri).

32329 **Trichobotrys effusa** 24 8
History: IFO (T. Ito; T. Ito S63-19-4; soil).

50154 **FBHE** 37 535
Depositor: RTCI (M. Sakaguchi).
50210 **L2C**
Depositor: Tokushima Univ. (F. Ota; leukemic cells).
50221 **N18-RE-105** 37
Depositor: RTCI (A. Nagaoka; mouse. rat hybridoma).
50248 **IT-79MTNC3** 37
Depositor: Tohoku Univ. (T. Ito; Thymic nurse cell).
50269 **GH1** 37
Depositor: RTCI (K. Igarashi).
50270 **6-23 clone 6** 37
Depositor: RTCI (K. Igarashi).
50271 **HUV-EC-C** 37
Depositor: RTCI (N. Suzuki).
50272 **CPA** 37 199
Depositor: RTCI (K. Kato, bovine). endothelium
50273 **CPAE** 37
Depositor: RTCI (K. Kato; bovine). endotherium
50275 **A10** 37
Depositor: RTCI (Y. Nozaki; DBIX rat). embryo; thoracic aorta
50278 **PC-12** 37
Depositor: RTCI (K. Nakahama; rat). adrenal pheochromocytoma
50279 **NKY113** 37
Depositor: I. Yamasina (Dept. Biochem., Fac. Pharm. Sci., Kyoto Univ.; NKY113; mouse
hybridoma). mouse X mouse (splenic lymphocytes X SP2/O-Ag24)
50282 **RAT-2** 37
Depositor: RTCI (Y. Kaisho).
50283 **IMR-32**
Depositor: RTCI (Y. Kaisho; human). neuroblastoma
50284 **A2B5 clone 105** 37
Depositor: RTCI (K. Igarashi).
50297 **HE-1** 36.5
Depositor: Osaka Oriental Medicine Inst. (A. Sato; human). embryo
50298 **Balb/c 3T3 A31-I-1** 37
Depositor: Inst. Mol. Cell Biol., Kyoto Pharm. Univ. (M. Tatsuka; mouse). embryo
50303 **T98G** 37
Depositor: RTCI (K. Igarashi). brain; glyoblastoma

ABSTRACTS 1989 - 1990

Microfungi from soils of bonfire sites at Mt. Daisen, Japan

T. Ito and T. Yokoyama

Trans. Mycol. Soc. Japan 29: 235-247 (1988)

A total of 35 species in 25 genera of microfungi were isolated from soils of bonfire sites at Mt. Daisen, Tottori Prefecture, Japan. Among these, Anixiella reticulata, Coniochaeta saccardoi, Gelasinospora reticulospora, Neurospora tetrasperma, Trichophaea abundans, Gilmaniella humicola, and G. subornata are pyrophilous fungi. Five noteworthy fungi were Cercophora coprohila, Coniochaeta sp., Gilmaniella subornata, Thielavia arenaria, and Wardomyces humicola, of which the first three are new to Japan including a hitherto undescribed species. Mycological notes and the geographical distributions of these ten fungi are given, and morphological characteristics of four of them are fully described.

A new species of Coniochaeta from Japanese soils

T. Yokoyama and T. Ito

Trans. Mycol. Soc. Japan 29: 319-322 (1988)

A new species of the genus Coniochaeta, C. cymbiformispora Yokoyama et T. Ito, sp. nov., is described based on two isolates obtained from soils collected in Nagano and Tottori prefecture, Japan. The new species resembles C. angustispora in the shape of its ascospores but differs in having larger ascospores.

A genetic analysis of taxonomic relation between Saccharomyces cerevisiae and Saccharomyces bayanus

I. Banno and Y. Kaneko

Yeast 5: special issue S373-S377 (1989)

Genetic analysis of the hybrid indicated that S. cerevisiae and S. bayanus are genetically isolated from each other. Inability of galactose fermentation in type strain of S. bayanus due to deficient in GAL4 gene only. GAL4-homologous gene found in S. bayanus was significantly different from GAL4 gene of S. cerevisiae in restriction map. The results support the idea that the two are distinct species, although they can not be phenotypically distinguished.

A new species of Oxydothis from the mangrove palm, Nypa fruticans

K. D. Hyde* and A. Nakagiri

Trans. Mycol. Soc. Japan 30: 69-75 (1989)

Decaying fronds of the mangrove palm Nypa fruticans collected from beaches and intertidal regions in Brunei and Thailand were examined for marine fungi. In this paper, Oxydothis nypae sp. nov. is described.

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Sensitivity to freezing storage of Pseudomonas aeruginosa phage 24 (IFO 20050)

Ko Imai

Jpn. J. Freez. Dry. 35: 21-27 (1989)

