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Data management	Masao TAKEUCHI, Itaru SUENAGA

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APPENDIX (Separate issue)

List of bacterial species approved and validated in the 20th century:

Y. NAKAGAWA, T. TAMURA and K. HATANO

発酵研究所スタッフ（2000年6月撮影）

前列左から：伊藤忠義、見方洪三郎、吉田正憲、竹内昌男、佐藤元信、波多野和徳.
中列左から：塩山絹子、吉田東歩、杉本美香、佐藤邦子、武内真理子、山下幸恵、山口やよい、末永　格、西井忠止.
後列左から：中川恭好、内野佳仁、中桐　昭、辻　憲悟、田村朋彦、西村久美子、岡根　泉、黒田暁子

IFO Res. Commun. **20**,
1-14, 2001 (March)

REPORT OF THE DIRECTOR

Masao TAKEUCHI

I. Overview of the IFO's Activities over Two Years

Since its establishment in 1944, the Institute for Fermentation, Osaka (IFO) has strongly endeavored to maintain a culture collection for over 56 years with the continuous financial support of Takeda Chemical Ind. Ltd. The activities of the IFO have developed year by year. Recently, many governments around the world have provided steady support for their own culture collections in obtaining, maintaining and using biological research resource materials. Another result is that a Convention on Microorganisms Diversity was recently concluded internationally and had a great influence on the traditional usage and transfer of all materials between researchers around the world.

To improve activities on culture collection, the IFO cooperated in the following public projects in Japan. Since 1995 the IFO, with the Japan Health Sciences Foundation, has managed a research resources bank for the Ministry of Health, Labour and Welfare. To reconstruct the IFO data management system, the IFO joined two research projects; the JST beginning in 1997, and NEDO beginning in 1998, established by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and Ministry of Economy, Trade and Industry, respectively. The NEDO's project was finished by March 2000. These three projects contributed to facilitating computerization and restructuring experimental equipments as well as improving the financial standing of the IFO.

To establish the IFO data management system, the IFO started to construct a computer networking system using a client-server to increase the efficiency of data management using the Internet, E-mail and common files on servers in 1998. Until the end of the 2000 fiscal year, the Home Page of the IFO was on the web site (http://www.ifo.or.jp). The database of biological research resource materials in the IFO (IFOBRDB) was constructed on the data base servers. I will describe the IFO data management system in a later section.

In the past two years, the IFO has supported two public projects; one was a memorial project to store microorganisms in the Time Capsule at the Expo'70, and the other was

joining the OECD task force project. I will also describe these projects in the later sections.

The financial situation of the IFO deteriorated between 1991 and 1995, but as a consequence of reorganizing the management, the situation has improved. Since 1995, the IFO has continuously shown a surplus and also expects to do so in 2000. The IFO has been in many researcher's confidence for half a century as a result of distributing many research resources to biologists. Recently, the financial supporter (Takeda Chemical Industries Ltd.) of the IFO announced that amount of annual donations will decrease to 125 million yen in 2001 and to 75 million yen in 2002. Because the work of the IFO is not always profitable, if the IFO loses financial support, it will be unable to operate. The IFO will have to be severe with economic management or will be in a so called "Endangered Culture Collection". In Europe, culture collection has over 100 years of history and over 75 years in the USA. In Japan the 56 years history of the IFO, however, is about to collapse. Why can't Japanese society understand the exact significance of such the activities of culture collection? Our mission is to keep the "Cultural Wealth" of the 17,000 strains stored in the IFO until the next-generation of researchers can benefit from then. I appreciate Dr. Cypess's proposal, the director of ATCC, that the ATCC will be available for co-operation with the IFO on the management of the IFO culture collection. The Dr. Cypess's proposal indicates the activities of IFO have internationally contributed to development culture collection for long period. We should consider every alternative options for survival of the IFO. In this report, I will outline the activities of the IFO over the past two years, and then describe the three special events in sections II to IV, which the IFO faces.

II. Conventional ordinary report

1. Personnel Affairs

At the 109th meeting of the Board of Trustees in June 1999, Dr. Kanji Meguro of Hamari Chemicals Ltd. was nominated as a councilors from June 1999. Dr. Masao Isono and Dr. Taiji Oshima were nominated as auditors at the 108th meeting of the Board of Trustees in March 1999, and at the 109th meeting in June 1999, respectively. Mr. Ryohei Kizaki and Dr. Toshio Miwatani retired as auditors in March and in June 1999, respectively.

Mr. T. Nishii and Mr. K. Mikata retired on July 1, 2000 and on January 20, 2000, respectively. Dr. Touho Yoshida and Ms. Akiko Kuroda moved to the Japan Health Sciences Foundation on December 1, 2000 and November 1, 2000, respectively. The IFO is grateful to then all for their dedication while working for the institute.

The new treasurer of the institute, Dr. Masanori Yoshida, joined the IFO in April 1999 and was appointed treasurer on April 1, 1999. Dr. Uchino and Ms. Mika Sugimoto joined the bacteria group and the yeast group on April 1, and February 1, 2000, respectively.

2. International Meetings

The staffs of the IFO have attended international meetings during the past two years,

presented scientific papers at these meetings and promoted mutual cooperation among collections around the world.

Dr. A. Nakagiri attended the 7th International Marine and Freshwater Mycology Symposium in Hong Kong in July 1999, and presented a paper on "Ecology and diversity of halophytophthoras". Tad. Ito and A. Nakagiri attended the symposium, and presented a paper on "Mycoflora of the rhizospheres of Japanese mangrove trees".

Mariko Takeuchi and K. Hatano attended the 9th International Congress of Bacteriology and Applied Microbiology in Sydney in August 1999, and presented a paper on "Proposal of the genus *Sphingomonas* sensu stricto and three new genera based on the analysis of 16S ribosomal RNA sequence and polyamine profile".

Dr. T. Tamura attended the 1st International Conference on Biology of Actinomycetes under Extreme Environments in China in August 1999, and he presented a paper on "Establishment of taxonomic status of the strains belonging to invalidated genera of the order *Actinomycetales*".

Mr. T. Tamura and Dr. K. Hatano attended the 11th International Symposium on the Biology of Actinomycetes in Greece in October 1999, and presented a paper on "The phylogenetic structure of the genus *Actinoplanes*". Dr. K. Hatano, et al. attended the same symposium, and they presented a paper on "Proposal of a new criterion for identification and classification of *Streptomyces* species: gyrB sequence".

A. Nakagiri and Tad. Ito et al. attended the 9th International Congress for Culture Collection in Brisbane in July 2000, and presented a paper on "Comparative biodiversity of fungi inhabiting tropical (Thailand) and subtropical (Japan) mangroves". Dr. K. Hatano et al. attended the same symposium, and presented a paper on "Classification and quality control of *Streptomyces* species by *gyrB*-based phylogenetic analysis". Masao Takeuchi and T. Yoshida et al. attended the same symposium, and a presented paper on "In vitro proplatelet formation of megakaryocytes generated from human cord blood CD (+) 34 cells". Drs. Y. Nakagawa and K. Hatano et al. attended the same symposium, and presented a paper on "L-drying method for preservation of bacteria in Institute for Fermentation, Osaka (IFO)".

Drs. I. Okane, A. Nakagiri and Tad. Ito attended the Asian Mycological Congress 2000 in Hong Kong in July 2000, and presented a paper on "Identity of two major endophytic ascomycetes of ericaceous plants"

3. Collection and Publications

The total number of cultures stored in the IFO culture collection reached 16,150 at the end of 1999 and 16,344 at the end of 2000. The newly accepted strains for each year are listed in the present issue of the IFO Research Communications in 2001. The total numbers of cultures distributed from the IFO culture collection were 7502 in 1999 and 8,072 in 2000. Of these, 404 and 310 strains were distributed abroad in 1999 and 2000, respectively.

The IFO Research Communications, No. 19 was published in March 1999, and the IFO List of Cultures, Animal Cell Lines, 6th edition, was published in March 2000. The catalog detail about 200 animal cell lines. The manuscripts for the catalogues were prepared with photographs of each cell lines and edited from the database stored in the

animal cell section of the IFO culture collection and processed by Drs. Touho Yoshida and Motonobu Satoh. Furthermore, the same full documents as in the catalogs of Animal Cell Lines and Microorganisms are shown on the website for animal cells and microorganisms on the IFO Home Page (http://www.ifo.or.jp). The IFO List of Cultures, 11th edition, Microorganisms, was published in January 2001. A CD-ROM catalogue of microorganisms was prepared with a special search system in January 2001.

4. Finances

Since the establishment of the IFO culture collection, Takeda Chemical Industries, Ltd. has promoted IFO activities by providing over 56 years of continuous financial support. The financial standing of the IFO over the past five years is summarized in Table 1, and shows the annual income and expenses of the IFO for each fiscal year for 1995 to 1999.

Takeda Chemical Industries Ltd. has continued to provide support for the IFO with annual donations of ¥175 million (¥195 million in 1995). "Interest and Dividends" is income acquired from a ¥10 billion endowment. "Cultures" indicates income acquired from the distribution fees for cultures. "Other services" includes income from commission of patent deposits or safety deposits. "Grants and Contracts" refers to a contract with the Japan Health Sciences Foundation for the management of the HSRRB bank and

Table 1. Closing Accounts of IFO

(100 thousand yen)

FISCAL YEAR		1995	1996	1997	1998	1999
INCOME	Donations	1,950	1,750	1,750	1,750	1,750
	Interest & Dividends	550	570	598	825	905
	Grants & Contracts	170	100	100	105	588
	Cultures	537	550	482	576	466
	Other Services	234	197	175	172	211
	TOTAL	3,441	3,237	3,110	3,428	3,921
EXPENSES	Personnel Expenses	2,439	2,110	1,820	1,746	1,922
	Supplies	316	214	352	682	1,489
	Facility Maintenance Expenses	398	375	278	239	372
	Other	267	101	88	218	136
	TOTAL	3,420	2,800	2,538	2,885	3,919
BALANCE		21	437	572	534	2

with the MITI for the long-term preservation of microorganisms in Japan. "Supplies" refers to expenses for the purchase of expendable supplies and equipment. "Facility Maintenance Expenses" is composed of expenses related to renting the facilities used by the IFO and paid to Takeda Chemical Industries Ltd.

The main cause of the decrease in annual income from 1991 to 1994, was a fall in the interest acquired from the endowment of ¥10 billion due to a reduction of the official rate. Since 1995, the fall in the interest seems to have stopped.

The IFO's expenses continued to increase by ¥10-15 million annually from 1991 to 1995. Increasing personnel expenses were the major contributory factor. A deficit occurred in 1992, reaching ¥43.8 million in 1994, as shown in the previous director's report in 1996. The main decrease in the expenses of 1996 was obtained by reducing personnel expenses, because employee numbers decreased to 60% of those in 1994. In fiscal year 1995, the budget showed a ¥2.1 million surplus as a result of increased income and decreased expenses, and was followed by ¥44 million, ¥57 million and ¥53 million in 1996, 1997 and 1998, respectively.

Culture collection has one particular characteristic, which may not necessarily be adapted to the economic principles of private enterprise. The IFO is the only privately supported, non-profit public service culture collection in the world. Government finances have supported some of main domestic and foreign culture collections for a long time. Therefore, intense competition between culture collections has increased. Therefore, in order to improve the IFO's activities we hope that a major financial supporter and the board members will show continued support in the future. At the same time, we should evaluate our attitude and ability in the field of culture collection.

Visitors

The IFO has welcomed a number of foreign visitors over the past two years. Some of these gave lectures or seminars. The titles of their informative speeches, below, had a great impression on us.

Dr. C. Kurtzman, NRRL, USA, on February 22, 1999. "Rapid identification of yeast by molecular methods and the use of molecular data for systematics".

Dr. N. L. Dung (Vietnam National Univ. and P.Van TY (National University of Hanoi), on February 9, 1999.

Dr. G. D. Castro-Bernas, Philipin Univ. on March 12, 1999, "A summary of the different biomolecular researches that are being done in my section"

Dr. Che Nyorya, Malaysia Univ., Dr. Dung and Dr. Ty (Vetonum Univ.), on March 5, 1999.

Dr. Judit Lehocki (NCIAM, Hungary), on October 26, 1999, "History and research activity of National Collection of Agricultural and Industrial Microorganisms".

Dr. Leka Manoch (Kasetsart Univ.), October 28, 1999, "Ecology and distribution of microfungi in Thailand".

Dr. Puspita Lisdiyanti (Indonesia Institute of Sciences), on June 28, 2000, "Systematic study of acetic acid bacteria".

Dr. P. A. R. Vandamme (Univ. Ghent, Belugium), on October 26, 2000, "Polyphasic

taxonomy in practice".

Hansjorgg Prilinger (Univ. Bodenkultur, Austria), on November 2, 2000, "Molecular phylogeny and systematics of the fungi with special reference to the Asco- and Basidiomycota".

III. Time Capsule at the Expo'70

To commemorate of Expo'70 held in 1970 in Osaka city, a time-capsule was stored under Osaka Castle. In the capsule several thousand industrial products and many other goods such as radio, photographs, medicines, etc. were stored. Three microorganisms were provided for storage in the capsule by the IFO. These microorganisms are useful for the food industry.

Aspergillus oryzae IFO 4290, *Penicillium roquefortii* IFO 5754, and *Bacillus natto* IFO 13169 from the opened capsule, were cultured in each appropriate medium on July 3, 2000 by Mr. Tad. Ito and Dr. Mariko Takeuchi. The cultivation conditions are indicated in Table 2. The three ampoules prepared in 1970 were prepared by a freeze-drying method. The three microorganisms from the capsule were active, and viable cells of *Aspergillus oryzae, Penicillium roquefortii* and *Bacillus natto* were 2.4×10^4, 1.7×10^5

Table 2. Media and Culture Conditions

Media	*Aspergillus oryzae,* *Penicillium roquefortii*	*Bacillus natto*
Count of Viable cells	No. 108 medium、 25℃、culture for 2 days	No.802 medium、 30℃、culture for 1 day
Acceleration test	37℃、30days	37℃、2 weeks
Suspension medium	3.0% Na-Glutamate 0.01% Actocole 0.1M Phosphate buffer pH 7.0	3.0% Na-Glutamate 1.5% Adonitol 0.05% Cystein-HCl (0.1M phosphate buffer、pH7)
Recovery medium	No. 707 medium: 0.5% peptone 0.3% Yeast extract 0.1% $MgSO_4\cdot7H_2O$ pH 7.0	No. 702 medium: 1% Polypepton 0.2% Yeast extract 0.1% $MgSO_4\cdot7H_2O$ pH7.0
Culture medium	No. 108 medium 1% glucose 5% peptone,0.3% yeast extract 0.3% malt extract, 1.5% agar pH 5.6	No. 802 medium 1% Polypepton 0.2% yeast extract 0.1% MgSO4·7H2O 1.5% agar pH 7.0

Suspension medium: A medium for suspending the cultured material before preparing the dried material. Recovery medium: A medium for suspending the dried material in ampoules.

and 2.0×10^5, respectively. On July 21, 2000 these three microorganism ampoules were prepared again for restorage in the Time Capsule. The new preparations (Figure 1) by the L-drying method were contained in 1.0×10^7 *Aspergillus oryzae*, 1.9×10^7 *Penicillium roquefortii*, and 4.8×10^7 *Bacillus natto*, and each viable ratio of the three preparations after accelerated preservation test was 30, 18 and 52% as shown in Table 3, respectively. The Time Capsule project plans to open the capsule 100 years later. The appropriate experimental conditions for the examination of the cultivated microorganisms in the ampoules stored in the Time Capsule on November 23, 2000 (see page 16) are shown in Table 2. When the capsule is opened again in 2100, we hope a researcher of the IFO continues the maintenance of these 3 microorganisms, according to our description in 2000.

The IFO prepared L-dried microorganisms for long-period storage in the Time Capsule or in cold room at the IFO. We have assumed the microorganisms will be viable after 30 years from the results of the accelerated preservation tests. After 100 years the IFO will estimate the viability of microorganisms stored by the two methods.

Table 3. Viable Cell Counts of the Three Microorganisms

	Recovered from capsule	Newly prepared		
		Before accelerated preservation test	After accelerated preservation test	Ratio
	(CFU/Ampoule)			(%)
Aspergillus oryzae IFO 4290	2.4×10^4	1.0×10^7	3.0×10^6	30
Penicillium roquefortii IFO 5754	1.7×10^5	1.9×10^7	3.5×10^6	18
Bacillus natto IFO 13169	2.0×10^5	4.8×10^7	2.5×10^7	52
Method of preparation	Freeze drying	L-drying		

***Aspergillus oryzae* IFO 4290**

***Penicillium roquefortii* IFO 5754**

***Bacillus natto* IFO 13169**

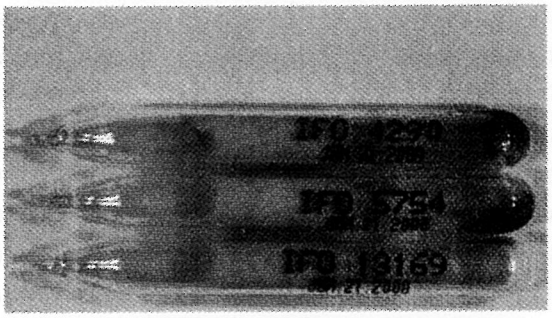

Fig. 1. L-dried microorganisms stored in the Time Capsule.

III. Answers to the OECD Task Force Questionnaire on BRCs

The OECD decided the task force groups to discuss the global problems related to the development and establishment of a biological resource center (BRC) in each nation of the world. To realize and understand the present economical conditions of a BRC in each nation, the task force groups gave a questionaire to eleven typical culture collections (synonym of BRC) in the world. The IFO received the questions in March 2000 as the representative organization in Japan. The questions (in italics) of the task force groups and the answers of the IFO are as follows:

Questionnaire and Answers-General

1. *What do you perceive as the most important challenges to the activity and maintenance of BRCs?*

To secure biological resources for academic and industrial uses in a state of advanced quality control over a prolonged period and to make them available to researchers in any country of the world.

2. *What strategies do you have in place locally, nationally and internationally to meet those challenges?*

We are collecting information concerning highly useful biological resources through the networks of various scientific associations such as the Japan Society for Culture Collections. We also evaluate the taxonomy of biological resources in our safekeeping by their subculturing and we examine the state of their contamination by other living organisms. In other words, we perform sufficient quality control of the biological resources, and provide them and information about them through the catalogues and our website. We also investigate the inventory of microbial strains (bacteria, fungi) in Japan, isolate and collect microbial strains, and classify and identify them to increase and improve our stocks of microbial strains. We also have joint research projects with overseas BRCs for the same purposes.

3. *Do you see the role of BRCs changing in the next decade?*

We do not think that the basic activities of BRCs will change. However, their activity patterns will change with the diversification of the research resources to be handled. Beyond the borders of the countries in which BRCs are located, new BRC groups will be formed in regions such as America, Europe, and Asia. In each of these regions, resources will be shared, and management methods suited to the unique situation of the region will be established. Problems such as the differences in the economic level, differences in the acquisition price due to the imbalance of the exchange rate, and geographic handicap for procurement of resources will be discussed in each region. The roles of the regions in the world, roles of national BRCs in each region, and division of work and responsibility among them will become clearer.

4. *What are the requirements to meet this changing role across the globe?*

Operations of BRCs in different countries must be linked, i.e. resources must be shared, communication among researchers promoted, and joint research projects powerfully progressed among BRCs internationally.

5. *What strategic alliances are needed for sustainability? Are they truly desirable?*

Biological resources must be defined as assets of common global ownership. The necessity of conservation operations must be appealed at the UNESCO and UN levels. The government of each country must be obligated to provide financial support to BRCs that are engaged in sharing operations.

6. *What is needed for the preservation and potential exploitation of the expanding biodiversity? How can those requirements be met?*

It will be important to evaluate joint ownership and disclosure of biological resources, information concerning them (genetic resources, information concerning genetics and biology, etc.) and work sharing of preservation operations on the global level. The preservation of all of the diverse biological resources by a single nation or a single organization is impossible. A BRC specializing in the preservation of biological resources must be established in each country, and a system for supporting such BRCs on the global level must be created.

Eight Special Questionnaire and Answers, - Special

1. *Define the nature, activity, and role of your BRC in terms of size (collection, staffing, facilities), breadth of mission, and foreseen changes with respect to its primary and essential functions. (In responding, please consider the extent of national and international collaboration, requests and distribution, and how future requirements for networking will be met.)*

IFO preserves around 16,000 strains of biological resources and has a staff of 18 (including 14 researchers). The biological resources handled at the IFO are bacteria, yeasts, molds, bacteriophages, and animal cells. The IFO distributes about around 8,000 strains annually, about 5% of which are distributed to foreign organizations. We preserve 80% of our biological resources by the liquid-drying method, which allows long-term preservation without changes in their biological properties. The rest of our biological resources are preserved by freezing (−80°C, or −150°C to −190°C). We have confirmed that each strain can survive for over 30 years without subculturing.

At the IFO, an appropriate system must be organized under the CBD for the procurement and distribution of strains established abroad. Providing and improving the service of classification and identification of biological resources and campaigning for the acquisition of the position of an international patent deposit organization based on the Budapest Treaty will be required to perform procedures related to the preservation of strains entrusted for patents in the IFO (Presently, The IFO belongs to EPO). Multiple patent deposit organizations are needed in Japan as in major foreign countries for the convenience of the users.

2. *Define current sources of funding, their reliability, and how future funding requirements will be met. (Please include details of any charges levied for accession or access to items, and how the imposition of charges influences policy and quality of service.)*

Of our funding, about 50% is from donations, 20% is income from the basic assets, 20% is income from the fees for the distribution of strains and for services, and 10% comes from

a grant contract with the government. Since it is difficult to anticipate increases in donations, we will try to increase the income from fees for distribution by increasing the value-added biological resources. The fee for distribution of a strain is 4,000 yen for public research organizations and 8,000 yen for private research organizations. Distributions are also made at the same fees to foreign institutions. For researchers at domestic universities, 4,000 yen is a reasonable charge for a strain. Distributions of strains among BRCs are made free of charge. We maintain an exchange system with domestic and foreign BRCs. Providing biological resources to researchers free of charge is not considered to be appropriate for improving the quality of a BRC. Researchers offered biological resources on payment strictly evaluate the biological quality of the resources provided and complain to the BRC if they find it questionable. These complaints are valuable information for the BRC. The BRC is compelled to reexamine and reconfirm the quality of the biological resources in question, leading eventually to improvements in the quality of its collection of biological resources.

We offer no special service to our sponsors because of their contributions. The IFO provides its services impartially to all researchers.

3. *Describe how quality assurance and quality control are applied and maintained. (Include details of accreditation, customer service response, and the numbers of complaints.)*

The IFO performs agreement tests with the species names in the literature, and identification of the species by morphological observation under a microscope and by gene analysis (analysis of base sequence, analysis of DNA by the RAPD method, and other methods) at subculturing. We collect information including research presentations concerned with the holding research resources, and accumulate biological information updating our taxonomic information such as changes in species and scientific names.

In response to inquiries from the users, we provide information concerning the state of presence and keeping of the strains, their culturing methods, properties, and literature.

Information concerning changes in species names is distributed through the IFO Research Communications, which is published every two years, or the IFO List of Culture (catalog), which is published every 4 years, and is on the website. We receive a few (two to three) complaints per year concerning the contamination of the distributed biological resource by other organisms. In such cases, the IFO repeatedly performs re-identification of the taxonomy and contamination tests of the biological resource in question. The results sometimes indicate errors on the users' side. If the errors are the IFO's, we newly procure the same strain from other culture collections, their original depositors, or researchers who have isolated and identified them, and preserve the renewed stock. We send the new strains to the users who have made the complaints and distribute this information to other users.

4. *Describe your current activities and infrastructure (staff, etc.) for technology and research, existing and projected integration with information technology, and your strategy for development. (Include details of R&D, education and training, requirements for specialists, and the need for expertise relevant to BRCs.)*

The IFO assigns 2-3 researchers or taxonomy experts with PhDs. to each of the fields

of biological resources (bacteria, actinomycetes, yeasts, molds, animal cells). The experts in each field are taxonomy researchers and have some knowledge of the technology of bioinformatics. Most researchers in the IFO major in microbiology, cell biology or molecular biology, and have Ms. or PhDs. They work actively through writing research papers and making presentations to many research societies. We hope not to be technicians but rather researchers operating and managing the collection, quality control, preservation and distribution of biological resources. In the past ten years, three members have moved to domestic universities as professors or associate professors.

The IFO has a facility that can handle level 2 biohazards, a facility that can handle level P2 DNA recombinants, a low-temperature preservation room (4 or 15 C), an L-dry preservation room, and a room for the preservation of frozen ampoules in liquid nitrogen. Our facilities are equipped with most instruments and devices needed for taxonomic studies (instruments for chemical analysis, DNA analysis, and morphological examination) and we are currently acquiring instruments for bioinformatics.

The work to construct a database concerning its holdings and the website on the internet are being carried out, to improve the techniques of all research staff members related to bioinformatics through these works. It is also advancing joint studies with overseas researchers at other culture collections to develop international cooperation among researchers on biological resources.

5. *How are bioinformatics and related systems utilised with respect to your holdings? (Include details of the extent and ease of access, and the quality of information and support. How will the impact of IT change the service with respect to integration and networking -- between live collections and databases, for example -- and to the need for improved technology and staffing expertise?)*

The IFO has created a database of about 16,000 strains of biological resources in its holdings. Concerning about 10,000 of them, the data of which can be disclosed to outside researchers, the website was arranged on the internet so that the users can read, search, and ask for the distribution of biological resources. Since the database and the website of the IFO are synchronized, the users can always access the latest information. A system that allows more detailed searches and is more convenient is being prepared.

We would like to hire an expert in bioinformatics as a researcher in the future.

6. *What are the extent and nature of existing strategic alliances and co-operative agreements in the context of national and international networks? (Include details of how such alliances will be developed to meet the future needs of BRCs.)*

The IFO has cooperated with other BRCs through the system of mutual exchange of biological resources. This system will be maintained in the future. To further strengthen the cooperation, preparations are being made to share databases among domestic BRCs. Internationally, the IFO is conducting joint research in the areas of isolation of microbial strains from nature, their identification, characterization and preservation. Closer and more concrete international collaboration and joint operations are needed in the future.

7. *How are biodiversity, specialisation and representation covered? What strategy do*

you have in place for representation in the light of exploration and exploitation? (Include details of how accession policy and access to holdings [strains, variants, libraries, clones or databases] might be restricted, and the rationale for maintaining or discarding material. Is there any intention to preserve mixed, biodiverse environmental samples and databases beyond the typical collection of pure, whole cells?)

The basic operation of the IFO is to hold microbial strains with scientific names and biological resources with added values about which such information as biological properties is available. With the exception of strains maintained on a contract such as patent deposits and strains in the process of quality control, the IFO opens all strains and their catalogue information to the public. However, detailed taxonomical information is not released. Strains that are taxonomically questionable and those with qualitative problems are discarded unless they have been distributed within the past 5 years. Those that have no quality control problems are maintained over a long period even if there has been no instance of distribution during the past 10 years. Strains that have high added economical values might be managed for a short period and used at each research organization (laboratories at universities and business firms). We consider that a BRC should perform the operations of isolation, quality control, preservation, and distribution of biological resources of scientific values in the longer perspective. We are making efforts to acquire new microorganisms from nature in Japan and abroad to increase the diversity of our microbial collection, active in taxonomical studies.

The IFO, with the current research staff and organization, has no capacity to be active in preserving "Mixed, Biodiverse Environmental Samples and Databases". However, we consider that we should act as a consultant in the planning of research by venture businesses and researchers of private firms, cooperate in the planning of basic research at universities, listen to requests to BRCs, and aggressively participate in new projects as long as they are useful for the essential activities of the BRC.

8. *Are the public currently aware of BRCs? Do they accept that BRCs are necessary? Do they place any importance on biological resources in general? (Include details of how accession policy and access to holdings [stains, variants, libraries, clones or databases] might be restricted, and the rationale for maintaining or discarding material. Is there any intention to preserve mixed, biodiverse environmental samples and databases beyond the typical collection of pure, whole cells?).*

We consider that researchers in general understand the necessity of BRCs, recognizing that biological resources are the basis for biosciences and have contributed to the development of science. However, researchers engaged in the most advanced research have no time to preserve biological resources while they may understand the importance of the constant accessibility to them. The public interest in the protection and preservation of living organisms including microorganisms as genetic resources appears to be growing due to their increasing awareness of environmental problems and the possibility of their future utility. Furthermore, the presence of highly effective BRCs is undoubtedly necessary for the development of science in a country. It is also obvious that any single

country or organization cannot integrate the preservation of all living biological resources or those being used in the country, given their diversity. Therefore, international mutual support of BRCs is clearly needed. Although many "Libraries of books" are established and managed on the national level, the development of BRCs, which may be regarded as "Libraries of Life", has been delayed. National policy makers and researchers in general must better recognize these facts. The industrial world also should not simply go after

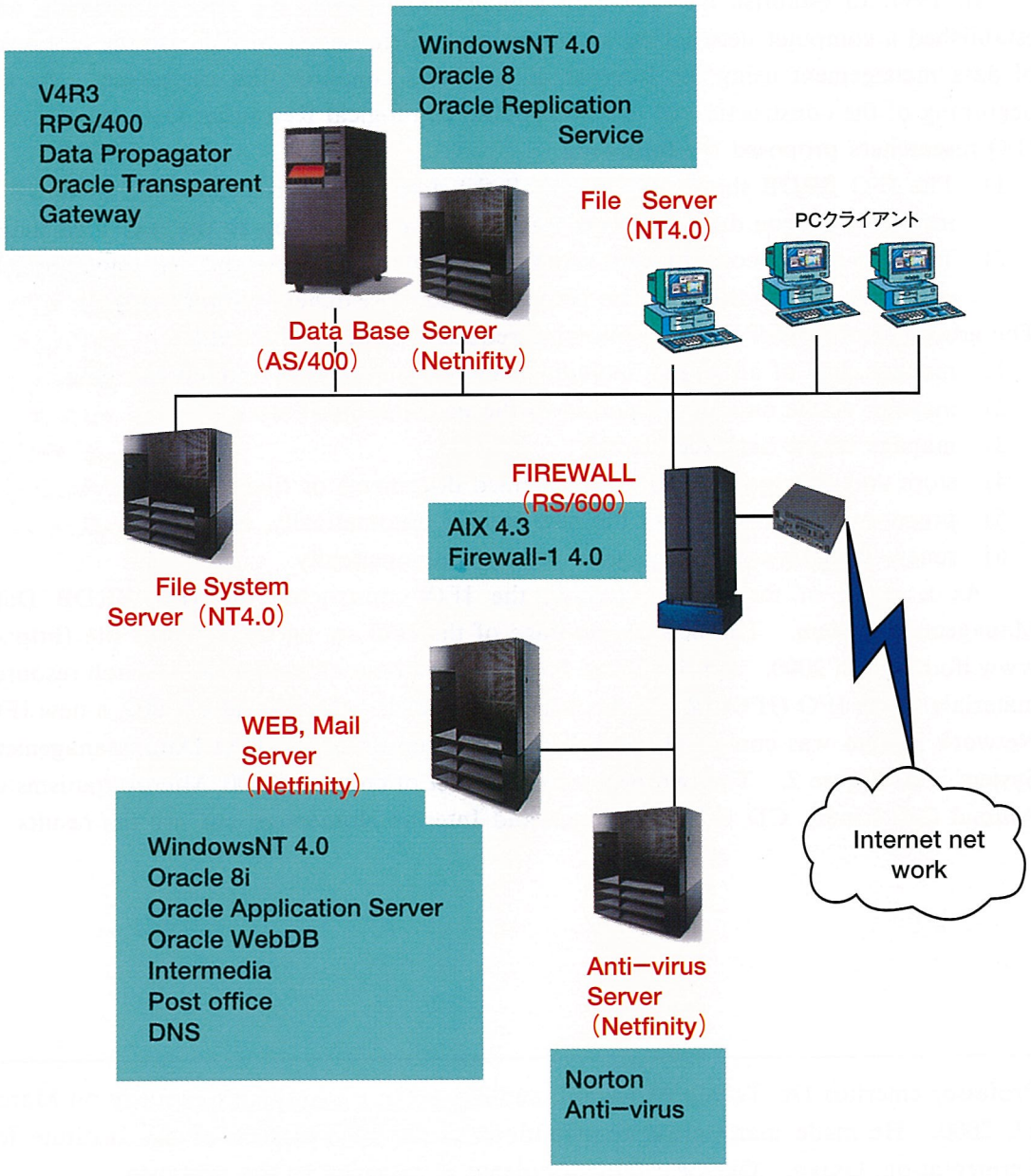

Fig. 2. Hard System of IFO Data Management System

immediate profits but extend financial support for developing organizations that preserve biological resources (BRCs), which provide the basis for industrial activities.

IV. Establishment of an IFO Data Management System

The establishment of an IFO data management system has been an important problem over the last ten years in our institute. Dr. Iijima (IFO Res. Common. 17, 44, 1995) created a prototype database of the IFO strain data in 1984 by use of an IBM/23 computer. Since then, information technology has developed greatly. We have been tackling this difficult problem since 1997.

In 1997, to establish an IFO data management system, the IFO constructed and established a computer networking system using a client-server to increase the efficiency of data management using the Internet, E-mail and common files on servers. At the beginning of the construction of the IFO BRDB (Biological Resources Data Base), some IFO researchers proposed the following.

1) The IFO BRDB should consist of all the data on strains preserving in the IFO, including picture data and form-attached data in addition to the text-filed data.
2) The IFO should collect useful data from outside researchers and distribute the data collected and modified by the IFO to outside researchers conveniently.

The administration staff had the following requests. The IFO should:

1) manage data of all strains including newly inputted data and storage data,
2) manage whole data with relation to the distribution of strains,
3) manage whole data keep secret,
4) store and arrange the many IFO-formed documents or files,
5) prepare the manuscripts of the new catalog automatically, and
6) renew the Internet Homepage of the IFO automatically.

As a result of the above requests, the IFO constructed the IFO BRDB Data Management System. The new Home Page of the IFO set up on the web site (http://www.ifo.or.jp) in 2000. On the Home Page, the database of biological research resource materials of the IFO (IFO BRDB) has been constructed. At the end of 2000, a new IFO Network System was completely established. A outline of the IFO Data Management System is in Figure 2. The two new catalogs (List of cultures, 2000, Microorganisms or Animal Cell Lines), CD-ROM catalogue and Internet Homepage, etc. are the results.

Professor emeritus Dr. Toshio Miwatani, auditor of the institute, passed away on March 23, 2000. He made many great contributions to the development of the Institute for Fermentation, Osaka. Our heartfelt condolence is extended to the bereaved.

IFO Res. Commun. **20**,
15-33, 2001 (March)

A History of Institute for Fermentation, Osaka

発酵研究所の歴史

Teiji IIJIMA

財団法人発酵研究所
理事　飯島貞二

1) はじめに

今から30年前，1970年に大阪千里丘陵で万国博覧会（EXPO '70）が「人類の進歩と調和」というテーマで開催された。世界の各国が，科学技術の進歩を携えて，競って当時の最新の技術を展示した。アメリカ館の「月の石」には長蛇の列ができ，入館まで2時間の待ち時間は普通という状況だった。それと対照的にスエーデン館の展示は，「公害問題」を取り上げ，科学技術の進歩と共にすすむ大気や水質の汚染というネガティブの面を取り上げて，技術の進歩と生活の向上との調和を強調していた。この万国博を記念して，松下電器と毎日新聞社との共同企画で，当時の文化，芸術，自然科学，社会状況を示す品々を5000年後の人々に伝えようと，大阪城公園内にタイムカプセルが2個（2セット）埋められた。今年西暦2000年はこのタイムカプセルの1セットを開封して暦年変化を検査し，再び埋め戻しをする最初の年に当たっている。今後は100年毎に同様に検査をして，西暦6970年まで続ける計画である（写真1）。発酵研究所は，日本の伝統的な醸酵食品を作るコウジカビ，アオカビ，納豆菌の標本作製に協力して，このタイムカプセルの中に封入した。この当時にこのサンプルを作った担当者（浅野勇所員，山内栄所員）も所長であった長谷川武治博士も退職されているが，この企画をした松下電器と毎日新聞社から竹内昌男現所長に，この第1回の試験の依頼があり，発酵研究所の現所員で試験を担当することになった。幸いと言うか予想通りというか，30年後の今日でも，当時のコウジカビ，アオカビ，納豆菌は元気に生育をし，おまけに美味しい酒もで

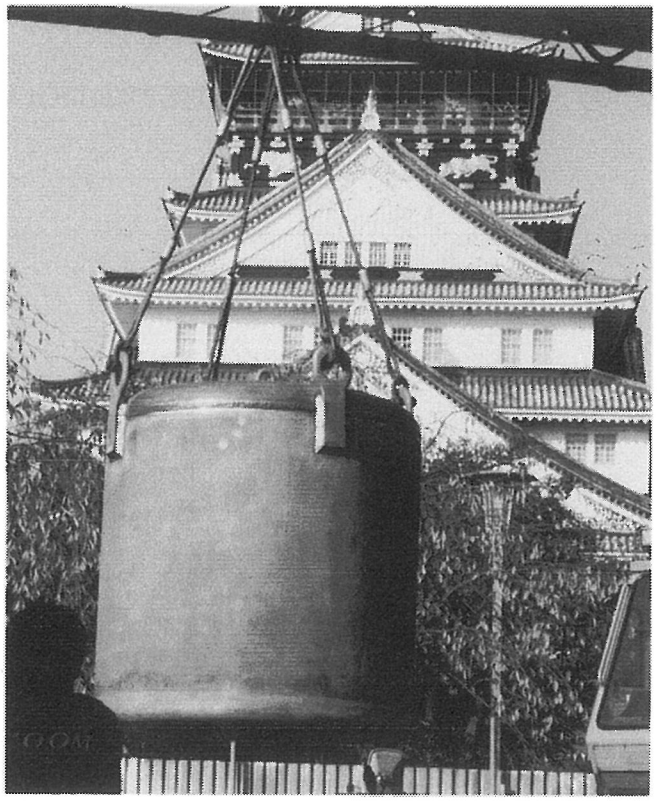

写真 1　タイムカプセルの埋め戻し（2000 年 11 月）

きたようである。これらの菌は，凍結乾燥状態でカプセルの中で 30 年間眠っていたことになる。30 年後に目覚めた菌たちの思いは，SF 小説で異なった空間と時間にタイムトラベルをする人の思いそのものではなかろうか。このタイムカプセルのテストを機会に，微生物保存機関はこのタイムトラベルを日常の業務としていることを今更感じさせてくれた。現在保存されている菌を，そのまま時間と空間を越えたところに送り，研究に産業に役立てているのである。発酵研究所の歴史という題目で筆を執ることになり，過去の資料を調べてみると，資料から当時の記憶が蘇って，その時代へタイムトラベルをするような気分である。

発酵研究所の資料として残っているものは，「発酵研究所事業報告書」(1)，「発酵研究所年報」（IFP Research Communications）(2) であり，また長谷川武治元所長が日本微生物資源学会誌に執筆された「日本の微生物株保存事業」(3－5) のなかに，創業時からの経緯が纏めてある。これらの資料と重複するところは，なるべく省いて，また私自身が所長として勤務した期間を主にして纏めてみたいと思う。発酵研究所の情報処理に関してはすでに発酵研究所年報に報告してあるので (6) これとの重複も避けたいと思う。また資料をあらためて記述するだけでなく，個人的な体験や見解を混ぜて記述することをお許し願いたい。

2)　発酵研究所設立の経緯

発酵研究所の設立は 1944 年（昭和 19 年）12 月に遡り，当時の国策に沿って，内閣技

術院と武田薬品工業株式会社との協同出資により，有用微生物の収集・保存・頒布と航空用燃料，航空用薬品そして航空用食品の開発生産を目指して財団法人「航空醱酵研究所」(理事長武田長兵衛，所長中澤亮治，基本財産5万円，運用資産5万円と内閣技術院からの研究補助金10万円)が設立されたのがもとである。日本の伝統技術である日本酒作りの技術を応用して，澱粉からアルコールを作り，当時血の一滴と言われたガソリンの使用を節約し，戦力の維持を目指したものであった。

しかし設立後まもなく，第二次世界大戦は終わり，1945年（昭和20年）11月に財団法人「醱酵研究所」と名前をあらためて，翌年4月から佐藤喜吉所長の下に，2研究室の構成で，武田薬品工業の資金援助による財団法人として出発した。戦後の抗生物質の開発研究の進展と共に，有用微生物の収集・保存・頒布，菌類の調査研究と並行して，菌類の応用研究部門が拡大して，武田薬品からの委託研究費を受けて，RNA分解酵素の微生物からの分離をはじめとして，工業生産研究の分野に研究範囲が広がってゆき(7−9)，公益法人としての業務の範囲を超えるまでに，応用開発研究の規模が拡大していった。1960年(昭和35年)に武田薬品研究所内に微生物部門が新設され，そこに醱酵研究所の応用研究部門が移されたのに伴って，翌年5月に財団法人「発酵研究所」と改称し，文部省の所管の財団として出発した。

理事会，評議員会のメンバーも一新され，日本の微生物株保存事業を担う新しい研究所として再出発することになった。1961年（昭和36年）6月の理事・評議員会では今後の発酵研究所の基本方針について討議が行われ，以下の基本方針が承認されている。

1. 発酵研究所は保存菌株の維持発展に必要な微生物分野の研究を行うこと。
2. 研究室は，菌学，細菌学，遺伝学の3部門から構成されることを目標として，それに必要な研究者などの増員を行うこと。
3. 研究室はそれぞれ基礎的研究を行うほか，それぞれの専門に従って，保存作業を分担すること。

事業報告書によると昭和36年度の研究テーマとして「微生物の代謝変異に関する研究」,「近畿地方を中心にした菌類の分布に関する研究」,「産業廃水の微生物学的研究」,「乳酸菌及びその応用に関する研究」,「菌類の保存に関する研究」などが列記されていて，この年から研究内容が大きく変化していることが伺われる。この頃奈良正倉院では御物収納の新収蔵庫が完成し，発酵研究所に庫内の微生物調査の依頼を受けたことも重要な事項である。

当時はまだ日本には国立の微生物保存機関はなく，各大学や国立研究機関に付置された施設によって，研究の合間に菌株保存の仕事が細々となされていたに過ぎない。微生物保存機関の役割は，国内，国外の微生物保存機関と協力して，分類学上の命名の根拠となった微生物の標本（基準または基準株）を収集し，保存し，国内外からの請求に応じてこれを分譲して，学問や産業の進展に寄与することを目的としている。そのため分類学の進展に応じた研究活動を維持して，基準となる菌株や標準となる株の品質を維持・保存していなければならない。また，保存菌株のリストの出版を通じて，菌株の情報を公開して，利用者の便を図らなければならない。当時このような活動を行っていたのは日本でもまた世界でも数少ないものであった。

