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REPORT OF THE DIRECTOR

Masao TAKEUCHI

Since its establishment in 1944, the Institute for Fermentation, Osaka (IFO) has strongly endeavored to maintain a culture collection for over 54 years with the continuously financial support by Takeda Chemical Ind. Ltd. 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 resources materials. Another result is that a Convention on Microorganisms Diversity was recently concluded internationally and had a great influence on 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. From 1995 the IFO joined with the Japan Health Sciences Foundation in managing a research resources bank for the Ministry of Health and Welfare. To reconstruct the IFO data management system, the IFO joined two research projects of the JST beginning in 1997 and NEDO beginning in 1998 established by the Science and Technology Agency and the Ministry of International Trade and Industry, respectively. These three projects contributed to facilitating computerization as well as improving the financial standing of IFO.

In 1998, the IFO constructed and established a computer networking system using a client-server to increase the efficiency of data management using Internet, E-mail and common files on servers. The Home Page of the IFO was set up on the web site (<http://wwwsoc.nacsis.ac.jp/ifo/index.html>). The database of biological research resources materials in the IFO is under constructing now on the Internet server.

In the past two years, the IFO organized the following meeting: The Autumn symposium of the Japan Tissue Culture Association on November 27, 1997.

Mr. Tadayoshi Ito was awarded a Prominent Study Award for Inhibition of sardine flesh lipoxygenase by a new antioxidant from *Aspergillus terreus* in 1997 by the Japanese Oil Chemistry Society.

The financial situation of IFO has deteriorated since 1991, but as a consequence of reorganizing the management of IFO, the economic situation was improved in 1995. Since 1995, the IFO has continuously shown a surplus and will also expect to show some surplus in 1998.

Personnel Affairs

At the 107th meeting of the Board of Trustees in June 1995, Dr. Masahiko Fujino of Takeda Chemical Industries Ltd., was nominated as a member of the Board of Trustees, while Dr. Ryoji Takenaka of Japanese Health Science Foundation and Dr. Takashi Nakase of RIKEN were nominated as councilors from June 1998.

In December 1997, Mr. Masayoshi Moriwaki, treasurer of the institute, retired; and Mr. Ryuji Takamaru, who then joined the IFO as treasurer, retired on January 1, 1999. The IFO is grateful to these persons for their dedication while working in the institute.

The new treasurer of the institute, Dr. Itaru Suenaga, joined the IFO in December 1998 and was appointed treasurer on January 1, 1999.

Ms. Akiko Kuroda of the Japan Health Sciences Foundation worked in the IFO from May 1996.

Mr. Kengo Tsuji, Ms. Yayoi Yamaguchi and Ms. Yukie Yamashita worked in the IFO part time from the summer of 1998. IFO was helped by their skilled services.

Dr. S. A. Alias (University Malaysia) joined to a fungi group for research on marine microbiology for two weeks.

International Meetings

The staffs of the IFO attended international meetings during these past two years, presented scientific papers at these meetings and promoted mutual cooperation among collections around the world.

Dr. Masao Takeuchi attended the first International Symposium on Preservation and Application of Microbial Resources, held in the CCRC of Taiwan in May 1997 and presented a paper on "Roles of animal cell banks in life sciences".

Mr. Tomohiko Tamura attended the 10th International Symposium for Biology Actinomycetes, held in China in May 1997 and presented a paper on "A new genus of the order *Actinomycetes*, *Kineopoolyspora* gen nov Roles".

Dr. Akira Nakagiri, attended the "Asian-Pacific Mycological Conference on Biochemistry and Biotechnology", held in Thailand in June 1998, and presented a paper on "Diversity of halophytophthoras in subtropical mangroves and factors affecting their distribution".

Dr. Mariko Takeuchi, attended the "First International Symposium on Identification of Microorganisms and their Secondary Metabolites", held in KCCM of Korea in August 1998, and presented a paper on "Actinobacteria in Mangrove Rhizospheres: Isolation, Identification and Classification".

Dr. Izumi Okane attended the Regional Workshop on Endophytic Microbes in Indonesia in November 1998, and they presented papers on "Assemblages of endophytic fungi in leaves of ericaceous plants and their successional change".