Freezing storage at -20 C was applied for eight bacteriophage strains for Pseudomonas aeruginosa, and their viability was examined. Of eight strains, seven were stably preserved both in the presence and absence of glycerol. An osmosensitive phage 24 (IFO 20050) was found to be sensitive to the freezing storage in the presence of 5 and 10% glycerol. Addition of sucrose or glucose made the same degree of loss in titer as that of glycerol. T-even phages of Escherichia coli, which are known to be osmosensitive, were also sensitive to the freezing storage in the presence of glycerol. When the phage lysate supplemented with 5% glycerol was cooled in dry ice-ethyl alcohol and stored at -80 C for 1 week, the loss in titer was diminished. However, storage at -20 C made remarkably the loss in titer. The unfrozen water content of the phage lysate

with or without sugars was examined by DSC. In the presence of sugars that caused the remarkable loss in titer, the amount of unfrozen water incresed at -20 C. On the other hand, the increasing of unfrozen water occurred at -7 C in the absence of sugars. After storage at -20 C for 1 week, the viability of lysate supplemented with both 5% glycerol and 1.7% methyl alcohol was 100 fold higher than that without methyl alcohol. Addition of 5% DMSO also markedly prevent the loss in titer by freezing storage at -20 C in the presence of 5% glycerol. Freezing storage of phage 24 in the presence of glycerol caused leakage of DNA from phage particles. Those findings suggest that sugars can affect on hydrophobic domains of a protein of the phage particle and change the stereostructure of proteins, and this change can trigger off the leakage of DNA from perticles.

(in Japanese)

Molecular characterization of a specific p-nitrophenylphosphatase gene, PHO13, and its mapping by chromosome fragmentation in Saccharomyces cerevisiae

Y. Kaneko, A. Toh-e[*1], I. Banno and Y. Oshima[*2]

Mol. Gen. Genet. 220: 133-139 (1989)

The structural gene, PHO13, for the specific p-nitrophenylphosphatase of Saccharomyces cerevisiae was cloned and its nucleotide sequence determined. The deduced PHO13 protein consists of 312 amino acids and its molecular weight is 34635. The disruption of the PHO13 gene produced no effect on cell growth, sporulation, or viability of ascospores. The PHO13 locus was mapped at 1.9 centimorgans from the HO locus on the left arm of chromosome IV. By chromosome fragmentation, the PHO13 locus was found to be located about 72 kb from the left-hand telomere of chromosome IV and distal to the HO locus.

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Succession of fungi in decomposing mangrove leaves in Japan

A. Nakagiri, S. Tokumasu*1, H. Araki*2, S. Koreeda*3 and K. Tubaki*4

Recent Advances in Microbial Ecology (ed. by T. Hattori et al.)
p. 297-301 (1989)

Fungal succession associated with mangrove leaves through senescence and decomposition was investigated in mangrove forests on the South-West Is. in Japan. Mangrove leaves still on the tree and fallen leaves were collected according to the stage of senesence and decay. Further decomposed leaf fragments were washed in a detergent solution and sterilized water, they were incubated on corn meal agar plates. The fungal community which appeared on the plates was recorded, and the occurrence frequency was calculated according to the stage of leaf decay. The sequence of fungal occurrence is summarized as follows: 1) Green leaves were infested with Pestalotiopsis sp., Cladosporium cladosporioides and other common primary saprophytes. The former fungus was prevalent throughout the leaf decaying process into fragments. 2) After defoliation, Phytophthora spp. infested the leaves and predomnated from an early stage in the decomposition to a fairly decomposed state. 3) Soil fungi, which are common in terrestrial soil, invaded the degrading fallen leaves and were prevalent in the decomposed leaf fragments. 4) A few higher marine fungi contributed to the decomposition in river mouth areas. 5) During the process of fungal succession, mycoparasitic relationships between previous colonizer and successor were observed.

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A possible role for acid phosphatase with thiamin-binding activity encoded by PHO3 in yeast

K. Nosaka*, Y. Kaneko, H. Nishimura* and A. Iwashima*

FEMS Microbiol. Lett. 60: 55-60 (1989)

Periplasmic soluble thiamin-binding protein in Saccharomyces cerevisiae (Iwashima, A. et al. (1979) Biochim. Biophys. Acta 577, 217-220) was demonstrated to be encoded by PHO3 gene that codes for thiamin repressible acid phosphatase (Schweingruber, M. E. et al. (1986) J. Biol. Chem. 261, 15877-15882) by genetic analysis. The pho3 mutant cells of S. cerevisiae in contrast to the parent cells have markedly reduced activity of the uptake of [^{14}C] thiamin phosphates, suggesting that thiamin repressible acid phosphatase plays a role in the hydrolysis of thiamin phosphates in the periplasmic space prior to the uptake of their thiamin moieties by S. cerevisiae.

* Department of Biochemistry, Kyoto Prefectural University of Medicine.

Damage of the cell membrane of Aquaspirillum metamorphum by L-drying and the role of ethylenediamine as a protectant

T. Sakane

Jpn. J. Freez. Dry. 35: 15-20 (1989)

Damage of the cell membrane by L-drying and the protective effect of ethylenediamine dihydrochloride (ED) in preventing the damage were studied. The strain examined was Aquaspirillum metamorphum IFO 13960, which is susceptible to damage by L-drying. When this strain was dried and rehydrated with phosphate buffer, a large amount of cell components, such as RNA, proteins and fatty acids were released into the rehydration fluid from the cells. Denatured or degraded proteins which were not found in vegetative cell envelopes appeared in the protein profiles of cell envelopes from rehydrated cells on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Furthermore, the amount of certain glycoproteins considered to be located on the cell surface markedly decreased. Addition of magnesium sulfate (0.1%) to the rehydration fluid prevented the release of cell components and damage of cell envelope proteins but not the degradation of glycoproteins. ED had strong protective effect on the survival of this strain after L-drying. Addition of ED (0.4%) to the medium for drying prevented the release of cell components and the damage of cell envelope proteins including the glycoproteins. These results indicate that ED prevents the damage of cell envelope protein at the

drying step, and magnesium sulfate prevents the damage occurring at the rehydration step.