3) 長谷川所長の時代（昭和36年から昭和50年）

1962年（昭和37年）4月に，私が大阪大学医学部遺伝学教室（主任教授吉川秀男）か

ら，発酵研究所に入所したときは，長谷川武治所長のもとに，事務部門3名と2つの研究室の総勢18名の研究所であった。第1研究室は長谷川武治所長が室長を兼任し室員11名，椿啓介副室長が糸状菌の分類と生態を担当し，酵母の分類を坂野勲研究員，微生物の変異を飯島が担当した。第2研究室は室長が児玉礼次郎博士，室員4名で，乳酸菌に関する研究，蚕の軟化病に関する微生物学的研究がテーマに取り上げられていた。菌株の保存は糸状菌と放線菌を浅野勇，古川政夫の2名の室員が，酵母と細菌の保存を山内栄，秦孝子の2名の室員が担当していた。当時の保存菌株数は5010株で地下の低温室内（摂氏5度）に斜面培地に増殖した状態で保存されており，湿度の関係で綿栓にかびが増殖するのを防ぐこと，ダニが綿栓につくことの防止対策など苦労が多いようであった。しかし担当者の努力で，年間4200株を超える信頼のある品質の菌株が日本の

表 1　発酵研究所の保存菌株数と分譲株数

年号	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S48	S49	S50
西暦	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975
保存菌株数	4338	5010	5485	5855	6270	6432	6719	7232	7413	7695	7943	8137	8541	8785	8952
真菌	2318	2895	3311	3566	3728	3813	3997	4174	4242	4419	4581	4698	4908	5029	5092
バクテリア	473	513	521	602	731	756	807	915	965	1007	1038	1069	1106	1131	1184
酵母	1372	1428	1479	1503	1577	1606	1652	1687	1705	1719	1759	1793	1792	1808	1831
酵母（遺伝子）															
放線菌	175	174	174	184	234	257	263	456	480	529	544	556	714	796	824
ファージ									21	21	21	21	21	21	21
動物細胞															
分譲総数	2082	4281	4086	5472	5415	3407	3024	2794	3181	3662	4097	3101	3067	6835	7115
国内	1983	4008	3871	5241	5094	3305	2780	2631	2985	3297	3741	2880	2794	6600	6851
国外	94	273	215	231	321	102	244	163	196	365	356	221	273	263	264

年号	S51	S52	S53	S54	S55	S56	S57	S58	S59	S60	S61	S62	S63	H1
西暦	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989
保存菌株数	9233	9650	9917	10390	10531	10635	10786	11563	11992	12384	12716	13000	13293	13442
真菌	5303	5475	5657	5969	6118	6128	6201	6480	6737	6977	7036	7049	7186	7093
バクテリア	1201	1231	1235	1264	1224	1232	1254	1525	1570	1605	1678	1769	1843	1965
酵母	1883	2033	2065	2094	2119	2162	2223	2289	2324	2366	2523	2616	2643	2677
酵母（遺伝子）														
放線菌	825	878	927	1030	1088	1080	1062	1216	1255	1263	1290	1340	1373	1409
ファージ	21	33	33	33	33	33	46	53	61	63	63	63	64	65
動物細胞									45	90	126	163	184	233
分譲総数	9497	13411	11857	10073	8946	9157	10691	7381	10862	8708	9948	9629	8353	10497
国内	9238	13117	11578	9826	8649	8834	10211	6935	10189	7820	9181	9076	7967	9762
国外	259	234	279	247	297	323	480	446	678	888	767	551	886	735

年号	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11
西暦	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
保存菌株数	13765	14015	14303	14775	15092	15441	15625	15828	16058	16238
真菌	7174	7221	7336	7396	7469	7601	7695	7778	7858	7885
バクテリア	2078	2175	2296	2497	2541	2621	2723	2785	2875	3000
酵母	2702	2750	2712	2780	2859	2918	2941	2931	2946	2949
酵母（遺伝子）				129	129	129	129	129	129	131
放線菌	1437	1452	1512	1566	1608	1645	1646	1663	1685	1696
ファージ	70	70	71	70	70	70	71	69	69	69
動物細胞	304	347	376	410	420	427	420	473	496	505
分譲総数	8962	8890	9274	9299	8708	8367	8528	8153	8753	7503
国内	8369	7742	8103	8269	7612	7688	7785	7586	8281	7098
国外	593	1148	1171	1030	1094	679	743	567	472	405

表 2　発酵研究所の理事，評議員の名簿（昭和 36 年）

理事長	武田長兵衛	武田薬品工業（株）社長
常務理事	長谷川武治	発酵研究所所長
理事	安東洪次	（財）実験動物中央研究所長
理事	今村荒男	大阪大学名誉教授
理事	片桐英郎	京都大学名誉教授
理事	坂口謹一郎	東京大学名誉教授
理事	照井堯造	大阪大学工学部教授
監事	三宅　馨	武田薬品工業（株）会長
評議員	赤井重恭	京都大学農学部教授
評議員	朝井勇宣	（財）醸造科学研究所長
評議員	有馬　啓	東京大学農学部教授
評議員	北原覚雄	東京大学応用微生物研究所所長
評議員	吉川秀男	大阪大学医学部教授
評議員	小林義雄	国立科学博物館植物学課長
評議員	佐々木酉二	北海道大学農学部教授
評議員	佐藤喜吉	武田薬品工業（株）顧問
評議員	立岡末雄	武田薬品工業（株）研究所長
評議員	中澤亮治	武田薬品工業（株）顧問
評議員	藤野恒三郎	大阪大学微生物研究所教授
評議員	本江元吉	九州大学農学部教授
評議員	茂木正利	（財）野田産業科学研究所長

各地の研究機関に無料で送付されていた（表 1）。研究所の運営方針を決める，理事会と評議員会の構成員は表 2 の通りであった。

発酵研究所は武田薬品大阪工場内の武田薬品研究所の 6 階建ての建物に同居しており，朝 8 時の工場操業開始のサイレンとともに業務が始まり，午後 4 時，工場の終業サイレンとともに終っていた。これはこの時間帯に，暖房や殺菌用の蒸気の供給があるということで，電気，ガスの供給は停まることはなく，研究者はこの時間外でも研究活動は出来るようになっていた。しかしまだ戦後の電力の不足は続いており，電力の余る日曜日に工場が操業したため，研究所も日曜に出勤して，別の週日に休日となる変則的な期間もあった。年に 3〜4 回の保存菌株の植え替え時期には，担当者はそれに掛かりきりの状態であった。研究室には空調はなく，真夏の暑い日でも，外部からの埃と雑菌の汚染防止のため，窓を閉めて仕事をしなければならなかった。それで微生物の実験は無菌箱の中でするのが通例で，その中に必要な実験器具を入れて紫外線殺菌灯で殺菌したのちに，更に消毒剤を噴霧して殺菌し，微生物の植替えや性質の検定実験をしていた。

このころはだんだんと世界の研究者同士の交流が盛んになる時期とも重なり，研究所への来訪者が多くなると共に，国際学会への参加が始まる時期でもあった。微生物保存機関は国内は言うまでもなく，外国の保存機関との間で自由に菌株の相互分譲を行っ

て，互いの活性を国際協力で高めようと努力がなされていた。長谷川所長は1962年カナダのオタワで開かれた微生物保存機関の専門者会議（Specialists' Conference on Culture Collections）に出席され，世界の保存機関の関係者と親交を深め，その後の発展に貢献された。この会議ではIAMS (International Association on Microbiological Society) のSectionにSection Culture Collectionを加えることが決まったのを始めとして，微生物保存機関の国際会議として，ICCC（International Conference on Culture Collections）がその後定例的に開催されるきっかけとなった。

第1回のICCC (ICCC-1) は1968年（昭和43年），日本ユネスコ国内委員会とJFCC（日本微生物株保存連盟）の協同主催で東京で開催されている。その後ICCCは微生物保存機関の重要な国際会議となり，現在も引き続いて世界各地でほぼ4年毎に開催されている。このICCC－1では，微生物保存機関を結ぶ国際的な機構の設立の必要性が決議され，1970年にはWFCC（World Federation for Culture Collections）を発足させた。長谷川武治所長は1951年のJFCC（Japan Federation for Culture Collections）の創設以来日本の保存機関の協力活動に貢献され，ICCC－1の際には，その運営とProceedings (10) の編集に携わり，WFCCの設立や運営に参画されて，副会長の要職を勤めている。発酵研究所の所員も，その後理事や各種委員会の委員として，運営に参画するとともに，ICCCに参加し，研究発表をして学問の進展の上で貢献が続いている。

1965年（昭和40年）に武田薬品中央研究所の第2棟が建設され，発酵研究所は新設の第2棟の1階部分に10部屋と6階に菌株保存室と移植用の部屋が割り当てられた。（その後1階の4室の増加割当てがあった。）それまでは地下室にあった菌株保存室が6階に移されたのは，第2室戸台風（1961年）で大阪大学の中之島キャンパスの地下室が高波で浸水したのを教訓として，実験室と隔たる不便を承知で6階に設置した。この第2棟では真菌の研究，酵母の研究，細菌の研究，変異の研究のそれぞれに適合した設備を備えた実験室が完成した。研究所は真菌研究室（椿主任研究員），細菌研究室（児玉主任研究員）酵母研究室（坂野研究員）微生物変異研究室（飯島研究員）の4部門となり，菌株の保存は2名が真菌と放線菌を担当し，2名が酵母・細菌を担当していた。この第2棟から全館空調となり（ただし勤務時間プラス2時間），また除菌した空気を送り，室内全体を無菌室として使う，ウオークイン式の微生物実験室も出来上がり，無菌箱は植菌用ドラフトチャンバーに代わり，研究の能率は格段と上がった。また研究室と同居していた保存株の移植と品質管理のための実験施設は6階の保存室に隣接して，菌種別に植菌室が設けられて相互の汚染を防止することにした。冷房のない時代，夏の暑い時期は窓を全部閉め，培養室に近い温度と湿度の中で汗まみれで実験したことなどは，昔の想い出だけとなった。真夏の暑い日でも，24度から26度という温度の中で実験ができ，窓から見える夏の青空の下が32度を超える酷暑とはとても信じられないようになった。実験室の設備の近代化と同時に面積にも余裕ができたために，微生物の変異株の取得実験に外部から発酵研究所に実習や研修に訪れる機会も増えた。これは外部との技術交流や情報交換にも極めて重要なことであった。

研究の環境だけでなく，技術の進歩も著しく，これまで一定期間に移植して保存していた菌株を，超低温で保存するか，あるいは凍結乾燥をして低温（5度から零度に）保存して，移植と再増殖による変化を少なくする方法が開発されてきた。超低温の保存法としては，液体窒素を使う方法（マイナス180度）や超低温槽（電気的にマイナス120度に下げる）での保存があったが，いずれもランニングコストや機器の価格の関係で，アメリカのATCC以外はあまり採用していなかった。凍結乾燥法は装置も操作も比較的

採用しやすく，発酵研究所でも 1961 年（昭和 36 年）から試験的に長期保存法として試みられていた（写真 2）。私の仕事の関係では，大腸菌の変異株を凍結乾燥法で保存することを試みていた。しかしこの方法は乾燥に時間がかかり，装置を自動化しない限り残業を必要としたので，ICCC－1 で発表された L－乾燥法 (11) を採用することにした。坂根健所員が単純な組成の分散媒を使うことから始め，乾燥の条件を詳細に検討した結果，きわめて生存率の高い L－乾燥標本が 2 時間程度の時間で出来上がるようになった。

1972 年（昭和 47 年）北海道大学低温研究所根井外喜男教授は日本学術振興会からの援助で，「凍結及び乾燥による微生物の保存法」をテーマに日米の研究者による合同セミナーを企画された。日本側からは凍結及び乾燥研究会の会員の中から，またアメリカ側は ATCC の所員を主に，アメリカ，ウエストヴァージニア州のバークレースプリングスの Cacapon 山荘に 5 日間の泊まり込み，英語による発表と討論で，この分野の問題点と今後の発展を共に討論することとなった。この会議の出席者と発表のテーマを表 3 に示した。初めてアメリカの地を踏み，日本で習った英語の力だけではどうなることかと不安に思ったが，幸い北海道大学出身の妙田俊夫博士（当時 du Pont 財団研究所）が終始会議に参加されて，言葉の足りないところを補って貰い，保存機関での菌株の保存方法についての情報の交換ができ，またそのスタッフとの個人的なつながりも始まり，以後の相互の交流の助けとなった。妙田博士はその後 USFCC の役員になり，たびたび ICCC には出席をされて，日本とアメリカの研究者の交流には大変な力になって貰って

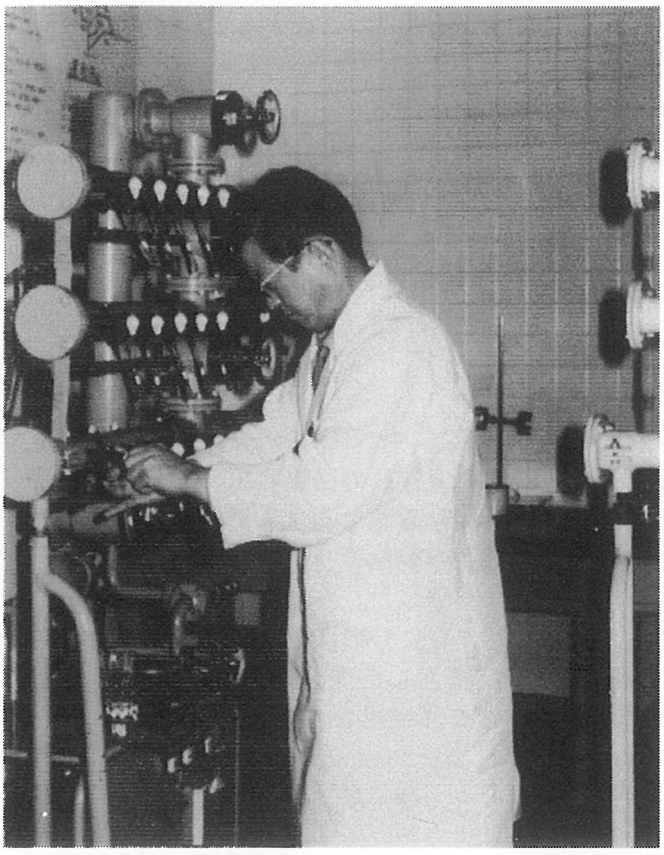

写真 2　凍結乾燥装置

表 3 日米合同セミナーへの出席者とテーマ

日本側 団長	根井外喜男 (北海道大学低温研)	
森地敏樹	(農林省畜産研)	乳酸菌の保存
鈴木正敏	(岐阜大農学部)	ワクシニアウイルスの保存
都留信也	(通産省微工研)	微細藻類の生理的変化
脇本 哲	(九州大農学部)	Bdelovibrio の凍結保存
高田信男	(大阪大工学部)	酵母の凍結乾燥保存
飯島貞二	((財) 発酵研)	L－乾燥法による細菌の保存
古屋 寛	(東京大応微研)	レプトスピラの保存法
僧都 博	(北大低温研)	凍結による傷害の解析
山里一英	(東京大応微研)	凍結乾燥法による細菌の保存法
長谷川篤彦	(東京大農学部)	皮膚病原菌の保存法
阿部重雄	(協和発酵)	微生物の凍結乾燥保存法

この他に米国留学中の次の２名が現地でシンポジウムのみに参加した。

高野光男	(大阪大工学部)
高野 勇	(サントリー研)

米国側 団長	W. A. Clark (ATCC)	
F. F. Busta	(Univ. Minesota)	凍結乾燥の際の傷害
J. L. Cunninngham	(ATCC)	液体窒素保存法
W. A. Daily	(Eli Lilly)	藻類の液体窒素による保存法
P. MacKenzie	(AFBR)	凍結乾燥の生存率に及ぼす条件検討
D. M. Robinson	(Red Cross)	動物細胞の凍結による傷害
Ellen Simon	(Univ. Illinois)	原虫の低温保存法
A. J. Sinsky	(MIT)	凍結傷害の特性
R. G. Zieg	(ATCC)	アメーバの保存法

いる。このセミナーの前後，ATCC や NIH の研究施設を実際に見学して，アメリカでは液体窒素の値段が安く，低温の保存法の冷媒に広く利用され，多くの微生物で生存率も良いことを教えられた。また保存菌株に関する情報処理の重要さを認識して，情報の機械処理によって，今日現在の保存菌株のリストを即座にプリントできると説明されたのには，われわれの現状の遅れを感じた。ちなみにこのL－乾燥法はその後大腸菌だけでなく，細菌一般にも適用でき，更に分散媒を変更することにより酵母にも適用できるようになり (12)，菌株の保存技術として発酵研究所では広く使われるようになった。1997 年日本微生物資源学会からこれに対して技術賞を受けることができた (13)。

4) ま と め

長谷川武治所長の時代（昭和 36 年から昭和 50 年まで）を簡略にまとめてみると，発

酵研究所が微生物保存機関としての基礎を築いた時代であり，この間に保存機関として不可欠な研究業績と情報の公開として，IFO Research Communications と IFO List of Cultures の定期的な発行が開始されている。これらの刊行物は，英文を主に編集されて，広く国外の研究機関にも配布され，研究所の国際的な活動として貢献した。実際このリストは，単に菌株のカタログとして利用されただけでなく，当時の菌の命名の根拠を知る手引きとしても利用されたようである (14)。研究所の研究室構成では糸状菌，酵母，細菌，微生物変異に加えて放線菌の研究室（中澤鴻一特別研究員）が加わり，完成した形となった。放線菌の国際的な標準化計画（ISP: International Streptomyces Project）では，世界の 4 カ所の微生物保存機関に約 400 株の放線菌標準株の保存が寄託され，IFO に寄託された標準株のチェックシステムが開始されたのも，保存機関における菌株の品質管理のあり方の一つとして注目に値することであった (15, 16)。

また 1974 年に菌株の有料化に踏み切ったことも特筆されるべきであろう。それまでは実験に使う菌株は，親しい研究者間で無料で交換されるのが常識となっていたが，菌株の分譲記録の責任が曖昧となり，菌株の同一性の証明が困難となる可能性があった。これを化学薬品や実験動物と同様に考えて有料にすることにはかなりの反論もあったようである。技術面では長期保存法の実用化があり，研究面での成果としては，*Rhodosporidium* の発見，蚕軟化病の菌の分離と応用，枯草菌の変異株の収集と分析などが IFO Res. Commun. から拾い出すことが出来る。

5) 飯島所長の時代（昭和 51 年より平成 1 年まで）

長谷川武治所長が 15 年の就任を終えて所長を退任されたのは 1976 年（昭和 51 年）4 月である。1974 年の 9 月に，椿啓介特別研究員の副所長への就任があり，研究所の全員はいずれ椿副所長が長谷川所長に代わり就任するものと思っていた。そこに突然椿副所長が退職して筑波大学へ転出と言う事態が起こり，私が所長という大任を引き受けることになった。微生物保存機関の研究の主体は，微生物の同定をし，命名規約に従って命名し，新種・新属などの分類群を提案し，標本として保存して，以後の利用に供することであり，これまで微生物遺伝学の分野を担当していた私には，全くの専門外であった。しかし私が発酵研究所に入所以来，まわりで行われている同定や分類に関する研究を見て，分類学に使われている同定の手法は遺伝子の発現形質の分析であり，近い将来には遺伝子そのものを基礎とした分類学が築かれるであろうということを感じていた。それはまた多くの人の思いでもあった。形態的な特徴の判定には経験が大切であり，微妙な点では主観の入る余地があった。形態の分化の少ない細菌や酵母では糖の利用性，発酵性，更に細胞組成である生体高分子の違いを分析して分類する化学分類と，さらにこれらの形質を数値化して主観の少ない数値分類を用いる方法が始まろうとしていた。さらに微生物保存機関にとって重要な変化は，1976 年の細菌命名規約の改訂によって (17)，それまでに発表されていた細菌の菌名（学名）の見直しがされ，正当な菌名として承認されたものを，細菌の承認名リスト（Approved Lists of Bacterial Names）(18) として，1980 年 1 月 1 日に IJSB（International Journal of Systematic Bacteriology）に公表されることになった。この日を命名の優先権主張のスタート時点として，その後の細菌の新種の発表記載論文は，IJSB に発表するか引用されることによって有効な発表と決められ，さらに発表論文の中に基準株（Type strain）を指定すると同時に，永続的な微生物保存機関に基準株を寄託し，寄託番号を公表することが推奨されることになっ

表 4 各菌株の保存業務担当者

年度	昭和３６年	昭和６１年	昭和６３年
糸状菌	浅野勇	伊藤忠義	伊藤忠義
酵母	山内栄	見方洪三郎	見方洪三郎
細菌	山内栄	坂根健	坂根健
放線菌	浅野勇	浅野勇	西井忠止

た。これは1980年の特許寄託制度に関するブタペスト条約の発効による微生物特許寄託制度(19)とともに，学問の上でも菌の寄託制度が微生物保存機関の機能の一つとして，要求される時代に入ってきたことを意味する。

そこで私が掲げた今後の研究所の目標は研究遂行の近代化であった。その内容はこのような周囲の変化に対応できる研究所の体制作りとして，(1)技術の革新，(2)情報の管理と充実，(3)研究設備の充実，(4)事務処理能力の向上とした。

(1) 技術の革新

技術の革新の内容としては，①保存と分譲方法の転換と②化学分類の導入を当面の目標としてあげた。

①保存と分譲方法の転換

保存機関でもっとも大切な菌株は，年に3回から数回，特に保存の難しいものは毎週移植が行われ，それに要する手間と時間，外部からの雑菌の混入に対しての配慮は大変なものであった。担当者4名(表4)はそれまでの経験，習慣をもとに，情熱を持って仕事にあたっていた。微生物の長期保存法の研究は世界の各保存機関で試みられていて，凍結乾燥標本による保存法，超低温の冷凍庫保存法(マイナス120度)，液体窒素による超低温保存法（マイナス180度）などアメリカでは費用の問題を解決して，実用化されていた。われわれもできるだけ早い時期に，移植による菌株の保存から，長期の保存法へ転換し，長期保存標本を分譲することによる省力化で，菌株の品質管理に人力をさいて，新しい命名規約の時代の要望に対応できる，保存機関になるのがねらいである(20)。

②化学分類の導入

1974年にBergey's Manual of Determinative Bacteriology 第8版が出版され(21)，ここで初めて化学分類に関するデータが取り上げられ，DNAのGC含量と細胞壁のペプチドグリカンのアミノ酸組成が記載され，化学分類に対する取り組みが始まった。またその後1984年には，Bergey's Manual of Systematic Bacteriology (22)が出版され，細胞の化学組成（細胞壁成分，脂肪酸，イソプレノイド・キノン，タンパク質など，さらにDNA塩基組成，塩基配列の類似性)を分析して，細菌の近縁性を考慮しようとする劇的な変動期に入った。分譲する菌株がその命名どおりの性質を保持しているかの検定に当たっては，このような大きな変化に対応できる研究所の体制を作ることが必要であり，また必要な機器の新設と分析の自動化による効率化を計ることも重要な目標となった。またパルス電気泳動法などの新しい技術の開発により，酵母の染色体の解析を行うことにより，近縁関係を推論することなどが試みられた(23, 24)。機器分析の導入は，コンピュータの導入とも関連し，研究室におけるコンピュータの取扱いは，研究に密着した日常的なものと変わってきた。これは情報の管理・処理とも関連して，研究所全体の近代化にも繋がっていった。

(2) 情報の管理と充実

微生物保存機関を支える柱は，研究，菌株情報，保存と配布である。情報の基本は菌株が実際にどれだけ保存されているかを確認することに始まる。発酵研究所の菌株はIFO番号という一連番号で管理されているが，日常の移植や，分譲用菌株の作成には，「菌株番号」という便宜的な番号を使って処理されていた。菌株の情報を組み立てて行くときに，まず作らなければならないものは，IFO番号順の菌株名リストである。コンピュータの発達した今日では，このようなリストを作ることはそれほど驚くことではないが，せいぜい電動タイプライターしか普及していない就任当時（昭和51年）では，8000株に近い数のこのようなリストを作ることはそれほど簡単なことでなかった。その上，基本となる研究所の菌株の元台帳は，アルファベット順の菌名台帳になっており，菌名が変わると当然別のページに移されていて，それを見つけるのに手間もかかった。

IFO番号順の菌株台帳が整備されたのは昭和31年からであるので，それ以前の菌株をまずIFO番号順にリスト化することから始め，半年ほどかかってIFO番号順のリストが出来上がった。仕上げてみると，番号だけで菌株がないもの，菌株があっても，品質管理が充分でなく，その菌名では信用のある菌株として分譲が出来ないものも混じっていた。しかしIFO番号は重複して使用されることはなく，一旦菌に付けられると，日常使用していた「菌株番号」と違い，菌名が変わってもIFO番号は変更されることがなく，また菌が死滅や廃棄されても，その番号は二度と使われることがない。このことは情報管理をコンピュータで処理するのには，都合の良いシステムであった。詳細については「発酵研究所における情報管理」(6) に述べたとおりであるが，1979年にIBM OS／6というデータ処理のできる機械がリース制度によって，比較的安価に利用できるようになったことは幸いであった。ここから先の情報管理は，8インチのフロッピーディスクに蓄えたデーターベースを，正確に次期のコンピュータIBM23，IBM36へと移行して，記憶容量の増加と処理の高速化へ進展して行くことであった。そして現在ではIBMAS400を中心に，研究室の各コンピュータを結ぶネットワークが構築されるまでになっている。

(3) 研究設備の充実

研究設備の充実と改善については，今になって振り返ってみると，昭和51年から平成1年は恵まれた時代であったといえる。世の中全体が設備の改善，近代化を推進したときであり，財団の運営についても，基本財産からの利息収入は確実な実績を残した。武田長兵衛（昭和36年から昭和55年），小西新兵衛（昭和56年から平成1年）両理事長のこの事業に対する熱意と理解は，理事・評議員の先生方の深い尊敬を受けていた。そのうえ武田薬品関係者の理解もあって，基本財産の増額（昭和51年5億円，昭和58年に6億5千万円に，更に昭和61年には10億円に増額された），運用財産への寄附金の増額などの（昭和51年から順次増額され，昭和59年からは1億7500万円となる）資金面での援助のみならず，武田薬品中央研究所第3棟の増設に際しては，菌株保存施設を拡張して，保存温度の違う保存室（摂氏5度と10度）を設けて，より有効な保存条件が実現できた。また1984年（昭和59年）の動物細胞株の保存事業の開始とともに，液体窒素による動物細胞株の保存設備と保存用タンクへの冷媒液体窒素の自動供給設備の新設など，新事業への進出とならんで設備の新設も行なわれた。

研究所経理状況の推移と理事・評議員の改選の大きい昭和53年と昭和60年を表5，表6，表7に示しておいた。

表 5 発酵研究所の経理状況

年号	S51	S52	S53	S54	S55	S56	S57	S58	S59	S60	S61	S62	S63	H1
西暦	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989
基本財産総額	5億	5億	5億	5億	5億	5億	5億	6.5億	6.5億	6.5億	10億	10億	10億	10億
年間収入 (千円)	140,166	153,449	149,627	175,622	236,407	202,037	238,821	318,208	285,689	436,051	447,519	290,931	293,480	315,788
年間支出 (千円)	135,666	148,096	152,854	160,554	237,125	189,175	230,478	227,009	289,583	268,749	264,367	274,115	288,585	288,582
年間収支差額	4,500	5,353	-3,227	15,068	-718	12,862	8,343	91,199	-3,894	167,302	183,152	16,816	4,895	27,206

年号	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11
西暦	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
基本財産総額	10億	10億	10億	10億	10億	10億	10億	10億	10億	10億
年間収入 (千円)	315,445	324,710	313,871	301,439	296,768	344,096	323,726	310,992	342,790	392,056
年間支出 (千円)	297,223	304,844	315,180	330,746	340,598	342,025	280,026	253,753	288,497	391,858
年間収支差額	18,222	19,866	-1,309	-29,307	-43,830	2,071	43,700	57,239	54,293	198

表 6 財団法人発酵研究所理事・評議員名簿（昭和 53 年）

理事長	武田長兵衛	武田薬品工業（株）会長
常務理事	飯島貞二	(財) 発酵研究所所長
理事	有馬　啓	東京大学名誉教授
理事	常松之典	東京大学名誉教授
理事	照井堯造	大阪大学名誉教授
理事	長谷川武治	日本微生物株保存連盟会長
理事	早石　修	京都大学教授
理事	本江元吉	九州大学名誉教授
理事	山田秀明	京都大学教授
監事	立岡末雄	武田薬品工業（株）専務取締役
評議員	飯塚　廣	東京理科大学教授
評議員	磯野正雄	(財) 磯野育英奨学会理事長
評議員	大村栄之助	武田薬品工業（株）常務取締役
評議員	岡見吉郎	(財) 微生物化学研究会理事
評議員	高尾彰一	北海道大学教授
評議員	武田六郎	武田薬品工業（株）取締役
評議員	田村学造	東京大学教授
評議員	椿 啓介	筑波大学教授
評議員	原田篤也	大阪大学教授
評議員	福井三郎	京都大学教授
評議員	本郷次雄	滋賀大学教授
評議員	松原謙一	大阪大学教授
評議員	三輪谷俊夫	大阪大学教授
評議員	與良 清	東京大学教授

表 7　財団法人発酵研究所理事・評議員名簿（昭和 60 年）

理事長	小西新兵衛	武田薬品工業（株）会長
常務理事	飯島貞二	（財）発酵研究所所長
理事	有馬　啓	東京大学名誉教授
理事	大村栄之助	武田薬品工業（株）顧問
理事	岡田善雄	大阪大学教授
理事	照井堯造	大阪大学名誉教授
理事	長谷川武治	理化学研究所研究顧問
理事	早石　修	大阪医科大学学長
理事	本江元吉	九州大学名誉教授
理事	山田秀明	京都大学教授
監事	木崎良平	弁護士
監事	三輪谷俊夫	大阪大学教授
評議員	飯塚　廣	東京理科大学教授
評議員	磯野正雄	（財）磯野育英奨学会理事長
評議員	岡見吉郎	（財）微生物化学研究会理事
評議員	高尾彰一	北海道大学教授
評議員	武田六郎	武田薬品工業（株）取締役
評議員	田村学造	東京大学教授
評議員	椿 啓介	筑波大学教授
評議員	原田篤也	大阪大学名誉教授
評議員	福井三郎	京都大学名誉教授
評議員	別府輝彦	東京大学教授
評議員	本郷次雄	滋賀大学教授
評議員	松原謙一	大阪大学教授
評議員	森田 桂	武田薬品工業（株）中央研究所長
評議員	與良 清	東京大学名誉教授

(4)　事務処理能力の向上と業務の集中化

研究機器の自動化に伴い，研究方法も大いに変化し，人材の活用，省力化が叫ばれる時代に入った。これまで各研究部門でそれぞれに遂行していた共通する業務の集中化についても検討の対象となった。坂野特別研究員を中心に，業務の集中化が検討され，菌株の分譲受付業務，発送業務がまず中央化され，ついで凍結乾燥標本の作製などの共通業務が集中化の対象となった。情報処理についても情報の変更，修正，その記録の保存などの付随する業務が，機械化に伴って増加していった。余談であるが，最初に設置したコンピュータ IBM-23 の使用マニュアルに「Housekeeping」という項目があり，「どこの家でも，毎日の家事のためにゴミも出るし，掃除も必要である。コンピュータも同じで，ディスクのなかに不要な情報が貯まってくるから，必ず整理整頓するように。」とい

う注意書きがあったのを思い出す。当時のように記憶容量がまだ大きくない機種では，とくに「Housekeeping」が必要であった。それと同時にデーターの変更が必要となったときに，その理由と訂正記録の保管，それに対する検索の方法なども確立された。

機械処理によって，分譲記録の集計が即時に印字できるようになると，ベストセラーの把握が容易になったほかに，分譲後のクレームの対処へのマニュアルづくり，分譲受付から発送までの期間の短縮などが実現できた。また対外文書の作成，特に英文の特許関係文書の作成や発刊物の原稿作成が容易となったことが効果としてあげられる。IFO List of Cultures の 7 版からは OS／6 で原稿を作り，菌名順の本文に加え，IFO 番号順の菌名索引を付けるようになった。8 版では IBM36 のデータを NEC/PC へ転送し NEC の中の文書作成ソフトウエアーで印刷原稿に仕上げる方法を今井紘主任研究員が構築してリストの印刷原稿作成までの時間と手間を省いてくれた。また研究所年報の論文原稿も，各自が印刷原稿まで作成し，写真による製版で印刷，製本まですすめる方式にしたために，出版までの時間の短縮となった。1974 年 (昭和 49 年)，日米セミナーの際に ATCC で聞かされた「今日現在の菌株リストの印刷」が IFO でも実現したことになる。

(5) 動物細胞部門の新設

昭和 59 年，当時の小西理事長は，日本における動物細胞の保存と分譲の状況を憂えて，動物細胞の保存と分譲を微生物保存機関として発展してきた発酵研究所の事業として実施可能かどうかを，大村栄之助理事（武田薬品専務取締役）に検討を依頼された。早速，当時武田薬品生物工学研究所杉野幸夫所長の協力により，所属の竹内昌男主任研究員のグループに白羽の矢が立ち，発酵研究所への出向が決定，昭和 59 年 3 月の理事会で承認のうえ，監督官庁の文部省の認可も受けて，動物細胞部門の設立が決定された。その後ヨーロッパ特許庁からの動物細胞の特許寄託機関としての承認などがあって，事業も順調にすすみ，発足当時は 45 株の保存規模が平成 2 年には 300 株以上に，平成 11 年には 500 株を越えるまでに発展した。特に品質の保持，マイコプラズマの汚染検査については利用者の信頼を得ている。動物細胞保存事業を始めるに当たって，小西理事長ほか関係者でその後の経費関係が検討され，基本財産の増額，運用資金の増額等の措置がなされ，設備関係では液体窒素保存の設備の新設が実現した。液体窒素の保存設備は，動物細胞保存では不可欠のものであるが，微生物の保存に関しても，これまで長期保存が困難と言われた糸状菌の一部の株の保存に適用できるようになり，研究所全体として保存方法の近代化の面で大きな力となった。これに関連して，これまで糸状菌の分譲に当たっては，新たに培地上で増殖を確かめて，分譲していたものを，液体窒素保存の標品（寒天培地上で増殖した菌を，保護剤を満たしたバイアルに入れ液体窒素中で凍結保存したもの）をそのまま郵送して分譲する方法に変更して，分譲業務の簡素化にも役立った。言うまでもなくこの方法に踏み切る前には，試験的に各地の研究施設にサンプルを送り，そのまま返送を依頼して，発酵研に到着したものから担当者が品質の検査をして，この方法で支障がないことを確かめる準備期間を設けた。

6) ま と め

昭和 51 年から平成 1 年までの期間をまとめると，微生物保存機関としての近代化への時期と言うことができよう。具体的には，

(1) 菌株情報データーベースの構築と情報処理の機械化の開始

(2) 新しい体制の保存機関への変化，その内容として，学術上の寄託機関（基準株の寄託機関）と特許制度による寄託機関（特許微生物寄託機関）として行う受入，保存，分譲業務と，品質管理，情報提供，その他のサービスなど，

(3) 分類学の中心となる研究機関としての活動

などへの歩みが，着実に進んでいったと感じている。

それには，これまでの習慣などにこだわらずに，このような変化を理解して対応した所員の努力によるものである。今まで永い間，日常の仕事の上で慣れていた「菌株番号」から「IFO 番号」に代えるのでも，現場においては戸惑いがあったが，業務の機械化を進める上で「菌株番号」を使い続けることは混乱を招く可能性があるということで理解して貰った。

また出版物として，定期的なものの他に，発酵研究所創立 40 周年記念として，1984 年に「Critical Problems of Culture Collections」(25) を出版し，1990 年には大阪で開かれた，IUMS の会議の際に，WFCC 主催の Král Symposium「100 Years of Culture Collections」の記録を出版した (26)。これは世界で初めて創られた微生物保存機関 Král Collection がサービスを開始して 100 年を迎えたのを記念して開かれたものである。

この間に忘れることが出来ないのは，1980 年（昭和 55 年）9 月の武田理事長の急逝である。朝日新聞には「武田薬品工業の次期社長といわれていた武田彰郎副社長の死去（今年 2 月）のショックもまだ充分癒えていないのに，武田薬品工業の象徴ともいえる武田長兵衛会長が突然他界。同社の社員たちは相次ぐ不幸に声をつまらせている」（朝日新聞

写真 3 発酵研究所に株式寄贈（於 武田薬品工業 本社）
（1980 年 7 月 1 日）

「鼓動」より)と報ぜられているように，1980年2月，武田彰郎副社長が急逝され，その心労もあってか，武田理事長がわずか半年の後に急逝された。武田理事長は，昭和20年の航空醱酵研究所創立時代から逝去の日まで理事長の重責を果たされ，常に「国のやるべきこの事業を，われわれ武田薬品がやらねばならないのは，文化国家として感心したことではない。」としばしば洩らされたことであった。武田会長の逝去を報じた新聞には「研究熱心な信頼置ける指導者」「業界のリーダー」「伝統ある老舗の当主」「長者の風あり」などの文字が残されている。武田理事長の後任には小西新兵衛社長が就任され，昭和56年から平成1年まで理事長をつとめられた。

1980年の武田彰郎副社長の逝去にあたって，ご遺族から発酵研究所の基本財産に武田薬品株式を寄贈いただくことになり，武田薬品本社において，伝達式があった(写真3)。この基金はその後の研究所の事業の発展に大いに力となったことも忘れてはならない。

1980年理化学研究所に微生物系統保存施設（JCM：Japan Collection of Microorganisms)が設置され，日本で初めての国立の微生物保存機関ができた。その名前JCMが示すとおり，日本の中心的な保存施設を目指したものである。JCMは設立以来，特に基準株を収集することに勢力を注ぎ，1982年に1000株の保存登録株数が，2000年（平成12年）には10000株を越え，分譲数も2000株を越えて，その所期の目的を達成している。

微生物の保存株は，一旦失われると回復が効かない歴史的な文化財ともいえるものであって，最近では世界の各国で，その国の貴重な生物資源，遺伝子資源として位置づけ，採集，持ち出し，利用には厳しい制限を付ける状況になってきた。そのため各保存機関では，それぞれに菌株を別の場所に二重保存して安全対策をたてると共に，国内でのバックアップ機構を考慮しなければならなくなっている。JCMと発酵研究所とは，これまで互いにその特徴を生かし，良きライバルとして，また時には貴重なバックアップ機関として機能してきた。

1986年，UNESCOは1万ドルの拠出金を用意して，JFCCに東南アジアの微生物保存機関の中堅技術者を対象としたトレーニング・コース，「カルチャーコレクションにおけるコンピュータの利用」の実施を提案してきた。これを受けて，JCMの駒形先生が組織委員長となり，JCMとIFOが協力して大阪と東京で合計10日間のコースを実施した。アジアの各地（スリランカ，マレーシア，フィリッピン，インドネシア，ネパール，香港，タイ，中国，韓国)から11名の研修生が参加して，最初の5日間は武田薬品研修所を会場に，あとの5日間は東京に移動して富士通とJCMで講義と実習をして，トレーニング・コースを終わった。このような研修を機会に，所員に国内の保存機関との協力と国際的な協力を意識させることになり，その後の外国との共同研究などにも良い影響を与えた。この研究に参加した研修生の中には，すでに日本の大学に留学した経験者や大阪大学の国際大学院コースを終了した人もあり，ここまでに至る日本の他の機関での人材の養成の成果も大きく感じられた。駒形先生から「カルチャーコレクションが機能するには，なにより人間関係を作ることが基本」と聞かされているが，その意味をあらためて認識させられた。

所長在職中はいろいろな人との出会いがあったが，その中で忘れることができない人の一人は，イギリスのケンブリッジ大学の名誉教授のコーナー先生（E. J. H. Corner）である。このことはすでにJCM Newsletter (27) に書いているが，ここに再録して，発酵研究所の歴史の筆を置きたい。

1983年11月4日研究所の見学を申し込まれて，コーナー先生はご夫人と永井進先生(奈良女子大名誉教授)と3人で訪問され，朝から3時間ぐらいゆっくりと研究所の中を

見学された。そして昼食を一緒にしてお別れしただけのことであったが，先生の穏やかな風貌と，いかにもイギリス紳士といった身のこなし，研究のことを熱心に聞き，質問される研究者としての一面のほかに，私が感銘を受けたのは先生の経歴であった。訪問前の紹介で先生がシンガポールの博物館に勤務されており，第2次世界大戦中の日本軍占領当時のことを「思い出の昭南博物館」(28) という本に纏めておられることを知った。この本は先生とお会いする1年前，昭和57年 (1982年) 8月に中公新書として出版されたもので，私の出会った本の中で，これも忘れることができない1冊である。

「思い出の昭南博物館」のプロローグに次のように書かれている。『日本がシンガポールを占領していた戦争中，あの南の小さな島の片隅に，徳川義親マライ軍政監部最高顧問(彼自身生物学者であった)を中心とする，敵と味方の入りまじった奇妙な学者グループが出現していた。世界中が戦火に燃え，地球が破壊されている最中に，島の文化遺産を守ることに奔走し，自然科学の研究にいそしんでいたのである。なぜそのようなことが可能であったのか，当事者の一人であった私（コーナー先生）にも今もって理解できぬ不思議な出来事であった。占領という非常事態のもとで，私たちは出会った。不信と敵意との燃える戦火の中から希望の光がかすかに光った。そしてそれが大きな炎となっても燃え始めたとき，私たちは国家のためでも勝敗のためでもなく，ただヒューマニティのために戦い，そしてそれに奉仕していた。』この著書によると，コーナー先生がシンガポールの土を踏まれたのは，1929年であった。それから1945年にイギリスに帰国されるまでの16年間を，副園長としてシンガポール植物園に奉職されている。シンガポール植物園と博物館はシンガポールを開いた，イギリスのラッフルズ卿の英知と勇気を讃えて，1887年にビクトリア女王の在位50年を記念して創立されたもので，広さ32ヘクタール，園内には，3000種にわたる熱帯，亜熱帯の植物が集められ，また付属の図書館，研究所を持っていて，この地域の文化と産業の中心ともなっていた。1942年 (昭和17年) 2月，シンガポールの英国防衛軍はマレー半島を南下した山下奉文将軍指揮下の日本軍に降伏して，シンガポールは日本の占領地となり，1945年8月に再び英国の統治下に戻るまで「昭南」と名前も変えられた。コーナー先生は日本の占領後すぐ，この島の博物館，植物園，図書館，研究成果の標本などの貴重な文化遺産を破壊から守るために，すでに施政権を失っている旧シンガポール総督を通じて，日本軍当局にこれらの保護を願い出た。その後の経過はコーナー先生自身不思議な出来事であったといわれるとおり，敵意に燃え不信感を持って戦っていた時に，敵味方を越えた協力が可能であったのは何故だったのであろうか。

山下・パーシバル降伏協定で，日本の行政官が到着するまでは，英国の行政官と技術者が復旧に協力すると取り決めてあったこと，豊田シンガポール元総領事と英国政庁関係者の間に交渉のパイプがあったこと，サイゴンからすぐ到着した田中館秀三教授が博物館，植物園および図書館などの資料に対して適切な処置をしたことなどの幸運が重なったことを挙げることができよう。そして陥落後1週間あとに，徳川義親侯爵がマレーやスマトラのサルタンとの親交があったことによって，マライ軍政監部最高顧問として赴任され，昭和17年9月から昭南博物館総長に就任したことにもよる。その後植物園長に郡場寛教授が，博物館長に羽根田弥太博士が赴任され，研究と文化遺産の保護は軌道に乗ったという。しかしこれらの人を動かしたのは，やはりコーナー先生の研究遺産の尊重への限りない熱意であったと思う。

戦争は非情である。友情も愛情も無視して，戦うことのみに目を向けさせる。しかしその戦いのさなかで，この戦いをどう処理し，この戦いの後の時代の人のために残さね

ばならない真に貴重なものはなにかを考えた人々がいたことは幸せであった。またそういう人の一人に親しく会い，話を聞くことが出来たことも幸せな出会いであった。コーナー先生は1986年（昭和61年），昭和天皇の在位60年を記念して創られた国際生物学賞の第1回受賞者となった。受賞対象は「熱帯植物の系統・分類学上の業績」である。

7) 終 わ り に

はじめにお断りしたように，私が発酵研究所を退職するまでのことについて纏めてみた。長谷川徹所長（平成2年から平成6年まで），竹内昌男所長（平成7年から現在）とこの後に続くことについては，機会が有れば適当な方に書いていただきたいと思っている。

ただ私の在任中のことで，薬剤安全関係の研究，微生物変異関係の研究，植物病原菌関係などについては記述する余地がなかったが，発酵研究所年報などには研究成果についての論文報告があるので，詳細はそれを参照していただくことにしたい。

最後に発酵研究所に在職した期間を振り返ってみると，微生物の系統保存という，地味であるが大切な仕事に携わる機会が与えられたことは，これも幸せな出会いであったと感じている。

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Sexual Compatibility and Asexual Reproduction of *Tremellochaete japonica*

Akira NAKAGIRI, Izumi OKANE and Tadayoshi ITO

Summary

A heterobasidiomycete, *Tremellochaete japonica*, was collected from the dead branch of a mangrove tree. Morphological and cultural studies revealed it has bifactorial mating system and produces conidia in annellidic conidiogenesis, a unique form of conidiogenesis among Exidiaceous fungi.