Collection and Publications

The total number of cultures stored in the IFO culture collection reached 15,784 at the end of 1997 and 15,943 at the end of 1998. The newly accepted strains during each year are listed in the present issue of the IFO Research Communications. The total number of cultures distributed from the IFO culture collection were 8,500 in 1997 and 9,032 in 1998. Of these, 300 and 452 strains were distributed abroad in 1997 and 1998, respectively.

IFO Research Communications No. 18 was published in March 1997, and the IFO List of Cultures, 5th edition, Animal Cell Lines, was published in March 1998. The catalogue involves about 200 animal cell lines. Manuscript for the catalogues was prepared and edited from the database stored in animal cell section of the IFO culture collection and processed by Drs. Touho Yoshida and Motonobu Satoh. Furthermore, the same full documents in the catalogue of animal cell lines are shown on the web site for animal cells in IFO Home Page (<http://wwwsoc.nacsis.ac.jp/ifo/index.html>).

Finances

Since the establishment of the IFO culture collection, Takeda Chemical Industries, Ltd. has promoted IFO activities by providing over 54 years of continuous financial support. The financial standing of the IFO in previous years (1991 to 1995) was

Table 1. CLOSING ACCOUNTS OF IFO

(100 thousand yen)

YEAR		1995	1996	1997	(%)
INCOME	Donations	1,950	1,750	1,750	56
	Interest & Dividends	550	570	598	19
	Grants & Contracts	170	100	100	3
	Cultures	537	550	482	16
	Other Services	234	197	175	6
TOTAL		3,441	3,237	3,110	100
EXPENSES	Personnel Expenses	2,439	2,110	1,820	72
	Supplies	316	214	352	14
	Facility Maintenance Expenses	398	375	278	11
	Other	267	101	88	3
	TOTAL	3,420	2,800	2,538	100
BALANCE		21	437	572	

summarized in the previous director's report published in 1997. The financial standing of the IFO in the recent two years is summarized in Table 1, which shows the annual income and expenses of the IFO for each fiscal year for 1996 and 1997.

Takeda Chemical Industries Ltd. has continued to provide support for 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 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 management of the HSRRB bank. "Supplies" refers to expenses for purchase of expendable supplies and equipment. "Facility Maintenance Expenses" is composed of expenses related to renting the facilities used by the IFO.

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

IFO's expenses continued to increase by ¥10–15 million annually from 1991 to 1994. Increasing personnel expenses were the major contributory factor. A deficit occurred in 1992, reaching ¥43.8 million in 1994, as shown the previous director's report. The main decrease in expenses was obtained by reducing personnel expenses, because the number of employees was decreased to 60% of that in 1994. In fiscal year 1995, the budget showed a ¥2.1 million surplus as a result of increased income and decreased expenses, and followed to ¥44 million and ¥57 million in 1996 and 1997, 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, public service, non-profit 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 IFO's activities we hope that the donor and board members will show invariable and continuous support in the future. At the same time, we should notice that we are evaluating 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 persons gave lectures or seminars. The titles of their speeches are shown. These informative speeches created a great impression on us.

Dr.J-D. Lee, Pusan Univ., Korea, on 7 February 1997.

Dr. K. Kersters, Director, BCCM, University Ghent, Belgium, on 7 April 1997.

Dr. M. Guangzhen, Director, Institute of Microbiology, Chinese Academy of Sciences, China, on 13 August 1997. "Biotechnology in China"

Dr. J-F. Bernardet, Centre de echerches INRA, France, on October 1997.

"Epidemiology of the salmonid fish pathogen *Flavobacterium psychrophilum*: outcome of molecular investigations"

Dr. S.Vikineswary, Univ. Malaya, Malaysia, on 12 November 1997.