(in Japanese)

Cell-wall polysaccharides in coryneform bacteria

Mariko Takeuchi and A. Yokota

J. Gen. Appl. Microbiol. 35: 233-252 (1989)

From the cell walls of 45 species (14 genera) of coryneform bacteria, we isolated polysaccharides and teichoic acids covalently linked to the peptidoglycan, and examined their chemical composition. Glycerol teichoic acid appeared in strains of the genera Brevibacterium, Pimelobacter, Rarobacter, in all of the species belonging to the "Arthrobacter nicotianae group" of the genus Arthrobacter, and in A. crystallopoietes belonging to the "A. globiformis group".

Strains of the genera Rubrobacter, Aureobacterium, Clavibacter, Brachybacterium, Exiguobacterium and species belonging to the "A. globiformis group" of the genus Arthrobacter contained neutral polysaccharide. Strains of the genera Corynebacterium and Caseobacter had arabinogalactan, as was already known.

The sugar composition of the cell-wall polysaccharides in the coryneform bacteria was neither uniform within the genus nor characteristic of each genus in the sugar profile, except for the genera Corynebacterium and Caseobacter which had arabinose and galactose as diagnostic sugars. On the other hand, the kind of cell-wall polymer, whether it is a neutral polysaccharide, teichoic acid or arabinogalactan, was homogeneous within each genus; hence, cell-wall polymers can be used as chemotaxonomic markers for classifying coryneform bacteria.

The phylogenetic relationship of the Conidium-forming anamorphic yeast genera Sterigmatomyces, Kurtzmanomyces, Tsuchiyaea and Fellomyces, and the teleomorphic yeast genus Sterigmatosporidium on the basis of the partial sequences of 18S and 26S ribosomal ribonucleic acids

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

Agric. Biol. Chem. 53: 2993-3001 (1989)

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

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The phylogenetic relationship of the Q9-equipped species of the heterobasidiomycetous yeast genera Rhodosporidium and Leucosporidium based on the partial sequences of 18S and 26S ribosomal ribonucleic acids: The proposal of a new genus Kondoa

Y. Yamada*, Y. Nakagawa* and I. Banno

J. Gen. Appl. Microbiol. 35: 377-385 (1989)

Eight strains of the Q9-equipped heterobasidiomycetous yeast species, Rhodosporidium malvinellum, Rhodosporidium toruloides, and Leucosporidium scottii and the Q9-equipped anamorphic yeast species, Rhodotorula lactosa were examined as to the partial sequence determinations of 18S rRNA and 26S rRNA. The position determined were 1451 through 1618 of 18S rRNA and 492 through 625 of 26S rRNA. The two determinations of the partial sequences of 18S rRNA and 26S rRNA showed that the four species mentioned above constituted their own separate clusters. Rhodosporidium malvinellum occupied a unique situation. The phylogenetic relationship between R. malvinellum and R. toruloides was more distant

than that between R. toruloides and L. scottii. The data indicated that these three heterobasidiomycetous yeast species are distinguished from each other at the generic level. The new genus Kondoa was proposed for R. malvinellum.

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The analysis of madurose, an actinomycete whole-cell sugar, by HPLC after enzymatic treatment

A. Yokota and Toru Hasegawa

J. Gen. Appl. Microbiol. 34: 445-449 (1989)

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

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

Streptomycete species with madurose (3-O-methyl-D-galactose) as a whole-cell sugar

A. Yokota, Y. Nakagaito and Toru Hasegawa

Arch. Microbiol. 152: 317-321 (1989)

Madurose, an actinomycete whole-cell sugar, was found in the strains of the genus Streptomyces: three strains of S. platensis, one strain each of S. platensis subsp. malvinus, and S. albus subsp. albus. The sugar was isolated from the hydrolysate of S. platensis IFO 14008 cells, and was identified as madurose (3-O-methyl-D-galactose) by chromatographic analyses, ^{1}H-NMR spectrometry, mass spectrometry as its alditol acetate, and demethylation with boron trichloride. Cell walls of the strain

contained peptidoglycan and teichoic acids. LL-Diaminopimelic acid, glycine, glutamic acid, and alanine were present in the peptidoglycan fraction in molar ratios of 1.0:1.3:1.2:2.3. Madurose was detected in the teichoic acid fraction, which was composed of phosphorus, glycerol, galactose, and madurose in molar ratios of 9.3:8.5:2.9:1.0. Thus, madurose was found in the glycerol teichoic acid moiety of the cell walls of this strain.

Regulation of thiamine biosynthesis in Saccharomyces cerevisiae

Y. Kawasaki*, K. Nosaka*, Y. Kaneko, H. Nishimura* and A. Iwashima*

J. Bacteriol. 172: 6145-6147 (1990)

A pho6 mutant of Saccharomyces cerevisiae, lacking a regulatory gene for the synthesis of periplasmic thiamine-repressible acid phosphatase activity, was found to be auxotrophic for thiamine. The activities of four enzymes involved in the synthesis of thiamine monophosphate were hardly detectable in the crude extract from the pho6 mutant. On the other hand, the activities of these enzymes and thiamine-repressible acid phosphatase in a wild-type strain of S. cerevisiae, H42, decreased with the increase in the concentration of thiamine in yeast cells. These results suggest that thiamine synthesis in S. cerevisiae is subject to a positive regulatory gene, PHO6, whereas it is controlled negatively by the intracellular thiamine level.