Keywords: bifactorial mating system, conidogenesis, heterobasidiomycete, *Tremellochaete japonica*.

A heterobasidiomycete, *Tremellochaete japonica* (Yasuda) Raitv. ("Tsubukikurage" in Japanese) (Exidiaceae, Auriculariales) has been often collected from dead branches of walnut in Japan, Russia and Papua New Guinea (1, 2) since its first collection from Japan in 1915 (14). However, because no cultural study of this fungus has been done, its no information is available about its pattern of sexual compatibility, the characteristics of its anamorphic state and cultural properties.

In the course of a biodiversity study of subtropical fungi at Amami Is. (28° 15′ N, 129° 24′ E), Kagoshima, Japan, we found basidiomata of *Tremellochaete japonica* on the dead branch of a mangrove tree. From the specimen, mass spore cultures and single basidiospore cultures were obtained by using a micro-manipulator. The pattern of sexual compatibility was examined by mating pairs of 10 monokaryotic strains. Secondary spore formation from basidiospores and conidium formation of the monokaryotic strains was examined by light and scanning electron microscopy. Both monokaryotic and dikaryotic stains were deposited in the IFO culture collection.

Materials and Methods

Specimen. Basidiomata of *Tremellochaete japonica* on an aerial dead branch of a mangrove tree, *Kandelia candel* Druce, which was overhanging a creek of the Sumiyo River, were collected on 4 March 2000 at Amami Island. Morphology of the basidiomata, basidia and basidiospores was compared with the previous description of this species. A dried specimen of the basidiomata was deposited in the herbarium of IFO.

Isolation. Spore-forming basidiomata were attached to the inside of the lid of a Petri dish containing corn meal agar (CMA) and set overnight. Basidiospores discharged from the basidiomata were isolated with the Skerman-type micro-manipulator. Ten single-basidiospore isolates were obtained and maintained on slants of YMC-1 agar (half-strength corn meal agar, 0.5 % malt extract, 0.1 % yeast extract) (1).

Sexual compatibility test. Pairs of the 10 strains were inoculated ca. 2 cm apart on plates of YMC-2 agar (half-strength corn meal agar, 0.1 % malt extract, 0.05 % yeast extract)(1) in 9-cm Petri dishes. The plates were incubated at room temperature (20-25°C) for 3 weeks. Mycelia in the contact zone of the paired colonies were examined under a light microscope for the presence of clamp connections and stained with alkaline Safranin O to check the nuclear phase (4).

Observation of conidia. Secondary spores produced directly from basidiospores were observed under light microscope. Conidia and conidiophores formed in the monokaryotic cultures on YMC-2 agar were observed under a scanning electron microscope (JSM 5400, JEOL).

Results

Morphology of the collected specimen

Tremellochaete japonica (Yasuda) Raitv., Eesti NSV TA Toimetised, biol. seer. 13: 30, 1964.

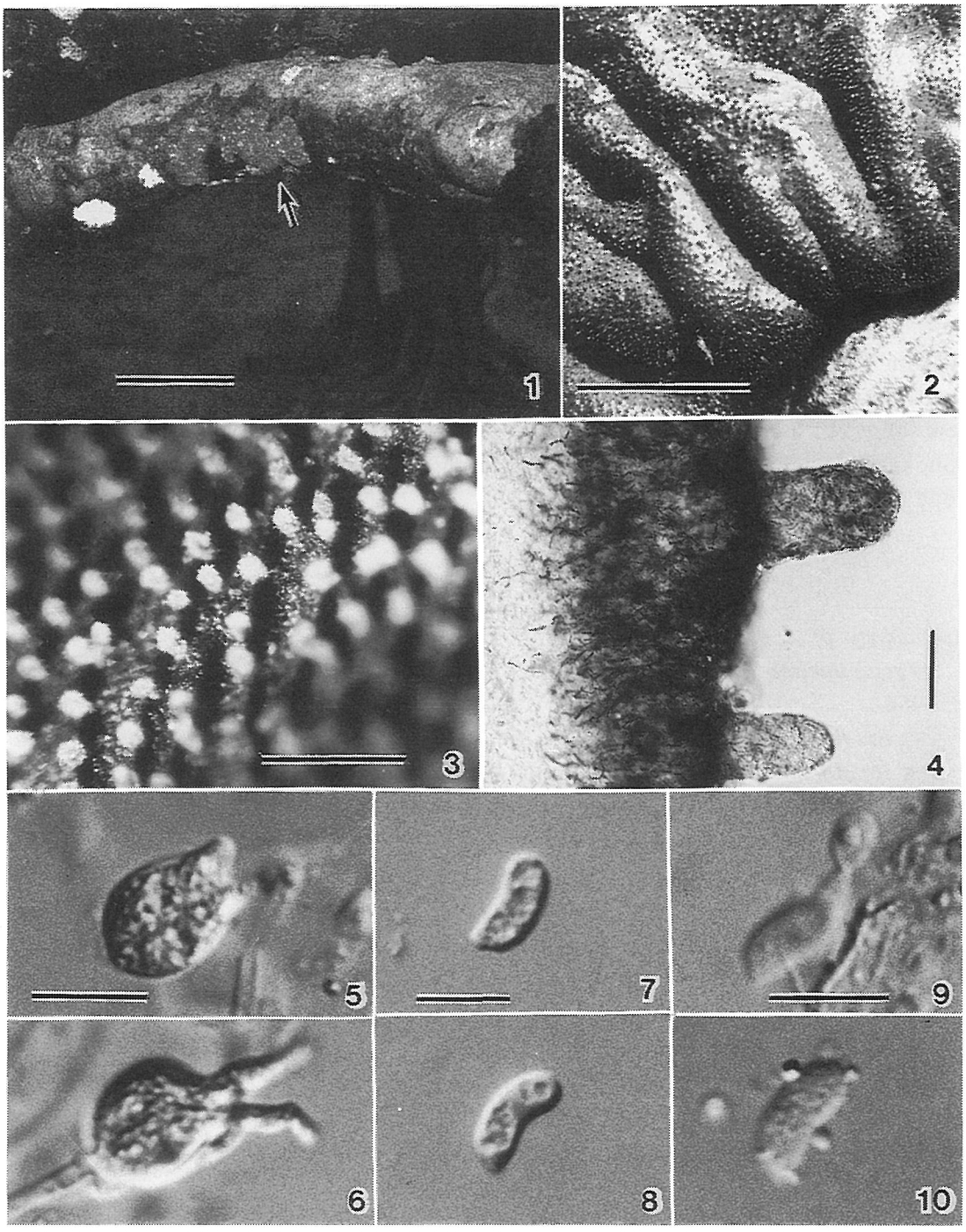
≡ *Exidia japonica* Yasuda in Lloyd, Myc. Writ. 5: 599, 1916.

≡ *Heterochaete japonica* (Yasuda) Kobayasi, Nagaoa 4: 40, 1954.

Basidiomata solitary or gregarious, effused, becoming confluent flat layer, 10×3 cm, fusing with continuous or discontinuous contact lines on the surface, sessile, firm to gelatinous, brownish gray (Figs. 1, 2). Hymenial surface plane to undulate, papillate with numerous hyphal pegs, 128-168 μm high, 56-104 μm in diam, composed of densely packed hyphae (Figs. 3, 4), covered with octahedral crystalline-like structures. Hymenia distributed over whole outer surface. Hypobasidia ovate to obpyriform, 10-14×8-11 μm, with cruciate longitudinal septa (Fig. 5). Epibasidia cylindric, tapering, 10-22 μm long, 2-4 μm in diam at the broadest point (Fig. 6). Basidiospores allantoid to reniform, 10-16×4-6 μm, hyaline, aseptate (Figs. 7, 8), germinating by germ tube or by secondary spore (ballistospore and microconidia) formation. Ballistospores were formed by repetition (Fig. 9). Microconidia (1×0.5 μm) were formed in blastic fashion from basidiospores (Fig. 10).

Colony of monokaryotic isolates reached 43-90 mm in diam on YMC-2 agar medium after 20 days of incubation at 20-25°C. Colonies hyaline on YMC-2 agar, but brown pigment was excreted on potato sucrose agar (PSA). Conidia were produced on a mononematous conidiophore, as described in detail below.

Specimen examined: IFO H-12240, on a dead branch of *Kandeliae candel*, Sumiyo River, Amami Is., Kagoshima, Japan, 4 March 2000.



Figs. 1–10. *Tremellochaete japonica*. 1. Basidiomata (arrow) on dead branch of *Kandelia candel* (in the field). 2. Surface of basidioma. 3. Close-up view of basidioma showing papillate surface with pegs. 4. Pegs. 5. Young basidium (hypobasidium). 6. Basidium with epibasidia. 7, 8. Basidiospores. 9. Basidiospore with a sterigma, from which a ballistospore was formed. 10. Basidiospore forming blastic microconidia. Scale bars: 1 = 10 cm; 2=5 mm; 3= 500μm; 4=50μm; 5 (=6), 7 (=8), 9 (=10)=10μm.

Table 1. Mating reactions among 10 monokaryotic strains of *Tremellochaete japonica*.

	Mating type	A1B1				A1B2	A2B2		A3B1		A3B2
Mating type	Tj-No.	3	7	8	9	2	4	10	1	5	6
A1B1	3		-	-	-	-	+	+	PC	PC	+
	7	-		-	-	-	+	+	PC	PC	+
	8	-	-		-	-	+	+	PC	-*	+
	9	-	-	-		-	+	+	PC	-*	+
A1B2	2	-	-	-	-		PC	PC	+	+	PC
A2B2	4	+	+	+	+	PC		-	+	+	PC
	10	+	+	+	+	PC	-		+	+	PC
A3B1	1	PC	PC	PC	PC	+	+	+		-	-
	5	PC	PC	-*	-*	+	+	+	-		-
A3B2	6	+	+	+	+	PC	PC	PC	-	-	

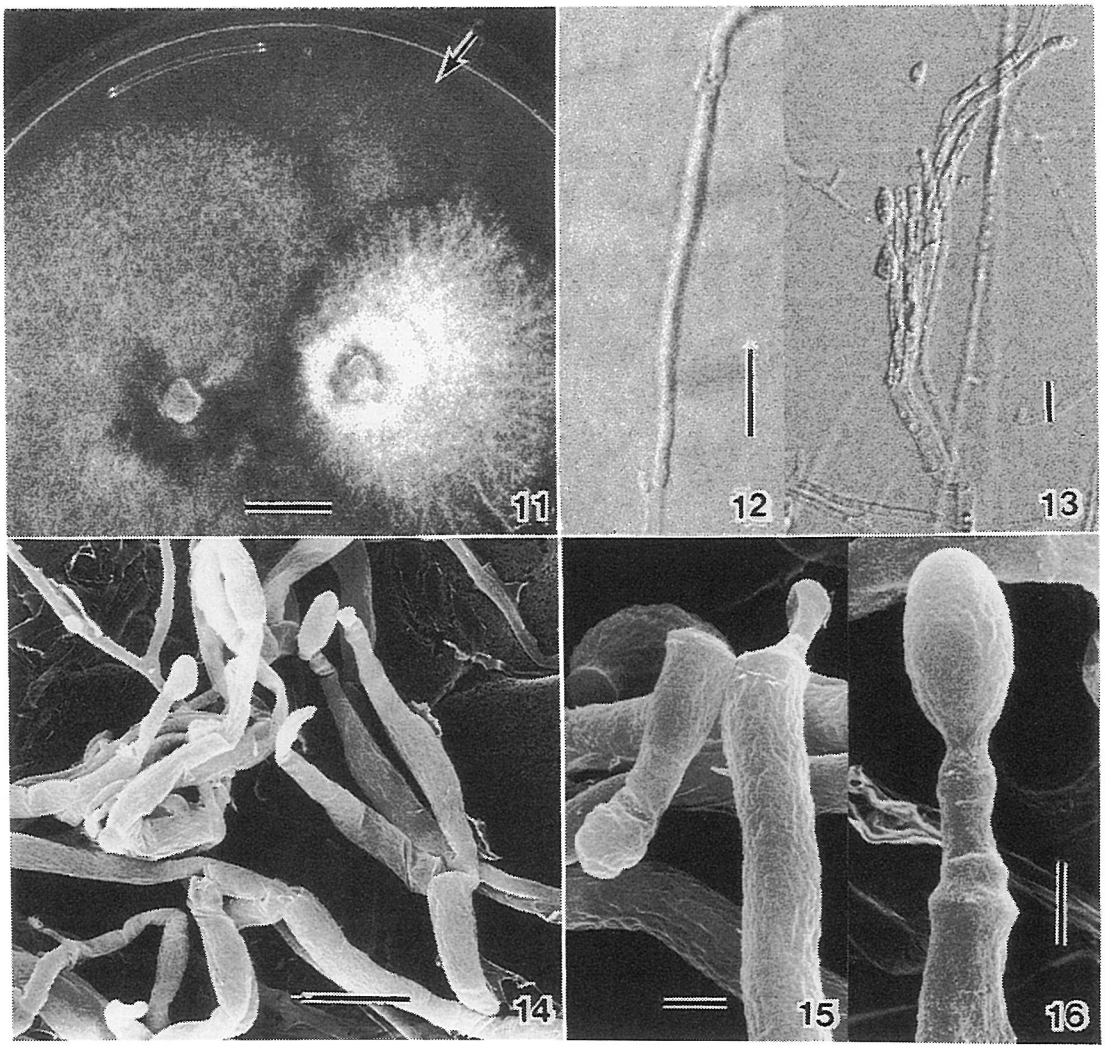
+, true-clamp formation; PC, pseudo-clamp formation; -, no reaction.
*, unexpected reaction.

Sexual compatibility

The mating tests showed this species has a bifactorial mating pattern, i.e., monokaryotic strains mated and formed true clamp connections on the secondary hyphae when both A and B mating factors were compatible, but they formed pseudoclamps when the factor A was compatible and the factor B was incompatible, and no clamps when the factor A was incompatible, even when the factor B was compatible (Table. 1). The 10 monokaryotic strains tested were found to include 4 strains of A1B1, 1 strain of A1B2, 2 strains of A2B2, 2 strains of A3B1 and 1 strain of A3B2. No strain of A2B1 type was detected, probably due to the small number of isolates tested. The presence of A3B1 and A3B2 type strains can be explained as the result of isolation of basidiospores from different basidomata. Typical clamp connections were observed on the hyphae in the contact zone of the matching pair. The secondary hyphae grew faster than the primary hyphae (Fig. 11). Incompatible mating resulted in pseudoclamp formation (Fig. 12) or no response. Since nuclear staining reaction with Saflanin O was not stable in this experiment, matching was confirmed mostly by repetitive examination of the presence of true or pseudoclamp connections for over one month. The following five representative strains of each mating type were deposited in the IFO culture collection: Tj-8=IFO 33184 (A1B1), Tj-2=IFO 33185 (A1B2), Tj-4=IFO 33186 (A2B2), Tj-1=IFO 33187 (A3B1), Tj-6=IFO 33188 (A3B2). A culture derived from mixed basidiospores was also deposited in the collection as IFO 33189 (=Tjmass-2).

Conidium formation

Conidia were produced in the monokaryotic cultures, among which the strain Tj-8 (IFO 33184) formed conidia abundantly. Conidiophores are mononematous, branched, 28-110 μm long, 2-6 μm in diam, and often the composing branches are detached from the septa (Figs. 13-15). Conidiogenous cells taper toward the apex and proliferate percurrently by successive formation of conidia, which leaves annellation at the apex of the cell (Fig. 16). Conidia are blastic, obovate, elliptical or reniform, aseptate, 6-13×3-7



Figs. 11-16. *Tremellochaete japonica* in culture. 11. A matching pair forming fast growing secondary hyphae (arrow). 12. Pseudoclamp connections. 13. Conidia and conidiophore. 14-16. Scanning electron micrographs of conidiophores and conidia. 14. Branching conidiophores. 15. Conidiogenous cell detaching at septum. Note a new conidiogenous cell is growing from the septum. 16. Conidiogenous cell showing annellation with a newly forming conidium at the apex. Scale bars: 11=1 cm; 12-14=10μm; 15, 16=2μm.

μm, hyaline.

Discussion

The morphology of the mangrove material of this fungus fits well with the previous descriptions (e.x., 1), though the pegs on the hymenial surface are larger than in the material observed by Yasuda (1917) and Kobayashi (1954) (100-150×80-200 μm and 90-

110×45-70 μm, respectively). This fungus has been reported from wide range of geography, i.e., Japan (Miyagi, Iwate, Nagano, Okinawa), Russia (Vladivostok) and Papua New Guinea (1). In Japan, it has often been found on broad-leaf trees, especially walnut, *Juglans mandshurica* Maxim. var. *sieboldiana* Makino (3, 14). This is the second report of this fungus from a mangrove tree since its collection by Nakagiri (T. Aoki bB1) from Iriomote Is., Okinawa in Feb. 1984 (1).

The present study revealed that *T. japonica* has a bifactorial mating system. Genera in the family Exidiaceae show unifactorial or bifactorial systems. *Exidia glandulosa* (Bull.) Fr., *E. recisa* Ditm., *E. saccharina* Fr. and *E. repanda* Fr. were found to have unifactorial systems (2, 5), whereas other species of *Exidia*, such as *E. candida* Klett, *E. populina* Klett and *E. pithya* Fr., were reportedly bifactorial (2). Bifactorial systems were also demonstrated in *Exidiopsis plumbescens* (Burt) Wells (12) and *Myxarium nucleatum* Wallr. (7). Among the order Auriculariales, species of the genus *Auricularia* Bull. ex Juss. were reported to have unifactorial or bifactorial mating systems (6, 13). Thus, both mating systems coexist in a genus and family within the Auriculariales.

Conidium formation on monokaryotic hyphae of *T. japonica* was observed for the first time in this study. This species showed annellidic conidiogenesis. Phialidic conidial formation has been observed among *Exidia* species and related heterobasidiomycetes. *Exidia glandulosa* and *E. thuretiana* (Lév.) Fr. were reported to produce C-shaped conidia in succession from a phialide (8, 9). This type of conidial formation was also observed in *E. uvapassa* Lloyd, *A. auricula* (Hook.) Underw. and *Heterochaete delicata* (Kl. ex Berk.) Bres. (1). On the other hand, *E. recisa* produces rod-shaped conidia on a phialide (10). Thus, *T. japonica* is unique in its conidiogenesis among Exidiaceous fungi and this may support delimitation of *Tremellochaete* from the allied genus *Exidia*, but further research and reexamination of conidiogenesis under a scanning electron microscope are necessary to clarify the taxonomic implication of conidiogenesis in this group of fungi.

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Assemblages of Endophytic Fungi on *Bruguiera gymnorrhiza* in the Shiira River Basin, Iriomote Is.

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Summary

Assemblages of endophytic fungi were investigated on the leaves of *Bruguiera gymnorrhiza* from the upper reach to the estuary of the Shiira River, Iriomote Is., Okinawa Pref., Japan. Few species were detected in summer (July 1997 and September 1998; 2-5 spp.) than in winter (February 1998; 5-9 spp.). The colonization frequency of fungi was higher on leaves collected in the upper reach than in the lower reach in each of the three surveys, but the number of species isolated was higher from leaves collected in the lower reach. *Colletotorichum* sp. was isolated in high colonization frequency from leaves collected at two upper stations, 33% at P-1 and 40% at P-3; but in low frequency from leaves collected at three lower stations, 5% at P-5, 4% at P-7 and 6% at P-9. *Phyllosticta* sp. and *Pestalotiopsis* sp. 1 were isolated in low frequency from leaves collected at all stations, and no difference in colonization frequency between the upper and lower reach was found. It is considered that assemblages of endophytic fungi of *B. gymnorrhiza* vary according to the surrounding vegetation.

Keywords: *Bruguiera gymnorrhiza*, endophytic fungi, fungal diversity, mangrove.

Endophytic fungi within tissues of aerial parts of vascular plants have been extensively studied over the past 15 years and found in a wide range of plants (17). In Japan, endophytic fungi have been investigated in conifers, grasses and several broad-leaf plants (3, 4, 5, 8, 9, 12, 13), but further investigations are necessary to clarify their ecological and species diversity on various plants. Because of its hot, moist climate during the growing season and diverse vascular plant flora, Japan is expected to have a very rich flora of endophytic fungi (1).

Most study on endophytic fungi has been carried out on plants from the temperate regions (17, 18), and it is only comparatively recently that the endophytic mycobiota of tropical ecosystems have been investigated. Petrini and Dreyfuss (19) isolated fungal endophytes of *Araceae, Bromeliaceae* and *Orchidaceae* from French Guyana, and from *Piperaceae* and *Crassulaceae* collected in Brazil and Colombia (2). Rodrigues and

Samuels (21) reported on the endophytes of the fan palm *Licuala ramsayi* (Muell.) Domin. from a tropical rain forest in Australia. However, studies on assemblages of endophytes in mangrove plants are very few (7, 20, 22), while mangrove regions preserve a great amount of biological resources including mycobiota.

In this study, we surveyed the assemblages of endophytic fungi within the leaves of *Bruguiera gymnorrhiza* Lamk. collected at five research stations along the Shiira River, Iriomote Is., Okinawa Pref.

Materials and Methods

Sample collection and fungal isolation. Young (new) and mature leaves (older than the third leaf) of *B. gymnorrhiza* were collected from trees at five stations (P-1, P-3, P-5, P-7 and P-9) along the Shiira River in Iriomote Is. (24N, 124E), Okinawa Pref., Japan. P-1 is 2.2 km from the river mouth, and at this uppermost point a stand of *B. gymnorrhiza* grows. P-3 is 400 m downstream from P-1, and P-5 is 400 m downstream from P-3 on the middle reach of the river, where *B. gymnorrhiza* forms dominant forest. P-7 is 400 m downstream from P-5, and P-9 is 400 m downstream from P-7 at the river mouth. Samples were collected in July 1997, February 1998 and September 1998. In Iriomote Is., the highest monthly average temperature occurs in July, the lowest in February. To detect fungi, two disks of 8 mm diam were punched out with a cork borer from 10 leaves of each leaf stage. The surface of the leaf disks was sterilized by immersion in 70% ethanol for 1 min and sodium hypochlorite solution (1% available chlorine) for 2 min. The disks were rinsed in sterile distilled water and put into sterile paper towels for 3 hr to remove water from the surface. They were then placed on half-strength corn meal seawater agar (CMSWA) (half-strength Corn Meal Agar with compensatory agar was dissolved in 15 ppt seawater) in 90-mm plates and incubated at 17°C for more than 3 months. The fungi growing out of the leaf disks were isolated and identified. The colonization frequency (CF) of individual fungi was calculated by the following equation:

CF (%) = (number of disks from which the fungus was detected/total number of disks examined in each sample) × 100.

Data analysis on species richness and diversity of endophytic fungi among seasons, stations, and dominant species. In this analysis, data of each leaf stage (young and mature) in three trials (July 1997, February and September 1998) were applied as one sampling unit. For analysis by station, six sampling units were applied as a data set of each station.

Species richness (number of species) was estimated by the jackknife estimate (10) by use of the following equation:

$$S = s + [(n-1)/n]\ k$$

where S = jackknife estimate of species richness, s = observed total number of species present at a station, n = total number of sampling units: 6 in this study, k = number of unique species, defined as species occurring in only one sampling unit.

Species diversity was measured by using Simpson's reciprocal index and the Shannon- Wiener function (10), as expressed by the following equation:

Simpson's reciprocal index : $1/D=1/\Sigma p_i^2$

where D=Simpson's index, p_i=Proportion of species i at a station

Shannon-Wiener function: $H'=\sum_{i=1}^{S}(p_i)(\log_2 p_i)$

where H'=information content of sample=index of species diversity, s=number of species, p_i=proportion of total sample belonging to ith species.

To test a difference in colonization frequency of all fungi at each station, a statistic for test for the proportion, $T(m_1, m_2)$, was calculated by using the following equation:

$$T(m_1, m_2)=[(m_1/N_1)-(m_2/N_2)-d_0]/\sqrt{p(1-p)(1/N_1-1/N_2)}$$

where m=number of leaf disks from which fungi were detected, N=total number of leaf disks, $d_0=p_1-p_2=0$ (no difference between p_1 ($=m_1/N_1$) and p_2 ($=m_2/N_2$) in statistical hypothesis), $p=(m_1+m_2)/(N_1+N_2)$

Differences in colonization frequency of three dominant fungi, *Colletotrichum* sp., *Phyllosticta* sp. and *Pestalotiopsis* sp. 1 at each station were also tested by using the above equation.

Results and Discussion

Several fungi including sterile mycelia were detected from the leaves of *B. gymnorrhiza* in the Shiira River basin. In the three years of this study, we isolated fungi from 296 of 600 leaf disks examined (ca. 50% colonization frequency). No significant difference was found in the colonization frequency of fungi between the surveys: 46% in July 1997, 51% in February 1998 and 52% in September 1998. However, the number of species isolated was different in summer (July 1997 and September 1998) and winter (February 1998): 8 spp. in July 1997, 6 spp. in September 1998 and 14 spp. in February 1998 (Table

Table 1. Colonization frequency (CF) (%) of the endophytic fungi on leaves (Y and M) of *B. gymnorrhiza* in July 1997.

Station	P-1		P-3		P-5		P-7		P-9	
Fungus	Y[a]	M[a]	Y	M	Y	M	Y	M	Y	M
Colletotrichum sp.	10	35	35	60	0	10	15	0	25	0
Pestalotiopsis sp.1	0	0	15	0	0	0	0	0	0	5
Phyllosticta sp.	0	0	0	0	20	35	0	30	0	15
Phomopsis sp.	15	20	5	0	0	0	0	0	0	0
xylariaceous fungi[b]	0	0	0	0	0	5	0	0	0	0
Surculiseries rugispora[c]	0	0	5	0	0	0	0	0	0	0
white sterile mycelia	5	15	10	15	10	15	10	10	0	10
colored sterile mycelia	0	0	0	0	0	0	0	15	0	10
CF in each leaf stage	30	70	45	75	30	70	25	50	25	35
CF in station	50		60		50		38		30	
Number of fungi detected	3		5		4		4		5	

[a] Y, young leaves; M, mature leaves.

[b] including *Geniculosporium* sp.

[c] new species (14).

1-3). *Pestalotiopsis* sp. 2, *Phoma* sp., *Acremonium* sp., an unidentified ascomycete and a coelomycete were only found in the survey of February 1998. The jackknife estimate put the number of species at 18.5 (in the range of 14-23) according to the actual number of species detected in February 1998, but 9.8 (7-13) in July 1997 and 6.9 (5-9) in September 1998 (shown in Table 5). Simpson's reciprocal index (1/D) and Shannon-Wiener function (H′) showed that the diversity of species inhabiting leaves of *B. gymnorrhiza* is higher in winter (1/D=7.21, H′=3.16) than summer (1/D=4.02, H′=2.31 in July 1997; 1/D=3.45, H′=1.99 in September 1998) (Table 5). The high temperature, high humidity and strong ultraviolet irradiation in summer in the Ryukyu Islands including Iriomote Is. may affect sporulation of the fungi, spore germination and invasion of the

Table 2. Colonization frequency (CF) (%) of the endophytic fungi on leaves (Y and M) of *B. gymnorrhiza* in February 1998.

Station	P-1		P-3		P-5		P-7		P-9	
Fungus	Y[a]	M[a]	Y	M	Y	M	Y	M	Y	M
Colletotrichum sp.	25	35	20	40	5	5	0	10	5	5
Pestalotiopsis sp.1	0	0	0	0	5	30	0	10	5	10
Pestalotiopsis sp.2	0	0	0	0	0	5	0	0	0	0
Phyllosticta sp.	5	15	0	0	0	5	0	0	5	10
Phomopsis sp.	0	10	0	0	0	0	10	0	0	10
Phoma sp.	0	15	5	0	0	0	0	5	0	15
Acremonium sp.	0	0	0	0	0	0	0	0	0	5
xylariaceous fungi[b]	0	0	5	0	50	15	5	0	10	5
Surculiseries rugispora[c]	0	0	0	0	0	5	0	0	0	0
ascomycete sp.	0	0	0	0	5	0	0	0	0	0
white sterile mycelia	15	15	0	30	5	15	10	20	5	0
colored sterile mycelia	0	0	0	45	0	25	0	0	0	0
coelomycete sp.	0	0	0	0	0	0	0	5	0	0
CF in each leaf stage	45	70	25	75	60	80	25	45	30	50
CF in station	58		50		70		35		40	
Number of fungi detected	5		5		9		7		9[b]	

[a] Y, young leaves; M, mature leaves.
[b] including *Geniculosporium* sp.
[c] new species (14).

Table 3. Colonization frequency (CF) (%) of the endophytic fungi on leaves (Y and M) of *B. gymnorrhiza* in September 1998.

Station	P-1		P-3		P-5		P-7		P-9	
Fungus	Y[a]	M[a]	Y	M	Y	M	Y	M	Y	M
Colletotrichum sp.	45	50	45	40	10	0	0	0	0	0
Pestalotiopsis sp.1	0	5	0	0	5	0	0	0	0	0
Phyllosticta sp.	0	0	35	40	0	25	10	0	5	5
xylariaceous fungi[b]	10	20	0	0	0	15	0	0	0	0
Pithomyces sp.	0	0	0	0	0	0	0	0	0	5
white sterile mycelium	5	25	5	35	10	0	10	55	5	30
CF in each leaf stage	60	90	75	100	25	40	20	55	15	40
CF in station	75		88		33		38		28	
Number of fungi detected	4		3		5		2		3	

[a] Y, young leaves; M, mature leaves.
[b] including *Geniculosporium* sp.

leaves of the host plants.

The colonization frequency of fungi was higher on the leaves collected from the upper reach than from the lower reach in all three surveys (Table 1-3). The colonization frequency increased as the leaves aged, and the number of fungal species isolated also tended to increase in most cases. The colonization frequencies at stations P-1 and P-3 were above 60%, and that at P-5 was 50.8%, while those at P-7 and P-9 were 36.7% and 32.5%, respectively (Table 4). The statistical tests supported a significant difference in colonization frequency of all fungi detected between the upper and lower stations (Table 6, (a)). While the colonization frequency increased from the lower to the upper reach, however, the number of fungal species isolated from leaves was higher in the lower reach than the upper: 12 spp. at P-9 as compared to 7 spp. at P-1 (Table 4). The number of species at each station estimated by the jackknife estimate are as follows: 8.67 (range, 6-11) at P-1, 13.99 (7-21) at P-3, 13.33 (7-20) at P-5, 13.99 (7-21) at P-7, 18.66 (11-26) at P-9 (Table 5). Both indexes of species diversity showed a richer diversity of fungal species harbored in *B. gymnorrhiza* in the lower reach than the upper (Table 5).

Colletotorichum sp., *Pestalotiopsis* sp.1 and *Phyllosticta* sp. were isolated from leaves at every station. *Colletotrichum* sp. was isolated more frequently from the leaves collected at the upper stations (33% at P-1, 40% at P-3) than the lower stations (5% at P-5, 4% at P-7, 6% in P-9) (Table 4 and Fig. 1). The statistical tests supported a significant difference in colonization frequency of *Colletotrichum* sp. between the upper stations, P-1 and P-3, and the lower stations P-5, P-7 and P-9 (Table 6, (b)). No such difference was found for the other dominant fungi, *Pestalotiopsis* sp.1 and *Phylllosticta* sp. (Table 6, (c)

Table 4. Colonization frequency (CF) (%) of the endophytic fungi on leaves of *B. gymnorrhiza* at five research stations.

Station / Fungus	P-1	P-3	P-5	P-7	P-9	No. of stations at which fungus was detected
Colletotrichum sp.	33.3	40	5	4.2	5.8	5
Pestalotiopsis sp.1	0.8	2.5	6.7	1.7	3.3	5
Pestalotiopsis sp.2	0	0	0.8	0	2.5	2
Phyllosticta sp.	3.3	12.5	14.2	6.7	6.7	5
Phomopsis sp.	7.5	0.8	0	1.7	1.7	4
Phoma sp.	2.5	0.8	0	0.8	2.5	4
Acremonium sp.	0	0	0	0	0.8	2
xylariaceous fungi[a]	5	0.8	14.2	0.8	2.5	5
Pithomyces sp.	0	0	0	0	0.8	1
Surculiseries rugispora[b]	0	0.8	0.8	0	0	2
ascomycete sp.	0	0	0.8	0	0	1
white sterile mycelia	13.3	15.8	9.2	18.3	8.3	5
colored sterile mycelia	0	7.5	4.2	2.5	1.7	4
coelomycete sp.	0	0	0	0.8	0	1
CF in station	60.8	65.8	50.8	36.7	32.5	
No. of fungi detected	7	9	10[a]	9	12[a]	

[a] including *Geniculosporium* sp.

[b] new species (14).

and (d), respectively).

In the upper reach of the Shiira River, *Barringtonia racemosa* Blume, *Mallotus*

Table 5. Species richness and diversity of endophytic fungi on *B. gymnorrhiza* in three surveys and at five research stations.

	Jul. 1997	Feb. 1998	Sep. 1998	P-1	P-3	P-5	P-7	P-9
Number of species detected	8	14	6	7	9	10	9	12
No. of species by jackknife estimate (S)	9.8	18.5	6.9	8.67	13.99	13.33	13.99	18.66
(range)	7-13	14-23	5-9	6-11	7-21	7-20	7-21	11-26
Simpson's reciprocal index (1/D)	4.02	7.21	3.45	3.12	3.22	6.21	3.32	7.54
Shannon-Wiener Function (H')	2.31	3.16	1.99	2.08	2.12	2.86	2.3	3.21

Note: the equations used to analyze species richness and diversity were adapted from Krebs (10).

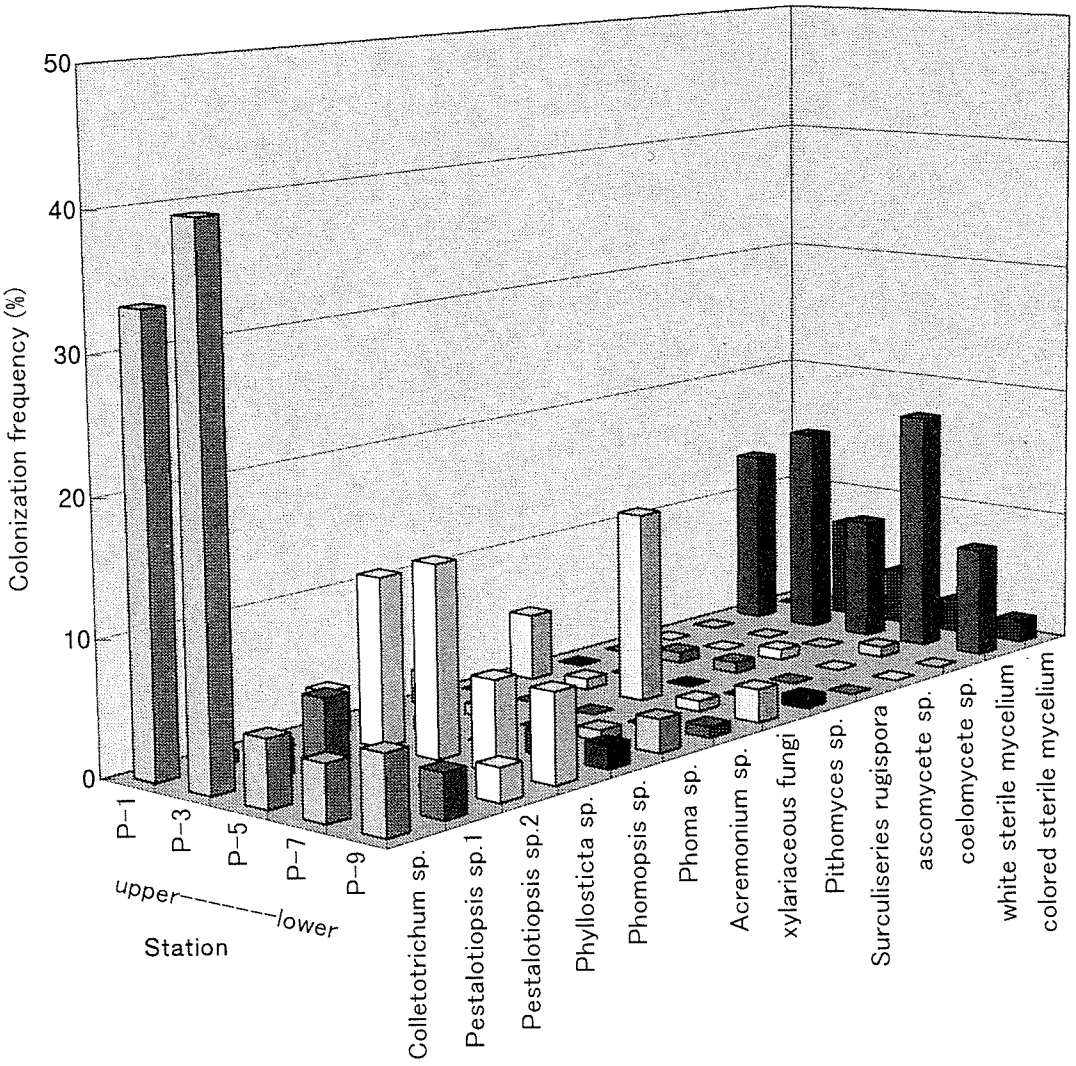

Fig. 1. Colonization frequency of endophytic fungi of *Bruguiera gymnorrhiza* at each station.

Table 6. Statistical tests for a difference in the colonization frequency of all fungi, and of three dominant fungi at each station.

(a) all fungi detected

	P-3	P-5	P-7	P-9
P-1	0.8	1.56	**3.73	**4.39
P-3		*2.36	**4.51	**5.16
P-5			*2.2	**2.88
P-7				0.68

(b) *Colletotrichum* sp.

	P-3	P-5	P-7	P-9
P-1	1.08	**5.57	**5.78	**5.37
P-3		**6.49	**6.69	**6.30
P-5			0.3	0.27
P-7				0.57

(c) *Pestalotiopsis* sp.1

	P-3	P-5	P-7	P-9
P-1	1.01	*2.38	0.58	1.36
P-3		1.54	0.46	0.39
P-5			0.39	0.95
P-7				0.41

(d) *Phyllosticta* sp.

	P-3	P-5	P-7	P-9
P-1	**2.64	**2.96	1.17	0.35
P-3		0.37	1.55	*2.34
P-5			1.9	*2.01
P-7				0.84

Note: *, significantly different at 0.05 level; **, significantly different at 0.01 level.

moluccanus Mueller-Arg. and *Trema orientalis* (L.) Blume are dominant plants around P-1, while *Helitiera littoralis* Ait., *Rhaphiolepis indica* (L.) Lindley and *Persea thunbergii* (Sieb. et Zucc.) Kostermans dominate the forest around P-3. Forest in the upper reach consists of many species of subtropical evergreen trees, which in places form a canopy over mangrove trees growing at the river's edge. In the lower reach, vegetation consisting of *B. gymnorrhiza, Kandelia candel* (L.) Druce and *Pandanus tectorius* Park. is dominant around P-5, while *B. gymnorrhiza* is dominant P-7. Around P-9, the lowest station in this study, *Rhizophora stylosa* Griff. predominates over *B. gymnorrhiza*. The richer vegetation in the upper reach suggests a more diverse fungal flora in the upper reach than the lower. However, fewer fungal species were found in leaves of *B. gymnorrhiza* collected in the upper reach, while the colonization frequency of fungi, especially *Colletotrichum* sp., was higher in the upper reach. In the case of apple bitter-rot and peach anthracnose caused by *Colletotrichum* spp., it has been reported that other trees growing in close proximity to these fruit trees appear to be major source of primary infection of these diseases (6, 11, 15). In the upper reach of the Shiira River, other trees growing near *B. gymnorrhiza* are likely to serve as an inoculum source of *Colletotrichum* sp., which is found in *B. gymnorrhiza* as an endophyte. This fungus may quickly infect the leaves and predominate over the other fungi, or prevent other fungi from invasion. In the lower basin, from around P-5 to the river mouth, *B. gymnorrhiza* and another mangrove trees form open forest, which is never covered by other trees throughout the year. This may be why the colonization frequency of *Colletotrichum* sp. decreased sharply in the lower forest of *B. gymnorrhiza*. It is considered that assemblages of endophytic fungi of *B. gymnorrhiza* vary according to the surrounding vegetation.