- Dr. Y-H. Park, Director, KCTC, Korea, on 13 November 1997
- Dr. P. Pitakpaivan, Dept. of Agriculture, Thailand, on 25 November 1997.
- Dr. R. A. Grzybowski, Director, Inst. Agricultural food biotechnology, Poland, on 26 November 1997.
- Dr. Y-K. Sin, KCTC, Korea, on 4 December 1997.
- Dr. M. Tanticharoen, Deputy Director, NCGEB, Thailand, on 10 December 1997.
- Dr. Z. Peijin, Chinese Academy Sciences, China, on 19 December 1997.
- Dr. J. Swings, Univ. Ghent., Belgium, on 27 April 1998.
- Dr. C-Y. Chen, Culture Collection & Research Center, Taiwan, on 31 July 1998.
"Quality control of animal cell lines at CCRC"
- Dr. M. C. Aime, Virginia Polytechnic and State Univ. USA, on 6 August 1998.
"Generic concepts in the Crepidotaceae (Imai)"
- Dr. L-M.Branchini, University of Campinas, UNICAMP. Brazil, on 3 September 1998.
- Dr. S. A. Alias, Univ. Malaysia, Malaysia, on 15 September 1998. "Biodiversity of tropical marine mangrove fungi in Malaysia"
- Dr. J. Kaiser, Max-Planck Institute, Germany, on 21 September 1998. "Sulfur isotopic exchange of thiosulfate"
- Dr. N. T. Thanh, Center for Mycological Research, National University of Hanoi, Vietnam, on 24 September 1998.

Professor emeritus Dr. Saburo Fukui, board member of the institute, passed away on January 11, 1998. He made great contributions to the development of the Institute for Fermentation, Osaka. Our heartfelt condolence is extended to the bereaved.

Establishment and characterization of a murine astroglial cell line

Motonobu SATOH, Nobuaki YANAI*, Touho YOSHIDA
and Masao TAKEUCHI

Summary

A murine cell line, designated as KT-5, was established from a brain tumor induced by the interplacental injection of *N*-nitrosomethyl urea. KT-5 cells grew *in vitro* with a doubling time of 17 h. The karyotype of KT-5 cells was pseudotriploid with a modal chromosome number of 61. KT-5 was tumorigenic when transplanted either intraperitoneally or subcutaneously into C3H/He mouse. Immunocytochemical analysis demonstrated that KT-5 was immunopositive for glial fibrillary acidic protein (GFAP) and S-100 protein but not for Thy-1, myelin basic protein, or neurofilament, suggesting that KT-5 cell derives from a murine astrocyte. Conditioned medium of KT-5 contained neurite-promoting activity for neurons and growth-promoting activity for KT-5 cells themselves.

Keywords: astrocyte, mouse, cell line

Three classes of glial cells, astrocytes, oligodendrocytes and microglia, are known in the vertebrate central nervous system (CNS). Each class is characterized and distinguished from others by cell morphology and several immunohistochemical markers (4). Among them, astrocytes are known to play roles in supplying glucose to neurons (11), in controlling the levels of neurotransmitters and ions in the brain environment through neuronal-glial signaling (1), and in forming a blood-brain barrier in cooperation with endothelial cells (8). In addition, astrocytes produce a variety of growth factors and cytokines including of neurite-promoting factor (15), glial growth factor (5), and neuroblast growth inhibitory factor (16), and they are thus suggested to regulate the proliferation and differentiation of cells in CNS (13).

Until recently, glial cells were more difficult to cultivate than cells originating from other organs, and therefore few immortalized glial cell lines have been reported. It is known that the interplacental administration of carcinogens such as *N*-nitrosomethyl urea

*Institute for Development, Aging and Cancer, Tohoku University

induces brain tumors in fetuses. A widely used glial cell line, C₆, was established from brain tumors induced by this method (2). Here we will report the establishment and characterization of a murine astroglial cell line from a *N*-nitrosomethyl urea-induced brain tumor.

Materials and Methods

Media and Reagents Ham's F12 medium, Dulbecco's modified Eagle's medium (DMEM), and Eagle's minimal essential medium (MEM) were purchased from Nissui Seiyaku; HEPES, Giemsa's solution, and kanamycin sulfate from Wako Junyaku; nerve growth factor and fibroblast growth factor from Takara Shuzo; dispase I from Sanko Junyaku, and fetal bovine serum (FBS) from M. A. Bioproducts. Antibodies used were as follows: rabbit anti-cow glial fibrillary acidic protein (anti-GFAP; Z334, Dakopatts), mouse monoclonal anti-GFAP (RPN1106, Amersham), rabbit anti-human brain myelin basic protein (A623, Dakopatts), rabbit anti-S100 protein (AB-1250, Didets), mouse monoclonal anti-Thy-1.2 (Becton Dickinson), mouse monoclonal anti-160 kD neurofilament polypeptide (RPN1104, Amersham), fluorescein-conjugated goat anti-rabbit IgG (S955, Miles), fluorescein-conjugated rabbit anti-mouse IgG (Cosmo Bio). All other reagents were purchased from Sigma.