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Induction of mutation in bacteria by drying and the preventive effects of thiourea, adonitol and cysteine

T. Sakane

Jpn. J. Freez. Dry. 36: 81-87 (1990)

Preservation of cells by L-drying or freeze-drying induced mutation in Escherichia coli, which depended on the temperature of preservation. When dried cells were preserved at a temperature between 10 C and 48 C, the number of mutants in the surviving fraction increased as the storage temperature increased. No mutation could be detected in the cells rehy-

drated immediately after drying or in dried cells preserved at temperatures below 5 C. Thiourea, adonitol and cysteine were found to be effective in preventing mutation, reducing the frequency of mutation during preservation. When added to the fluid for drying, these compounds improved survival and/or prevented the induction of mutation, even when the dried cells were preserved at high temperature. It seems that the role of thiourea and adonitol in protecting cells from mutation is to prevent DNA-strand breakage by acting as radical-scavengers during preservation of dried cells, and that of cysteine is to protect the DNA-repair system enzyme(s) during preservation of dried cells.

(in Japanese)

Comparative structures of the cell-wall polysaccharides of four species of the genus Microbacterium

Mariko Takeuchi, A. Yokota and A. Misaki*

J. Gen. Appl. Microbiol. 36: 255-271 (1990)

Cell-wall polysaccharides purified from four species of the genus Microbacterium were characterized in relation to their taxonomy, based on ^{1}H-NMR and ^{13}C-NMR spectrometry, methylation analysis and Smith degradation.

The polysaccharide isolated from M. imperiale IFO 12610 contained a backbone consisting of heptasaccharide repeating-units, to which side chains of L-rhamnopyranosyl-(1→2)-D-mannopyranosyl-(1→2)-D-mannopyranose, as branches were attached to position 6 of the 3-linked D-galactopyranose residues. The polysaccharide of M. lacticum IFO 141315 contained hexasaccharide repeating-units, to which disaccharide groups of L-rhamnopyranosyl-(1-2)-L-rhamnopyranose were attached to position 6 of the 3-linked D-galactopyranose residues to form branches. The polysaccharide of M. arborescens IFO 3750 consists of pentasaccharide repeating-units, and single D-mannopyranosyl groups were attached as branches to position 6 of the 3-linked D-galactofuranose residues. The polysaccharide of M. laevaniformans IFO 14471 had a more complicated structure consisting of decasaccharide repeating-units, and branches occurs at three different positions.

Thus, the cell-wall polysaccharides of the former three species were

similar in their basic structures, but that of M. laevaniformans has a significantly different structure. These results indicated that the structures of cell-wall polysaccharides, are heterogeneous in the genus Microbacterium and may characterize the species in the genus.

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Taxonomy of "Protaminobacter alboflavus": Reclassification as Mycobacterium diernhoferi

Mariko Takeuchi, A. Yokota, S. Mizuno*1, I. Yano*1 and M. Tsukamura*2

J. Gen. Appl. Microbiol. 36: 195-202 (1990)

The taxonomic position of "Protaminobacter alboflavus" IFO 3707, which has been shown to belong to the genus Mycobacterium, was studied. Its chemotaxonomic and physiological characteristics were compared with those of M. diernhoferi. Both strains contained α-mycolic acids of the same molecular species composition. The G+C content of DNA was 67.6 mol% for "P. alboflavus" IFO 3707 and 67.2-68.1 mol% for M. diernhoferi. The cellular fatty acid compositions of the two strains were identical. Numerical analysis of their taxonomic properties also showed a high degree of similarity.

From these data, it was concluded that "P. alboflavus" IFO 3707 should be classified as M. diernhoferi. The present study eliminates the only remaining species classified as belonging to the genus "Protaminobacter" den Dooren de Jong 1927.

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Amino acid composition and partial sequences of two types of alkaline serine proteases from Nocardiopsis dassonvillei subsp. prasina OPC-210

H. Tsujibo*, K. Miyamoto*, Toru Hasegawa and Y. Inamori*

Agaric. Biol. Chem. 54: 2177-2179 (1990)

An alkalophilic actinomycete, Nocardiopsis dassonvillei subsp. prasina OPC-210, produced two types of alkaline serine proteases (NDP-I and NDP-II). NDP-I is classified as a chmotrypsin-type serine protease. The amino-terminal amino acid sequence of BDP-II was analyzed and compared with those of subtilisin BPN, elastase YaB, thermitase, proteinase K and aqualysin I. And the partial amino acid sequence of NDP-II showed striking homology with that of aqualysin I (65% homology). This is the first reported example of an aqualysin I-like alkaline serine protease produced by an alkalophilic actinomycete. N. dassonvillei subsp. prasina OPC-210 and NDP-II seems to be a good candidate for protein engineering.

* Osaka University of Pharmaceutical Sciences.