On the other hand, we should also consider the physiological nature of *B. gymnorrhiza*. Tsukamoto and Nakanishi (23, 24) reported that inorganic salt and chemical

element contents of the leaves of *B. gymnorrhiza* and *R. stylosa* at the Shiira River basin, which control osmotic pressure inside plant tissue to restore moisture and nutrition, change according to the concentration of salt in water. They also reported that a positive correlation between manganese and tannin contents is an important factor controlling oxidation-reduction potential in these mangrove plants. With the increase in manganese content that accompanies the decrease salt concentration in river water in upper reach, oxidative stress inside the leaves builds up. Conversely, reductive stress inside the leaves increases in the lower reach because of a sharp drop of manganese content. Further study on the influence on fungi of chemical elements and the internal environment of plant tissues is necessary to clarify the factors involved in establishment of assemblages of endophytes.

Finally, in the course of this study, an unknown imperfect fungus was isolated from the leaves collected at P-3 and P-5, which we accommodated in a new genus and named *Surculiseries rugispora* Okane *et al.* (14). This fungus is considered to belong to Xylariaceae based on sequence analysis of 18S ribosomal DNA. Xylariaceous fungi have been found from some plant families, especially in tropical regions, and they are considered to appear commonly on census lists of endophytes (16, 19). Discovery of this new fungus from *B. gymnorrhiza* indicates that mangrove forests preserved in the Ryukyu Islands harbor unknown fungi and encourages further investigation of fungal diversity in this region.

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Mycobiota of Mangrove Forest Soil in Thailand

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Summary

To examine the distribution and ecology of the fungi inhabiting mangrove forest soil in a tropical region, 20 samples from mangrove sites and 3 from terrestrial sites were collected in Thailand in March 1999. Soil fungi were isolated by heat incubation at 45°C and the dilution plate method at 25°C. Comparison of the findings with those of an earlier survey of subtropical mangroves in Okinawa, Japan revealed the following. 1. The average numbers of colonies and species per sample from the Thai mangroves were smaller than in samples from Japanese mangroves. 2. *Talaromyces byssochlamydoides*, a thermophilic fungus, and *Aspergillus aculeatus* were frequently detected in the Thai samples but were not found in Japanese mangroves. 3. Species of the mitosporic genera *Coniothyrium, Phialophora*, and *Phoma* occurred less frequently in the Thai than the Japanese mangroves. 4. Species of the genus *Aspergillus* were abundantly detected in the Thai samples. The smaller population and lower ecological diversity of fungi in the Thai mangrove soil might be due to the lower nutrient content and anaerobic nature of the soil, which is mostly composed of clay particles. However, *Aspergillus* species, which adapt to high temperature, showed higher diversity in the Thai soil.

Keywords: diversity, fungal populations, mangrove forest, mycobiota, Thai soil.

Mangrove forest is distributed along estuaries and rivers in tropical and subtropical regions. As an ecosystem, it has unique characteristics and is one of the most diverse reserves of biological organisms. Recent attention has focused on the diversity of microorganisms and the important role that they play in the dynamics of the mangrove ecosystem. The soils of mangrove forests in tropical and subtropical regions are reported to be semi-aerobic, low in nutrients, and to have higher concentrations of heavy metals and higher salinity than terrestrial soils (10). Mycobiota on mangrove and its mud have been investigated in the subtropical zone in Japan (4, 5), and tropical regions (7, 8, 9).

To examine the distribution and ecology of the fungi inhabiting mangrove forest soil in a tropical region, soil samples were collected from mangroves in Thailand. Fungi were

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isolated and identified, and several isolates were tested for optimum growth temperature and tolerance to sodium chloride. The results were compared with those of a study conducted in 1997 on the mycobiota of the mangrove rhizosphere in the subtropical region of Okinawa (4, 5).

A part of this study was carried out as a co-operative project of the IFO (Japan), Kasetsart University (Thailand), and National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand.

Materials and Methods

Sampling sites. Twenty soil samples from the rhizosphere of eight mangrove species were collected from the Ranong Research Center of Kasetsart University and Phang-nga forest in southern Thailand, and three samples were collected from the campus of Kasetsart University (K.U.) in Bangkok on March 1999 as a terrestrial soil samples (Table 1). The mangrove collection sites were in the estuaries of two rivers debouching into the Andaman Sea. This region has a tropical monsoon climate, with annual rainfall of 2800 mm, an average annual temperature of 27°C, and a hot season (March) temperature of 32-36°C. Water temperature is 25-26°C, salinity is 5-30 ppt, and depth is 1-2 m. The mangrove species *Rhizophora mucronata* Lum., *R. apiculata* Bl., *Xylocarpus obovatus* A. Juss., *Avicennia alba* Bl., *Lumnitzera racemosa* Willd., *Nypa fruticans* Wurmb., *Bruguiera gymnorrhiza* (L.) Lam., *B. indica, Kandelia candel* Druce, and *Sonneratia alba* J.A. Sm. dominate the vegetation of these estuaries. Only two species of collected species, *Bruguiera gymnorrhiza* and *Sonneratia alba*, were common in Japan. Table 2 compares the mangrove forest environments of Thailand and Japan.

Isolation methods. The isolation methods adopted are the same as in the survey of Japanese mangrove forests reported previously (4, 5). The mud samples were suspended in physiological salt solution containing 0.85 % sodium chloride. Two isolation methods

Table 1. Soil samples of mangrove rhizosphere collected in Thailand.

Sample No.	Date sampled	Locality	Vegetation of Mangrove tree
Th- 1 - Th- 2	18/3/'99	Ranong	*Avicennia alba*
Th- 3 - Th- 4	18/3/'99	Ranong	*Ceriops tagal*
Th- 5 - Th- 6	18/3/'99	Ranong	*Rhizophora mucronata*
Th- 7 - Th- 8	18/3/'99	Ranong	*Xylocarpus obovatus*
Th- 9 - Th-10	18/3/'99	Ranong	*Bruguiera sexangula*
Th-11 - Th-12	18/3/'99	Ranong	*Rhizophora apiculata*
Th-13 - Th-14	18/3/'99	Ranong	*Sonneratia alba*
Th-15 - Th-16	19/3/'99	Phang-nga	*Avicennia alba*
Th-17 - Th-18	19/3/'99	Phang-nga	*Bruguiera sexangula*
Th-19	19/3/'99	Phang-nga	*Sonneratia alba*
Th-20	19/3/'99	Phang-nga	*Bruguiera gymnorrhiza*
Th-21 - Th-23	22/3/'99	Kasetsart Univ.	terrestrial soil

Table 2. Comparison of mangrove forest environments in Thailand and Japan.

	Thailand	Japan
Locality	Rannong	Okinawa
	Phang-nga	Iriomote and Ishigaki Is.
	99° E, 9° N	127° E, 26° N
Climate	tropical	subtropical
	27℃ (Mar.)	17-19℃ (Mar.)
	2800 mm	2000 mm
Mangrove	*Avicennia alba*	*Avicennia marina*
	Bruguiera gymnorrhiza	*Bruguiera gymnorrhiza*
	Bruguiera sexangula	*Kandelia candel*
	Ceriops tagal	*Lumnitzera racemosa*
	Rhizophora apiculata	*Rhizophora stylosa*
	Rhizophora mucronata	*Sonneratia alba*
	Sonneratia alba	
	Xylocarpus obovatus	
Soil type	Clay	Sandy loam
Salinity	15-30‰	15-25‰

were applied: incubation at 45°C and the standard dilution plate method with × 10 and × 100 dilution. Two plates were used for each sample and method. All fungi appearing from each sample were incubated at 45°C and 25°C for 2 and 4 days, respectively. Single colonies were picked up from the plates under a dissecting microscope and transferred to a half-strength malt extract agar slant.

Isolation medium. Malt extract-yeast extract-agar (per liter: 100 g glucose, 5 g peptone, 3 g malt extract, 3 g yeast extract and 20 g agar) containing 50 μg/ml of tetracycline antibiotic was used.

Identification of isolates. Isolates were inoculated on the plates of the appropriate medium for identification, such as oatmeal, potato carrot, cornmeal and potato sucrose agar, and incubated at 45°C and 25°C for appropriate periods. To identify the isolates, one representative strain of each species was used. Most were identified at K. U., and the remainder were identified at IFO. For species identification, some strains of IFO Culture Collection were used as reference.

Results and Discussion

Fungal population density

The number of fungi found in each sample by the dilution plate method was counted

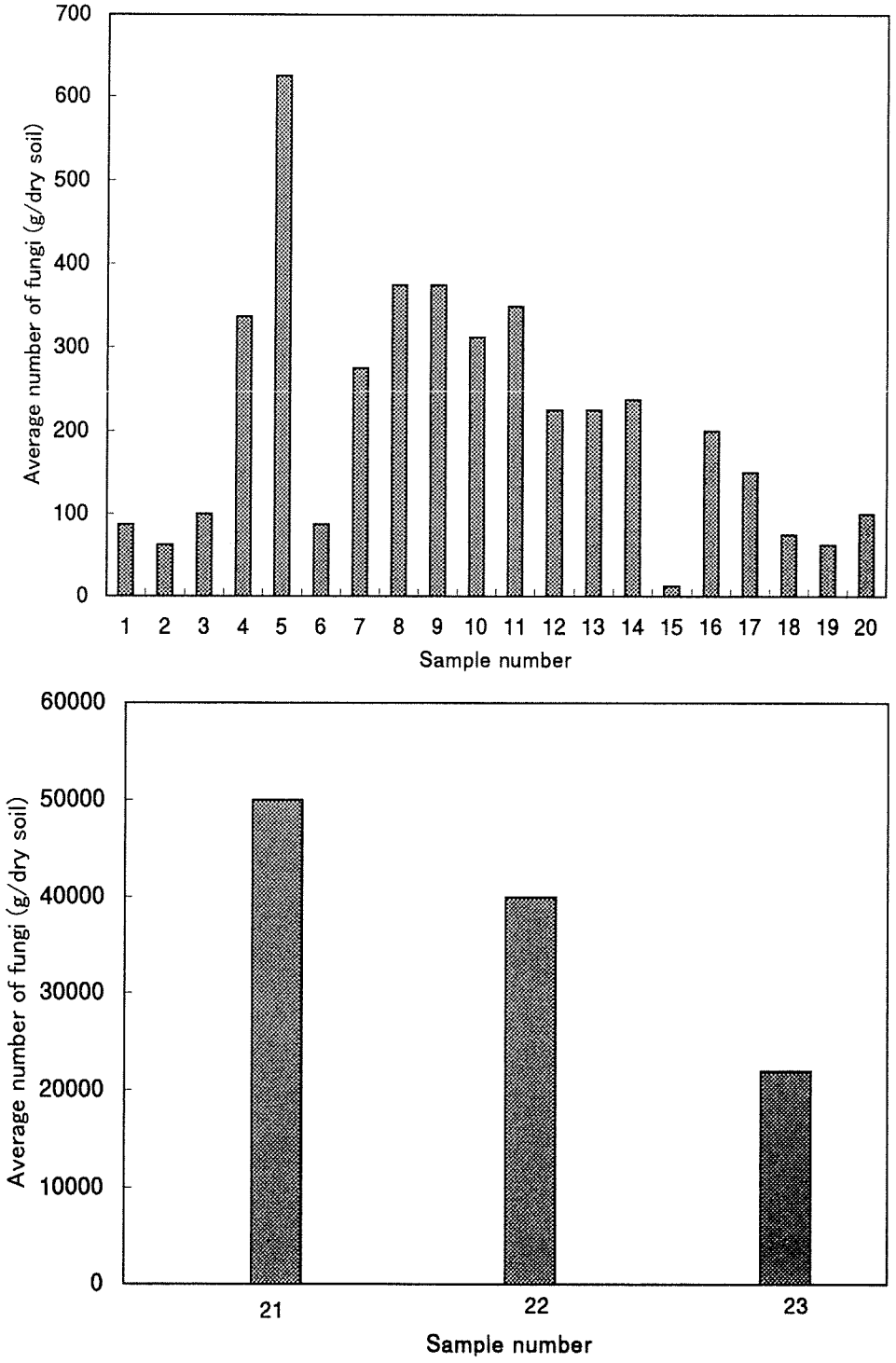

Fig. 1. Number of fungi isolated per sample by the dilution plate method.

under dissecting microscope, and the results are shown in Figure 1. The average number of fungi found for each mangrove species ranged from 2.0×10^{1} to 6.2×10^{2} cfu/g of dry

Table 3. Fungi isolated by two isolation methods from rhizosphere soil, by mangrove species.

Fungus	Mangrove species								Frequency
	Avicennia alba	*Ceriops tagal*	*Rhizophora mucronata*	*Xylocarpus obovatus*	*Bruguiera sexangula*	*Rhizophora apiculata*	*Sonneratia alba*	*Bruguiera gymnorrhiza*	(%)[a]
Acremonium curvulum W. Gams							1		5
Acremonium spp.		1	1		3		1		30
Aspergillus aculeatus Iizuka	1	1	1		1			2	30
Aspergillus clavatus Desmaz.	2								10
Aspergillus fumigatus Fres.		1	1	1					15
Aspergillus niger van Tiegh.				1	1				10
Aspergillus niger aggr.	2	1					2		25
Aspergillus spp.		2			1	1			20
Aspergillus terreus Thom		2	1				1		20
Cladosporium cladosporioides (Fres.) de Vries		1	1	1		1			20
Coniothyrium sp.			1						5
Cylindrocladium parvum Anders.			1						5
Eupenicillium javanicum (van Veyma) Stolk & Scott	1	1	1	1	1				25
Eupenicillium sp.	1							1	10
Fusarium oxysporum Schlecht. emend. Sny. & Hans.	1								10
Fusarium solani (Mart.) Appel & Woll. emend. Sny. & Hans.	1	1							10
Fusarium sp.		1							5
Geotrichum candidum Link: Pers. emend. Carmich.					1				5
Gongronella butleri (Lend.) Peyr. & Dal Vesco		1			1				10
Metarhizium anisopliae (Metschnik.) Sorok.	1		1					1	15
Microascus cinereus (Emil.-Weil & Gand.) Curzi			1						5

a: Number of positive samples/total number of samples x 100.

(Continued).

Fungus	Mangrove species								Frequency
	Avicennia alba	***Ceriops tagal***	***Rhizophora mucronata***	***Xylocarpus obovatus***	***Bruguiera sexangula***	***Rhizophora apiculata***	***Sonneratia alba***	***Bruguiera gymnorrhiza***	(%)[a]
Mucor sp.			1			1			10
Neosartorya fischeri (Fenn. & Raper) Mall. & Cain var. *glabra* (Wehm.) Mall. & Cain							1		5
Paecilomyces variotii Bain.		1	2				1		20
Paecilomyces sp.		1							5
Penicillium citrinum Thom	1						1		10
Penicillium funiculosum Thom				1					5
Penicillium purpurogenum Stoll							1		5
Penicillium verruculosum Peyr.		1	1	1					15
Penicillium sp. -1	1	1			1				10
Penicillium sp. -2	1				1	1			15
Penicillium spp.		1	2	2	3		1	1	50
Pestalotiopsis sp.			1						5
Phoma sp.		1							5
Talaromyces byssochlamydoides Stolk & Samson	1		2			1	1		25
Talaromyces flavus (Klock.) Stolk & Samson var. *flavus*		1			1				10
Talaromyces wortmannii C.R. Benj. in Stolk & Samson	1								5
Trichoderma harzianum Rifai	2	2		2		1	1		40
Trichoderma koningii Oudem.		1							5
Trichoderma pseudokoningii Rifai	1	1	1			1			15
Trichoderma spp.		2			1	1		1	25
Unidentified strains			2	2			2		30
Total number of strains	18	26	22	12	16	8	14	6	
Number of samples	4	2	2	2	4	2	3	1	20

a: Number of positive sample/total number of samples x 100.

Table 4. Fungi isolated from soils of campus of Kasetsart univesity.

Acremonium sp.	1[a]
Aspergillus awamori Nakazawa	1
Aspergillus candidus Link	1
Aspergillus fumigatus Fresen.	1
Aspergillus niger aggr.	3
Aspergillus sp.	3
Aspergillus tamarii Kita	1
Aspergillus terreus Thom	3
Cladosporium cladosporioides (Fres.) de Vries	3
Coelomycetes	1
Curvularia lunata (Wakker) Boed.	1
Emericella nidulans (Eidam) Vuill.	1
Eupenicillium sp.	1
Fusarium oxysporum Schlecht. emend. Snyd. & Hans.	1
Fusarium solani (Mart.) Appel & Woll. emend. Snyd. & Hans.	1
Fusarium sp.	1
Gliocladium roseum (Link) Bain.	1
Mucor spp.	3
Myceliophthora thermophila (Apinis) van Oorsch.	1
Myrothecium cinctum (Corda) Sacc.	1
Neurospora crassa Shear & Dodge	1
Nigrospora sphaerica (Sacc.) Mason	1
Penicillium citrinum Thom	1
Penicillium funiculosum Thom	3
Penicillium spp.	3
Pithomyces graminicola R.Y. Roy & Rai	1
Rhizomucor sp.	1
Scolecobasidium sp.	1

a: Number of positive samples (3 samples tested).

soil, being lower than that in the subtropical zone of Okinawa (2.3×10^3 cfu/g of dry soil). On the other hand, campus soil samples from K. U. gave counts ranging from 2.5×10^5 to 5.0×10^5 cfu/g of dry soil, similar to those of agricultural soil in subtropical Japan (2.1×10^5 cfu/g of dry soil) (4). These findings indicate a poor distribution of fungal propagules in the tropical mangrove mud. The reason may be the semi-anaerobic condition of the mud, which is more clayey than mangrove soil in Japan.

Mycobiota of mangrove rhizosphere soil

The fungi isolated from mangrove soil in Thailand were compared with those isolated in Japan. Eighty percentage of the Thai isolates were also found in Japan, including less dominant species (4), and almost all of them are known as typical soil-borne fungi (1, 2, 3, 6). Table 3 lists all the species of fungi isolated from mangrove soil samples by the two isolation methods and their frequency. The number of species found per sample was

Table 5. Fungi isolated from soil of washed mangrove roots by the dilution plate method in Japan.

Species	No. of positive samples	Frequency (%)[a]
Acremonium spp.	11	50.0
Cladosporium cladosporioides (Fres.) deVries	6	27.3
Coniothyrium spp.	12	54.5
Exophiala sp.	5	22.7
Fusarium spp.	8	36.4
Gliocladium roseum (Link) Bain.	3	13.6
Metarhizium anisopliae (Metschn.) Sorok.	3	13.6
Nodulisporium sp.	3	13.6
Paecilomyces lilacinus (Thom) Samson	8	36.4
Paecilomyces spp.	5	22.7
Penicillium citrinum Thom	6	27.3
P. crustosum Thom	3	13.6
P. janthinellum Biour.	4	18.2
P. purpurogenum Stoll	8	36.4
Penicillium spp.	3	13.6
Phialophora fastigiata (Langerb. & Melin) Conant	5	22.7
Phialophora spp.	6	27.3
Phoma spp.	13	59.1
Scopulariopsis spp.	5	22.7
Trichoderma harzianum Rifai	10	45.5
T. koningii Oudemans	3	13.6
Trichoderma spp.	3	13.6
Sterile mycelium	16	72.7

a: Number of positive samples/total number of samples x 100 (22 samples).
Mangrove species: *Avicennia marina, Rhizophora stylosa, Sonneratia alba, Bruguiera gymnorrhiza, Kandelia candel, Lumnitzera racemosa.*

fewer than in the Japanese mangrove (2.1 species/sample). Isolates mainly belong to the mitosporic fungi. Fungi isolated by the heat incubation method were mainly *Aspergillus fumigatus* Fresenius, *A. terreus* Thom, *A. aculeatus* Iizuka, *Paecilomyces variotii* Bainier and *Talaromyces byssochlamydoides* Stolk & Samson. The latter three of these species were dominant in the Thai samples but were not detected in the subtropical zone of Japan. Conversely, *Acremonium alabamense* Morgan-Jones, which was dominant in Japan (4), was not detected in the Thai samples. This may reflect a geological difference in fungal diversity between tropical and subtropical zones.

Species of the genera *Penicillium* and *Trichoderma* were dominantly detected by the dilution plate method. These species, which decompose plant debris, are commonly isolated from subtropical soil. Species of the mitosporic genera *Coniothyrium, Phialophora*, and *Phoma* occurred less frequently than in the subtropical mangroves (4). The reason is suggested to be differences in the mangrove environment. The fungi detected in this survey are typical terrestrial soil fungi that are considered to have been carried in from

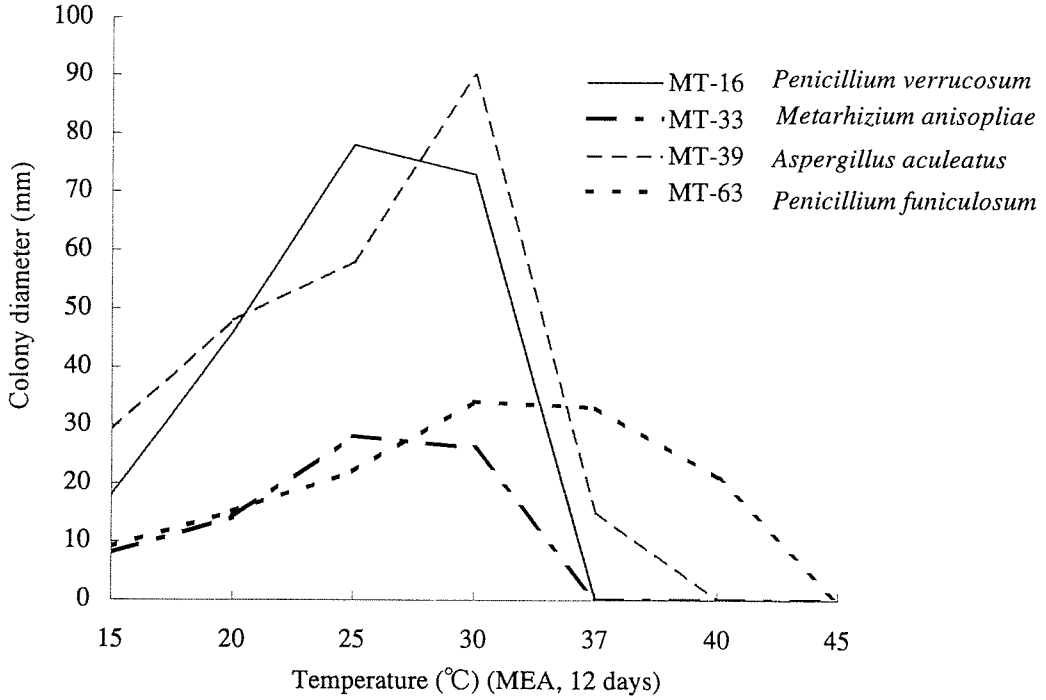

Fig. 2. Hyphal growth of four strains

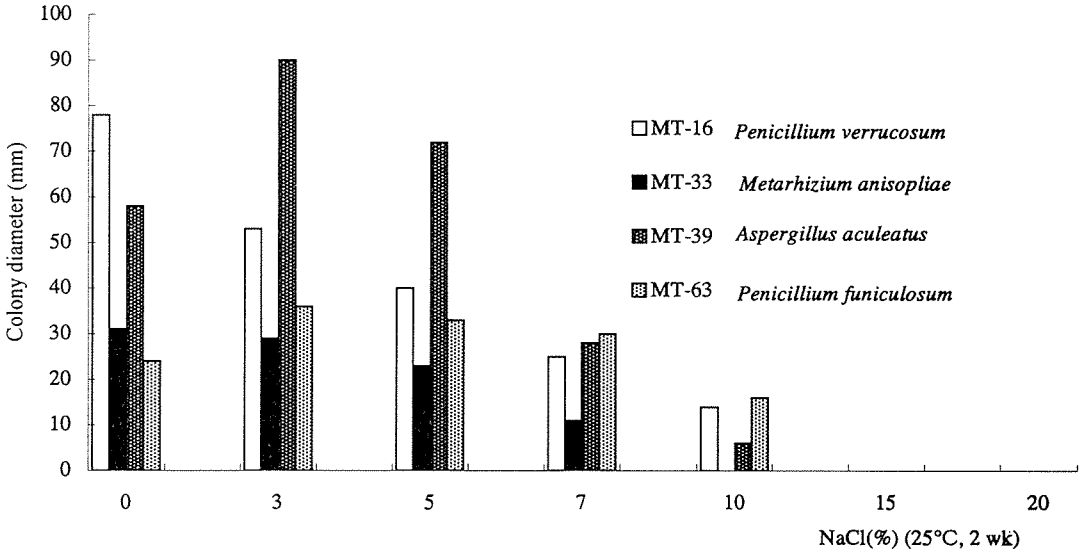

Fig. 3. Salinity range for growth of four strains.

surrounding substrates and adapted to the mangrove environment.

Aspergillus species were dominantly isolated from campus soil of K.U.: *A. terreus, A. tamarii* Kita, *A. awamori* Nakazawa, *A. candidus* Link, and *A. fumigatus* (Table 4).

Except for *A. terreus* and *A. fumigatus* (4), these species are less dominant in subtropical zone. This difference represents a difference in the fungal diversity in terrestrial soil between tropical and subtropical zones. *Penicillium funiculosum* was characteristically isolated from soil of campus of K.U., but it was not predominantly isolated in the subtropical zone. To confirm why do such differences occur, research into mangrove rhizosphere mycobiota should continue.

Table 5 shows the fungi dominantly detected in Japanese mangrove forest soils by the dilution plate method (5).

Optimum growth temperature

The effect of temperature on hyphal growth was investigated for four of the isolated fungi: *A. aculeatus, Metarhizium anisopliae* (Metschnikoff) Sorokin, *Penicillium verruculosum* Peyronel, and *P. funiculosum*. Figure 2 shows the results. The optimum temperature for *A. aculeatus, M. anisopliae* and *P. verruculosum* was 25°C to 30°C, and that for *P. funiculosum* was 30°C to 35°C. These fungi may be adapted to the conditions of the mangrove rhizosphere.

Tolerance to sodium chloride

Tolerance to sodium chloride was tested for the same four strains. Except for *M. anisopliae*, the fungi grew at NaCl concentrations of up to 10 % (Fig. 3). These fungi have been isolated from natural substrates and soil (1). Their salt tolerance means that these fungi can withstand the high osmotic pressure if the soil in which mangrove is growing dries up or if they are carried into salt water by the tide. That is, they are probably able to adapt to the mangrove environment.

The smaller population and lower diversity of fungi in the Thai mangrove soil might be due to the lower nutrient content of soil, which is mostly composed of clay particles. However, the *Aspergillus* species, which adapt to high temperature, showed higher diversity in the Thai soil.

We thank Dr. Sanit Aksornkaew (Faculty of Forestry, Kasetsart University) for identification of mangrove species and for offering valuable suggestions.

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Phylogenetic Diversity of *Cytophaga*-like Strains Isolated from the Sub-tropical Zone of Japan

Yasuyoshi NAKAGAWA, Makoto SUZUKI* and Kazunori HATANO

Summary

The phylogenetic diversity of the *Cytophaga*-like strains isolated from Amami-O-Shima (Amami Islet) and Iriomote Shima (Iriomote Islet) in the sub-tropical zone of Japan was examined. Partial sequencing of 16S rDNA revealed that 39 strains belonged to the cytophaga-flavobacteria-bacteroides (CFB) phylum and could be divided into 15 groups (cfb 1 to 15) including 15 sub-groups. The precise phylogenetic position of each group and sub-group was inferred from the almost complete 16S rDNA of a representative strain. Fourteen of the groups were located in the family *Flavobacteriaceae* and were affiliated with various taxa: cfb 2 clustered with the genus *Salegentibacter* and *Cytophaga latercula*; cfb 4 clustered with *Psychroserpens*; cfb 6, 7, and 8 clustered with *Cellulophaga*; cfb 9 clustered with *Cytophaga marinoflava*; cfb 11 clustered with *Polaribacter*; cfb 12 clustered with the new genus "*Tenacibaculum*"; cfb 13, 14, and 15 clustered with *Flavobacterium*; cfb 3 formed a sister group of *Psychroflexus*; cfb 5 formed a sister group of *Capnocytophaga* and *Coenonia*; and cfb 10 formed a sister group of *Cellulophaga* and *Cytophaga marinoflava*. The remaining group, cfb 1, was not included in the family *Flavobacteriaceae* and constituted a distinct phylogenetic lineage as a sister group of *Cyclobacterium marinum*. Only cfb 8 of the fifteen groups exhibited an exact match with any of the 16S rRNA sequences deposited in the databases. Almost all groups were considered to represent new genera and/or species. From these results, it is clear that the biodiversity of the CFB phylum is much wider than was previously thought. Unknown microbiological genetic resources are buried in sub-tropical areas such as Amami-O-Shima and Iriomote Shima, Japan.

Keywords: Cytophaga-flavobacteria-bacteroides phylum, biodiversity, microbiological genetic resource.

The cytophaga-flavobacteria-bacteroides (CFB) phylum, one of main phyla of the domain *Bacteria*, is composed of the genera *Cytophaga, Flavobacterium, Flexibacter* and other genera including anaerobes such as *Bacteroides* (32). The taxonomy of the genera *Cytophaga, Flavobacterium, Flexibacter* has a turbulent history. These genera have been called the *Flavobacterium-Cytophaga* complex, because they share similar phenotypic

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characteristics (7, 8, 14, 22, 26). Phylogenetic analyses based on the 16S rRNA sequences have also shown a huge degree of biological diversity (10, 20). In the last decade, the understanding and acceptance of the diversity of the CFB phylum have lead to emendation and reclassification of some genera. The genus *Cytophaga* was emended to include only two species, *Cytophaga hutchinsonii* and *Cytophaga aurantiaca* (21); and *Flavobacterium* was emended to include *Flavobacterium aquatile* and its phylogenetic relatives (2). Some misnamed *Cytophaga* species were allocated to new genera, *Marinilabilia, Flammeovirga, Persicobacter*, and *Cellulophaga* (15, 19, 21, 27), and some misnamed *Flavobacteirum* species were classified into *Chryseobacterium, Empedobacter*, and *Myroides* (30, 31). In addition, new bacteria of the CFB phylum have been isolated from marine environments of the Arctic and the Antarctic, and have been assigned to several new genera, *Psychroserpens, Gelidibacter, Psychroflexus, Polaribacter*, and *Salegentibacter* (4, 5, 11, 17). The biodiversity of the CFB phylum is expanding.

In this study, the CFB group of *Bacteria* was isolated from coastal sea regions in the sub-tropical zone of Japan. The 16S rDNA sequences of the isolates were determined to investigate their phylogenetic positions and the biodiversity of the CFB phylum.

Materials and Methods

Sampling. Water samples were collected from mangrove areas in Iriomote Shima (Iriomote Islet), Okinawa Prefecture, Japan (24°15′ N, 123°50′ E) in February 1998 (Table 1). Rocks, seaweed, seawater, and sponges were collected from the seashore, and water and fallen leaves from mangrove in Amami-O-Shima (Amami Islet), Kagoshima Prefecture, Japan (28°15′ N, 129°25′ E) in March 2000 (Table 1). Both islands are located in the sub-tropical zone.

Isolation and cultivation of strains. Samples were suspended in 5 ml of 0.85 % NaCl solution and homogenized. The homogenates, seawater and water samples were decimally diluted to 10^{-3}. One hundred μl of each dilution was plated onto the following media

Table 1. Source of isolates.

Name of source[1)]	Source	Environment	Location	Media used for isolation
AM1	Sea weed	Sea shore	Amami	SP5
AM2	Sea weed	Sea shore	Amami	SP5
AM3	Sea water	Sea shore	Amami	SP5
AM4	Sponge	Sea shore	Amami	SP5
AM5	Sea weed	Sea shore	Amami	SP5
AM8	Fallen leaf	Mangrove	Amami	SP5
AM9	Sea weed	Mangrove	Amami	SP5
AM10	River water	Mangrove	Amami	SP5
AM11	Sea water	Sea shore	Amami	SP5
AM12	Rock	Sea shore	Amami	SP5
P7	River water	Mangrove	Iriomote	PY, PYSW
P12	River water	Mangrove	Iriomote	PY, PYSW

[1)] The first half of strain no. corresponds to the name of source.

to isolate bacterial strains: PY medium, containing 0.5 % of Polypepton (Wako Pure Chemical Industries, Osaka, Japan), 0.25 % of yeast extract (Difco Laboratories, Detroit, Mich., USA), 0.01 % of $MgSO_4 \cdot 7H_2O$, and 1.5 % of agar in 1 L of distilled water (pH 7.0); PYSW medium, containing the same components as PY medium in 750 ml of artificial sea water (Jamarin S, Jamarin Laboratories, Osaka, Japan) and 250 ml of distilled water (pH 7.2–7.4); SP5 medium (23), containing 0.9 % of Casitone (Difco), 0.1 % of yeast extract in 1 L of artificial sea water. The plates were incubated at 25°C. Colonies appearing on the plates were purified and preserved by freezing at −80°C and/or L-drying.

Preparation of DNA template for PCR. A sample of a colony taken by pricking it with a sterilized toothpick was suspended in 50 μl of sterile and distilled water. The suspension was boiled for 10 min, then immediately transferred into ice water. After centrifugation at maximum speed for 10 min, 1 μl of the supernatant containing crude DNA was used as a DNA template for PCR (24).

PCR amplification and sequencing of 16S rDNA. The almost complete 16S rRNA gene (16S rDNA) was amplified by PCR with the primers 9F (5′-GAGTTTGATCCTGGCTCAG, the same sequence as positions 9 to 27 [*Escherichia coli* numbering system of Brosius *et al.* (6)]) and 1541R (5′-AAGGAGGTGATCCAGCC, complementary to positions 1525 to 1541). The 1.5-kb amplified 16S rDNA fragment was purified by using a GFX PCR DNA and gel band purification kit (Amersham Pharmacia Biotech, Japan, Tokyo, Japan) or by agarose gel electrophoresis and a QIAquick gel extraction kit (Qiagen, Hilden, Germany). The PCR products were directly sequenced by using a BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The partial 16S rDNA sequences (ca. 500 bp) were determined with the oligonucleotide primers 9F and 536R (5′-GTATTACCGCGGCTGCTG, complementary to positions 519 to 536) for initial search of the taxonomic position of the isolates. The oligonucleotide primers used to sequence the full length of amplified 16S rDNA were 339F (5′- CTCCTACGGGAGGCAGCAG, the same sequence as positions 339 to 357), 785F (5′- GGATTAGATACCCTGGTAGTC, same as positions 785 to 805), 1099F (5′-GCAACGAGCGCAACCC, same as positions 1099 to 1115), 802R (5′-TACCAGGGTATCTAATCC, complementary to positions 785 to 802), 1115R (5′-AGGGTTGCGCTCGTTG, complementary to positions 1100 to 1115) and 1541R, in addition to 9F and 536R.

Phylogenetic analysis. The 16S rDNA sequences were assembled with the AutoAssembler program (Applied Biosystems). Partial 16S rDNA sequences of the isolates were compared with sequences in the available DNA databases using the BLASTN algorithm (1) to search for close evolutionary relatives. The isolates that were found to relate with the CFB phylum were divided into fifteen groups including fifteen sub-groups based on the sequence similarities. The almost complete 16S rDNA sequences determined for a representative strain of each group were aligned with the sequences of reference organisms derived from databases using the CLUSTAL W ver. 1.8 software package (29). The alignments were modified manually against the 16S rRNA secondary structure of *Escherichia coli* (12). Phylogenetic relationships were inferred from the phylogenetic tree based

on the neighbor-joining method (25) and the K_{nuc} values (16) by using the CLUSTAL W. Positions at which secondary structures varied between strains and caused an ambiguous alignment (142-220, 447-487, 841-845, 991-1045 and 1134- 1140), positions before 111 and after 1376, and all sites for which sequences were not determined in any reference organisms were excluded from the analysis. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (9) with 1,000 replicates.

Table 2. Grouping of isolates.

Group	Sub-group	Strain no.[1]	Sea water requirement	Isolated from:
cfb 1		AM8-1, AM8-3[2]	−	Amami
cfb 2		AM2-1[2]	+	Amami
cfb 3		AM3-2[2]	−	Amami
cfb 4	4-1	AM1-8[2]	+	Amami
	4-2	AM8-7[2]	+	Amami
cfb 5		P7-7[2]	+	Iriomote
cfb 6	6-1	AM2-2S[2], AM5-4, AM11-1S, AM11-10, AM11-15S	W[3]	Amami
	6-2	AM11-7[2]	+	Amami
cfb 7		AM3-1[2], AM8-6, AM10-3, AM11-8, AM12-2	−	Amami
cfb 8		AM8-4S[2]	+	Amami
cfb 9		AM4-3[2]	−	Amami
cfb 10		AM2-4[2]	+	Amami
cfb 11	11-1	AM1-3[2], AM11-12	+	Amami
	11-2	AM11-3[2], AM11-9	W	Amami
cfb 12		AM1-1[2], AM1-7	+	Amami
cfb 13		AM10-1[2]	−	Amami
cfb 14	14-1	P12-11[2]	−	Iriomote
	14-2	P12-22[2]	−	Iriomote
	14-3	P12-27[2]	−	Iriomote
cfb 15	15-1	AM8-2[2], AM8-5	−	Amami
	15-2	AM9-3[2], AM11-16	−	Amami
	15-3	AM9-4S[2]	−	Amami
	15-4	AM10-8[2]	−	Amami
	15-5	AM11-4S[2]	−	Amami
	15-6	AM12-3[2]	−	Amami

[1] Strains in same group or sub-group show the identical partial sequence of 16S rDNA.

[2] Representative of each group or sub-group of which almost full length of 16S rDNA sequence was determined.

[3] W, weakly positive.

Fig. 1. Phylogenetic positions of isolated strains in the cytophaga-flavobacteria-bacteroides phylum. *Agrobacterium tumefaciens, Bacillus subtilis*, and *Escherichia coli* were used as the root organisms. Accession numbers of the sequences are shown in parenthesis. All data except for *Blattabacterium cuenoti* (strain has not cultivated yet), *Cyclobacterium marinum, Empedobacter brevis, Persicobacter diffluens, Spirosoma linguale*, and *Thermonema lapsum* are derived from the type strain. Scale bar = 0.02 K_{nuc} in nucleotide sequences. The numbers of the branches are the confidence limits estimated by a bootstrap analysis. The lengths of the vertical lines are not significant.

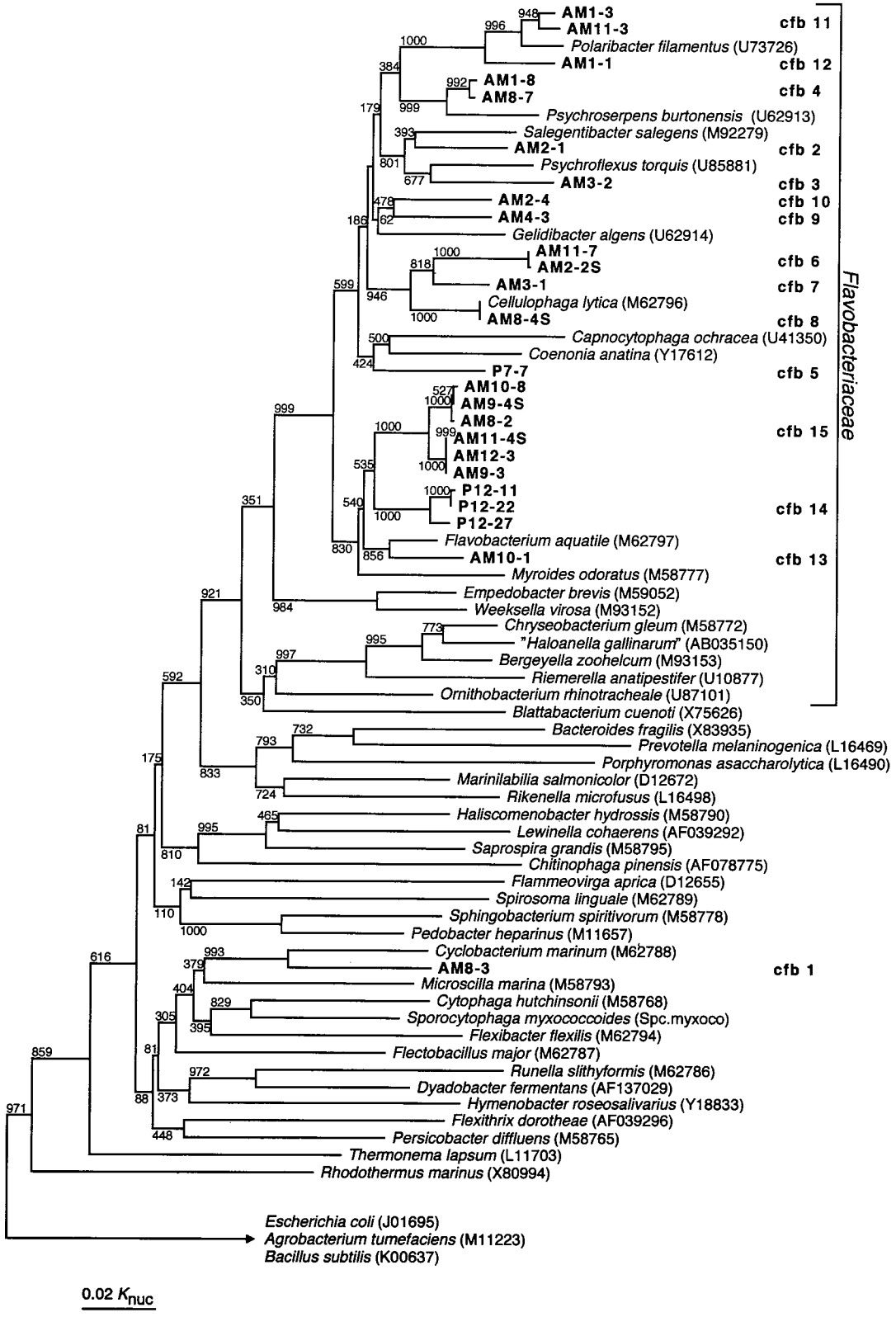
AM1-3
AM11-3
Polaribacter filamentus (U73726)
AM1-1
AM1-8
AM8-7
Psychroserpens burtonensis (U62913)
Salegentibacter salegens (M92279)
AM2-1
Psychroflexus torquis (U85881)
AM3-2
AM2-4
AM4-3
Gelidibacter algens (U62914)
AM11-7
AM2-2S
AM3-1
Cellulophaga lytica (M62796)
AM8-4S
Capnocytophaga ochracea (U41350)
Coenonia anatina (Y17612)
P7-7
AM10-8
AM9-4S
AM8-2
AM11-4S
AM12-3
AM9-3
P12-11
P12-22
P12-27
Flavobacterium aquatile (M62797)
AM10-1
Myroides odoratus (M58777)
Empedobacter brevis (M59052)
Weeksella virosa (M93152)
Chryseobacterium gleum (M58772)
"Haloanella gallinarum" (AB035150)
Bergeyella zoohelcum (M93153)
Riemerella anatipestifer (U10877)
Ornithobacterium rhinotracheale (U87101)
Blattabacterium cuenoti (X75626)
Bacteroides fragilis (X83935)
Prevotella melaninogenica (L16469)
Porphyromonas asaccharolytica (L16490)
Marinilabilia salmonicolor (D12672)
Rikenella microfusus (L16498)
Haliscomenobacter hydrossis (M58790)
Lewinella cohaerens (AF039292)
Saprospira grandis (M58795)
Chitinophaga pinensis (AF078775)
Flammeovirga aprica (D12655)
Spirosoma linguale (M62789)
Sphingobacterium spiritivorum (M58778)
Pedobacter heparinus (M11657)
Cyclobacterium marinum (M62788)
AM8-3
Microscilla marina (M58793)
Cytophaga hutchinsonii (M58768)
Sporocytophaga myxococcoides (Spc.myxoco)
Flexibacter flexilis (M62794)
Flectobacillus major (M62787)
Runella slithyformis (M62786)
Dyadobacter fermentans (AF137029)
Hymenobacter roseosalivarius (Y18833)
Flexithrix dorotheae (AF039296)
Persicobacter diffluens (M58765)
Thermonema lapsum (L11703)
Rhodothermus marinus (X80994)
Escherichia coli (J01695)
Agrobacterium tumefaciens (M11223)
Bacillus subtilis (K00637)
cfb 11
cfb 12
cfb 4
cfb 2
cfb 3
cfb 10
cfb 9
cfb 6
cfb 7
cfb 8
cfb 5
cfb 15
cfb 14
cfb 13
cfb 1
Flavobacteriaceae
0.02 K_{nuc}

Fig. 1

Results and Discussion

Grouping of isolates

The initial phylogenetic analyses to search for the taxonomic positions of all isolates were examined with the partial sequences of 16S rDNA (ca. 500 bp). Homology search based on these sequences using BLASTN revealed that 39 strains belonged to the CFB phylum. These strains were divided into fifteen groups by the sequence similarity, and five of these groups were subdivided into fifteen sub-groups by sequence identity (Table 2). Strains in the same group or sub-group showed the identical partial sequences of 16S rDNA. The almost complete sequence of 16S rDNA for a representative strain of each group was determined to clarify their precise taxonomic positions. The length of sequences determined ranged from position 29 to 1524. The phylogenetic tree depicted by the neighbor-joining method and the K_{nuc} values based on the almost complete sequences showed that fourteen groups were assigned to the family *Flavobacteriaceae* (Fig. 1, 2). The taxonomic position of each group is discussed below.

The cfb 1 group

The cfb 1 group is the only group that was not included in the family *Flavobacteriaceae*. It formed a distinct lineage in the CFB phylum as the sister group of *Cyclobacterium marinum*. This group will be classified as a new genus from the point of view of the sequence difference.

The cfb 2 group

The cfb 2 group constituted a cluster with [*Cytophaga*] *latercula* (square brackets indicate a misnamed organism). [*Cytophaga*] *latercula* was not included in the genus *Cytophaga* as emended by Nakagawa and Yamasato (21). Its taxonomic position remains unsettled. The phylogenetic neighbor of cfb 2 is the genus *Salegentibacter*, which was proposed for [*Flavobacterium*] *salegens* (17). The cfb 2 strain is a halophilic bacterium, as are [*Cytophaga*] *latercula* and *Salegentibacter*. *Phylogenetic* analysis suggested that cfb 2 and [*Cytophaga*] *latercula* should be classified in a new genus or a new species of the genus *Salegentibacter*.

The cfb 3 group

The cfb 3 group was a sister group of the genus *Psychroflexus*. The genus *Psychroflexus* accommodates two species, *Psychroflexus gondowanensis* and *Psychroflexus*

Fig. 2. Phylogenetic positions of isolated strains in the family *Flavobacteriaceae*. All available data of the species belonging to the *Flavobacteriaceae* are included (data for *Myroides odoratimimus* and *Riemerella columbina* are not available). Data for all strains except *Empedobacter brevis*, "*Flavobacterium xylanivorum*" (strain number is unknown), and "*Sporocytophaga cauliformis*" are derived from the type strain. The details are described in the legend to Fig. 1.

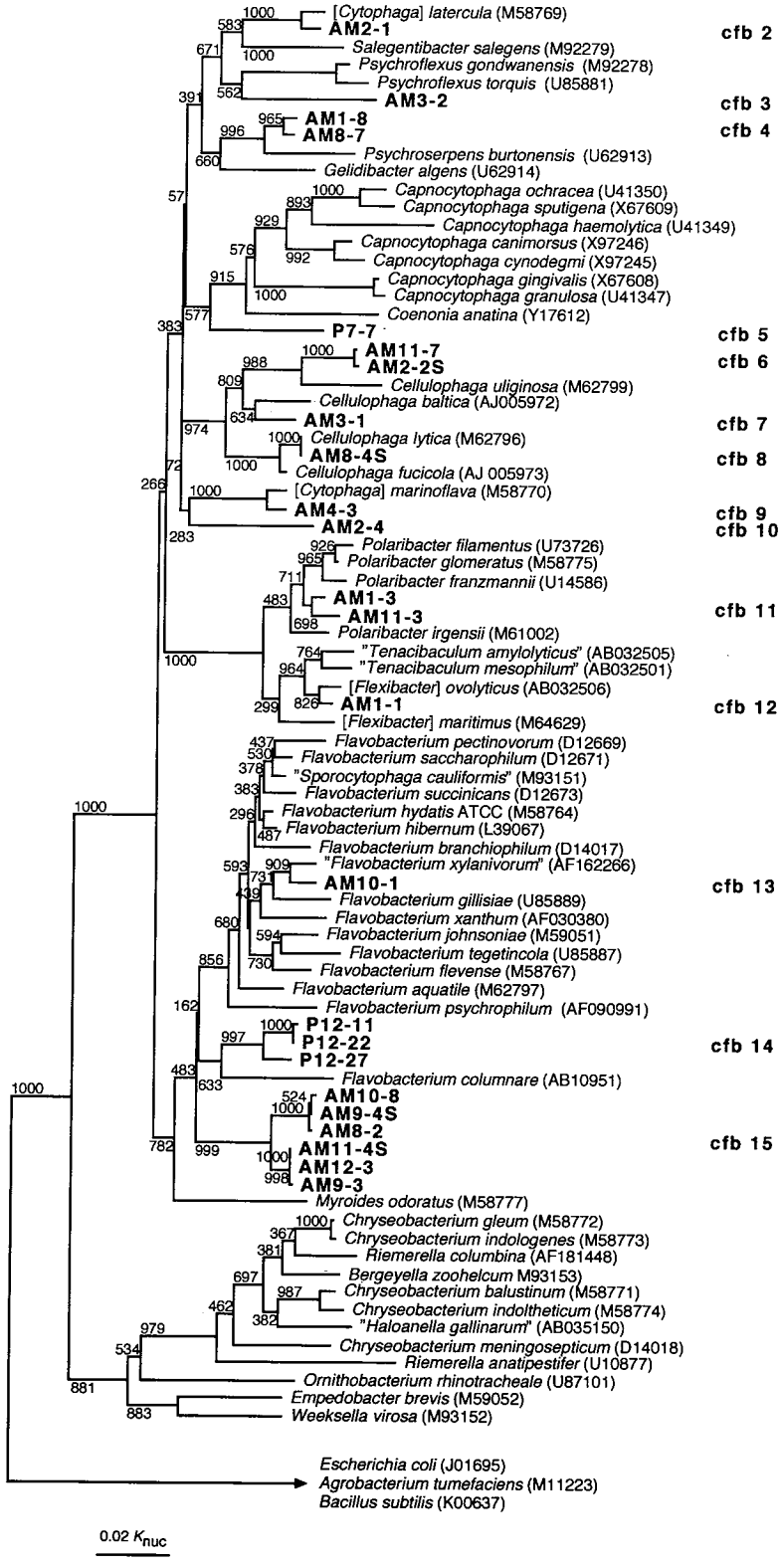
[Cytophaga] latercula (M58769)
AM2-1
cfb 2
Salegentibacter salegens (M92279)
Psychroflexus gondwanensis (M92278)
Psychroflexus torquis (U85881)
AM3-2
cfb 3
AM1-8
AM8-7
cfb 4
Psychroserpens burtonensis (U62913)
Gelidibacter algens (U62914)
Capnocytophaga ochracea (U41350)
Capnocytophaga sputigena (X67609)
Capnocytophaga haemolytica (U41349)
Capnocytophaga canimorsus (X97246)
Capnocytophaga cynodegmi (X97245)
Capnocytophaga gingivalis (X67608)
Capnocytophaga granulosa (U41347)
Coenonia anatina (Y17612)
P7-7
cfb 5
AM11-7
AM2-2S
cfb 6
Cellulophaga uliginosa (M62799)
Cellulophaga baltica (AJ005972)
AM3-1
cfb 7
Cellulophaga lytica (M62796)
AM8-4S
cfb 8
Cellulophaga fucicola (AJ 005973)
[Cytophaga] marinoflava (M58770)
AM4-3
cfb 9
AM2-4
cfb 10
Polaribacter filamentus (U73726)
Polaribacter glomeratus (M58775)
Polaribacter franzmannii (U14586)
AM1-3
AM11-3
cfb 11
Polaribacter irgensii (M61002)
"Tenacibaculum amylolyticus" (AB032505)
"Tenacibaculum mesophilum" (AB032501)
[Flexibacter] ovolyticus (AB032506)
AM1-1
cfb 12
[Flexibacter] maritimus (M64629)
Flavobacterium pectinovorum (D12669)
Flavobacterium saccharophilum (D12671)
"Sporocytophaga cauliformis" (M93151)
Flavobacterium succinicans (D12673)
Flavobacterium hydatis ATCC (M58764)
Flavobacterium hibernum (L39067)
Flavobacterium branchiophilum (D14017)
"Flavobacterium xylanivorum" (AF162266)
AM10-1
cfb 13
Flavobacterium gillisiae (U85889)
Flavobacterium xanthum (AF030380)
Flavobacterium johnsoniae (M59051)
Flavobacterium tegetincola (U85887)
Flavobacterium flevense (M58767)
Flavobacterium aquatile (M62797)
Flavobacterium psychrophilum (AF090991)
P12-11
P12-22
P12-27
cfb 14
Flavobacterium columnare (AB10951)
AM10-8
AM9-4S
AM8-2
AM11-4S
AM12-3
AM9-3
cfb 15
Myroides odoratus (M58777)
Chryseobacterium gleum (M58772)
Chryseobacterium indologenes (M58773)
Riemerella columbina (AF181448)
Bergeyella zoohelcum M93153)
Chryseobacterium balustinum (M58771)
Chryseobacterium indoltheticum (M58774)
"Haloanella gallinarum" (AB035150)
Chryseobacterium meningosepticum (D14018)
Riemerella anatipestifer (U10877)
Ornithobacterium rhinotracheale (U87101)
Empedobacter brevis (M59052)
Weeksella virosa (M93152)
Escherichia coli (J01695)
Agrobacterium tumefaciens (M11223)
Bacillus subtilis (K00637)
0.02 K_{nuc}

Fig. 2

torquis, that were isolated from Antarctica. *Psychroflexus torquis* is a halophilic and psychrophilic bacterium that requires NaCl for growth and cannot grow at above 20°C (5). In contrast, the cfb 3 strain grew at 25°C without NaCl. It will probably be classified in a new genus, considering the phylogenetic distance between cfb 3 and *Psychroflexus*, although detailed studies will be needed.

The cfb 4 group

The cfb 4 group was a sister group of the genera *Psychroserpens* and *Gelidibacter*. Both genera were proposed for strains isolated from Antarctica (4). *Psychroserpens burtonensis*, the sole species of the genus, requires sea water for growth and cannot grow at above 20°C. The cfb 4 group also needs sea water for growth, but growth occurred at 25°C. Further taxonomic studies may place it in a new genus.

The cfb 5 group

The cfb 5 group formed a distinct lineage at the periphery of the cluster composed of the genera *Capnocytophaga* and *Coenonia*. Strains of *Capnocytophaga* and *Coenonia* are associated with mammals and birds. The cfb 5 strain is a marine bacterium that requires sea water for growth; furthermore, the 16S rRNA analysis clearly separated cfb 5 from both *Capnocytophaga* and *Coenonia*.

The cfb 6, 7, and 8 groups

The cfb 6, 7 and 8 groups were included in the genus *Cellulophaga*. *Cellulophaga* was proposed to accommodate three species, *Cellulophaga lytica* (formerly [*Cytophaga*] *lytica*) as the type species, *Cellulophaga baltica*, and *Cellulophaga fucicola* (15). Bowman (3) added two species, *Cellulophaga uliginosa* transferred from the genus *Cytophaga*, and *Cellulophaga algicola*. The closest phylogenetic neighbors of cfb 6 and 7 were *Cellulophaga uliginosa* and *Cellulophaga baltica*, respectively. However, both groups were distant from the known species in the genus and probably constitute independent species. The cfb 8 strain showed identical sequences with *Cellulophaga lytica*, suggesting that it should be identified as *Cellulophaga lytica*.

The cfb 9 and 10 groups

The cfb 9 strain was related with [*Cytophaga*] *marinoflava* and formed a distinct cluster with cfb 10. It has been suggested that [*Cytophaga*] *marinoflava* is misplaced in the genus *Cytophaga* and should be classified in a new genus (20). Based on 16S rRNA sequence analysis, the cfb 9 strain will be placed in the same genus as [*Cytophaga*] *marinoflava*. The cfb 10 strain is comparatively remote from them, and it is unclear whether it should be classified in the same genus as cfb 9 and [*Cytophaga*] *marinoflava*.

The cfb 11 group

The cfb 11 group was placed in the genus *Polaribacter* proposed by Gosink *et al.* (11). *Polaribacter* includes four species that were isolated from the Arctic and the Antarctic and cannot grow at above 25°C. The cfb 11 isolates are the first *Polaribacter*

strains that are not psychrophiles, being isolated from the sub-tropical zone.

The cfb 12 group

The cfb 12 group constituted a cluster with the new genus "*Tenacibaculum*". Suzuki *et al.* (28) proposed establishing the new genus "*Tenacibaculum*" with two new species, "*Tenacibaculum amylolyticus*" and "*Tenacibaculum mesophilum*", and transferring [*Flexibacter*] *ovolyticus* and [*Flexibacter*] *maritimus* into "*Tenacibaculum*". Phylogenetic analysis clearly showed that cfb 12 should be classified in "*Tenacibaculum*". Its phylogenetic neighbor is [*Flexibacter*] *ovolyticus*, a known fish pathogen (13). Detailed taxonomic investigation should confirm the taxonomic position of cfb 12 in the genus "*Tenacibaculum*".

The cfb 13, 14, and 15 groups

The cfb 13, 14 and 15 groups were affiliated with the genus *Flavobacterium*, but each of them formed a distinct lineage in the genus. The cfb 13 strain was related with the invalid taxon, "*Flavobacterium xylanivorum*", of which the 16S rRNA sequence is available. It is clear from phylogenetic analysis that cfb 13 should be classified in *Flavobacterium*, while cfb 14 and 15 were located at the periphery of *Flavobacterium*. The bootstrap values for clustering of cfb 14 and 15 with *Flavobacterium* are low, indicating that the classification of these groups in *Flavobacterium* is uncertain. Chemotaxonomic and phenotypic analysis should clarify the taxonomic allocation of these bacteria.

As discussed above, we have isolated bacterial strains belonging to the CFB phylum from the sub-tropical zone of Japan. These strains are divided into fifteen groups, and most of them appear to represent novel genera and/or species based on phylogenetic analyses derived from 16S rRNA sequences. It is evident that the biodiversity of the CFB phylum is much wider than was previously thought. Further investigation will be needed to clarify the biodiversity of the CFB group of Bacteria, but it is clear that the sub-tropical zone of Japan, including Amami-O-Shima and Iriomote Shima, is a treasure house of new microbiological genetic resources.

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Taxonomic significance of 2-hydroxy fatty acid profiles of the species in the genus *Sphingomonas* and related taxa

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Summary

Cellular fatty acid compositions of 29 strains of the genus *Sphingomonas* and related taxa grown on different media and at different temperatures was examined from the viewpoint of taxonomy. These bacteria contained a large amount of straight-chain saturated and unsaturated acids of $C_{16:0}$, $C_{18:1}$ or $C_{17:1}$, 2-hydroxy fatty acids of $C_{14:0}$ or $C_{15:0}$, and a minor amount of other acids. The total compositions of saturated and unsaturated fatty acids were affected by the culture temperature, and the minor components or second most common type of whole-cell or glycosphingolipid (GSL) fatty acids were affected by the composition of growth media. However, no noticeable differences were found in the primary components of whole-cell or GSL fatty acids with changes in culture conditions. 2-Hydroxy fatty acid profiles of the GSLs of the strains grown on the rich medium (R medium) at 30°C characterized the sphingomonads of each of four phylogenetic clusters. These results show that when the culture conditions are standardized, the fatty acid profile provides reliable information for identification of these bacteria.

Keywords: glycosphingolipid, fatty acid profile, Sphingomonas.

Since the proposal of the genus *Sphingomonas* by Yabuuchi et al. (18), large numbers of new species isolated from various environments have been added, and it has come to encompass a relatively broad range of species with respect to physiology and ecology. These species are also genetically relatively diverse and can be classified into at least four clusters within the α-4 subgroup of the *Proteobacteria* (12), as reported previously (11, 21). However, remarkable differences in morphological and physiological characteristics that distinguish these clusters have not been found. *Segers et al.* (11) suggested that *S. yanoikuyae* and *S. capsulata* belong to a genus other than the genus *Sphingomonas*, and this suggestion was supported by polyamine profiling (2, 7, 9). Kawahara *et al.* (5)

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suggested that oligosaccharide types in the glycosphingolipids (GSLs) are different among species in the genus *Sphingomonas* and should be useful for taxonomy of this genus. The GSLs are the most important characteristic component of the genus *Sphingomonas* and are composed of the long-chain base dihydrosphingosin, 2-hydroxy (2-OH) fatty acids, and oligosaccharides. The 2-OH fatty acid compositions of GSLs also differ with species in the genus *Sphingomonas*: most species have 2-hydroxymiristic acid (2-OH $C_{14:0}$) as the primary component, but *S. macrogoltabidus* and *S. terrae*, which are included in the phylogenetic subclass 2 of the genus *Sphingomonas* (15, 21), contain 2-OH $C_{16:0}$ in addition to 2-OH $C_{14:0}$ or 2-OH $C_{15:0}$. Thus, the chemotaxonomic and phylogenetic heterogeneity existing in the genus *Sphingomonas* calls for taxonomic improvement regarding this genus and related sphingomonads.

To determine the reliability and usefulness of fatty acid profiles in the taxonomy of sphingomonads, we investigated the effects of culture conditions on cellular fatty acid composition. This paper describes the characterization of the fatty acid profiles of sphingomonads grown on different media and at different temperatures, and the usefulness of this character for classification of species in the genus *Sphingomonas* and related taxa.

Materials and Methods

Bacterial strains and cultivation. The strains used in this study are shown in Table 1. They are divided into four clusters based on 16S rDNA sequences as reported previously (16). All strains were cultured aerobically at 24°C, 30°C, and 37°C in peptone-yeast medium (PY medium), which contains, per liter, 10 g of peptone, 2 g of yeast extract, and 1 g of $MgSO_4 \cdot 7H_2O$ (pH 7.0); and at 30°C in rich medium (R medium)(19), which contains, per liter, 10 g of peptone, 5 g of yeast extract, 5 g of malt extract, 5 g of Bacto-casamino acids, 2 g of beef extract, 2 g of glycerol, 50 mg of Tween 80, and 1 g of $MgSO_4 \cdot 7H_2O$ (pH 7.0).

Cellular fatty acid analysis. Cells were harvested at the mid-exponential phase of growth, washed with distilled water, and freeze-dried. Cellular fatty acids were extracted from dried cells (50 mg) by acid methanolysis, purified (20), and analyzed by GLC-MS with a Shimadzu GCMS-QP 5000 spectrometer combined with a CLASS-5000 MS Workstation computer system. Gas-liquid chromatography (GLC) was performed with a Shimadzu GC-17A gas chromatograph equipped with a BPX 70 capillary column (SGE) containing 70% cyanopropyl equivalent modified siloxane (50 m × 0.25 mm). Components were separated with helium as carrier gas at a flow rate of 1.4 ml min^{-1} under the following thermal conditions: 80°C for 2 min, 80-150°C at 15°C min^{-1}, 150-250°C at 8°C min^{-1}, and 250°C for 5 min.

Glycosphingolipid analysis. GSLs were extracted from dried cells (50 mg) with chloroform-methanol (1:3, v/v), treated with 0.1 N NaOH at 100°C for 1 h, and extracted twice with the chloroform-methanol mixture (6). The lipids were separated by TLC with Merck Kieselgel $60F_{254}$ plates using chloroform-methanol-acetic acid-H_2O (100 : 20 : 12 : 5, v/v) as the developing solvent (6). The major spot, corresponding to the monosaccharide- type GSL, was scraped off from the plate and subjected to fatty acid

Table 1. Bacterial strains used in this study.

Phylogenetic cluster and species		Strain (IFO no.)	Other designations *	Sources
Cluster I	*Sphingomonas adhaesiva*	15099^T	ATCC51229^T, DSM 7418^T, GIFU 11458^T	Sterile water
	Sphingomonas asaccharolytica	15499^T	ATCC 51839^T	Apple tree roots
	Sphingomonas echinoides	15742^T	ATCC 14820^T, DSM 50409^T, NCIB 9420^T	Plate contaminant
	Sphingomonas mali	15500^T	ATCC 51840^T, DSM 10565^T, JCM 10193^T	Apple tree roots
	Sphingomonas parapaucimobilis	15100^T	ATCC 51231^T, DSM 7463^T, GIFU 11387^T	Urine
	Sphingomonas paucimobilis	13935^T	ATCC 2983^T, DSM 1098^T, NCTC 11030^T	Hospital respirator
	Sphingomonas pruni	15498^T	JCM 10277^T	*Prunus persica* roots
	Sphingomonas sanguinis	13937^T	JCM 7514^T	Blood
	Sphingomonas trueperi	16157^T	ATCC 12417^T, DSM 7225^T, LMG 2142^T	Soil
	Sphingomonas sp. OGS-47	15495		Paddy soil (10)
	Sphingomonas sp. Y-22	15496		Paddy soil (10)
	Sphingomonas sp. Y-39	15497		Paddy soil (10)
	Sphingomonas sp. Sus-A	15914		Corrosion of stainless steel welds (8)
Cluster II	*Sphingomonas chlorophenolica*	16172^T	ATCC 33790^T, JCM 10275^T	Soil
	Sphingomonas herbicidovorans	16415^T	DSM 11019^T	Soil
	Sphingomonas yanoikuyae	15102^T	ATCC 51230^T, DSM 7462^T, GIFU 9882^T	Clinical specimen
Cluster III	*Sphingomonas aromaticivorans*	15084^T	CIP 105152^T, SMCC F199^T	Coastal plain sediments
	Sphginomonas capsulata	12533^T	ATCC 14666^T, DSM 30196^T, GIFU 11526^T	Stocked distilled water
	Sphingomonas rosa	15208^T	NCPPB 2661^T, IAM 14222^T	*Rosa* sp. roots
	Sphingomonas stygia	16085^T	ATCC 70280^T, CIP 10514^T, SMCC B0712^T	Coastal plain sediments
	Sphingomonas subarctica	16058^T	DSM 10700^T, JCM 10398^T	Fluidized-bed reactor
	Sphingomonas subterranea	16086^T	ATCC 70279^T, CIP 105153^T, SMCC B0478^T	Coastal plain sediments
Cluster IV	*Sphingomonas macrogoltabidus*	15033^T	ATCC 51380^T, DSM 17324^T	Soil
	Sphingomonas terrae	15098^T	ATCC 51381^T, CIP 10419^T, JCM 10195^T	Activated sludge
	Sphingomonas sp. Cu-B	15915		Underground water (8)
	Sphingomonas sp. Cu-D	15917		Underground water (8)
Blastomonas natatoria		15649^T	ATCC 35951^T, DSM 3183^T, JCM 10396^T	Fresh-water swimming pool
Erythrobacter longus		14126^T	ATCC 33941^T, DSM 6997^T, JCM 6170^T	Seaweed *Enteromorpha linza*
Rhizomonas suberifaciens		15211^T	ATCC 49355^T, JCM 8521^T	Corked lettuce root

* For abbreviation see facing page.

analysis as noted above.

Results

Whole-cell fatty acid profiles

The whole-cell fatty acid compositions of 29 strains grown at 30°C on PY medium are shown in Table 2. Most strains of the genus *Sphingomonas* contained straight-chain unsaturated acids of $C_{18:1}$ and 2-hydroxy acids of $C_{14:0}$ as the primary component, and straight-chain saturated acids of $C_{16:0}$ as the second most common type of the whole-cell fatty acids, but other cellular fatty acid components varied among strains. *S. asaccharolytica* IFO 15499^T contained 2-OH $C_{15:0}$ as the primary component, and *Sphingomonas* sp. IFO 15914, *S. terrae* IFO 15098^T and *Sphingomonas* sp. IFO 15917 contained $C_{17:1}$ and 2-OH $C_{15:0}$ as the primary components of whole-cell fatty acids.

Effect of culture temperature on whole-cell fatty acids

Whole-cell fatty acid compositions of the representative strains of the genus *Sphingomonas* and related taxa grown at different temperatures are shown in Table 3. The compositions of unsaturated acids of $C_{18:1}$ in all strains are lower in each strain grown at 37°C than at 24°C. On the other hand, the compositions of unsaturated acids of $C_{17:1}$ of *Sphingomonas* sp. IFO 15914, *S. terrae* IFO 15098^T, *B. natatoria* IFO 15649^T, *E. longus* IFO 14126^T and *R. suberifaciens* IFO 15211^T, did not change with culture temperature. However, the total compositions of unsaturated acids of $C_{16:1}$, $C_{17:1}$ and $C_{18:1}$ of the whole-cell fatty acids of all strains except *Sphingomonas* sp. IFO 15914 decreased with the increase in culture temperature. On the contrary, the compositions of each saturated acid and total compositions of 2-OH $C_{14:0}$ and 2-OH $C_{15:0}$ of all strains except *Sphingomonas* sp. IFO 15914 were higher in each strain grown at 37°C than at 24°C. *Sphingomonas* sp. IFO 15914 did not grow well at 24°C, and the fatty acid composition of this strain grown at 24°C was not reliable.

Effect of the composition of the culture medium on the whole-cell fatty acids

Whole-cell fatty acid compositions of the representative strains of the genus *Sphingomonas* and related taxa grown on different culture media are shown in Table 4. Many minor components such as $C_{15:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:0}$, $C_{18:2}$, 2-OH $C_{15:0}$, 2-OH $C_{16:0}$,

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* Abbreviations: American Type Culture Collection, Manassas, VA, USA; CIP, Collection of the Institute Pasteur, Paris, France; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germeny; GIFU, Department of Microbiology, Gifu University School of Medicine, Gifu, Japan; IAM, Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan; IFO, Institue for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Hirosawa, Wako, Japan; LMG, Laboratorium voor Microbiologie, Universiteit

Gent, Belgium ; NCIB, National Collection of Industrial Bacteria, NCIMB (National Collection of Industrial and Marine Bacteria) Ltd., Aberdeen, Scotland, UK.; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpendern, UK.; NCTC, National Collection of Type Cultures, Central Public Health Lab. Service, London, UK.; SMCC, Subsurface Microbial Culture Collections, Florida State University, USA.

Table 2. Whole-cell fatty acid compositions of the species in the four clusters of *Sphingomonas* and related microorganisms.

Phylogenetic cluster and species	IFO no.	Fatty acid composition (%)																	
		Nonpolar														2-OH			
		14:0	14:1	14:2	15:0	15:1	16:0	16:1	16:2	17:0	17cy	17:1	18:0	18:1	18:2	14:0	15:0	16:0	16:1
Sphingomonas Cluster I (genus *Sphingomonas* sensu stricto)																			
S. adhaesiva	15099^T						13	7				6		47		28			
S. asaccharolytica	15499^T						6			4		16	12	22		15	24		
S. echinoides	15742^T				4		11	2		2		15	4	33		23	6		
S. mali	15500^T						10					10	16	36		20	8		
S. parapaucimobilis	15100^T						7	6						56		32			
S. paucimobilis	13935^T						10	2						50		38			
S. pruni	15498^T						10	2						50		27			
S. sanguinis	13937^T	1					11	6	1					50		31			
S. trueperi	16157^T			8			14		2			2		53		25			
Sphingomonas sp.	15495			8	8		15			3		8	6	31		21	4		
Sphingomonas sp.	15496			7	3		17			2		'	7	32		22	4		
Sphingomonas sp.	15497			5			24			1		2	9	33		25	1		
Sphingomonas sp.	15914										8	47		12	4	5	20		
Sphingomonas Cluster II																			
S. chlorophenolica	16172^T						7	9	3			3	2	46		26	1	2	3
S. herbicidovorans	16415^T						5	7					5	49		28		3	3
S. yanoikuyae	15102^T			8			10	8	2				4	32		34		3	
Sphingomonas Cluster III																			
S. aromaticivorans	16084^T						23					3		42		23	4		
S. capsulata	12533^T						5	3				3		52		35	2		
S. rosa	15208^T						13	7						50		33			
S. stygia	16085^T						25					11		36		24	3		
S. subarctica	16058^T						14	10						52		20			
S. subterranea	16086^T						6	11				16		38		21	9		
Sphingomonas Cluster IV																			
S. macrogoltabidus	15033^T	2	2		13	3	10	21	6				2	23		10		5	
S. terrae	15098^T				4		5	11		2	5	32		18		7	18		
Sphingomonas sp.	15915						15	20				11	3	18		10	8	11	
Sphingomonas sp.	15917				3		4	4		4	6	34	2	16		4	24		
B. natatoria	15649^T						13	8				16		35		9	6	4	7
E. longus	14126^T				3		6	8		5	6	31	2	18	4	18	2		
R. suberifaciens	15211^T				3		5	5		4	6	31	2	16	4	21	2		

Table 3. Whole-cell fatty acid compositions of the sphingomonads grown at different temperatures.

Phylogenetic cluster and species	Temp.	Fatty acid composition (%)																	
		Nonpolar													2-OH				
		14:0	14:1	14:2	15:0	15:1	16:0	16:1	16:2	17:0	17cy	17:1	18:0	18:1	18:2	14:0	15:0	16:0	16:1
Sphingomonas Cluster I (genus *Sphingomonas* sensu stricto)																			
S. paucimobilis 13935^T	37°C						9	2				2		57		30			
	30°C						10	2						50		38			
	24°C						6	3				2		64		25			
Sphingomonas sp. 15914	37°C						2				8	47		17		6	20		
	30°C										8	47		12	4	5	20		
	24°C				2		4			1	4	21		21		9	23		
Sphingomonas Cluster II																			
S. yanoikuyae 15102^T	37°C			3			9	4	3				3	35		36		4	3
	30°C			8			10	8	2				4	39		34		3	
	24°C						9	6	2				4	42		36		1	
Sphingomonas Cluster III																			
S. subterranea 16086^T	37°C						6	17				5		43		27	3		
	30°C						6	11				16		38		21	9		
	24°C						1	19				5		49		24	2		
Sphingomonas Cluster IV																			
S. macrogoltabidus 15033^T	37°C				5		7	17						40		10		16	5
	30°C	2	2		13	3	10	21	6				2	23		10		5	
	24°C						8	28						41		14		9	
S. terrae 15098^T	37°C				4		4	12		2	4	27		15		10	22	1	
	30°C				4		5	11		2	5	32		18		7	18	2	
	24°C				1		5	13			4	24		29		8	12	3	
B. natatoria 15649^T	37°C				4		6	5		6	4	32		13		19	7	4	
	30°C						13	8				16		35		10	6	4	7
	24°C				1		4	9			6	31		34		5	8	2	
E. longus 14126^T	37°C				8		10	6		8	4	31		14		2	17	1	
	30°C				3		6	5		5	6	31	2	18		4	18	2	1
	24°C				1		6	8		2	6	31		29		5	10	2	
R. suberifaciens 15211^T	37°C				6		7	5		7	5	32		12		22	3	1	
	30°C				3		5	5		4	6	31	2	16		21	4	2	
	24°C				2		6	9		2	6	33		22		10	4	1	

Table 4. Whole-cell fatty acid compositions of sphingomonads grown on different media.

Phylogenetic cluster and species	Medium	Fatty acid composition (%)																	
		Nonpolar														2-OH			
		14:0	14:1	14:2	15:0	15:1	16:0	16:1	16:2	17:0	17cy	17:1	18:0	18:1	18:2	14:0	15:0	16:0	16:1
Sphingomonas Cluster I (genus *Sphingomonas* sensu stricto)																			
S. echinoides 15742^T	PY				4		11	2		2		15	4	33		23	6		
	R						19					9	3	42		27			
S. mali 15500^T	PY						10					10	16	36		20	8		
	R						9					9	19	31		24	8		
S. paucimobilis 13935^T	PY						10	2						50		38			
	R						17	3						57		25			
Sphingomonas sp. 15914	PY										8	47		12	4	5	20		
	R				4		3			6	5	41		21		5	15		
Sphingomonas Cluster II																			
S. chlorophenolica 16172^T	PY						7	9	3			3	2	46		26	1	2	3
	R						9	10	2					48		29		1	
S. herbicidovorans 16415^T	PY						5	7					5	49		28		3	3
	R	1	3	12			7	10	4					46		15			
S. yanoikuyae 15102^T	PY			8			10	8	2				4	32		34		3	
	R						10	3						45		42			
Sphingomonas Cluster III																			
S. capsulata 12533^T	PY						5	3				3		52		35	2		
	R						10					2		60		28			
S. stygia 16085^T	PY						25					11		36		24	3		
	R						18	8				5	13	36		20			
S. subterranea 16086^T	PY						6	11				16		38		21	9		
	R						3	20				2		45		26	1		
Sphingomonas Cluster IV																			
S. macrogoltabidus 15033^T	PY	2	2		13	3	10	21	6				2	23		10		5	
	R						12	31						36		13		8	
S. terrae 15098^T	PY				4		5	11		2	5	32		18		7	18		
	R				2		6	15		2	4	23		26		9	12	2	
B. natatoria 15649^T	PY						13	8				16		35		9	6	4	7
	R						4	4		4	8	39		20		8	15	1	
E. longus 14126^T	PY				3		6	8		5	6	31	2	18	4	4	18	2	
	R				3		5	6		3		35		20		3	14	2	
R. suberifaciens 15211^T	PY				3		5	5		4	6	31	2	16	4	21	2		
	R				5		7	8		4		34		18		13	3		

Table 5. 2-Hydroxy fatty acid composition of sphingolipids of species of the genus *Sphingomonas* and related genera grown on different media.

Phylogenetic cluster and species	Strain	Composition (%)						
		PY Medium				R medium		
		14:0	15:0	16:0	16:1	14:0	15:0	16:0
Cluster I (genus *Sphingomonas*)								
S. adhaesiva	IFO 15099^T	100				100		
S. asaccharolytica	IFO 15499^T	39	61			18	82	
S. echinoides	IFO 15742^T	79	21			100		
S. mali	IFO 15500^T	71	29			75	25	
S. parapaucimobilis	IFO 15100^T	100				100		
S. paucimobilis	IFO 13935^T	100				100		
S. pruni	IFO 15498^T	86	14			100		
S. sanguinis	IFO 13937^T	100				100		
S. trueperi	IFO 16157^T	98	2			100		
Sphingomonas sp.	IFO 15495	81	19			100		
Sphingomonas sp.	IFO 15496	84	16			100		
Sphingomonas sp.	IFO 15497	96	4			100		
Sphingomonas sp.	IFO 15914	18	82			25	75	
Cluster II (genus *Sphingobium*)								
S. chlorophenolica	IFO 16172^T	84	3	5	8	100		
S. herbicidovorans	IFO 16415^T	83		8	9	100		
S. yanoikuyae	IFO 15102^T	92		8		100		
Cluster III (genus *Novosphingobium*)								
S. aromaticivorans	IFO 16084^T	87	13			100		
S. capsulata	IFO 12533^T	96	4			100		
S. rosa	IFO 15208^T	100				100		
S. stygia	IFO 16085^T	89	11			100		
S. subarctica	IFO 16058^T	87		13		100		
S. subterranea	IFO 16086^T	70	30			100		
Cluster IV (genus *Sphingopyxis*)								
S. macrogoltabidus	IFO 15033^T	67		33		62		38
S. terrae	IFO 15098^T	32	51	12	5	39	52	9
Sphingomonas sp.	IFO 15915	34	27	28	1		nd	
Sphingomonas sp.	IFO 15917	13	75	7	5		nd	
Blastomonas natatoria	IFO 15649^T	35	23	15	27	16	79	5
Blastomonas ursincola	DSM 9006	60	26	14		59	22	19
Erythrobacter longus	IFO 14126^T	13	75	6	6	16	74	10
Porphrobacter neustonensis	DSM 9434^T	54	24	22			nd	
Rhizomonas suberifaciens	IFO 15211^T	90	10			84	16	

nd, not determined.

and 2-OH $C_{16:1}$ that were detected in the strains grown on PY medium were not found in the strains grown on R medium. However, there was little difference in the primary components of the whole-cell fatty acids of the strains grown on different media.

2-OH fatty acids of glycosphingolipid

The fatty acid compositions of GSLs extracted from the cells grown on different media at 30°C were examined (Table 5). 2-Hydroxy fatty acids of $C_{14:0}$ and $C_{15:0}$ were detected in *S. echinoides* IFO 15742^T, *S. mali* IFO 15500^T, *S. pruni* IFO 15498^T,

Sphingomonas trueperi IFO 16157^T, *Sphingomonas* sp. IFO 15495, *Sphingomonas* sp. IFO 15496, and *Sphingomonas* sp. IFO 15497 as the primary components (71–98 %) or as the second most common type of fatty acids (2–29 %) when they were grown on PY medium. However, when they were grown on R medium, only 2-hydroxy acids of $C_{14:0}$ were detected in these strains. The sole exception was *S. mali* IFO 15500^T, which contained 2-OH $C_{14:0}$ and 2-OH $C_{15:0}$ as the primary (75 %) and the second most common type of fatty acid (25 %), respectively, regardless of the medium composition. On the other hand, *S. asaccharolytica* IFO 15499^T and *Sphingomonas* sp. IFO 15914 also contained 2-hydroxy acids of $C_{15:0}$ and $C_{14:0}$ as the primary (61–82 %) and the second most common type of fatty acid (18–39 %), respectively, regardless of the medium composition. These results showed that two types of GSLs exist in the strains of cluster I: GSLs composed of only 2-OH $C_{14:0}$, or 2-OH $C_{14:0}$ with small amounts of 2-OH $C_{15:0}$; and GSLs composed of 2-OH $C_{15:0}$ with small amounts of 2-OH $C_{14:0}$.

On the other hand, minor or second most common type of fatty acids of 2-hydroxy acids of $C_{15:0}$, $C_{16:0}$, or $C_{16:1}$ (3–30 %) were detected in the strains of clusters II and III when they were grown on PY medium. However, when they were grown on R medium, only 2-OH $C_{14:0}$ was detected in these strains. These results showed that the GSLs of the strains in clusters II and III are composed of 2-OH $C_{14:0}$ only. On the contrary, strains in cluster IV contained 2-hydroxy acids of $C_{14:0}$, $C_{15:0}$, or $C_{16:0}$ as the primary components of fatty acids of GSLs, regardless of the medium composition. These results showed that the GSLs of the strains in cluster IV are composed of 2-hydroxy acids of $C_{14:0}$, $C_{15:0}$, or $C_{16:0}$.

Discussion

Bacterial fatty acid composition was considered to be useful for the study of interrelations and for rapid identification of *Pseudomonas* species (4), bacteriochlorophyll-containing bacteria, which include *Rhodospirillaceae* genera and *Erythrobacter* (17), and coryneform bacteria (13). It has been suggested that the properties of fatty acid synthase are useful for the taxonomic characterization of corynebacteria (1).

To clarify the usefulness of fatty acid compositions for taxonomic purpose and for identification of the sphingomonads, we investigated the effects of culture conditions on fatty acid composition. In addition, we examined the GSL fatty acid profiles of sphingomonads grown on different media, because GSL is the most important component for characterizing sphingomonads taxonomically.

In general, when cells are grown at higher temperatures, the composition of saturated fatty acids increases and the composition of unsaturated fatty acids decreases (1, 13). In this study, we confirmed that the culture temperature affected the total compositions of saturated and unsaturated fatty acids. On the other hand, contrary to the report that the composition of the culture medium did not affect the fatty acid composition (13), we found that minor components or second most common type of whole-cell or GSL fatty acids were affected by the medium composition.

It is evident that the taxonomic comparisons of fatty acid composition should only be

done with data obtained from cells grown under the same culture conditions. In this study, we found that the fatty acid profiles of cells grown on rich medium (R medium) appeared to be simpler and more useful for taxonomic purposes than those obtained with peptone-yeast medium (PY medium). Strains of the four clusters of the genus *Sphingomonas* can be characterized by their GSL fatty acid profiles: cluster I strains have GSL fatty acids of 2-hydroxy acids of $C_{14:0}$ and/or $C_{15:0}$; cluster II and cluster III strains have GSL fatty acids of only 2-OH $C_{14:0}$; and cluster IV strains have GSL fatty acids of 2-hydroxy fatty acids of $C_{14:0}$, $C_{15:0}$, and $C_{16:0}$.

Species in the genus *Sphingomonas* are also characterized by their polyamine profiles: members of cluster I contain homospermidine, while members of clusters II, III and IV all have spermidines as the primary components of polyamine (16). The results obtained in this study confirmed that the species in cluster IV can be separated from members of clusters I, II, and III by the 2-hydroxy fatty acids of $C_{14:0}$, $C_{15:0}$ and $C_{16:0}$ in their GSLs. We believe that the four clusters of the genus *Sphingomonas* can be taxonomically distinguished from each other by a combination of phylogenetic data based on 16S rDNA analyses and chemotaxonomic characteristics including polyamine profiles and 2-OH fatty acid profiles of GSLs.