Purification and characterization of two types of alkaline serine proteases produced by an alkalophilic actinomycete

H. Tsujbo*, K. Miyamoto*, Toru Hasegawa and Y. Inamori*

J. Appl. Bacteriol. 69: 520-529 (1990)

Two types of alkaline serine proteases were isolated from the culture filtrate of an alkalophilic actinomycete, Nocardiopsis dassonvillei OPC-210. The enzymes (protease I and protease II) were purified by acetone precipitation, DEAE-Sephadex A-50, CM-Sepharose CL-6B, Sephadex G-75 and phenyl-Toyopearl 650 M column chromatography. The purified enzymes showed a single band on sodium dodecyl sulfate polyacrylamide gel electrophoresis. The molecular weights of proteases I and II were 21,000 and 36,000, respectively. The pIs were 6.4 (protease I) and 3.8 (protease II). The optimum pH levels for the activity of two proteases were pH 10-12 (protease I) and pH 10.5 (protease II). The optimum temperature for the activity of protease I was 70 C and that for protease II was 60 C. Protease I was stable in the range of pH 4.0-8.0 up to 60 C and protease II was stable in the range of pH 6.0-12.0 up to 50 C.

* Osaka University of Pharmaceutical Sciences.

Purification and properties of three types of xylanases produced by an alkalophilic actinomycete

H. Tsujibo*, T. Sakamoto*, N. Nishino*, Toru Hasegawa and Y. Inamori*

J. Appl. Bacteriol. 69: 398-405 (1990)

Three types of xylanases (1, 4-β-D-xylan xylanohydrolase, EC 3. 2. 1. 8.) were isolated from the culture filtrate of an alkalophilic actinomycete, Nocardiopsis dassonvillei subsp. alba OPC-18. The enzymes (X-I, X-II and X-III) were purified by acetone precipitation, chromatographies of DEAE-cellulofine A-800, Sephadex G-75 and preparative isoelectric focusing. The purified enzymes showed single bands on sodium dodecyl sulphate polyacrylamide gel electrophoresis. The molecular weights of X-I, X-II and X-III were 23, 000, 23, 000 and 37, 000, respectively. The pIs were 4. 9 (X-I), 5. 3 (X-II) and 4. 1 (X-III). The optimum pH levels for the activities of X-I and X-II were pH 7. 0. X-III was also most active at pH 7. 0, but 62. 5% of the activity remained even at pH 11. The optimum temperatures for the activities of X-I and X-II were 60 C and that of X-III was 50 C. X-I and X-II were stable in the range of pH 6-10, and X-III was stable in the range of pH 8-12 until 40 C for 30 min.

* Osaka University of Pharmaceutical Sciences.

Isolation of cytotoxic substance, kalafungin from an alkalophilic actinomyete, Nocardiopsis dassonvillei subsp. prasina

H. Tsujibo[*1], T. Sakamoto[*1], K. Miyamoto[*1], G. Kusano[*1], M. Ogura[*2], Toru Hasegawa and Y. Inamori[*1]

Chem. Pharm. Bull. 38: 2299-2300 (1990)

An alkalophilic actinomycete, strain OPC-553 regarded as Nocardiopsis dassonvillei subsp. prasina, produced the cytotoxic substance, TS-1, which showed a marked inhibitory activity against L5178Y mouse leukemic cell in vitro. The cytotoxicity of TC-1 on this cell was very strong and its ID_{50} was 0. 018 μg/ml. Through direct comparison of its spectral data with those of an authentic sample, TS-1 was identified as the antifungal antibiotic, kalafungin, already isolated from the culture broth of Streptomyces tanashiensis. However, the isolation of kalafungin from an alkalophilic actinomycete and its cytotoxicity are reported for the first time in this paper.

*1 Osaka University of Pharmaceutical Sciences.

*2 Yamasa Shoyu Co., Ltd.

Identification of 38 brewering yeasts maintained in IFO Collection.

Yoriko Yamada, Y. Kaneko and K. Mikata

Bull. JFCC 6: 76-85 (1990)

Saccharomyces cerevisiae sensu Yarrow (1984) has recently been recognized to be separated at least into the three species: S. cerevisiae, S. bayanus, S. paradoxus. Therefore the strains of Saccharomyces sensu stricto preserved in Institute for Fermentation, Osaka (IFO) have been re-examined. The present paper deals with re-identification of 4 baker's yeasts, 12 brewery yeasts, 9 distillery yeasts, 8 sake yeasts and 5 wine yeasts on the basis of morphological and physiological properties, karyotype in pulsed field gel electrophoresis (PFG), G+C content of DNA, and DNA similarity estimated by fluorometric microplate hybridization.

In physiological tests, 29 strains showed cerevisiae-biotype; eight strains uvarum-biotype; and one strain bayanus-biotype. The chromosomal DNA band patterns in PFG of 38 strains are similar to one another and to that of the type strain of S. cerevisiae, being included in chromosome length polymorphism of S. cerevisiae. G+C content ranged from 36 to 40 mol% among 38 strains. In DNA hybridization, 31 strains showed high similarity value to the type strain of S. cerevisiae, and one showed high similarity value to the type strain of S. bayanus. Six strains of 12 brewery yeasts were of intermediate similarity value both to S. cerevisiae and S. bayanus, and considered to be a hybrid between the two species.