Thus, when the culture conditions are standardized, the fatty acid profiles provide reliable information for the taxonomy of sphingomonads and are useful for their identification.

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Taxonomic studies on *Streptomyces violaceoruber* group and related species based on *gyrB* sequences

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Summary

To determine the taxonomic status of 23 strains of *Streptomyces violaceoruber* group and related species, phylogenetic analysis of partial sequences of *gyrB*, which encodes DNA gyrase subunit B protein, was performed. *S. violaceoruber* group (20 strains) including "*S. lividans*" and *S. coelicolor* A3 (2), which are hosts for gene manipulation of the genus *Streptomyces*, have been generally characterized as follows. Spore chains form spirals and the spore surface is smooth. Aerial mass is gray or brownish gray, and substrate mycelium is pale yellow or brown to purple or violet. Soluble pigments are none, or blue, red, violet or purple. Melanin formation is negative. All strains belonging to this group formed a tight cluster with high similarity value (99.2 %) of *gyrB* sequences, suggesting that they are synonymous. On the other hand, *S. althioticus*, which has a spiny spore surface, *S. tuirus*, which is positive in melanin formation and utilizes raffinose and sucrose for growth, and *S. violaceus*, which has a spiny spore surface, is positive in melanin formation and utilizes raffinose and sucrose, had similarity values of 93.1, 93.5 and 90.5 % with the type strain of *S. violaceoruber*.

Keywords: *gyrB* gene, phylogenetic analysis, *Streptomyces violaceoruber*, taxonomy.

The genus *Streptomyces* contains nearly 500 validly described species which are basically defined by their phenotypic characteristics such as morphology of spore chains and spore surface, aerial mass and substrate mycelium color, formation of melanin pigment, diffusible pigments, and utilization of carbohydrates and DNA homology. Therefore, classification and identification of new isolates are laborious and time-consuming work, and in addition require experience. We have been searching for a simple, accurate and simple method for classification and identification of *Streptomyces* species.

Kataoka *et al.* (7) employed a variable region (120 bp) of 16S rDNA for clustering 89 strains of *Streptomyces* species belonging to category I in Bergey's Manual of Systematic Bacteriology (21). Rainey *et al.* (12) also established a phylogenetic classification of more than 400 species of the genus *Streptomyces* using three highly variable regions (about

800 bp) of 16S rDNA. Both studies concluded that the phylogenetic analysis of 16S rDNA sequence is a useful tool for classification and identification of *Streptomyces* species, and additionally for constructing a database for identification of *Streptomyces* species. Recently, Yamamoto and Harayama, Yamamoto *et al.* and Kasai *et al.* reported that phylogenetic analysis of *gyrB*, which encodes DNA gyrase subunit B protein, is a useful tool for discrimination of species level in *Acinetobacter* (22, 24), *Pseudomonas* (23, 25) and *Micromonospora* (6), respectively. Phylogenetic analysis of *gyrB* gives a higher degree of resolution than that of 16S rDNA because of the rapid evolutionary substitution of nucleotides in *gyrB* gene. We attempted to employ this method for classification at the species level in the genus *Streptomyces*.

This paper deals with classification of *Streptomyces violaceoruber group and related species based on phylogenetic analysis of gyrB* sequences.

Materials and Methods

Strains and culture conditions. Table 1 lists 23 strains which were considered to belong to *S. violaceoruber* and related species used in this study. All strains were incubated in YG medium (1 % glucose and 1 % yeast extract, pH 7.0) in a test tube for two days at 28°C on a reciprocal shaker. Incubated cells were harvested, washed three times with sterile distilled water, and stocked in a refrigerator (−20°C) until used.

DNA extraction. DNA was extracted from washed cells by the method of Saito and Miura (14).

PCR amplification of the gyrB gene. The *gyrB* gene was amplified by PCR (13) using TaKaRa Taq LX (Takara Shuzo, Japan) and a pair of primers as follows: PF-1 (5′-GAGGTCGTGCTGACCGTGCTGCACGCGGGCGGCAAGTTCGGC-3′), and PR-2 (5′-GTTGATGTGCTGGCCGTCGACGTCGGCGTCCGCCAT-3′), which were newly designed from the conserved regions of the *gyrB* sequence of *S. coelicolor* A3(2) (1). The *gyrB* gene was amplified in a total volume of 50 μl containing 10 pmol of each primer, 0.1 μg of target DNA, 50 μl of 10×buffer and 2.5 units Taq polymerase (LX, Takara Shuzo) in a 0.5-ml microtube. The DNA was amplified under the following conditions: 95°C for 3 min for denaturation of target DNA, then 30 cycles of denaturation at 95°C for 0.5 min, primer annealing at 65°C for 0.5 min, and primer extension at 72°C for 1 min, and finally 72°C for 4 min to complete amplification. The products was cooled to 4°C.

Sequencing of gyrB. After purifying PCR product, it was subjected to cycle sequencing by using a BigDye Terminator Cycle Sequencing kit by Amplitaq FS (PE Applied Biosystems) and a Gene Amp PCR System 9700 (Perkin Elmer) according to the manufacturer's protocol with the following seven primers: F-1 (5′-GAGGTCGTGCTGACCGTGCTGCA-3′, position 355 to 378), F-352 (5′-TACCACTACGAGGGCGGCATC-3′, position 779 to 799), F-701 (5′-AGCCGCAGTTCGAGGGCCAGAC-3′, position 1128-1149), R-1 (5′-GTTGATGTGCTGGCCGTCGACGT-3′, position 1637 to 1659), R-996 (5′-CTCGACGATGAAGATCTCGCAC-3′, position 1393-1414), R-728 (5′-GTCTTGGTCTGGCCCTCGAACTG-3′, position 1133 to 1155) and R-4 (5′-

Table 1. List of strains used in this study.

Organism	Other designation
S. anthocyanicus IFO 14892^T	ATCC 19821
"*S. caesius*" IFO 13376^T	ISP 5419
S. coelescens IFO 13378^T	ISP 5421
S. coelicolor IFO 3114	formerly *Actinomyces violaceus-ruber*
S. coelicolor IFO 3176	ATCC 10147, Waksman strain 3034
S. coelicolor IFO 3504	Ogata strain (IFO)
"*S. cyanocolor*" IFO 13034^T	ISP 5425
"*S. cyanogenus*" IFO 13035^T	ISP 5426
S. humiferus IFO 12244^T	formerly *Actinopycnidium caeruleum*
"*S. lazureus*" IFO 13384^T	ISP 5433
"*S. lividans*" IFO 13385^T	ISP 5434
"*S. lividans*" 66	Hopwood strain 1326
S. rubrogriseus IFO 15455^T	JCM 6927
S. tricolor IFO 15461^T	JCM 5085
"*S. tumuli*" IFO 13492^T	ISP 5605
S. violaceolatus IFO 13101^T	ISP 5434
S. violaceoruber IFO 12826^T	ISP 5049
S. violaceoruber IFO 15146	formerly *S. coelicolor* A3(2), (a single spore of Waksman 3443)
S. violaceoruber IFO 15731	JCM 4691;Hopwood strain 749
S. violaceoruber IFO 15732	JCM 4979; G. Sermonti A3(2) (Waksman's 3443)
Related species	
S. althioticus IFO 12740^T	ISP 5092
S. tuirus IFO 15617^T	ISP 5505
S. violaceus IFO 13103^T	ISP 5082

Abbreviations: ATCC, American Type Culture Collection; IFO, Institute for Fermentation, Osaka; JCM, Japan Collection of Microorganisms; ISP, International *Streptomyces* Project; *S.*, *Streptomyces*; T, type strain.

CGCTCCTTGTCCTCGGCCTC-3′, position 866-885). The conditions for thermal cycling were 25 cycles of denaturation at 96°C for 10 sec, primer annealing at 50°C for 5 sec, and primer extension at 60°C for 4 min. The products were analyzed with a model ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems).

Phylogenetic analysis. GyrB sequences translated from *gyrB* sequences were aligned by use of CLUSTAL W ver. 1.7 software (20) and manually corrected. The sequences of *gyrB* were aligned according to the alignments of GyrB sequences. After gaps and insertion nucleotides were omitted, the evolutionary distances and the similarity values based on Kimura's two-parameter model (8) were calculated by use of CLUSTAL W. The neighbor-joining (15) tree was constructed by using the Njplot (10). The stability of the tree was assessed by bootstrap analysis using the resampling method of Felsenstein (3) with 1000 replications by using CLUSTAL W.

Observation of phenotypes. Morphological, cultural and physiological characteristics of the strains were observed and described according to the method of the International *Streptomyces* Project (ISP) (16).

DNA-DNA hybridization. DNA-DNA hybridization was performed by the method of Ezaki *et al.* (2) at a temperature of 55°C in 2×SSC (1×SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0) containing 50 % formamide. The experiment was performed in at least triplicate and the level of DNA relatedness was expressed as a percentage of the homologous DNA binding value.

Results and Discussion

Phenotypes of Streptomyces violaceoruber group and related species

Table 2 shows phenotypes of *S. violaceoruber* group and related species. All strains belonging to *S. violaceoruber* group exhibited similar phenotypes except for "*S. tumuli*" IFO 13492^T, which lost the ability to form aerial mycelium. Spore chains form spirals and spore surface is smooth. Aerial mass is white to gray, substrate mycelium is yellowish brown, red to blue or reddish purple and melanin formation is negative. Glucose, arabinose, fructose, inositol, mannitol, rhamnose and xylose are utilized for growth, raffinose and sucrose are not. On the other hand, *S. althioticus* IFO 12740^T exhibits almost same phenotype as *S. violaceoruber* IFO 12826^T except that it has a spiny spore surface, *S. tuirus* IFO 15617^T is melanin-positive and utilizes raffinose and sucrose, and *S. violaceus* IFO 13103^T has a spiny spore surface, is melanin-positive, and utilizes raffinose and sucrose.

Phylogenetic analysis of gyrB sequences

The DNA-fragment length of *gyrB* amplified from *S. violaceoruber* group and the related species was 1227 bp. The phylogenetic tree and similarity matrix of this group and related species based on *gyrB* sequences are shown in Fig. 1 and Table 3, respectively. The strains in the following five subgroups had the same *gyrB* sequence: *S. violaceoruber* IFO 12826^T, "*S. tumuli*" IFO 13492^T, *S. coelicolor* IFO 3114 and IFO 3176; *S. violaceolatus* IFO 13101^T, *S. coelescens* IFO 13378^T and "*S. lividans*" IFO 13385^T; *S. humiferus* IFO 12244^T, *S. tricolor* IFO 15461^T, "*S. cyanocolor*" IFO 13034^T and "*S. lazureus*" IFO 13384^T; "*S. lividans* 66" IFO 15675 and "*S. cyanogenus*" IFO 13035^T; *S. violaceoruber* IFO 15146 (=*S. coelicolor* A3(2)) and *S. violaceoruber* JCM 4979 (=IFO 15732=*S. coelicolor* A3(2)). All strains belonging to *S. violaceoruber* group clustered tightly with more than 99.2 % similarity values of *gyrB* sequences. On the other hand, similarity values between the type strain of *S. violaceoruber* IFO 12826^T and *S. althioticus* IFO 12740^T, *S. tuirus* IFO 15617^T and *S. violaceus* IFO 13103^T were 93.1, 93.5 and 90.5 %, respectively. This result indicates that *gyrB* sequences can distinguish these phenotypic differences.

Monson *et al.* (9) reported that levels of DNA relatedness between *S. violaceoruber* 14980 (type strain) and *S. violaceoruber* 3443, which is the strain of origin of *S. coelicolor* A3(2), and *Actinopycnidium caeruleum*, which is reclassified as *S. humiferus*, were 97 and

Table 2. Phenotypes of *Streptomyces violaceoruber* group and its related species.

Organism	Morphology of spore chain	Spore surface	AM	Rev	SP	Utilization of carbohydrates										References
						Mel	Glc	Ara	Fru	Ino	Man	Raf	Rha	Suc	Xyl	
S. anthocyanicus IFO 14892^T	Spira	SM	W, G	p-Y, R, V	N	-	++	++	++	++	++	-	++	-	++	11, this study
"*S. caesius*" IFO 13376^T	Spira	SM	G	R, Pur	R, bl-V	-	++	++	++	++	++	v	++	++	++	19
S. coelescens IFO 13378^T	Spira	SM	G	Pur	R, Bl, V	-	++	++	++	++	++	+/-	++	+/-	++	19
S. coelicolor IFO 3114	Spira	SM	W, G	p-Y, R, V	N	-	++	++	+	++	++	-	++	-	++	this study
S. coelicolor IFO 3176	Spira	SM	G	p-Y, R, V	N	-	++	++	++	++	++	-	++	-	++	this study
S. coelicolor IFO 3504	Spira, RA	SM	G	p-Y, R, V	N	-	++	++	++	++	++	+/-	++	-	++	this study
"*S. cyanocolor*" IFO 13034^T	Spira, RA	SM	G, R, Bl	R, Bl, V	R, Bl, V	-	++	++	++	++	++	+/-	++	+/-	++	18
"*S. cyanogenus*" IFO 13035^T	Spira	SM	G, R	R, Bl	R, Bl, V	-	++	++	++	++	++	++	++	+	++	18
S. humiferus IFO 12244^T	Spira, RF	SM	W, G	p-Y, R, V	N	-	++	++	++	++	++	-	++	-	++	4
"*S. lazureus*" IFO 13384^T	Spira, (RF)	SM	R, G	Bl, Pur, R	R, Bl, V	-	++	++	++	++	++	+/-	++	-	++	19
"*S. lividans*" IFO 13385^T	Spira	SM	G	y-Br, Bl, Pur	N or f-Bl	-	++	++	++	++	++	-	++	-	++	5, 19
"*S. lividans*" 66	Spira	SM	G	y-Br, r-Pur	N or f-R	-	++	+	++	+	++	-	++	-	+	5
S. rubrogriseus IFO 15455^T	Spira	SM	G	R, pur-R	N	-	++	++	++	++	++	+	++	-	+	this study
S. tricolor IFO 15461^T	Spira	SM	G	r- or bl-Pur	bl-Pur, Bl	-	++	++	++	++	++	-	+	-	++	11, this study
"*S. tumuli*" IFO 13492^T	Spira	nd	W, G	p-Y	N	-	++	++	++	++	++	+/-	++	+/-	++	19
S. violaceolatus IFO 13101^T	Spira	SM	G, (R)	Bl, Pur, R	Bl, V, R	-	++	++	++	++	++	++	++	+	++	19
S. violaceoruber IFO 12826^T	Spira	SM	G	R, Bl or V	Bl, V	-	++	+	++	+	++	-	++	-	+	5, 17
S. violaceoruber IFO 15146	Spira	SM	G	R, Bl or V	Bl, V	-	++	+	++	+	++	-	++	-	+	5, this study
S. violaceoruber IFO 15731	Spira	SM	G	bl-Pur or V	f-Pur	-	nd	nd	nd	nd	nd	nd	nd	nd	nd	this study
S. violaceoruber IFO 15732	Spira	SM	G	R, Bl or V	Bl, V	-	++	+	++	+	++	-	++	-	+	this study
Related species																
S. althioticus IFO 12740^T	Spira	Spiny	G	Bl, V	Bl, V	-	++	++	++	++	++	-	++	v	++	17
S. tuirus IFO 15617^T	Spira	SM	G	r-Br, R	R, r-Br, V	+	++	++	++	++	++	++	++	++	++	19
S. violaceus IFO 13103^T	Spira	Spiny	W, R	pur-P, p-Pur	R, V	+	++	++	++	++	++	++	++	++	++	18

Abbreviations: AM, aerial mass color; Rev, reverse side color; SP, soluble pigments; Mel, melanin formation; Glc, glucose; Ara, arabinose; Fru, fructose; Ino, inositol; Man, mannitol; Raf, raffinose; Rha, rhamnose; Suc, sucrose; Xyl, xylose; ++, strongly positive; +, positive; +/-, doubtful; -, negative; v, variable; nd, not determined; RF, Rectus-Flexibilis; RA, Retinaculum-Apertum; SM, smooth; G, gray; R, red; W, white; Pur, purple; Bl, blue; V, violet; Br, brown; p-Y, pale yellow; f-, faint; bl-Pur, bluish purple; bl-V, bluish violet; p-Pur, pinkish purple; pur-P, purplish pink; r-Br, reddish brown; r-Pur, reddish purple; y-Br, yellowish brown; N, none.

Table 3. Similarity matrix for *gyrB* sequences of *Streptomyces violaceoruber* group and related organisms.

Organism	% Similarity																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 *S. anthocyanicus* IFO 14892^T																						
2 "*S. caesius*" IFO 13376^T	99.8																					
3 *S. coelescens* IFO 13378^T	99.7	99.8																				
4 *S. coelicolor* IFO 3114	99.8	99.8	99.9																			
5 *S. coelicolor* IFO 3176	99.8	99.8	99.9	100																		
6 *S. coelicolor* IFO 3504	99.8	99.8	99.9	99.8	99.8																	
7 "*S. cyanocolor*" IFO 13034^T	99.8	99.9	99.8	99.9	99.9	99.9																
8 "*S. cyanogenus*" IFO 13035^T	99.7	99.8	99.7	99.8	99.8	99.8	99.8															
9 *S. humiferus* IFO 12244^T	99.8	99.9	99.8	99.9	99.9	99.9	100	99.8														
10 "*S. lazureus*" IFO 13384^T	99.8	99.9	99.8	99.9	99.9	99.9	100	99.8	100													
11 "*S. lividans*" IFO 13385^T	99.7	99.8	100	99.9	99.9	99.9	99.8	99.7	99.8	99.8												
12 "*S. lividans*" 66	99.7	99.8	99.7	99.8	99.8	99.8	99.8	100	99.8	99.8	99.7											
13 *S. rubrogriseus* IFO 15455^T	99.1	99.2	99.1	99.2	99.2	99.2	99.3	99.1	99.3	99.3	99.1	99.1										
14 *S. tricolor* IFO 15461^T	99.8	99.9	99.8	99.9	99.9	99.9	100	99.8	100	100	99.8	99.8	99.3									
15 "*S. tumuli*" IFO 13492^T	99.8	99.8	99.9	100	100	99.8	99.9	99.8	99.9	99.9	99.9	99.8	99.2	99.9								
16 *S. violaceolatus* IFO 13101^T	99.7	99.8	100	99.9	99.9	99.9	99.8	99.7	99.8	99.8	100	99.7	99.1	99.8	99.9							
17 *S. violaceoruber* IFO 12826^T	99.8	99.8	99.9	100	100	99.8	99.9	99.8	99.9	99.9	99.9	99.8	99.2	99.9	100	99.9						
18 *S. violaceoruber* IFO 15146	99.6	99.7	99.9	99.8	99.8	99.8	99.8	99.6	99.8	99.8	99.9	99.6	99.0	99.8	99.8	99.9	99.8					
19 *S. violaceoruber* IFO 15731	99.3	99.4	99.7	99.6	99.6	99.6	99.5	99.3	99.5	99.5	99.7	99.3	98.8	99.5	99.6	99.7	99.6	99.8				
20 *S. violaceoruber* IFO 15732	99.6	99.7	99.9	99.8	99.8	99.8	99.8	99.6	99.8	99.8	99.9	99.6	99.0	99.8	99.8	99.9	99.8	100	99.8			
21 *S. althioticus* IFO 12740^T	92.9	93.0	93.1	93.1	93.1	93.0	93.1	92.9	93.1	93.1	93.1	92.9	93.0	93.1	93.1	93.1	93.1	93.1	92.9	93.1		
22 *S. tuirus* IFO 15617^T	93.4	93.5	93.6	93.5	93.5	93.6	93.6	93.4	93.6	93.6	93.6	93.4	94.0	93.6	93.5	93.6	93.5	93.5	93.2	93.5	91.4	
23 *S. violaceus* IFO 13103^T	90.6	90.7	90.6	90.7	90.7	90.7	90.8	90.7	90.8	90.8	90.6	90.7	91.0	90.8	90.7	90.6	90.7	90.5	90.3	90.5	90.6	94.0

S. althioticus, S. tuirus and *S. violaceus,* listed below the dotted line, are related organisms of *S. violaceoruber* group.

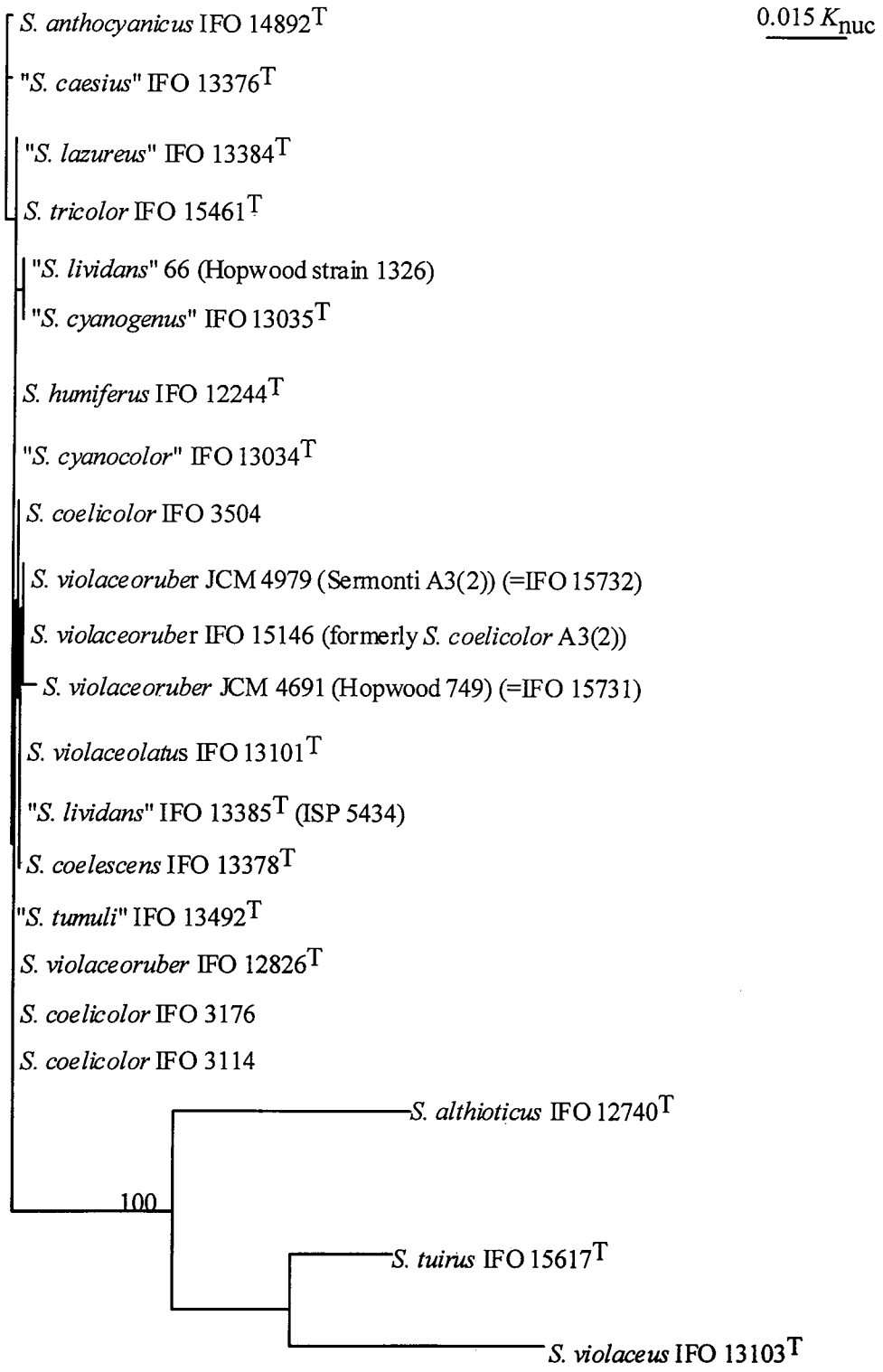

Fig. 1. Phylogenetic tree of *S. violaceoruber* group and the related organisms based on *gyrB* sequences. The tree was depicted by the neighbor-joining method. Scale bar=0.015 K_{nuc} in nucleotide sequences.

82%, respectively (9). Hatano *et al.* (5) also reported that levels of DNA relatedness among *S. violaceoruber* IFO 12826^T and *S. violaceoruber* IFO 15146 (=*S. coelicolor* A3(2)), and two strains of "*S. lividans*" are 81–103 %, indicating that these strains belong to the same species. These results were congruent with those of phylogenetic analysis based on *gyrB* sequences. Furthermore, Kasai *et al.* (6) reported in reclassification of *Micromonospora* species using *gyrB* sequences and DNA-DNA hybridization that 0.014 of genetic distance based on *gyrB* sequence corresponded with 70 % of DNA relatedness, which is minimum value for determination to belong to the same species. The genetic distance (0.014) roughly corresponds with 98.5 % of similarity value of *gyrB* sequence. Consequently, we concluded that the following strains, which have more than 99.2 % of similarity value with *S. violaceoruber*, and closely resemble it in phenotypes, are synonyms of *S. violaceoruber*: *S. anthocyanicus* IFO 14892^T, *S. coelescens* IFO 13378^T, *S. humiferus* IFO 12244^T, *S. rubrogriseus* IFO 15455^T, *S. tricolor* IFO 15461^T, *S. violaceolatus* IFO 13101^T, "*S. caesius*" IFO 13376^T, "*S. cyanocolor*" IFO 13034^T, "*S. cyanogenus*" IFO 13035^T, "*S. lazureus*" IFO 13384^T, "*S. lividans*" IFO 13385^T, "*S. lividans*" 66 and "*S. tumuli*" IFO 13492^T. *S. coelicolor* IFO 3114, *S. coelicolor* IFO 3176, and *S. coelicolor* IFO 3504 were identified with *S. violaceoruber*.

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Reexamination of *Dipodascus* and *Geotrichum* Strains by DNA-DNA Hybridization

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Summary

In this study, the identity of *Dipodascus ambrosiae* IFO 10801^T and *Dipodascus ovetensis* IFO 1201 and IFO 10827, and *Dipodascus spicifer* IFO 10809^T and *Geotrichum clavatum* IFO 10824^T were investigated in respect of G+C content and DNA similarity. *D. ambrosiae* and *D. ovetensis* could not be distinguished by G+C content and DNA-DNA hybridization test, and it is suspected, therefore, that *D. ambrosiae* might be a synonym of *D. ovetensis*. DNA similarity values between *D. spicifer* IFO 10809^T and *G. clavatum* IFO 10824^T were unusual: one showed high (92%) and the other showed intermediate (66%) homology. Therefore, it is suggested that *G. clavatum* might be a hybrid between *D. spicifer* and another species.

Keywords: *Dipodascus*, DNA-DNA hybridization, *Geotrichum*.

The genus *Dipodascus* Lagerheim comprises ascomycetous yeast-like fungi, and its anamorphic genus is *Geotrichum* Link:Fries. In 1986, de Hoog *et al.* (1) reported *Dipodascus ambrosiae, Dipodascus capitatus, Dipodascus geniculatus, Dipodascus spicifer*, and *Geotrichum clavatum* as new species. The report stated that *D. ambrosiae* was superficially similar to *Dipodascus ovetensis*, a telepmorph of *Geotrichum sericeum*, but differed considerably in cell size, the G+C content, and assimilation of D-mannitol and D-sorbitol, and that *D. spicifer* and *G. clavatum* were similar but distinguished by growth on D-xylose. *D. capitatus* was reported to be a teleomorph of *Geotrichum capitatum*. Guého *et al.* (4) reported that the distinction of *G. clavatum* and *D. spicifer* was justified by their intermediate DNA similarity values (47-64%).

On the basis of 26S rRNA gene (rDNA) domain D1/D2 sequence comparison, Kurtzman and Robnett (7) suggested that *D. ovetensis* and *D. ambrosiae* were conspecific because of their identical sequence; and that *G. clavatum* was an anamorph of *D. spicifer* because it contained only one base substitution. Ueda-Nishimura and Mikata (10) showed that *D. ambrosiae* IFO 10801^T and *D. ovetensis* IFO 1201 and IFO 10827 had identical 18S rDNA sequences, and that only one base substitution was found between *D. spicifer* IFO 10809^T and *G. clavatum* IFO 10824^T. It was also shown that neither *D.*

spicifer nor *G. clavatum* could assimilate D-xylose and, therefore, that they could not be distinguished by any physiological characters (10). Phylogenetic analyses using 26S rDNA domain D1/D2 and 18S rDNA sequences showed that *D. capitatus* was closely related to *D. spicifer* and *G. clavatum* (7, 10).

In this study, the identities of *D. ambrosiae* IFO 10801^T and *D. ovetensis* IFO 1201 and IFO 10827, and of *D. spicifer* IFO 10809^T and *G. clavatum* IFO 10824^T were investigated in respect of G+C content and DNA similarity.

Materials and Methods

Yeast strains and DNA preparation. The strains studied are listed in Table 1. The strains were cultured as described by Mikata and Ueda-Nishimura (8). Protoplasts were prepared and DNA was extracted following the protocol of Holm *et al.* (5) modified by Kaneko and Banno (6).

G+C contents determination and DNA-DNA hybridizations. G+C contents were determined by the HPLC method of Tamaoka and Komagata (9). DNA-DNA hybridizations were studied by the photobiotin microplate method of Ezaki *et al.* (2, 3) as modified by Kaneko and Banno (6). Calculations were based on the average of five measurements.

Results and Discussion

Dipodascus ambrosiae and Dipodascus ovetensis

The G+C contents of DNAs from *D. ambrosiae* IFO 10801^T and *D. ovetensis* IFO 1201 and IFO 10827 were identical at 47 mol%, although different from the data of de

Table 1. Strains studied.

Species	Strain		
	IFO	CBS	
Dipodascus ambrosiae	10801	749.85	Type strain
Dipodascus capitatus	10819	197.35	
Dipodascus capitatus	10820	580.82	Authentic culture of *Geotrichum linkii*
Dipodascus ovetensis	1201	634.85	Authentic culture of *Geotrichum sericeum*
Dipodascus ovetensis	10827	635.85	
Dipodascus spicifer	10809	244.85	Type strain
Geotrichum clavatum	10824	425.71	Type strain

Table 2. Similarity and G+C content of DNA of *D. ambrosiae* and *D. ovetensis*.

Species	IFO	G+C content (mol%)		DNA similarity (%)	
				10801^T	1201
D. ambrosiae	10801^T	47	45.2*	100	112
D. ovetensis	1201	47	51.2*	86	100
D. ovetensis	10827	47	—	92	106
S. cerevisiae	10217^T	—	—	15	21

*Data from de Hoog *et al.* (1) using *Tm* method.

Hoog *et al.* (1) (Table 2). Therefore, it was shown that they could not be distinguished by G+C content.

DNA-DNA hybridization was tested at 46°C with DNA from the three strains and photobiotin-labelled DNA from *D. ambrosiae* IFO 10801^T and *D. ovetensis* IFO 1201. The results are shown in Table 2. DNA similarity values among them were 86-112%. These data showed that *D. ambrosiae* IFO 10801^T and *D. ovetensis* IFO 1201, corresponding to an authentic culture of the anamorph *Geotrichum sericeum*, and IFO 10827 should be considered to be conspecific. Thus the two species were distinguishable only by the cell size and assimilation of D-mannitol and D-sorbitol. In this study, it was shown that *D. ovetensis* IFO 1201, corresponding to an authentic culture of the anamorph *Geotrichum sericeum*, and *D. ambrosiae* were conspecific. Therefore, it is suspected that *D. ambrosiae* might be a synonym of *D. ovetensis*, according to the priority of naming. Nevertheless, since the type strain of *D. ovetensis* was not tested in this study, *D. ambrosiae* will be temporarily left as is.

Dipodascus spicifer, Geotrichum clavatum, and Dipodascus capitatus

The G+C contents of DNAs from *D. spicifer* IFO 10809^T, *G. clavatum* IFO 10824^T and *D. capitatus* IFO 10819 and IFO 10820 were 32-34 mol% (Table 3). DNA-DNA hybridization was tested two times at 40 and 47°C with DNA from the four strains and photobiotin-labelled DNA from *D. spicifer* IFO 10809^T, *G. clavatum* IFO 10824^T and *D. capitatus* IFO 10819. The results did not vary so much with hybridization temperature; therefore, the results by 47°C as representative are shown in table 3. *D. capitatus* IFO 10819 and IFO 10820 were confirmed to be conspecific by their homology level of 94%. DNA similarity values between *D. spicifer* IFO 10809^T and *G. clavatum* IFO 10824^T were unusual: one showed high (92%) and the other showed intermediate (66%) homology. Kaneko and Banno (6) reported that a hybrid between *S. bayanus* and *S. cerevisiae* constructed by mating showed high DNA similarity to its parents. On the basis of this

Table 3. Similarity and G+C content of DNA of *D. spicifer, D. capitatus*, and *G. clavatum*.

Species	IFO	G+C content (mol%)		DNA similarity (%)		
				10809^T	10824^T	10819
D. spicifer	10809^T	34	30.6*	**100**	**92**	66
G. clavatum	10824^T	32	31.1*	66	**100**	42
D. capitatus	10819	34	36.3*	63	59	**100**
D. capitatus	10820	33	-	58	59	**94**
S. cerevisiae	10217^T	-	-	13	12	13

*Data from de Hoog *et al.* (1) using *Tm* method.

report, it is suggested that *G. clavatum* might be a hybrid between *D. spicifer* and another species. Therefore, it is supposed that *G. clavatum* is not an anamorph of *D. spicifer*.

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IFO Res. Commun. **20**,
97-100, 2001 (March)

Unique Shape of the Ascospores of *Pichia sporocuriosa*

Kozaburo MIKATA

Summary

The surface structure of the ascospores of the ascomycetous yeast *Pichia sporocuriosa* IFO 10941 was examined. Under the light microscope, distinct ledges were not observed on the surface of ascospores. However, by scanning electron microscopy (SEM), frill-like ledge of S-shape or irregular shape was found turning two or three times on the spore surface and having two end.

Keywords: *Pichia sporocuriosa*, surface structure of ascospore, Unique shape of the ascospore.

Péter *et al.* isolated a new yeast from rambutan (*Nephelium lappaceum*), and named it *Pichia sporocuriosa* (8). By transmission electron microscopy (TEM), the ascospores were seen to be spheroidal in cross section, rough-walled, and surrounded by several ledges that were difficult to observe under the light microscope. The ledges were seen to be randomly located and some of them were uncircumfluent. Ledges numbered at least three, whereas in hat-shaped or saturn-shaped ascospores, only two ledges should have been observed by cross section TEM (2, 9). The observation of several ledges suggested that these ascospores had a unique surface structure. We therefore examined the surface structure of the ascospores of *Pichia sporocuriosa* IFO 10941 (NCAIM Y01078).

A mass of cells harvested from a colony on a YM agar slant was transferred to a corn meal agar slant and incubated for 1 or 2 weeks at 24°C to induce sporulation. Asci contained one or two spheroidal ascospores. Ascospores were prepared for SEM by the method of Mikata and Nakase (7).

Under the light microscope, distinct ledges were hardly observed on the ascospores of *Pichia sporocuriosa* (Fig 1). By SEM observation, the surface structure displayed frill-like ledge of S-shape or irregular shape with two or three turns. This ledge did not form a ring, but had two ends (Fig. 2).

In hat-shaped *Pichia membranifaciens* having ledge on the lower part of the ascospore (6) and saturn-shaped *Satunispora aheanii* having ledge on the equatorial surface of the ascospore (5), each ledge formed an unbroken ring. Ascomycetous yeasts known

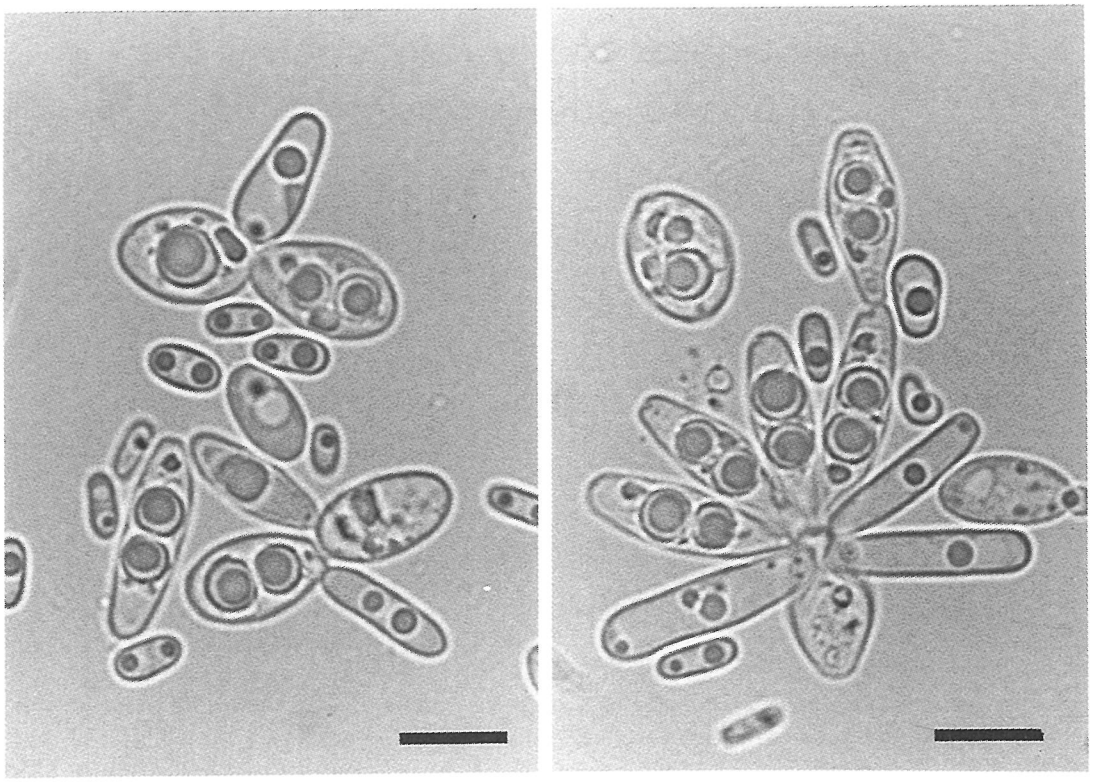

Fig. 1. Light micrographs of the ascospores of *Pichia sporocuriosa* IFO 10941. Scale bars: 10 μm

to have ledges on ascospores include the genera *Hanseniaspora, Pachysolen, Pichia, Saccharomycopsis, Satrunispora, Stephanoascus, Wicherhamia*, and *Williopsis*, all of which have unbroken annular ledges.

The ascospores of *Pichia sporocuriosa* displayed a new type of surface structure. It should be noted that the genus *Pichia* includes species with various kinds of ascospore surfaces.

Kawakami *et al.* reported that, in *P. farinosa*, ascospores had thin subequatorial ledges visible under an electron microscope (3); and in *P. fluxuum*, in addition to the roughening, electron microscopy revealed the existence of spores with a thin subequatorial ring (1, 4). In *P. ohmeri*, Wickerham and Burton observed an unusual phenomenon: crosses between different pairs of haploid strains produced either spheroidal or hat-shaped spores (10).

Although most ascospores of the genus *Pichia* are hat-shaped, the above three species and *P. sporocuriosa* showed unique surface structures, indicating that this genus is rich in variety. In partial 18S rDNA sequence, *Pichia sporocuriosa* showed a close lineage relation to *P. membranifaciens* and *Candida krusei* (8).

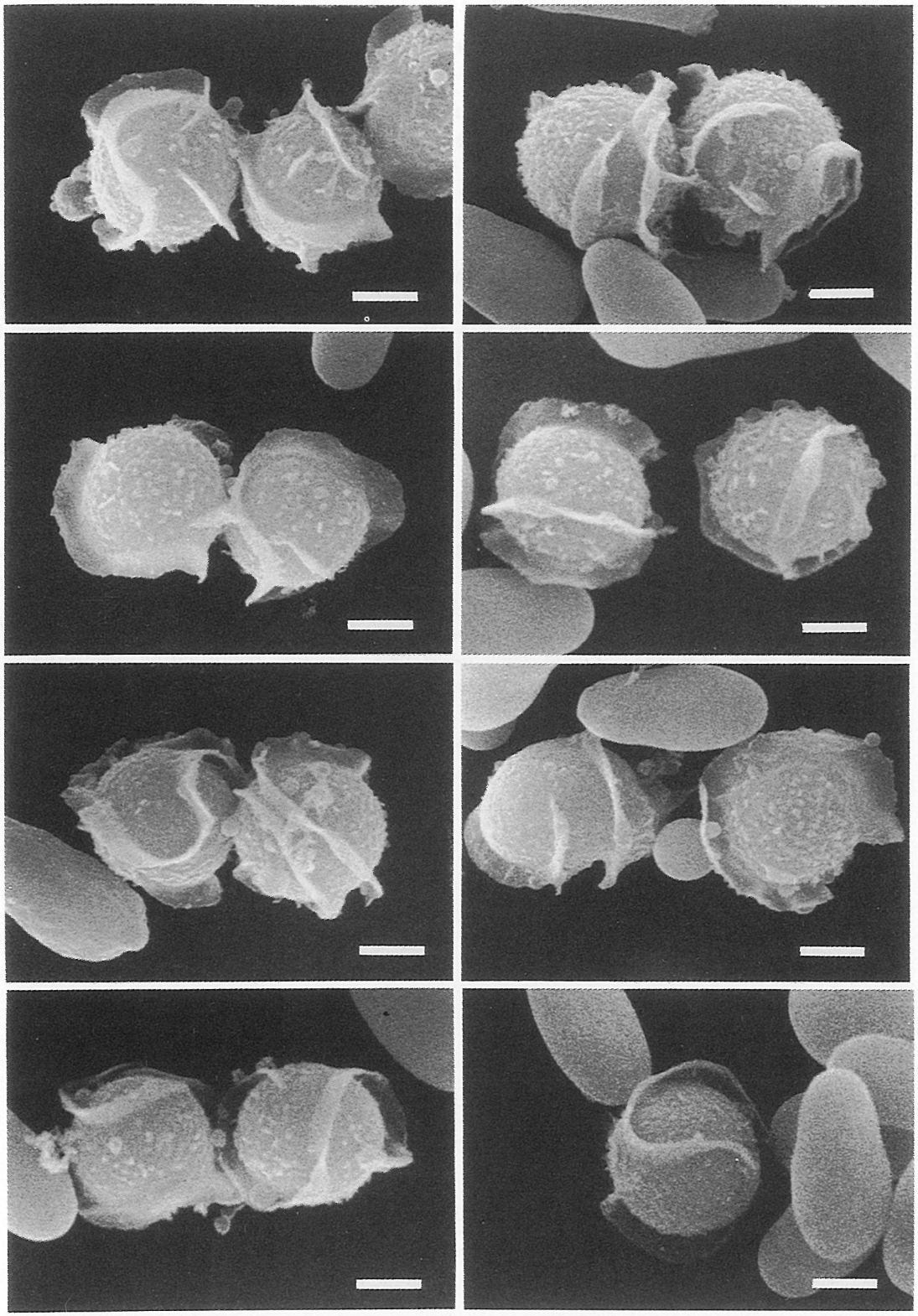

Fig. 2. Scanning electron micrographs of the ascospores of *Pichia sporocuriosa* IFO 10941. Scale bars: 1 μm

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IFO Res. Commun. **20**,
101-107, 2001 (March)

Descriptive Catalogue of IFO Fungus Collection XVII

In the routine identification work on fungi isolated in Japan, and checks of the list of the fungal taxa preserved in the IFO Culture Collection for published records of their occurrence in Japan, many taxa have been found to be either new to Japan or obscurely or insufficiently described. In some cases, the first record of a fungus in Japan gives only the name of its 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 our knowledge of the fungal flora of Japan.

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

108. ***Calcarisporiella thermophila*** (Evans) de Hoog (Figs. 1-2) Hyphomycetes

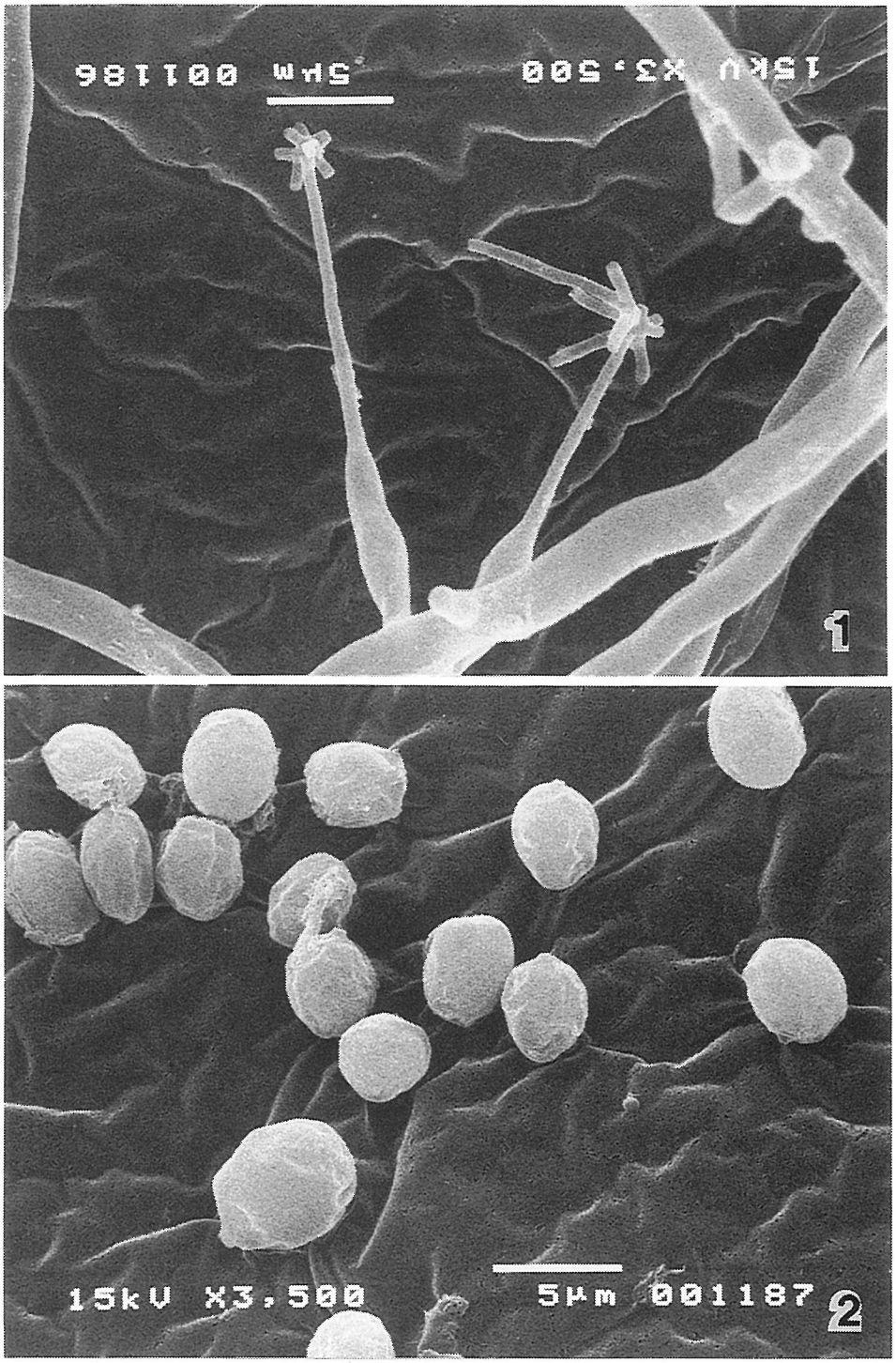
Stud. Mycol. 7: 68 (1974).
Basinonym: *Calcarisporium thermophilum* Evans (as "*C. thermophile*"); Evans, H. C. Trans. Br. Mycol. Soc. 57: 247 (1971).

Colonies on malt agar, potato sucrose agar, potato carrot agar, oatmeal agar, and cornmeal agar growing moderately, reaching diameter of 45-50 mm in 4 wk at 28°C, white, floccose, sporulating abundantly. Reverse uncolored. No exudates and odor. Vegetative hyphae hyaline, smooth- and thin-walled, septate. Conidiophores erected solitary or in pairs, arising orthotropically from undifferentiated aerial hyphae, with a transverse septum at the base and an inflated basal part, 25-50 μm long, 1.5-2.0 μm wide at the septum, 2.5-3.5 μm wide at the inflated middle part. Conidial formation by sympodial growth, consisting of 3-5 conidium-bearing denticles, with small geniculate denticulate rachis. Denticles cylindrical, one to five elongate, 3-6 μm long, 1.0-1.5 μm width. Conidia hyaline, smooth- and thin-walled, ovoid to ellipsoidal, base truncate or rounded, 6-9$\times$3-5 μm. Teleomorph unknown.

Growth of this fungus occurs between 20°C and 45°C, with an optimum at 40°C. Thermotolerant fungus.

Hab.: soil, natural forest park, Ryugo-cho, Ooshima-gun, Kagoshima, 5 March 2000. (IFO 33169=Tad. Ito H1245-6-3, IFO H-12238).
Distribution: England, Japan

During a survey of the soil-borne fungi in southern Japan, a noteworthy strain appeared. The fungus is apparently identical with *Calcarisporiella thermophila* (Evans)



Figs. 1-2. *Calcarisporiella thermophila.* Fig.1. Conidiophore on aerial hyphae. Fig. 2. Conidia. Bars: Figs. 1-2=5 μm.

de Hoog (de Hoog, 1974). This is only the second finding of this fungus after the ex-type strain (CBS 279.70=ATCC 22718=IMI 144750). This species was originally described

by Evans (Evans, 1971) from coal-spoil tip soil at Keele, Staffordshire, England, March 1968. He described it as a new species *Calcarisporium thermophilum* Evans (Evans, 1971, as "*C. thermophile*"). However, de Hoog established the new genus *Calcarisporiella* and moved it to this genus as *C. thermophila* (de Hoog, 1974), because it differs from *Calcarisporium* in its wide, undulating, fragile hyphae, the shape of the conidiogenous cells, the wide conidium-bearing denticles, and the shape and size of the conidia.
The fresh isolate (Tad. Ito H1245-6-3) of *C. thermophila* was collected from soil at the natural forest park, Ryugo-cho, Ooshima-gun, Kagoshima, on 5 March 2000. Cultural and morphological characteristics of this strain agreed well with the de Hoog's description (de Hoog, 1974).

(Tad. Ito, I. Okane, and A. Nakagiri)

109. *Cladobotryum verticillatum* (Link ex S.F. Gray) Hughes

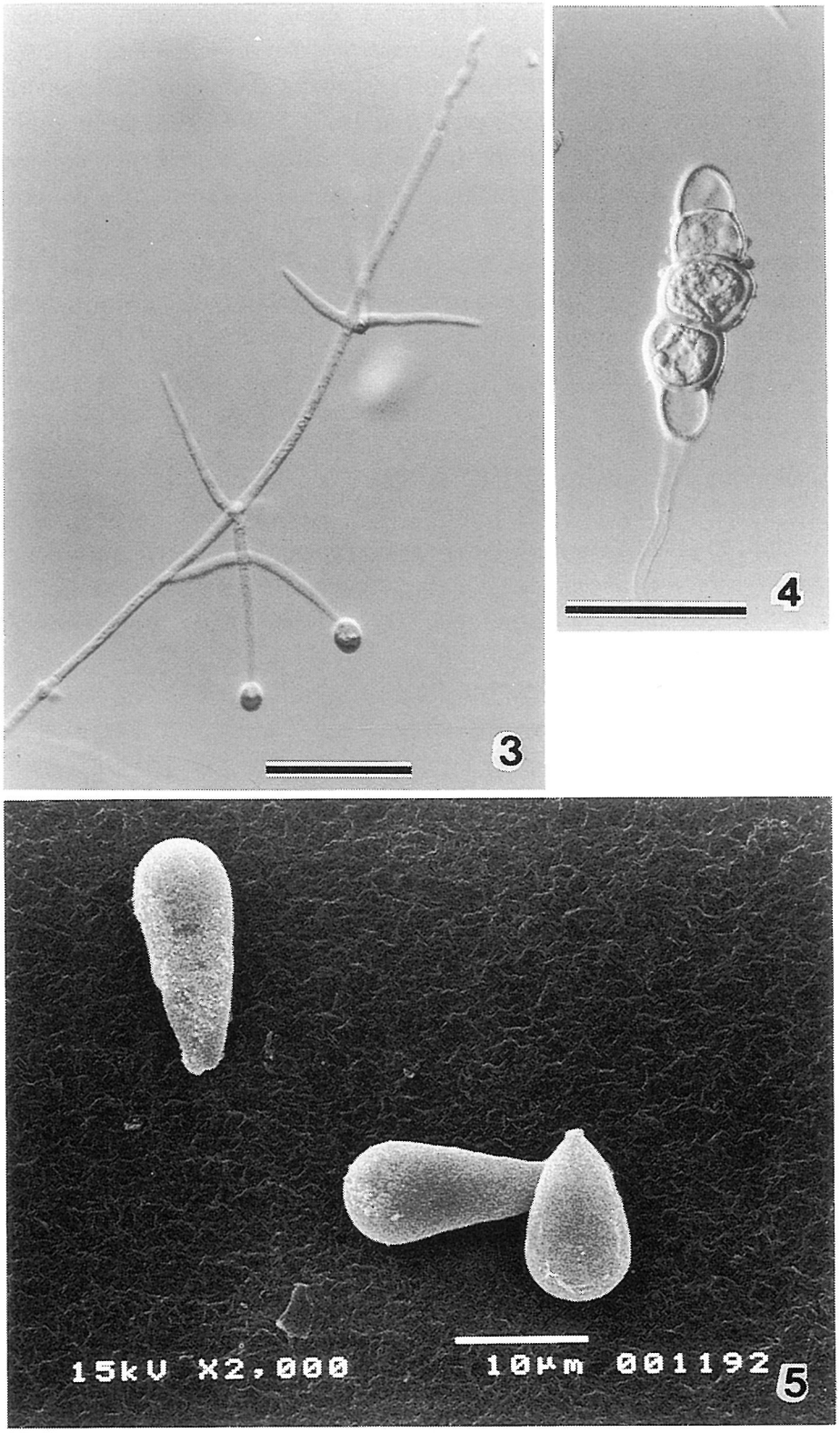
(Figs. 3-5) Hyphomycetes

Can. J. Bot. 36: 750 (1958). Gams & Hoozemans, Persoonia 6: 95-110 (1970).
Teleomorph: *Hypomyces armeniacus* Tulasne, Selecta Fungorum Carpologia 1: 1-242 (1861), Paris; Selecta Fungorum Carpologia 3: 1-206 (1865), Paris; Rogerson & Samuels, Mycologia 86: 839 (1994).
Syn.: *Acremonium verticillatum* Link, Mag. Ges. Naturf. Fr. Berl. 3: 15 (1809).
Mycogone verticillata (Link ex S.F. Gray) Spreng., Linn. Syst. Ver. 4: 555 (1827).
Verticillium lactarii Peck, Rep. N.Y. St. Mus. Nat. Hist. 35: 140 (1882).

Colonies on potato carrot agar with a daily growth rate of 20-23 mm at 25°C, creeping on agar surface. Pure white, fluffy, 10-15 mm high. Sporulation abundant after 2 wk of incubation. Reverse uncolored. On oatmeal agar 18-21 mm/day growth, pure white, fluffy, 10-15 mm high. Reverse uncolored. Vegetative hyphae hyaline, smooth- and thin-walled, partly immersed, 5-7 μm wide. Conidiophores erect on aerial hyphae or substrate, hyaline, smooth- and thin-walled, verticillately branched, mostly bearing whorls of 2-4 phialides. Conidiogenous cells monoblastic, orthotropic, tapering into a narrow neck, with a septum near the base, up to 60 μm long, 5 μm wide at the base, 2 μm wide at the apex. Conidia holoblastic, pyriform to obconic, non-septate or rarely septate in old cultures, with minutely truncate base, 14-18×8-11 μm. Chlamydospores produced mostly in terminal position, immersed, thick-walled, straight to curved, chains of 3-8 cells, each cell ovoid to ellipsoid, hyaline to pale brown, smooth to minutely roughed, 70-120 μm long, the widest part 20-22 μm.
At 37°C, growth is nil.

Hab.: decayed mushroom, Okutu-cho, Tomata-gun, Okayama, Japan, 19 Aug., 2000. (IFO 33178=Tad. Ito H12-81, IFO H-12239).

The connection of the anamorph and teleomorph in this species was not proven until 1994. Rogerson and Samuels (1994) confirmed the connection between *C. verticillatum*



Figs. 3-5. *Cladobotryum verticillatum.* Fig. 3. Conidiophore and conidia on main stalk. Fig. 4. Chlamydospore. Fig. 5. Conidia. Bars: Figs. 3-4=50 μm, Fig. 5=10 μm.

and *Hypomyces armeniacus* Tul. by germinating ascospores of two collections.

The present species is known to be distributed in Europe, North America and Colombia as a fungicolous Hyphomycetes. According to Gams and Hoozemans (1970), this species grows on the basidiomata of Agaricales and Russulales, and the teleomorph is formed only on decaying wood, litter or the ground.

This strain was isolated from a very decayed mushroom, the species of which is difficult to identify.

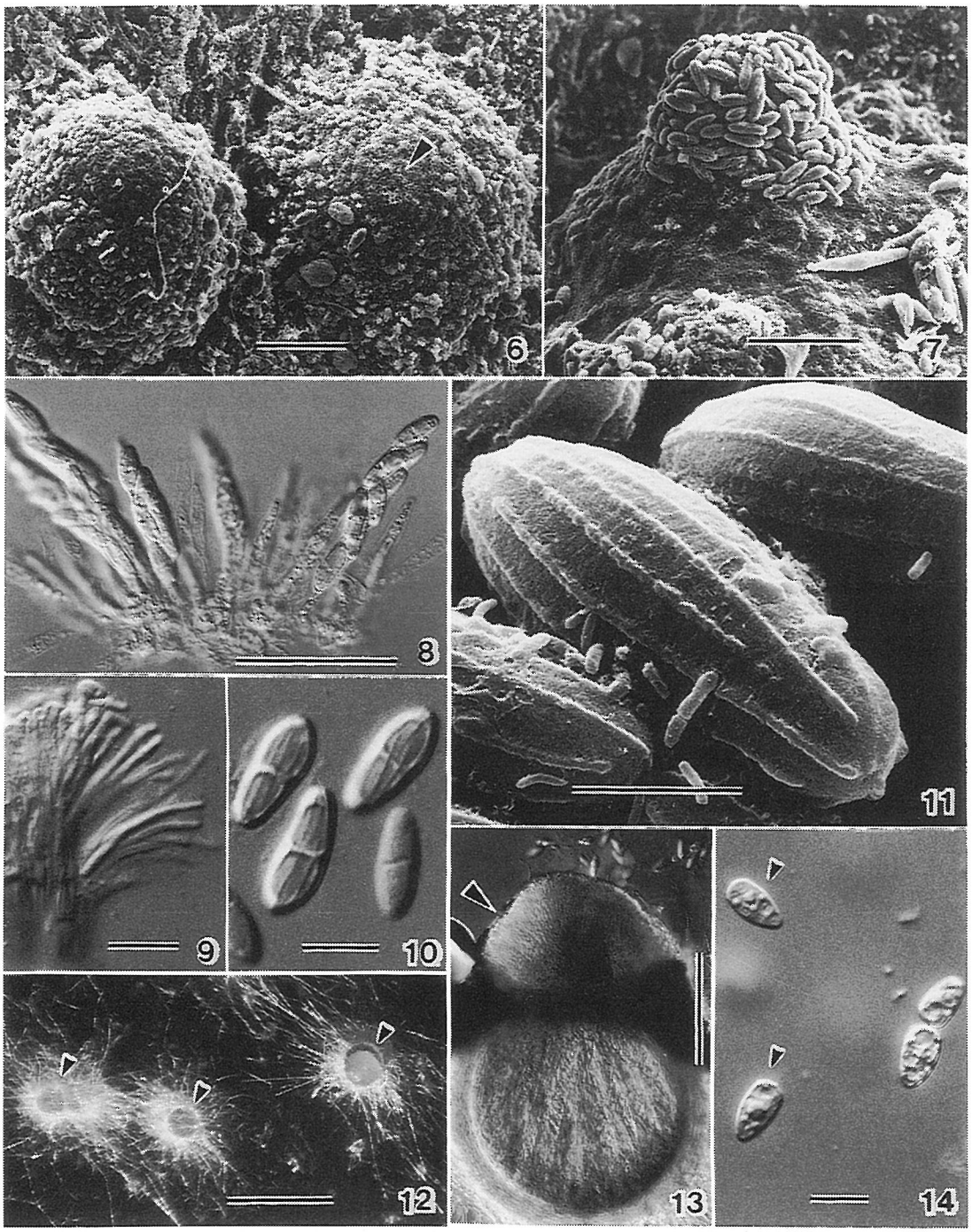
(Tad. Ito)

110. *Kallichroma tethys* (Kohlm. & Kohlm.) Kohlm. & Volkm.-Kohlm.
(Figs. 6-14) (Hypocreales)

Mycol. Res. 97: 759 (1993)
Basionym: *Hydronectria tethys* Kohlm. & Kohlm., Nova Hedwigia 9: 95 (1965)

Ascomata 250-400 μm high, 280-400 μm in diam, globose to depressed-subglobose, party immersed, ostiolate, epapillate or inconspicuously papillate, dark orange, gregarious. Peridium 50-60 μm thick, leathery. Ostiolar canal lined with periphyses, 6-8$\times$1.5-2 μm, septate, simple, tapering, merging into apical paraphyses 24-26$\times$1.5-2 μm. Asci 70-94$\times$9-16 μm eight-spored, clavate, short pedunculate, unitunicate, thin-walled, without apical apparatus, developing seccessively at the base of the ascoma venter. Ascospores 18-24$\times$7-10 μm, uni- or biseriate, obovate to ellipsoidal, one-septate somewhat below the center, slightly constricted at the septum, hyaline, with 10-12 longitudinal ridges running from one pole to the another.

Colonies of single ascospore isolates growing moderately on cornmeal seawater agar (cornmeal agar dissolved in 20 ppt salinity artificial seawater, Jamarin S (Jamarin Lab., Osaka), 26-31 mm in diam at 25°C in 4 weeks, hyaline, sparse, with less aerial hyphae, forming ascomata initials on and in agar medium. After 3 months incubation, ascomata became yellow-orange in color and produced ascospores inside. Thus, this fungus is homothallic. On other media, such as vegetable juice seawater agar (10% vegetable juice agar with 10 ppt salinity sea water), colonies attaining 27-28 mm in diam at 25°C in 4 weeks, white, with thick aerial hyphae, forming abundant ascomata initials, but on cornmeal agar (with fresh water), colonies growing slowly (24-25 mm in diam), forming ascomatal initials but not maturing after prolonged incubation. Thus, this fungus adapts to marine conditions in growth and reproduction. Morphology of ascomata produced in culture is similar to that on natural substrate; ascomata 360-380 μm high, 260-280 μm in diam, yellow-oragne, papillate with thick surrounding hyphae (Fig. 13); peridium 30-40 μm thick with surface textura epidermoidea to intricata; papillae 100-120 μm long, 180-200 μm in diam, composed of densely gathered short hyphae spreading outward, ostiolar canal 30-40 μm in diam; periphyses merging into apical paraphyses 20-45 μm$\times$2-3 μm; asci 70-80$\times$14-18 μm, ascospores 21-23$\times$7-8 μm, with longitudinal ridges. A mass of ejected ascospores was deposited at the apex of ascoma through the ostiole (Fig. 12). The ascospores in the spore mass were often separated at the septum into two cells (Fig. 14), which was not observed in the natural materials.



Figs. 6-14. Light and SEM micrographs of *Kallichroma tethys*. 6. Ascomata. Note an ostiolar opening (arrowhead). 7. A mass of ascospores discharged and deposited on an ascoma. 8. Aaci. 9. Periphyses. 10. ascospores. 11. Ascospores with longitudinal ridges on the surface. 12. Ascomata produced in culture on cornmeal seawater agar. Note spore drops (arrowheads) discharged and deposited on the top of ascomata. 13. Ascoma formed in culture, showing a papilla (arrowhead) surrounded by densely gathered short hyphae. 13. Ascospores formed in culture, separating into two cells (arrowheads). Scale bars: 6-8=50μm; 9, 10, 14=10μm; 11=5μm; 12=500μm; 13=100μm.

Hab.: decomposing tree of *Pittosporum tobira* Ait., submerged in sea water at the intertidal zone of Kashima Is. (33° 42′N, 135° 21′E), Tanabe, Wakayama Pref., 9 Nov. 1999. Single ascospore isolate from the material (IFO H-12241) was deposited in IFO culture collection as IFO 33109 (=AN-1660).

This species has been reported frequently from subtropical and tropical areas around the world, especially from mangroves as a colonizer of decomposing mangrove wood (Kohlmeyer and Kohlmeyer, 1979; Kohlmyer and Volkmann-Kohlmeyer, 1993). This is the first report of this fungus from a temperate coast and also from Japan. The collection site, Kashima Is., is influenced by the Kuroshio (Japan Current), a warm sea current. The warm climate (average air temp., 6.2-27.1°C) and the sea current may account for the distribution of this fungus up to the temperate region.

Collection of the material was done by the courtesy of Mr. Noboru Goto and staffs of the Board of Education of Tanabe City and supported by the grant from Fujiwara Natural History Foundation to A. N. through Dr. Keisuke Tubaki (the leader of the project: Reinvestigation of cryptogamic flora around Kishu-Kumano.)

(A. Nakagiri)

IFO Res. Commun. **20**,
108-109, 2001 (March)

Descriptive Catalogue of IFO Bacteria Collection XI

In the identification of newly isolated strains, interesting strains worth adding to the IFO bacteria collection have been found. The purpose of this catalogue is to provide descriptions of taxonomical characteristics of these strains. Below, the descriptions are arranged in alphabetical order of the scientific name of the strains. The authors of the descriptions are shown in parentheses.

The following two strains were isolated from the grave of Margrave Jošt Lucemburský, an important Czech ruler who reigned over Moravia at the turn of the 14th and the 15th century, by Dr. Ivo Sedlácek and Dr. Zdena Pácová, Czech Collection of Microorganisms, Mararyk University Brno, Faculty of Science, Brno, Czech Republic. The grave was opened for the second time in 1999 (for the first time in the 17th century) for the purpose of archaeological and anthropological research by the Brno City Museum.

91. *Arthrobacter sulfureus* Stackebrandt *et al.* 1984

IFO 16294

The strain CCM 4759 (=IFO 16294) was isolated from the tombstone of the grave of Margrave Jošt Lucemburský as a coryneform bacterium.

Cells are Gram-positive, non-motile, non-spore forming, short rods and cocci. Colonies are yellowish or whitish, smooth convex with a diameter of approx. 1 mm after 48 hr on peptone- yeast medium (IFO medium no. 802). Growth occurs at 4-30°C, and in 1-10 % (w/v) NaCl. The strain is strictly aerobic and catalase- and oxidase- positive. By using the API CORYNE system, pyrazinamidase and urease are positive, but nitrate reductase, *N*-acetyl-β- glucosaminidase, esculinease (β-glucosidase) and gelatinase are negative. Using the API 50CH system, most of the substrates are not used as a sole carbon source.

The cell wall murein type is thought to be A4α from the molar ratio of amino acids in the cell wall (glutamic acid: glycine : alanine : lysine=1.00 : 1.15 : 1.00 : 1.0). The G+C content of DNA is 64.4 mol %. The major cellular fatty acids are *iso*-$C_{15:0}$ and *anteiso*-$C_{15:0}$. The predominant menaquinone is MK-9. Mycolic acids are not present.

The 16S ribosomal DNA sequence similarity between IFO 16294 and *A. sulfureus* DSM 20167^T (X83409) is 99.3 %. The level of DNA/DNA relatedness between strain IFO 16294 and *A. sulfureus* IFO 12678^T is 83-85 %.

On the basis of morphological, physiological and chemotaxonomic characteristics, as well as DNA-DNA hybridization data, this strain was identified as a strain of *Arthrobacter sulfureus*.

(Mariko. Takeuchi and K. Hatano)

92. *Rhodococcus erythropolis* (Gray and Thornton 1928) Goodfellow and Alderson 1979

IFO 16296

The strain CCM 4761 (=IFO 16296) was isolated from the femur of remains of Jošt Lucembursk$\acute{y}$ in the grave as irregular rods which show "nocardioform" morphological features.

Cells are Gram-positive, non-motile rods. Cocci germinate to give filaments which show elementary branching. The growth cycle is completed by the appearance, through fragmentation, of cocci. Colonies are light pink, opaque and convex with slightly irregular edges on peptone- yeast agar (IFO medium no. 802). Growth occurs at 10-40°C but not at 45°C. Catalase is positive. By using the API CORYNE system, esculinase and urease are positive, but nitrate reductase, pyrazinamidase, pyrorydonilalyramidase, alkaliphosphatase, β-glucuronidase, β- galactosidase, α-glucosidase, and gelatinase are negative. Acid is produced from glucose, glycerol, sorbitol, sucrose, and trehalose but not from glycogen, rhamnose, ribose, or xylose. *N*- Acetylglucosamine, citrate, fumarate, D-galactose, gluconate, glycerol, maltose, succinate, L- leucinse and quinate are utilized as a sole carbon and energy source, but inositol, lactate, malate, mannitol, L-rhamnose, D-ribose, DL-tartrate, trehalose, proline or L-serine are not. Ethanol, sorbitol, and sucrose are used weakly.

N-Glycolated muramic acid moieties of peptidoglycan are present. The cell wall contains *meso*-diaminopimelic acid, arabinose and galactose (wall chemotype IV). The predominant menaquinone is MK-8(H_2). Mycolic acids are present. The major fatty acids of $C_{16:0}$, $C_{16:1}$, and 10-methyl $C_{18:0}$ (TBSA) are found. The G+C content of the DNA is 62.0 mol%.

The 16S ribosomal DNA sequence similarity between IFO 16296 and *R. erythropolis* DSM 43066^T is 99.8 %. The level of DNA/DNA relatedness between strain IFO 16296 and *R. erythropolis* IFO 15567^T is 74-98 %.

On the basis of morphological, physiological and chemotaxonomic characteristics, as well as DNA-DNA hybridization data, this strain was identified as a strain of *Rhodococcus erythropolis*.

(Mariko. Takeuchi and K. Hatano)

IFO Res. Commun. **20**,
110–111, 2001 (March)

Descriptive Catalogue of IFO Actinomycetes Collection V

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

21. ***Streptomyces violaceoruber*** (Waksman and Curtis 1916) Pridham 1970
Skerman, V.B.D., V. McGowan, and P.H.A. Sneath, Int. J. Syst. Bacteriol., 30: 225–420 (1980)

IFO 3114

Strain IFO 3114 was deposited under the name of *Streptomyces coelicolor* derived from CBS as *Actinomyces violaceus-ruber*. Its phenotypic characteristics closely resemble those of *Streptomyces violaceoruber* IFO 12826^T (T=type strain). Spore chains form spirals and the spore surface is smooth. Aerial mass is white to brownish gray and substrate mycelium is pale yellow to reddish brown or purplish red (Cherry in Color Harmony Manual). Soluble pigments are not produced. Melanin formation is negative on International *Streptomyces* Project (ISP) media 1, 6 and 7. Glucose, arabinose, fructose, inositol, mannitol, rhamnose, xylose and glycerol are utilized for growth, but raffinose and sucrose are not. The similarity value of *gyrB* sequences (1227 bp) is 100 % with *Streptomyces violaceoruber* IFO 12826^T. From these results, IFO 3114 is identified as a strain of *Streptomyces violaceoruber*.

(K. Hatano and T. Nishii)

IFO 3176

Strain IFO 3176 was deposited under the name of *Streptomyces coelicolor*, derived from ATCC as *Streptomyces coelicolor* ATCC 10147, which was derived from IMRU (Waksman Institute of Microbiology, Rutgers, the State University of New Jersey) as Waksman strain 3034. Its phenotypic characteristics closely resemble those of *Streptomyces violaceoruber* IFO 12826^T (T=type strain). Spore chains form spirals and the spore surface is smooth. Aerial mass is brownish gray and substrate mycelium is pale yellow to brown or reddish purple (Rose Wine). Soluble pigment is not produced. Melanin formation is negative on International *Streptomyces* Project (ISP) media 1, 6 and 7. Glucose, arabinose, fructose, inositol, mannitol, rhamnose, xylose and glycerol are utilized for growth, but raffinose and sucrose are not. The similarity value of *gyrB* sequences (1227 bp) is 100 % with *Streptomyces violaceoruber* IFO 12826^T. From these results, IFO 3176 is identified as a strain of *Streptomyces violaceoruber*.

(K. Hatano and T. Nishii)

IFO 3504

Strain IFO 3504 was deposited under the name of *Streptomyces coelicolor* isolated by Ogata (IFO). Its phenotypic characteristics closely resemble those of *Streptomyces violaceoruber* IFO 12826^T (T=type strain). Spore chains form spirals and the spore surface is smooth. Aerial mass is brownish gray and substrate mycelium is brown to purplish red (Light Coral Red). Soluble pigment is not produced. Melanin formation is negative on International *Streptomyces* Project (ISP) media 1, 6 and 7. Glucose, arabinose, fructose, inositol, mannitol, rhamnose, xylose and glycerol are utilized for growth, but raffinose is doubtfully utilized and sucrose is not. The similarity value of *gyrB* sequences (1227 bp) is 100 % with *Streptomyces violaceoruber* IFO 12826^T. From these results, IFO 3504 is identified as a strain of *Streptomyces violaceoruber*.

(K. Hatano and T. Nishii)

Catalogue of Newly Accepted Strains

January 1999 – December 2000

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

IFO No (T=Type strain)	Name	Temp	Med
0099	*Pichia membranifaciens* HUT (Naganishi) ← GIB.	24	108
0137	*Pichia membranifaciens* Nippon Syurui Co. ← HUT (Naganishi) ← GIB.	24	108
0158	*Kodamaea ohmeri* GRIF ← CLMR ← GIB (Takahashi, Mycoderma sp. N).	24	108
0159	*Candida valida* GRIF ← CLMR ← GIB (Takahashi, Mycoderma sp. Q).	24	108
0188	*Pichia membranifaciens* HUT (Naganishi) ← GIB.	24	108
0191	*Pichia manshurica* HUT (Naganishi) ← CLMR. Source: Manchurian sorghum spirits	24	108
0348	*Schizosaccharomyces pombe* FAT (Sakaguchi). Source: fermenting molasses	28	108
0461	*Pichia membranifaciens* HUT (Naganishi).	24	108
0546	*Pichia manshurica* HUT (H. Naganishi, Kahmehefe No.12) ← GIB.	24	108
0572	*Pichia membranifaciens* FAH (Y. Sasaki) ← F. Hemmi.	24	108
0812	*Pichia membranifaciens* Kodama Shuzo Co. (Kodama, 10). Source: pressed juice of sweet potato	24	108
0813	*Pichia manshurica* Kodama Shuzo Co. (Kodama, 38).	24	108
0814	*Pichia manshurica* Kodama Shuzo Co. (Kodama, 20).	24	108
1244	*Bulleromyces albus* FMJ 31-004 (Tsuchiya) ← CBS 500.	24	108

IFO No (T=Type strain)	Name	Temp	Med
1943 T	*Saccharomyces pastorianus* NCYC 392 ← CBS 1538 ← O. Winge.	28	108
10237	*Candida zeylanoides* ATCC 26318 ← C. Ramirez. Source: Spanish sausage	24	108
10398	*Lipomyces kononenkoae* subsp. *kononenkoae* CBS 8113 ← J.P. van der Walt. Source: soil	24	108
10399	*Lipomyces kononenkoae* subsp. *kononenkoae* CBS 8114 ← J.P. van der Walt. Source: soil	24	108
10931 T	*Candida kofuensis* CBS 8058 ← RIFY 4841 (S. Goto). Source: berry	24	108
10932	*Candida kofuensis* JCM 2321 ← S. Goto, No. 611.	24	108
10933	*Saturnispora zaruensis* JCM 10341 ← NRRL Y-17640 ← CBS 8055 ← S. Goto, No. 611.	24	108
10935	*Metschnikowia* sp. IFO (K. Nishimura, U96-417-5). Source: flower	24	108
10936	*Metschnikowia* sp. IFO (K. Nishimura, U96-402-2). Source: flower	24	108
10937	*Saccharomyces bayanus* Dept. of Biotechnol., Osaka Univ. (Y. Kaneko, B19-3C, a segregant of hybrid between B1-5A & B18-2A).	28	109
10938	*Saccharomyces bayanus* Dept. of Biotechnol., Osaka Univ. (Y. Kaneko, B19-3D, a segregant of hybrid between B1-5A & B18-2A).	28	109
10940	*Candida novakii* NCAIM Y 00987. Source: decyaing of wood	24	108
10941 T	*Pichia sporocuriosa* NCAIM Y 01078. Source: fermenting rambutan	24	108
10942 T	*Saturnispora ahearnii* JCM 10726 ← NRRL Y-7555 ← D.G. Ahearn.	24	108

IFO No (T=Type strain)	Name	Temp	Med
10943 T	*Candida novakii* NCAIM Y 00986. Source: decaying wood	24	108
10944	*Saccharomyces* sp. Dept. Biotech., Fukuyama Univ. (T. Hisatomi, THE1-16B, a heterothallic segregant of DL-EH1).	24	108
10945	*Saccharomyces* sp. Dept. Biotech., Fukuyama Univ. (T. Hisatomi, THE1-16C, a heterothallic segregant of DL-EH1).	24	108
10946 T	*Saccharomyces turicensis* CBS 8665 ← M.-T. Wyder, HBU3. Source: kefyr grain	24	108
10947 T	*Saccharomyces cariocanus* G.I. Naumov, N 50816-4B, single spore isolate of UFRJ 50816 ← P.B. Morais, UFRJ 50816. Source: fruit fly	24	108
10948	*Saccharomyces cariocanus* G.I. Naumov, N 50791-2D, single spore isolate of UFRJ 50791 ← P.B. Morais, UFRJ 50791. Source: fruit fly	24	108
13296	*Bacillus vitellinus* IFO (T. Sakane) ← RTCI (S. Tanida, Z1159).	30	802
15965 T	*Cryptosporangium arvum* IFO (T. Tamura) ← Yamanashi Univ. (M. Hayakawa, YU 629-1). Source: soil	28	266
15966 T	*Cryptosporangium japonicum* IFO (T. Tamura) ← Yamanashi Univ. (M. Hayakawa, YU 636-3). Source: soil	28	266
16267 T	*Acrocarpospora pleiomorpha* IFO (T. Tamura) ← Tanabe Seiyaku Co., Ltd. (S. Suzuki, R-31). Source: soil	28	245
16274	*Detolaashinbacter* sp. Yokohama Plant Protection Service (T. Tsukamoto, CM-01). Source: cultivated mushroom	30	802
16275	*Detolaashinbacter* sp. Yokohama Plant Protection Service (T. Tsukamoto, CM-02). Source: cultivated mushroom	30	802

IFO No (T=Type strain)	Name	Temp	Med
16276	*Detolaashinbacter* sp. Yokohama Plant Protection Service (T. Tsukamoto, CM-03). Source: cultivated mushroom	30	802
16277	*Detolaashinbacter* sp. Yokohama Plant Protection Service (T. Tsukamoto, CM-05). Source: cultivated mushroom	30	802
16278	*Detolaashinbacter* sp. Yokohama Plant Protection Service (T. Tsukamoto, CM-10). Source: cultivated mushroom	30	802
16279	*Detolaashinbacter* sp. Yokohama Plant Protection Service (T. Tsukamoto, CM-14). Source: cultivated mushroom	30	802
16280	*Detolaashinbacter* sp. Yokohama Plant Protection Service (T. Tsukamoto, CM-15). Source: cultivated mushroom	30	802
16281	*Detolaashinbacter* sp. Yokohama Plant Protection Service (T. Tsukamoto, CM-16). Source: cultivated mushroom	30	802
16282	*Detolaashinbacter* sp. Yokohama Plant Protection Service (T. Tsukamoto, CM-20). Source: cultivated mushroom	30	802
16283	*Detolaashinbacter* sp. Yokohama Plant Protection Service (T. Tsukamoto, CM-21). Source: cultivated mushroom	30	802
16294	*Arthrobacter sulfureus* CCM 4759 (Z. Pacova). Source: tombstone	30	802
16295	*Rhodococcus* sp. CCM 4760 (Z. Pacova). Source: femur in the grave	30	802
16296	*Rhodococcus erythropolis* CCM 4761 (Z. Pacova). Source: femur in the grave	30	802
16317 T	*Verrucosispora gifhornensis* DSM 44337 ← R.M. Kroppensstedt, HR1-2. Source: peat bog	30	227
16318 T	*Kocuria palustris* DSM 11925 ← R.M. Kroppenstedt, TAGA 27. Source: rhizoplane of the narrow-leaves cattail	30	802

IFO No (T=Type strain)	Name	Temp	Med
16319 T	*Kocuria rhizophila* DSM 11926 ← R.M. Kroppensteedt, TAGA 68. Source: rhizoplane of the narrow-leaved cattail	30	802
16320 T	*Gordonia polyisoprenivorans* DSM 44302. Source: fouling tyre water	30	802
16322 T	*Streptomyces thermoalcalitolerans* DSM 41741 ← M. Goodfellow, TA56. Source: soil	45	227
16323 T	*Streptomyces thermocarboxydus* DSM 44293 ← S.B. Kim (M. Goofellow), AT37. Source: allotment garden soil	45	227
16324 T	*Streptomyces thermocarboxydovorans* DSM 44296 ← S.B. Kim (M. Goodfellow), AT52. Source: allotment garden soil	45	227
16347	*Serratia marcescens* Res. Lab. Appl. Biochem., Tanabe Seiyaku Co. Ltd. (H. Matsumae) ← Dept. Bacteriol., Fac. Med., ShinSnshu Univ. (H. Matsumoto, Sr41). Source: sewage	30	802
16353	*Streptomyces* sp. JCM 8963 ← M. Hamada, MA 267-A1. Source: soil	28	228
16357	*Clostridium sporogenes* JCM 7850 ← IAM 19235 ← S. Sugama ← ATU ← National Cancer's Association, Washington.	37	802
16364	*Microbacterium* sp. VKM Ac-1389. Source: steam gall induced by Anguina agropyri	24	802
16365	*Microbacterium* sp. VKM Ac-1391. Source: seed gall induced by Anguina agrostis	24	802
16366	*Microbacterium* sp. VKM Ac-1781. Source: seed gall induced by Anguina agrostis	24	802
16367	*Microbacterium* sp. VKM Ac-1782 ← T.G. Dobrovolskaya, strain 3G1a. Source: root gall induced by Sabanguina radicicola	24	802

IFO No (T=Type strain)	Name	Temp	Med
16368	*Microbacterium* sp. VKM Ac-1807. Source: leaf gall induced by Anguina graminis	24	802
16369	*Microbacterium* sp. VKM Ac-1808. Source: leaf gall induced by Anguina graminis	24	802
16370	*Microbacterium* sp. VKM Ac-2011. Source: permafrost (depth 23m, 1.8-3.0 million years ago)	24	802
16371	*Microbacterium* sp. VKM Ac-2012. Source: permafrost (depth 23m, 1.8-3.0 million years ago)	24	802
16372	*Microbacterium* sp. VKM Ac-2013. Source: permafrost (depth 23m, 1.8-3.0 million years ago)	24	802
16373	*Microbacterium* sp. VKM Ac-2014. Source: permafrost (depth 48.8 m, 1.8-3.0 million years ago)	24	802
16374	*Microbacterium* sp. VKM Ac-2015. Source: permafrost (depth 32.2 m, 1.8-3.0 million years ago)	24	802
16375	*Microbacterium* sp. VKM Ac-2016. Source: permafrost (depth 23m, 1.8-3.0 million years ago)	24	802
16376	*Microbacterium* sp. VKM Ac-2047. Source: phylloplane	24	802
16377	*Microbacterium* sp. VKM Ac-2048. Source: phyllosphere	24	802
16378	*Microbacterium* sp. VKM Ac-2049. Source: phyllosphere	24	802
16379	*Microbacterium* sp. VKM Ac-2050. Source: rhizosphere of Carex pachysylis	24	802