PRESENTATION OF PAPERS AT SCIENTIFIC MEETINGS 1989 - 1990

Agricultural Chemical Society of Japan (April, 1989, Niigata)

H. Tsujibo*, T. Sakamoto*, N. Nishino*, Y. Inamori* and Toru Hasegawa
Purification and properties of xylanases produced by an alkalophilic actinomycete.
*Osaka University of Pharmaceutical Sciences

H. Tsujibo*, K. Miyamoto*, Y. Inamori* and Toru Hasegawa
Purification and characterization of alkaline proteases produced by an alkalophilic actinomycete.
*Osaka University of Pharmaceutical Sciences

A. Yokota, Y. Nakagaito and Toru Hasegawa
Streptomyces species with madurose as a whole-cell sugar.

Mariko Takeuchi and A. Yokota
Cell-wall polysaccharides in coryneform bacteria.

Japanese Society for Research of Freezing and Drying (April, 1989, Tokyo)

K. Imai
Sensitivity to freezing storage of Pseudomonas aeruginosa phage 24 (IFO 20050).

T. Sakane
Damage of cell membrane of Aquaspirillum metamorphum as a result of L-drying and a role of ethylenediamine as a protectant.

Mycological Society of Japan (May, 1989, Higashiosaka)

A. Nakagiri
Phytophthora spp. isolated from decomposing mangrove leaves.

T. Ito and T. Yokoyama
Microfungi from soils of bonfire sites at Mt. Daisen, Tottori Prefecture.

Japanese Society of Mycoplasmology (May, 1989, Tokyo)

T. Sakane, T. Yoshida, Masao Takeuchi and K. Yamamoto*
Preservation of mycoplasma by L-drying.
*National Institute of Animal Health, Japan

Japanese Tissue Culture Association (June, 1989, Yokohama)

T. Yoshida and Masao Takeuchi
Cryopreservation of mouse astroglial cells in primary culture under serum-free conditions.

K. Takeuchi*1, M. Ogura*2, H. Saito*3, M. Satoh and Masao Takeuchi
Cytoskeletal organization in MEG-01 cell.
*1 Ehime College of Health Science
*2 Aichi Cancer Center Research Institute
*3 School of Medicine, Nagoya University

Japan Federation for Culture Collection (July, 1989, Suita)

T. Ito and T. Yokoyama
Survival test after mailing of cultures thawed from preservation of frozen state at -80 C.

K. Mikata and I. Banno
Preservation of yeast cultures by freezing at -80 C: II, Viability after 5 years storage.

T. Sakane, T. Yoshida and Masao Takeuchi
Preservation of cell wall-less bacteria by L-drying.

Masao Takeuchi, T. Yoshida, M. Satoh and T. Sakane
Activities of IFO on "biological contamination" in animal cell lines.

Hakkoukougaku Gijutsu Koshuukai (July, 1989, Osaka)

Y. Kaneko

An analytical technique of giant size DNA.

Joint Meeting of The Society for Actinomycetes, Japan and The Society for Applied Genetics of Actinomycetes, Japan (July, 1989, Tokyo)

Y. Nakagaito, A. Yokota and Toru Hasegawa

Madurose-containing actinomycete strains having cell-wall types other than type III/B.

The 5th International Syposium on Microbial Ecology (August, 1989, Kyoto)

A. Nakagiri, S. Tokumasu[*1], H. Araki[*2], S. Koreeda[*3] and K. Tubaki[*4]

Succession of fungi in decomposing mangrove leaves in Japan.

*1 Institute of Biological Sciences, University of Tsukuba

*2 Institute of Biological Sciences, University of Tsukuba; present address, Mitsubishi Gas Chemical Co.

*3 Institute of Biological Sciences, University of Tsukuba; present address, University of Tokyo

*4 Institute of Biological Sciences, University of Tsukuba; present address, Nihon University

H. Oyaizu[*1], Y. Oyaizu-Masuchi[*1], A. Yokota and S. Takakuwa[*2]

Phylogenetic study of the genus Thiobacillus with 16s rRNA partial sequencing.

*1 Department of Biology, Toyama University

*2 Department of Biology, Kyoto Women's University

Joint Meeting on the 30th Anniversary of Japanese Society for Research of Freezing and Drying, The Annual meeting of Japan Society for Cryosurgery, and The Annual Meeting of Japan Society for Low Temperature Medicine (October, 1989, Tokyo)

T. Sakane

Induction of mutation by drying in bacteria and effects of thiourea, adonitol and cysteine on preservation of induction of mutation.

The Genetic Society of Japan (October, 1989, Sapporo)

Y. Kaneko, I. Banno, A. Toh-e[*1] and Y. Oshima[*2]

DNA sequencing and mapping of the PHO13 gene encoding p-nitrophenylphosphatase in Saccharomyces cerevisiae.

*1 Department of Biology, Faculty of Science, University of Tokyo

*2 Department of Fermentation Technology, Faculty of Engineering, Osaka University

Japan Society for Cell Biology (October, 1989, Kyoto)

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

Immunofluorescent observation on platelet production from human megakaryoblastic leukemia cell line (MEG-01).

*1 Ehime College of Health Science

*2 Aichi Cancer Center Research Institute

*3 School of Medicine, Nagoya University

Japanese Society for Alternatives to Animal Experiments (October, 1989, Yokohama)

T. Ohno[*1], H. Mizusawa[*2] and Masao Takeuchi

Banking of cell lines and database in Japan.

*1 Riken Gene Bank

*2 National Institute of Hygienic Sciences

Annual Meeting on Microbial Chemotaxonomy (December, 1989, Kobe)

S. Takakuwa[*1], A. Yokota, H. Oyaizu[*2] and Y. Masuchi-Oyaizu[*2]

Phylogenetic study of mesophilic sulfur-oxidaizing bacteria with 16S rRNA partial sequencing.

*1 Department of Biology, Kyoto Women's University

*2 Department of Biology, Toyama University

A. Yokota and T. Sakane

Taxonomic significance of cellular fatty acid composition in Rhizobiaceae.

The Japan Hematological Society (March, 1990, Tokyo)

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

Platelet production from MEG-01 cell.

*1 Ehime College of Health Science
*2 Aichi Cancer Center Research Institute
*3 School of Medicine, Nagoya University

Japanese Society for Bacteriology (March, 1990, Tokushima)

Toru Hasegawa
Actinomycetes with madurose as a whole-cell sugar.

Japan Society for Bioscience, Biotechnology, and Agrochemistry (March-April, 1990, Fukuoka)

Y. Yamada*, Y. Nakagawa* and I. Banno
Phylogenetic relationships among yeasts of Rhodosporidium and allied genera based on partial 18S rRNA sequence.
* Department of Applied Biological Chemistry, Shizuoka University

T. Nakase*1, M. Itoh*1, A. Takematsu*1, Y. Yamada*2 and I. Banno
Kockovaella gen. nov.., ballistospore forming yeasts isolated from plants in Thailand.
*1 Japan Collection of Microorganisums, the Institute of Physical and Chemical Research (RIKEN)
*2 Department of Applied Biological Chemistry, Shizuoka University

K. Imai and Y. Yamada
DNA homologies among LPS-specific Pseudomonas aeruginosa phages.

A. Yokota and H. Oyaizu*
Taxonomic study of facultative chemolithotrophic Thiobacillus species.
*Department of Biology, Toyama University

J. K. Shin*, A. Yokota, J. Sugiyama* and K. Komagata*
Chemical composition of lipopolysaccharides and their taxonomic significance in Pseudomonas species.
* Institute of Applied Microbiology, University of Tokyo

Mariko Takeuchi and A. Yokota
Reidentification of Cytophaga heparina IFO 12017^{T} and "Cytophaga keratolytica" IFO 14087.

VAAM-DGHM Spring Meeting (April, 1990, Berlin, Germany)

S. A. Campos-Portuguez*, A. Yokota and H. Mayer*
Different lipid A constituents in lipopolysaccharides of Thiobacillus species.
* Max-Planck-Institut für Immunbiologie, Freiburg i. Br., F. R. G.
VAAM: Vereinigung für Allgemeine und Angewandte Mikrobiologie
DGHM: Deutsche Gesellschaft für Hygiene und Mikrobiologie

The 5th international symposium of Research Center for Pathogenic Fungi and Microbial Toxicoses (May, 1990, Chiba)

A. Nakagiri and K. Tubaki*
Mode of life and taxonomy of marine fungi.
* Nihon University

Japanese Tissue Culture Association (May, 1990, Kyoto)

Masao Takeuchi, T. Yoshida, H. Mizusawa*1 and T. Ohno*2
Activities of Japanese Cell Banks on mycoplasmal contamination problems.
*1 National Institute of Hygienic Sciences
*2 Riken Cell Bank

H. Mizusawa*1, M. Honma*1, E. Kataoka*1, Y. Shinagawa*2, N. Nomura*3, Masao Takeuchi and T. Ohno*4
Application of the DNA profiling method for quality control of cell lines.
*1 National Institute of Hygienic Sciences
*2 Toyama Institute of Health
*3 Nippon Veterinary and Zootechnical College
*4 Riken Cell Bank

The 9th Yeast Symposia Japan (June, 1990, Tokyo)

Y. Kaneko
Chromosome fragmentation at the target locus.

Mycological Society of Japan (June, 1990, Shizuoka)

A. Nakagiri
Basidiocarp structure of a marine basidiomycete, Halocyphina villosa.

K. Mikata, I, Bannǫ, T. Nakase*1 and Y. Yamada*2
Ballistospore and stalked conidium formation of Kockovaella and allied yeasts.
*1 Japan Collection of Microorganisumus, the Institute of Physical and Chemical Research (RIKEN)
*2 Department of Applied Biological Chemistry, Shizuoka University

Japan Federation for Culture Collection (June, 1990, Hachioji)

I. Banno
An introduction to USFCC culture collection guidelines.

Y. Yamada, Y. Kaneko and K. Mikata
Re-identification of brewering yeasts preserved in Institute for Fermentation, Osaka.

T. Nishii and A. Nakagiri
Cryopreservation of Oomycetous fungi in liquid nitrogen.

The 9th Tsukuba Microbiol. Seminor (July, 1990, Tsukuba)

A. Nakagiri
Fungi inhabiting marine environments.

Joint Meeting of The Society for Actinomycetes, Japan and The Society for Applied Genetics of Actinomycetes, Japan (July, 1990, Nagoya)

Y. Nakagaito and Toru Hasegawa
A rare actinomycete isolated from fallen leaf in mangrobe region.

Pharmaceutical Society of Japan (August, 1990, Sapporo)

H. Tsujibo*, Y. Yoshida*, Y. Inamori* and Toru Hasegawa
Purification and properties of chitinase from an alkalophilic actinomycete OPC-131.
* Osaka University of Pharmaceutical Sciences

H. Tsujibo*, T. Kuda*, Y. Inamori* and Toru Hasegawa
Purification and properties of xylanases from a thermophilic actinomycete, Streptomyces thermoviolaceus OPC-520.
* Osaka University of Pharmaceutical Sciences

H. Tsujibo*, K. Miyamoto*, Y. Inamori* and Toru Hasegawa
Partial amino acid sequences of alkaline serine proteases from an alkalophilic actinomycete.
* Osaka University of Pharmaceutical Sciences

The Genetic Society of Japan (October, 1990, Tokyo)
Y. Kaneko and I. Banno
Electrophoretic Karyotype and DNA similarity of Saccharomyces bayanus.