IFO No (T=Type strain)	Name	Temp	Med
16380	*Microbacterium* sp. VKM Ac-2051 ← INMI VKM B-1201 ← IBPM B-159 ← V.L.Belikova, strain 19-2. Source: rhizosphere of Poa bulbosa	24	802
16381	*Microbacterium* sp. VKM Ac-2053. Source: phylloplane of Mentha sp.	24	802
16384	*Curtobacterium* sp. VKM Ac-1376. Source: root gall induced by Subanguina radicicola	24	802
16385	*Curtobacterium* sp. VKM Ac-1386. Source: seed gall induced by Anguina agrostis	24	802
16386	*Curtobacterium* sp. VKM Ac-1387. Source: leaf gall induced by Mesoanguina picridis	24	802
16387	*Curtobacterium* sp. VKM Ac-1393. Source: steam gall induced by Heteroanguina graminophila	24	802
16388	*Curtobacterium* sp. VKM Ac-1395. Source: leaf gall induced by Anguina graminis	24	802
16389	*Curtobacterium* sp. VKM Ac-1397. Source: leaf gall induced by Anguina graminis	24	802
16390	*Curtobacterium* sp. VKM Ac-1399. Source: leaf gall induced by Anguina graminis	24	802
16391	*Curtobacterium* sp. VKM Ac-1794. Source: seed gall induced by Anguina agrostis	24	802
16392	*Curtobacterium* sp. VKM Ac-1795. Source: seed gall induced by Anguina agrostis	24	802
16393	*Curtobacterium* sp. VKM Ac-1796. Source: leaf gall induced by Mesoanguina picridis	24	802

IFO No (T=Type strain)	Name	Temp	Med
16394	*Curtobacterium* sp. VKM Ac-1806. Source: leaf gall induced by Anguina graminis	24	802
16395	*Curtobacterium* sp. VKM Ac-1809. Source: leaf gall induced by Anguina graminis	24	802
16396	*Curtobacterium* sp. VKM Ac-1811. Source: steam gall induced by Heteroanguina graminophila	24	802
16397	*Curtobacterium* sp. VKM Ac-1840. Source: seed gall induced by Anguina agrostis	24	802
16398	*Curtobacterium* sp. VKM Ac-2052. Source: leaf gall induced by Heteoanguina graminophila	24	802
16399	*Curtobacterium* sp. VKM Ac-2054. Source: steam gall induced by Heteroanguina graminophila	24	802
16400	*Curtobacterium* sp. VKM Ac-2055. Source: seed	24	802
16401	*Curtobacterium* sp. VKM Ac-2056. Source: seed	24	802
16402	*Curtobacterium* sp. VKM Ac-2057. Source: phyllosphere	24	802
16403	*Curtobacterium* sp. VKM Ac-2058. Source: phyllosphere	24	802
16404	*Curtobacterium* sp. VKM Ac-2059. Source: seed	24	802
16405	*Curtobacterium* sp. VKM Ac-2060. Source: seed	24	802
16406	*Curtobacterium* sp. VKM Ac-2061. Source: seed	24	802

IFO No (T=Type strain)	Name	Temp	Med
16407	*Curtobacterium* sp. VKM Ac-2062. Source: seed	24	802
16408 T	*Actinoplanes capillaceus* Kitasato Inst. (S. Omura, K95-5561). Source: soil	28	227
16411 T	*Clostridium sporogenes* ATCC 7955 ← NCA 3679 (putrefactive anaerobe).	37	802
16412 T	*Bacillus subtilis* subsp. *subtilis* JCM 1465 ← IAM 12118 ← ATCC 6051 ← H.C. John (strain Marburg).	30	357
16414 T	*Microbacterium kitamiense* JCM 10270 ← H. Matsuyama Kitami C-2. Source: waste water of a sugar-beet factory	25	802
16415 T	*Sphingomonas herbicidovorans* DSMZ 11019 ← H.P.E. Kohler ← F. Streichsbier, TU Vienna,MH. Source: soil	30	802
16416 T	*Microbispora corallina* JCM 10267 ← V. Kitpreechavanich, DF-32. Source: soil	28	231
16432 T	*Beutenbergia cavernae* DSM 12333 ← I. Groth, Hans-Knoll Inst., Jena, HKI 0122. Source: soil	30	230
16433 T	*Gordonia alkanivorans* DSM 44369 ← C. Kummer, Hans-Knoll Inst., Jena. HKI 0136. Source: tar and phenol contaminated soil	30	230
16434 T	*Ornithinicoccus hortensis* DSM 12335 ← I. Groth, Hans-Knoll Inst., Jena, HKI 0125. Source: garden soil	30	230
16435 T	*Rhodobacter capsulatus* IAM 14232 ← Y. Hoshino ← ATCC 11166 ← C.B. van Niel, ATH 2.3.1.	28	360
16436 T	*Rhodobacter azotoformans* IAM 14814 ← JCM 9340 ← A. Hiraishi, KA25.	28	360
16437 T	*Rhodobacter blasticus* DSM 2131 ← C.S. Dow. Source: eutrophic pond	30	360
16444 T	*Bacillus lentus* R & D Dept., Higeta Shoyu Co., Ltd. (O. Shida, HSCC 187) ← JCM 2511 ← CCM 2214 ← NCIB 8773 ← NCTC ← T. Gibson, 165. Source: soil	30	802

IFO No (T=Type strain)	Name	Temp	Med
16446 T	*Streptomyces malaysiensis* DSM 41697 ← M. Goodfelow, ATB-11 ← A. Al-Tai, Univ. Malaya. Source: soil	28	231
16449	*Bacillus subtilis* subsp. *subtilis* Shizuoka Univ. (Y. Tahara) ← Kikkoman Co. Source: natto	30	802
16458 T	*Rhodobacter veldkampii* DSM 11550 ← ATCC ← J.F. Imhoff, 51 ← T.A. Hansen, CIP 103912. Source: freshwater pond	25	802
16462 T	*Nonomuraea longicatena* NRRL 15532 ← Kyowa Hakko Co., Ltd., K-252, soil. Source: soil	28	266
16470 T	*Acetobacter tropicalis* NRIC 0312. Source: coconut juice	30	804
16471 T	*Acetobacter indonesiensis* NRIC 0313. Source: rotten zirzak (fruit)	30	804
16472	*Acetobacter aceti* NRIC 0471 ← IFO 14818.	30	804
16473	*Acetobacter aceti* NRIC 0470 ← IFO 14818.	30	804
16482	*Flexibacter tractuosus* NCIMB 12854 ← R.A. Lewin, EE-13 ← W. Gunkel. Source: red-brown mud	25	335
16486 T	*Flexibacter roseolus* NCIMB 1433 ← R.A. Lewin. Source: hot spring	25	341
32594	*Thielavia aurantiaca* IFO (T. Ito, T. Ito H2-2-5-13). Source: field soil	24	2
32595	*Thielavia aurantiaca* IFO (T. Ito, T. Ito H2-4-10-21). Source: field soil	24	2
32596	*Thielavia aurantiaca* IFO (T. Ito, T. Ito H2-5-10-18). Source: field soil	24	2
32625	*Discostroma corticola* IMI 079706 (W.G. Bramley).	24	1

IFO No (T=Type strain)	Name	Temp	Med
32626	*Discostroma tostum* IMI 188376 (T.G. Mitchell).	24	1
32705	*Discostroma tricellulare* IFO (I. Okane; IOC-203). Source: healthy leaf of Satsuki	24	8
32706	*Discostroma tricellulare* IFO (I. Okane; IOC-807). Source: healthy leaf of Mochitsutsuji	24	8
32707	*Discostroma tricellulare* IFO (I. Okane; IOC-892). Source: healthy leaf of Kirishimatsutsuji	24	8
33057	*Acremonium borodinense* IFO (T. Ito, T. Ito H8-16-2). Source: soil	24	8
33058	*Acremonium cavaraeanum* IFO (T. Ito) ← Takeda Chem. Ind., Ltd. (T. Kusaka, 2-2). Source: wall of wooden house	24	8
33082	*Aspergillus sojae* Kikkoman Co. (Y. Hanya) ← NISL 1478A. Source: seed koji of soy sauce	30	1
33083	*Aspergillus sojae* Kikkoman Co. (Y. Hanya) ← NISL 1478B. Source: seed koji of soy sauce	30	1
33084	*Aspergillus sojae* Kikkoman Co. (Y. Hanya) ← NISL 1905A. Source: seed koji of soy sauce	30	1
33085	*Aspergillus sojae* Kikkoman Co. (Y. Hanya) ← NISL 1909. Source: seed koji of soy sauce	30	1
33086	*Aspergillus sojae* Kikkoman Co. (Y. Hanya) ← NISL 1920A. Source: seed koji of soy sauce	30	1
33087	*Aspergillus sojae* Kikkoman Co. (Y. Hanya) ← NISL 1939A. Source: seed koji of soy sauce	30	1
33088	*Aspergillus sojae* Kikkoman Co. (Y. Hanya) ← NISL 1939B. Source: seed koji of soy sauce	30	1

IFO No (T=Type strain)	Name	Temp	Med
33089	*Geosmithia virida* ATCC 56972 ← FRR 1863 (A.D. Hocking, Australia). Source: creek bank soil	24	1
33090	*Paecilomyces pascus* ATCC 56973 ← FRR 1925 (G.C.M. Latch, New Zealand). Source: pasture grass	24	1
33091	*Penicillium oblatum* ATCC 56979 ← FRR 2234 (A.D. Hocking, Australia). Source: spoiled baby food	24	1
33092	*Penicillium sabulosum* ATCC 56984 ← FRR 2743 (A.D. Hocking, Australia). Source: pasteurized fruit juice	24	1
33093	*Nigrospora sacchari* Life Web, Graduate School of Human and Environmental Studies, Kyoto University (M. Okada, 98170804). Source: living leaf of reed	24	1
33094	*Nigrospora sphaerica* Life Web, Graduate School of Human and Environmental Studies, Kyoto University (M. Okada, 98320101). Source: living leaf of reed	24	1
33095	*Phaeosphaeria nigrans* Life Web, Graduate School of Human and Environmental Studies, Kyoto University (M. Okada, 9612A0603). Source: living leaf of reed	24	8
33096	*Rosellinia* sp. Meiji Seika Kaisha Ltd. (S. Miyadoh, PF1022). Source: leaf	24	1
33097	*Phycomyces blakesleeanus* Institute of Genetic Ecology, Tohoku University (A. Miyazaki, IGE 1101) ← Caltech ← NRRL 1555.	24	1
33098	*Crocicreas epifagicola* CUP 63663 (R.P. Korf). Source: old stems	20	1
33099	*Clonostachys rosea* Tanabe Seiyaku Co. (T. Okuda, TC 1294). Source: soil	24	1
33100	*Clonostachys rosea* Tanabe Seiyaku Co. (T. Okuda, TC 1297). Source: living plant leaf	24	1

IFO No (T=Type strain)	Name	Temp	Med
33101	*Clonostachys compactiuscula* Tanabe Seiyaku Co. (T. Okuda, TC 1292). Source: bark	24	1
33102	*Gliocladium catenulatum* Tanabe Seiyaku Co. (T. Okuda, TC 1280). Source: soil	24	1
33103	*Gliocladium* sp. Tanabe Seiyaku Co. (T. Okuda, TC 1052 (F2665)). Source: leaf	24	1
33104	*Gliocladium* sp. Tanabe Seiyaku Co. (T. Okuda, TC 1282). Source: fruit body	24	1
33105	*Mariannaea elegans var. punicea* Tanabe Seiyaku Co. (T. Okuda, TC 1322). Source: soil	24	1
33106	*Mariannaea camptospora* Tanabe Seiyaku Co. (T. Okuda, TC 1287). Source: soil	24	1
33109	*Kallichroma tethys* IFO (A. Nakagiri, AN-1660). Source: decomposing wood in seawater	24	15
33112	*Penicillium digitatum* Lab. Plant Pathol., Dept. Agric. Environ. Biol., The Univ. Tokyo (T. Hibi; PD5). Source: domestic satsuma mandarin	24	1
33113	*Penicillium digitatum* Lab. Plant Pathol., Dept. Agric. Environ. Biol., The Univ. Tokyo (T. Hibi; DF1). Source: imported orange	24	1
33114	*Penicillium digitatum* Lab. Plant Pathol., Dept. Agric. Environ. Biol., The Univ. Tokyo (T. Hibi; U1). Source: domestic satsuma mandarin	24	1
33115	*Penicillium digitatum* Lab. Plant Pathol., Dept. Agric. Environ. Biol., The Univ. Tokyo (T. Hibi; LC2). Source: imported lemon	24	1
33116	*Penicillium digitatum* Lab. Plant Pathol., Dept. Agric. Environ. Biol., The Univ. Tokyo (T. Hibi; M1). Source: imported lemon	24	1
33117	*Penicillium digitatum* Lab. Plant Pathol., Dept. Agric. Environ. Biol., The Univ. Tokyo (T. Hibi; I1). Source: imported lemon	24	1

IFO No (T=Type strain)	Name	Temp	Med
33118	*Penicillium digitatum* Lab. Plant Pathol., Dept. Agric. Environ. Biol., The Univ. Tokyo (T. Hibi; LC2M).	24	1
33126	*Pythium porphyrae* CBS 369.79 ← IFO 30347 ← Coll. Univ. Osaka Pref. (M. Takahashi; UOP 166). Source: diseased thallus of cultivated Susabinori	24	15
33128	*Halophytophthora vesicula* Marine Inst., Univ. Georgia (S. Newell, SAP 127). Source: submerged (seawater) decyaing leaves	24	15
33130	*Colletotrichum orbiculare* Miyagi Pref. Hort. Exp. Stn. (H. Kanno, MCK-1). Source: netted melon	24	1
33131	*Morchella esculenta* Fac. Agr., Shinshu Univ. (A. Yamada, 102).	24	1
33132	*Morchella esculenta* Fac. Agr., Shinshu Univ. (A. Yamada, 551).	24	1
33133	*Lyophyllum shimeji* Fac. Agr., Shinshu Univ. (A. Yamada, 608).	24	7
33134	*Lyophyllum decastes* Fac. Agr., Shinshu Univ. (A. Yamada, 506).	24	1
33135	*Lyophyllum semitale* Fac. Agr., Shinshu Univ. (A. Yamada, 586).	24	7
33136	*Tricholoma matsutake* Fac. Agr., Shinshu Univ. (A. Yamada, Y-1).	20	7
33137	*Tricholoma matsutake* Fac. Agr., Shinshu Univ. (A. Yamada, F).	20	7
33138	*Tricholoma bakamatsutake* Fac. Agr., Shinshu Univ. (A. Yamada, B-1).	20	7
33139	*Tricholoma ustale* Fac. Agr., Shinshu Univ. (A. Yamada, 610).	24	7
33140	*Tricholoma ustale* Fac. Agr., Shinshu Univ. (A. Yamada, 611).	24	7
33141	*Tricholoma ustale* Fac. Agr., Shinshu Univ. (A. Yamada, 612).	24	7
33142	*Tricholoma flavovirens* Fac. Agr., Shinshu Univ. (A. Yamada, 613).	24	7
33143	*Tricholoma flavovirens* Fac. Agr., Shinshu Univ. (A. Yamada, 614).	24	7

IFO No (T=Type strain)	Name	Temp	Med
33144	*Tricholoma portentosum* Fac. Agr., Shinshu Univ. (A. Yamada, 615).	24	7
33145	*Tricholoma saponaceum* Fac. Agr., Shinshu Univ. (A. Yamada, 616).	24	7
33146	*Leucopaxillus giganteus* Fac. Agr., Shinshu Univ. (A. Yamada, 571)	24	7
33147	*Suillus granulatus* Fac. Agr., Shinshu Univ. (A. Yamada, 108).	24	7
33148	*Suillus granulatus* Fac. Agr., Shinshu Univ. (A. Yamada, 577).	24	7
33149	*Suillus luteus* Fac. Agr., Shinshu Univ. (A. Yamada, 524).	24	7
33150	*Suillus bovinus* Fac. Agr., Shinshu Univ. (A. Yamada, 606).	24	7
33151	*Rhizopogon rubescens* Fac. Agr., Shinshu Univ. (A. Yamada, 517).	24	7
33152	*Rhizopogon rubescens* Fac. Agr., Shinshu Univ. (A. Yamada, 630).	24	7
33153	*Rhizopogon rubescens* Fac. Agr., Shinshu Univ. (A. Yamada, 632).	24	7
33154	*Lactarius hatsudake* Fac. Agr., Shinshu Univ. (A. Yamada, 584).	24	7
33155	*Lactarius hatsudake* Fac. Agr., Shinshu Univ. (A. Yamada, 124).	24	1
33156	*Lactarius akahatsu* Fac. Agr., Shinshu Univ. (A. Yamada, 561).	24	7
33157	*Lactarius akahatsu* Fac. Agr., Shinshu Univ. (A. Yamada, 583).	24	7
33163	*Lysurus mokusin* IFO (T. Ito, Kumada, ground). Source: ground	25	1
33164	*Halophytophthora vesicula* Marine Inst., Univ. Georgia (S. Y. Newell, SAP 86). Source: submerged decyaing leaves of black mangrove	24	15
33165	*Halophytophthora vesicula* Marine Inst., Univ. Georgia (S. Y. Newell, SAP 100). Source: submerged decyaing leaves of black mangrove	24	15

IFO No (T=Type strain)	Name	Temp	Med
33166	*Halophytophthora vesicula* Marine Inst., Univ. Georgia (S. Y. Newell, SAP 114). Source: submerged decyaing leaves of black mangrove	24	15
33169	*Calcarisporiella thermophila* IFO (T. Ito, H1245-6-3). Source: soil	37	2
33176	*Mycogone cervina* IFO (Tad. Ito, Tad. Ito H11-21). Source: fruit body	24	8
33177	*Mycogone cervina* IFO (Tad. Ito, Tad. Ito H12-78). Source: fruit body	24	8
33178	*Cladobotryum verticillatum* IFO (Tad. Ito, Tad. Ito H12-81). Source: decayed mushroom	24	8
33180	*Phytophthora cinnamomi* Kochi Pref. Pl. Prot. Of. (T. Kobayashi, Hp. Phy2SH). Source: root of tutsan	24	1
33181	*Phytophthora cinnamomi* Kochi Pref. Pl. Prot. Of. (T. Kobayashi, Hp. Phy4SH). Source: root of tutsan	24	1
33182	*Phytophthora cinnamomi* Kochi Pref. Pl. Prot. Of. (T. Kobayashi, Hp. Phy5-2SH). Source: root of tutsan	24	1
33183	*Phytophthora cinnamomi* Kochi Pref. Pl. Prot. Of. (T. Kobayashi, Hp. Phy6SH). Source: root of tutsan	24	1
33184	*Tremellochaete japonica* IFO (A. Nakagiri, Tj-8). Source: dead branch of mangrove	24	1
33185	*Tremellochaete japonica* IFO (A. Nakagiri, Tj-2). Source: dead branch of mangrove	24	1
33186	*Tremellochaete japonica* IFO (A. Nakagiri, Tj-4). Source: dead branch of mangrove	24	1
33187	*Tremellochaete japonica* IFO (A. Nakagiri, Tj-1). Source: dead branch of mangrove	24	1

IFO No (T=Type strain)	Name	Temp	Med
33188	*Tremellochaete japonica* IFO (A. Nakagiri, Tj-6). Source: dead branch of mangrove	24	1
33189	*Tremellochaete japonica* IFO (A. Nakagiri, Tjmass-2). Source: dead branch of mangrove	24	1
33190	*Phytophthora nicotianae* Kochi Pref. Pl. Prot. Of. (T. Kobayashi, C.C. Phy 1-1SZ). Source: leaf of Chinese chive	24	1
33191	*Phytophthora nicotianae* Kochi Pref. Pl. Prot. Of. (T. Kobayashi, W.O.Phy 1-1SZ). Source: leaf of Welsh onion	24	1
33192	*Phytophthora nicotianae* Kochi Pref. Pl. Prot. Of. (T. Kobayashi, G. Phy 1-1SZ). Source: bulb	24	1
33193	*Phytophthora nicotianae* Kochi Pref. Pl. Prot. Of. (T. Kobayashi, L. Phy 1SZ). Source: stem of lily	24	1
50513	MTA natural killer (NK)-like T cell leukemia/lymphoma	37	
50514	ISO-HAS hemangiosarcoma arizing on scalp, metastasis to auricle	37	
50515	ISOS-1 poorly differentiated angiosarcoma at skin	37	
50516	TMD5 acute lymphoblastic leukemia	37	
50518	KAI3 severe chronic active Epstein-Barr virus infection , hypersensitivity to mosquito bite	37	
50519	A1 Dept. Neurochem., Natl. Inst. Neurosci. (K. Ohsawa).	37	
50520	MG5 Dept. Neurochem., Natl. Inst. Neurosci. (K. Ohsawa).	37	
50524	NCB chronic myelogenous leukemia, blast crisis	37	
50525	NC02 chronic myelogenous leukemia, blast crisis	37	

List of Excluded Strains

January 1999 - December 2000

IFO No.	NAME OF SPEICES
5822	*Phycomyces blakesleeanus*
5871	*Phycomyces blakesleeanus*
6927	*Tricholoma matsutake*
14292	*Clostridium sporogenes*

List of Media Newly Registered

353 SSW Medium

Sodium malete	1	g
Sodium succinate	1	g
KH_2PO_4	0.2	g
K_2HPO_4	0.9	g
$(NH_4)_2SO_4$	1	g
$MgSO_4 \cdot 7H_2O$	0.2	g
NaCl	20	g
$CaCl_2 \cdot 2H_2O$	0.05	g
$Na_2S_2O_3 \cdot 5H_2O$	0.1	g
$MgCl_2 \cdot 6H_2O$	2	g
Yeast extract	0.1	g
Vitamin mixture*	1	ml
Trace element solution**	1	ml
Distilled water	1	L
	pH 7.2	

* Vitamin mixture

Thiamine-HCl	50	mg
Niacine	50	mg
p-Aminobenzoic acid	30	mg
Pyridoxal-HCl	10	mg
Biotin	5	mg
Vitamin B_{12}	5	mg
Distilled water	100	ml

** Trace element solution

EDTA-2Na	1	g
$FeCl_3 \cdot 6H_2O$	2	g
$ZnCl_2$	0.1	g
$MnCl_2 \cdot 2H_2O$	0.1	g
H_3BO_3	0.1	g
$CoCl_2 \cdot 6H_2O$	0.1	g
$Na_2MO_4 \cdot 2H_2O$	20	mg
$CuCl_2 \cdot 2H_2O$	10	mg
$NiCl_2 \cdot 6H_2O$	10	mg
Na_2SeO_3	5	mg
Distilled water	1	L

354 (for IFO 3836)

Polypepton*	10	g
Yeast extract	2	g
$MgSO_4 \cdot 7H_2O$	1	g
Soil extract**	100	ml
Distilled water	900	ml
	pH 7.2	

* Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

** 500 g of soil in 500 ml of water was autoclaved at 121℃ for 30 min and filtered.

355 (for IFO 15890)

R-CW Agar

Peptone	5	g
Tryptone	5	g
Yeast extract	5	g
KH_2PO_4	5	g
$CH_3COONa \cdot 3H_2O$	5	g
$(NH_4)_2 \cdot HC_6H_5O_7$	2	g
$MgSO_4 \cdot 7H_2O$	0.5	g
$MnSO_4 \cdot nH_2O$	0.5	g
Tween 80	1	ml
Cheese whey*	1	L
	pH 6.0	

* Add HCl to warmed milk, and coagulate. After coagulation, centrifuge and use the supernatant.

356 (for IFO 14606, 14607)

Polypepton*	10	g
Yeast extract	2	g
$MgSO_4 \cdot 7H_2O$	1	g
N-Acetyl-D-glucosamine	50	mg
Distilled water	1	L
Agar	15	g
	pH 7.0	

* Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

357 (for IFO 14412, 14413)

Polypepton*	10	g
Yeast extract	2	g
$MgSO_4 \cdot 7H_2O$	1	g
D-Alanine	50	mg
Distilled water	1	L
Agar	15	g
	pH 7.0	

* Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

358 (for IFO 14414)

Polypepton*	10	g
Yeast extract	2	g
$MgSO_4 \cdot 7H_2O$	1	g
Thymine	100	mg
Distilled water	1	L
Agar	15	g
	pH 7.0	

* Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

359 (for IFO 14419)

Polypepton*	10	g
Yeast extract	2	g
$MgSO_4 \cdot 7H_2O$	1	g
Cytidine	50	mg
Distilled water	1	L
Agar	15	g
	pH 7.0	

*Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

360 SA Medium

Sodium acetate	1	g
Sodium succinate	1	g
KH_2PO_4	0.5	g
K_2HPO_4	0.6	g
$(NH_4)_2SO_4$	1	g
$MgSO_4 \cdot 7H_2O$	0.2	g
NaCl	0.2	g
$CaCl_2 \cdot 2H_2O$	0.05	g
$Na_2S_2O_3 \cdot 5H_2O$	0.1	g
Yeast extract	0.1	g
Vitamin mixture*	1	ml
Trace element solution**	1	ml
Distilled water	1	L
Agar	15	g
	pH 6.7	

* Vitamin mixture

Thiamine-HCl	500	mg
Niacine	500	mg
p-Aminobenzoic acid	300	mg
Pyridoxal-HCl	100	mg
Biotin	50	mg
Vitamin B_{12}	50	mg
Distilled water	1	L

** Trace element solution

EDTA-2Na	1	g
$FeCl_3 \cdot 6H_2O$	2	g
$ZnCl_2$	0.1	g
$MnCl_2 \cdot 2H_2O$	0.1	g
H_3BO_3	0.1	g
$CoCl_2 \cdot 6H_2O$	0.1	g
$Na_2MoO_4 \cdot 2H_2O$	20	mg
$CuCl_2 \cdot 2H_2O$	10	mg
$NiCl_2 \cdot 6H_2O$	10	mg
Na_2SeO_3	5	mg
Distilled water	1	L

361 Rhodobacter veldkampii Medium

Sodium acetate	1	g
Sodium succinate	1	g
KH_2PO_4	0.5	g
K_2HPO_4	0.6	g
$(NH_4)_2SO_4$	1	g
$MgSO_4 \cdot 7H_2O$	0.2	g
NaCl	0.2	g
$CaCl_2 \cdot 2H_2O$	0.05	g
$Na_2S_2O_3 \cdot 5H_2O$	0.1	g
Yeast extract	0.1	g
Vitamin mixture*	1	ml
Trace element solution**	1	ml
Sulfide solution***	50	ml
Distilled water	1	L
	pH 6.7	

Sterilize Vitamin mixture and Sulfide solution separately, and add after autoclaving.

* Vitamin mixture

Thiamine-HCl	50	mg
Niacine	50	mg
p-Aminobenzoic acid	30	mg
Pyridoxal-HCl	10	mg
Biotin	5	mg
Vitamin B_{12}	5	mg
Distilled water	100	ml

** Trace element solution

EDTA-2Na	1	g
$FeCl_3 \cdot 6H_2O$	2	g
$ZnCl_2$	0.1	g
$MnCl_2 \cdot 2H_2O$	0.1	g
H_3BO_3	0.1	g
$CoCl_2 \cdot 6H_2O$	0.1	g
$Na_2MoO_4 \cdot 2H_2O$	20	mg
$CuCl_2 \cdot 2H_2O$	10	mg
$NiCl_2 \cdot 6H_2O$	10	mg
Na_2SeO_3	5	mg
Distilled water	1	L

*** Sulfide solution

Na_2S	3	g
Distilled water	200	ml

Autoclave with a magnetic-stirrer rod in the flask. Partially neutralize the sterilized sulfide solution by adding, on a magnetic stirrer, drop by drop, sterile 2 M H_2SO_4.

362 (for IFO 12172)

Polypepton*	5	g
Yeast extract	5	g
Glucose	5	g
$MgSO_4 \cdot 7H_2O$	1	g
NaCl	80	g
Distilled water	1	L
	pH 9.5	

* Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

363 (for IFO 3896)

Peptone	10	g
Meat extract	10	g
Yeast extract	5	g
Glucose	20	g
Tween 80	1	g
K_2HPO_4	2	g
Sodium acetate	5	g
Diammonium citrate	2	g
$MgSO_4 \cdot 7H_2O$	0.2	g
$MnSO_4 \cdot nH_2O$	0.05	g
Mevalonic acid	50	mg
Distilled water	1	L
	pH 5.5	
or		
SI medium dehydrated*	50	g
Distilled water	1	L
	pH 5.5	

* Nippon Jozo Kyokai, 6-30, Takinogawa 2 chome, Kita-ku, Tokyo 114.

364

Polypepton*	5	g
Yeast extract	5	g
Glucose	5	g
$MgSO_4 \cdot 7H_2O$	1	g
Distilled water	1	L
Agar	15	g
	pH 8.0	

* Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

365

Polypepton*	5	g
Yeast extract	5	g
Glucose	5	g
$MgSO_4 \cdot 7H_2O$	1	g
Distilled water	1	L
	pH 5.5	

* Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

366 For Lactic Acid Bacterium

Peptone	10	g
Meat extract	10	g
Yeast extract	5	g
Glucose	20	g
Tween 80	1	g
K_2HPO_4	2	g
Sodium acetate	5	g
Diammonium citrate	2	g
$MgSO_4 \cdot 7H_2O$	0.2	g
$MnSO_4 \cdot nH_2O$	0.05	g
Distilled water	1	L
Agar	15	g
	pH 5.5	

367 mjYPGS Medium

NaCl	3	g
K_2HPO_4	14	mg
$CaCl_2 \cdot 2H_2O$	14	mg
NH_4Cl	12.5	mg
$NaNO_3$	12.5	mg
$MgSO_4 \cdot 7H_2O$	0.34	g
$MgCl_2 \cdot 6H_2O$	41.8	mg
KCl	33	mg
$NiCl_2 \cdot 6H_2O$	0.05	mg
$Na_2SeO_3 \cdot 5H_2O$	0.05	mg
Fe(III)-citrate	5	mg
Trace mineral solution*	1	ml
$Na_2S_2O_3 \cdot 5H_2O$	2	g
$Na_2SiO_3 \cdot 9H_2O$	1	g
Yeast extract (Difco)	1	g
Peptone (BBL)	1	g
Glucose	0.2	g
Sodium succinate	0.2	g
Distilled water	1	L
	Adjust pH 7.5-8.0 with H_2SO_4	

* Trace mineral solution

Nitlirotriacetic acid	1.5	g
$MnSO_4 \cdot 2H_2O$	0.5	g
$CoSO_4 \cdot 7H_2O$	0.5	g
$ZnSO_4 \cdot 7H_2O$	0.18	g
$CuSO_4 \cdot 5H_2O$	0.01	mg
$KAl(SO_4)_2 \cdot 12H_2O$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \cdot 2H_2O$	0.01	mg
Distilled water	1	L

702 (for rehydration of dried culture)

Polypepton*	10	g
Yeast extract	2	g
$MgSO_4 \cdot 7H_2O$	1	g
Distilled water	1	L
	pH 7.0	

* Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

807 Thioglycolate Medium*

Polypepton**	15	g
Yeast extract	5	g
Glucose	5	g
NaCl	2.5	g
L-Cystine	0.5	g
Sodium thioglycolate	0.5	g
Resazurin	1	mg
Distilled water	1	L
Agar	15	g
	pH 7.0 - 7.2	

* Thioglycollate Medium I <Daigo> for JP general test, Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

** Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

Presentation of Papers at Scientific Meetings, 1999-2000

Nishi-Nippon Division of The Mycological Society of Japan, 6th Mycology Course (January, 1999, Kyoto)

K. Mikata
The latest taxonomy of the ascomycetous yeasts

The Committee of Information, The Japan Health Science Foundation (March, 1999, Osaka)

Masao Takeuchi
Biological Research Resources in Japan

Japan Society for Bioscience, Biotechnology and Agrochemistry (March, 1999, Fukuoka)

T. Tamura, S. Suzuki[1)] and K. Hatano
New actinomycetes belonging to the family *Streptosporangiaceae*
1) Tanabe Seiyaku Co., Ltd.

Y. Nakagawa, K. Kuroshima[1)], T. Sakane[1)] and K. Hatano
Phylogenetic positions of the three strains isolated from silkworm larvae
1) Takeda Chem. Ind.

K. Ueda-Nishimura and K. Mikata
A new species of ascomycetous yeast from leaves of mangrove *Kandelia candel*

Mycological Society of Japan (May, 1999, Hirosaki)

Tad. Ito, I. Okane, A. Nakagiri and W. Gams[1)]
Three species of the genus *Acremonium* of Deuteromycotina
1) CBS, The Netherlands

I. Okane, A. Nakagiri and Tad. Ito
Secondary conidium-like structures of *Monochaetia*

A. Nakagiri, I. Okane and Tad. Ito
Diversity and ecology of halophytophthoras (Oomycetes) inhabiting mangrove brackish water

Japan Society for Culture Collection (June, 1999, Chiba)

A. Nakagiri

Exchange of cultures between culture collections

K. Mikata, K. Ueda-Nishimura, S. Goto[1)], C. P. Kurtzman[2)], M. Suzuki[3)], D. Yarrow[4)] and T. Nakase[3)]
The new species *Candida kofuensis* sp. nov., isolated from wild grapes
1) Tokyo Univ. of Agric.
2) NRRL, USA
3) Japan Collection of Microorganisms, RIKEN
4) CBS, The Netherlands

The Annual Meeting of the Society for Actinomycetes Japan (June, 1999, Kitamoto)
T. Tamura and K. Hatano
The phylogenetic structure of the genus *Actinoplanes* on the basis of 16S rRNA

The 7th International Marine and Freshwater Mycology Symposium (July, 1999, Hong Kong)
A. Nakagiri
Ecology and diversity of halophytophthoras

Tad. Ito and A. Nakagiri
Mycoflora of the rhizospheres of Japanese mangrove trees

D. Honda[1,2)], T. Yokochi[2)], T. Nakahara[2)], S. Raghukumar[3)], A. Nakagiri, K. Schaumann[4)] and T. Higashihara[2)]
18S rDNA phylogeny of labyrinthulids and thraustochytrids with molecular floristic investigation
1) Marine Biotech. Inst.
2) Natl. Inst. Biosci. Human-Tech.
3) Natl. Inst. Ocean., India
4) Alfred-Wegener-Inst. fur Polar-Meeresforsch., Germany

9th International Congress of Bacteriology and Applied Microbiology (August, 1999, Sydney)
Mariko Takeuchi, A. Hiraishi[1)], K. Hamana[2)] and K. Hatano
Proposal of the genus *Sphingomonas sensu stricto* and three new genera based on the analysis of 16S ribosomal RNA sequence and polyamine profile
1) Toyohashi Univ. of Technol.
2) Sch. of Health Sci., Gunma Univ.

1st International Conference on Biology of Actinomycetes under Extreme Environments (August, 1999, Kunming, China)
T. Tamura
Establishment of taxonomic status of the strains belonging to invalidated genera of

the order *Actinomycetales*

The Society for Bioscience and Bioengineering, Japan (September, 1999, Osaka)

A. Nakagiri
Diversity of mangrove inhabiting fungi and their application

Y. Nagatsuka[1)], H. Kawasaki[1)], L. Savitree[2)], K. Mikata and T. Seki[1)]
Citeromyces siamensis sp. nov., a new species of halotolerant yeasts isolated in Thailand
1) IC Biotech, Osaka Univ.
2) Kasetsart Univ., Thailand

Annual Meeting on Microbial Taxonomy (October, 1999, Osaka)

Y. Nagatsuka[1)], H. Kawasaki[1)], L. Savitree[2)], K. Mikata and T. Seki[1)]
Citeromyces siamensis sp. nov., a new species of halotolerant yeasts isolated in Thailand
1) IC Biotech, Osaka Univ.
2) Kasetsart Univ., Thailand

A. Nakagiri
Studies on the diversity and collection of micro-organisms inhabiting mangroves

D. Honda[1,2)], T. Yokochi[2)], T. Nakahara[2)], S. Raghukumar[3)], A. Nakagiri, K. Schaumann[4)] and T. Higashihara[2)]
Molecular phylogeny of labyrinthulids and thraustochytrids based on the sequencing of 18S ribosomal RNA gene
1) Marine Biotech. Inst.
2) Natl. Inst. Biosci. Human-Tech.
3) Natl. Inst. Ocean., India
4) Alfred-Wegener-Inst. fur Polar-Meeresforsch., Germany

Mariko Takeuchi, Y. Hamana[1)] and A. Hiraishi[2)]
Proposal of the genus *Sphingomonas sensu stricto* and three new genera on the basis of phylogenetic and chemotaxonomic analyses
1) Sch. of Health Sci., Gunma Univ.
2) Toyohashi Univ. of Technol.

11th International Symposium on the Biology of Actinomycetes (October, 1999, Crete, Greece)

T. Tamura and K. Hatano
The phylogenetic structure of the genus *Actinoplanes*

K. Hatano, T. Nishii and H. Kasai[1)]

Proposal of a new criterion for identification and classification of *Streptomyce* species: *gyrB* sequence
1) Marine Biolotech. Inst.

Riken Symposium (November, 1999, Wako)
A. Nakagiri
Autecology of *Halophytophthora* species inhabiting mangrove brackish water

Japan Science and Technology Corporation (JST) Symposium (December, 1999, Tokyo)
Masao Takeuchi and K. Hatano
A database for the classification and identification of *Streptomyces* strain

Japan Society for Bioscience, Biotechnology and Agrochemistry (April, 2000, Tokyo)
M. Suzuki[1,2], Y. Nakagawa, S. Yamamoto[1] and S. Harayama[1]
Phylogenetic analysis of marine *Cytophaga*-like bacteria based on the *gyrB* sequence: Proposal of new genus *Haerentibaculum*
1) Marine Biotech. Inst.
2) Kyowa Hakko Kogyo

Mycological Society of Japan (May, 2000, Nara)
Tad. Ito, A. Nakagiri, M. Tantichaoren[1] and L. Manoch[2]
Mycobiota of the mangrove forest soil in Thailand
1) National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand
2) Kasetsart Univ., Thailand

I. Okane, A. Nakagiri and Tad. Ito
Identity of *Guignardia* sp. inhabiting ericaceous plants

A. Nakagiri, Tad. Ito, Leka Manoch[1] and Morakot Tanticharoen[2]
A new species of *Halophytophthora* (Oomycota) from intertidal fallen leaves in mangroves of Iriomote Is. and Thailand
1) Kasetsart Univ., Thailand
2) National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand

T. Oda[1], C. Tanaka[1], P. Liu[2], A. Nakagiri and M. Tsuda[1]
Molecular phylogeny of *Termitomyces* species
1) Grad. Sch. Agric., Kyoto Univ.
1) Kunming Inst. Bot., Academia Sinica

Japan Society for Culture Collection (June, 2000, Sendai)
K. Ueda-Nishimura and K. Mikata

A new genus of *Saccharomyces* complex, *Tetrapisispora*

Symposium on labyrinthulids and related eukaryotic micro-organisms (June, 2000, Kobe)

A. Nakagiri
Methods for culture and preservation of labyrinthulids and taxonomy and ecology of marine fungi

The 9th International Congress for Culture Collection (July, 2000, Brisbane)

A. Nakagiri, Tad. Ito, L. Manoch[1)] and M. Tanticharoen[2)]
Comparative biodiversity of fungi inhabiting tropical (Thailand) and subtropical (Japan) mangroves
1) Kasetsart Univ., Thailand
2) National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand

K. Hatano and T. Nishii
Classification and quality control of *Streptomyces* species by *gyrB*-based phylogenetic analysis

Kikuko Takeuchi[1)], T. Yoshida and Masao Takeuchi
In vitro proplatelet formation of megakaryocytes generated from human cord blood CD(+)34 cells
1) Osaka Pref. Coll. of Health Sci.

T. Sakane[1)], Y. Nakagawa and K. Hatano
L-drying method for preservation of bacteria in Institute for Fermentation, Osaka (IFO)
1) Takeda Chem. Ind.

Asian Mycological Congress 2000 (July, 2000, Hong Kong)

I. Okane, A. Nakagiri and Tad. Ito
Identity of two major endophytic ascomycetes of ericaceous plants

10th International Symposium on Yeasts, The Rising Power of Yeasts in Science and Industory (August, 2000, Arnhem)

T. Hisatomi[1)], T. Kodama[1)], K. Takahashi[1)], M. Tsuboi[1)], K. Ueda-Nishimura and K. Mikata
Construction of host-vector systems and characterization of chromosome organization in a new species "*Saccharomyces naganishii*"
1) Dept. Biotechnol., Fac. Engineering, Fukuyama Univ.

M. T. Smith[1)], A.W.A.M. de Cock[1)], Y. van de Peer[2)], K. Ueda-Nishimura and G.

S. de Hoog[1)]
Phylogeny and biodiversity in *Geotrichum* Link: Fries
1) CBS, The Netherlands
2) Univ. of Konstanz, Germany

The 20th Annual Meeting on Microbial Systematics -Microbial Systematics, Today and Tomorrow- (October, 2000, Tokyo)

K. Hatano
Classification of *Streptomyces* species based on *gyrB* sequences -Comparative studies of phylogenetic classification based on *gyrB* sequences and phenotypes in the genus *Streptomyces*-

Nishi-Nippon Division of The Mycological Society of Japan (November, 2000, Tsu)

Tad. Ito
Long-term preservation of basidiomycetous cultures

Scientific Papers, 1999-2000

The phylogeny of species of the ascogenous teleomorphic yeast genera *Ambrosiozyma, Hormoascus, Hyphopichia, Arthroascus*, and *Botryoascus* based on the partial sequences of 18S and 26S ribosomal RNAs

Y. Yamada[1)], T. Higashi[1)] and K. Mikata

Bull. Fac. Agric. Shizuoka Univ. 48: 1-13 (1998)

1) Dept. of Appl. Biol. Chem., Fac. of Agric., Shizuoka Univ.

Replacement of ATCC 21273, The current type strain of *Streptomyces rameus* Shibata 1959, with IFO 3782: Request for an opinion

K. Hatano

Int. J. Syst. Bacteriol. 49: 931-932 (1999)

Molecular phylogeny of labyrinthurids and thraustochytrids based on the sequencing of 18S ribosomal RNA gene

D. Honda[1,2)], T. Yokochi[2)], T. Nakahara[2)], S. Raghukumar[3)], A. Nakagiri, K. Schaumann[4)] and T. Higashihara[2)]

J. Eukaryot. Microbiol. 46: 637-646 (1999)

1) Marine Biotech. Inst.

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Information

Information on the cultures maintained in the IFO collection is available in two forms.

One is the *List of Cultures* (11th edition, 2000), in which a total of 11,688 strains of bacteria including actinomycetes, yeast, filamentous fungi, and bacteriophages are listed. This publication also includes the IFO strain database on a CD-ROM operating on a standard browser, IE 4.0 or NN 6.0. The price is ¥ 5,000 (plus tax in Japan) excluding postage. For overseas delivery, additional shipping and handling charges are requested. It is available through the Academic Society Japan, 16-19, Honkomagome 5-chome, Bunkyo- ku, Tokyo 113-8622.

The second source of information is the IFO home page (http://www.ifo.or.jp), which gives access to the IFO biological resources database, the order sheet, and information on publications, services etc. This on-line system allows the user to search for scientific names, IFO numbers, other culture collection designations, strain data, and medium data of microorganisms, and to order cultures and deposit strains.

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