Japan Society for Cell Biology (October, 1990, Tokyo)
T. Yoshida and Masao Takeuchi
Production of fibronectin and laminin by mouse astroglial cells under serum-free conditions.

Japanese Society for Bacteriology (November, 1990, Osaka)
Toru Hasegawa
Trends in actinomycete taxonomy.

Congress of Asian-Pacific Organization for Cell Biology (November, 1990, Shanghai, China)
K. Takeuchi*1, M. Ogura*2, H. Saito*3, M. Satoh, M. Takeuchi
Platelet-like particles released from human megakaryoblastic leukemia cell line (MEG-01).
*1 Ehime College of Health Science
*2 Aichi Cancer Center Research Institute
*3 School of Medicine, Nagoya University

Annual Meeting on Microbial Ecology of Japan (November, 1990, Tsukuba)
M. Furuki*, M. Kuroda* and A. Yokota
Ecology of the bacteria which decompose Chattonella.
* The Environmental Science Institute of Hyogo Prefecture

Society of Fermentation Technology, Japan (November, 1990, Osaka)

M. Furuki*, A. Yokota and Toru Hasegawa

Isolation, Identification and physiological characteristics of the bacteria which decompose Chattonella antiqua cells.

* The Environmental Science Institute of Hyogo Prefecture

A. Yokota

Syposium on Bergey's Manual of Systimatic Bacteriology Vol. 3 and Vol. 4. Aerobic chemolithotrophic bacteria and associated organisms.

Nihon Archaebacteria Kenkyu-kai (November, 1990, Osaka)

T. Sakane, I. Fukuda*, T. Itoh* and A. Yokota

Preservation of archaebacteria by L-drying.

* College of Liberal Arts and Sciences, Kiatasato University

Annual Meeting on Microbial Chemotaxonomy (November, 1990, Ooarai-cho)

Y. Kaneko and I. Banno

Reexamination of Saccharomyces bayanus strains by electrophoretic karyotyping and DNA similarity.

MISCELLANEOUS SCIENTIFIC PAPERS

Toru Hasegawa. 1989. Trends in microbiological taxonomy. 4. Actinomycetes. Bioscience and Biotechnology 27: 129-136. [in Japanese]

Toru Hasegawa, M. P. Lechevalier and H. A. Lechevalier. 1989. Section 31. Thermomonospora Genus Actinosynnema Hasegawa, Lechevalier and Lechevalier, 1978a, 304[AL]. In S. T. Williams (ed.) Bergey's Manual of Systematic Bacteriology, vol. 4, p. 2560-2562, Williams and Wilkins, Baltimore.

K. Suzuki[*1] and Toru Hasegawa. 1989. A classified table of bacteria. In the Japanese Association of Microbiology (ed.) Dictionary of Microbiology, p. 1208-1214. Gihodo Shuppan Co., Ltd., Tokyo. [in Japanese]

A. Yokota. 1989. Analysis of reducing sugars with HPLC. In Methods for Identification of Actinomycetes. Qualitative and quantitative estimation of cell components with HPLC. Japan Association of Actinomycetes, p. 9-23. [in Japanese]

H. Mayer[*2], J. H. Krauss[*2], A. Yokota and J. Weckesser[*3]. 1990. Natural variants of lipid A. In Endotoxin, ed. by H. Friedman, T. W. Klein, M. Nakano and A. Nowotny, Plenum Publishing Corporation, Stuttgart, p. 45-70.

A. Nakagiri. 1990. Fungi inhabiting marine environments. Setouchi Kagaku 1: 72-79. [in Japanese]

A. Nakagiri. 1990. Marine fungi. Biseibutsu 6: 67-71. [in Japanese]

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*1 Japan Collection of Microorganisms, RIKEN

*2 Max-Planck-Institut für Immunbiologie, Freiburg i. Br., F. R. G.

*3 Institut für Biologie II, Mikrobiologie, der Universität, Freiburg i. Br., F. R. G.

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CORRECTIONS

In the issue of IFO Research Communications No. 14, the following corrections should be made.

Page	Line	Type	Should read
137	15	marqundii	marquandii
137	49	*Since only a single colony of each species was isolated from an isolation medium, the number of strains isolated is equal to the number of samples from which a given fungus was detected.	**Since we isolated only a single colony of each species from each two duplicate isolation plates of four isolation methods, "8 isolates" means that we detected this fungus totally on 8 isolation paltes among the total of 656 isolation plates examined, excluding duplicate isolation plates.
137	52	**Ordinal numbers preceding fungus name are the numbers ascribed to the species isolated in this work.	*Ordinal numbers preceding fungus name are the numbers ascribed to the species isolated in this work.

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