Cell Cultures A murine brain tumor induced by *N*-nitrosomethyl urea was transplantable *in vivo* when injected intraperitoneally into C3H/He mice. The tumor in the 8th passage *in vivo* was obtained from Department of Neurosurgery, Kyorin University. The tumor was excised from the host mouse under sterile conditions, and cut into 1 mm³ pieces. The pieces were treated with 0.2% trypsin in phosphate-buffered saline (PBS) for 30 min at 37 °C, then suspended in Ham's F12 medium supplemented with 10% FBS and 50 µg/ml kanamycin. The resultant cell suspension was seeded onto plastic culture dishes (Primaria # 3802, Falcon) and cultured in humidified air/5% CO₂ at 37 °C. At subcultivation, the cells were harvested by treatment with 500 units/ml dispase I and resuspended in a culture medium consisting of Ham's F12, 10% FBS, and 4 mM HEPES (pH 7.3). After several passages, seven clones were isolated by cloning. Two morphologically different types of clones were observed. One was fibroblastic, and was later revealed to be fibroblasts, possibly derived from meninges. The other had epithelial to crescent-shaped morphology with several processes. One of the latter type of clones, designated KT-5, was investigated. In this study, KT-5 cells were cultivated using Ham's F12 as a basal medium. This cell line was also cultivable in such media as MEM, DMEM, Iscov's modification of MEM, HB101 medium (Irvine Scientific), and GIT medium (Wako).

Plating efficiency KT-5 cells were seeded onto 35-mm culture dishes at 100 - 680 cells/dish and cultivated for 4 weeks. After Giemsa staining, the number of colonies was counted. The plating efficiency was calculated from the formula: (number of colonies/number of cells seeded) x 100 (%).

Karyotyping Cells were treated with 0.1 µg/ml colcemid for 2 h, harvested, treated

with 75 mM KCl, fixed with Carnoi's fixative, and spread over a slide glass. Chromosome specimens were stained with Giemsa's solution.

Tumorigenicity analysis KT-5 cells were intraperitoneally or subcutaneously injected into 5-week-old female C3H/Hej mice at 10^2 - 10^6 cells/animal. After 4 weeks, the mice were examined for tumor formation.

Isoenzyme analysis The electrophoretic migration patterns of isoenzymes were determined using an isoenzyme profiling kit (Authentikit, Corning).

Immunohistochemistry Cells were fixed with acetic acid : acetone = 1 : 9 mixture at -20°C , treated serially with acetone and ethanol each for 5 min at -20°C , and finally with PBS at room temperature. Fixed cells were incubated in primary antibody solution (1 : 2-1 : 200 dilution in PBS), then in fluorescein-conjugated secondary antibody solution (1 : 200 dilution in PBS) each for 1 h at room temperature. The fluorescein-labeled specimens were observed under a fluorescent microscope (Olympus BH2).

FACS analysis Cell suspension (2×10^6 cells/ml) was mixed with fluorescein-conjugated anti-Thy-1 monoclonal antibody (1 : 100 dilution), incubated for 30 min at 4°C , washed with PBS containing 0.1% sodium azide, and applied to FACS IV (Becton Dickinson) for analysis of Thy-1 expression.

Preparation of conditioned Media Confluent cultures of KT-5 were washed three times with PBS and once with MEM, and further cultivated in serum-free MEM (200-300 ml/850-cm² roller bottle) for 3 days. The conditioned culture medium was centrifuged at $2,500 \times g$, 4°C for 20 min, and the supernatant was filtered through $0.45\text{-}\mu\text{m}$ pore and then $0.22\text{-}\mu\text{m}$ pore Millipore filters. The resultant medium (fraction 1) was further separated by ultrafiltration through Amicon YM10 membrane into a 50 x concentrated fraction with molecular weight > 10 kDa (fraction 2) and a pass-through fraction (fraction 3).

Primary culture of chick forebrain neurons The forebrains of 8-day chick embryos were excised and treated with 0.25% trypsin and $80 \mu\text{g/ml}$ DNase in PBS for 20-25 min at 37°C . After terminating the trypsin reaction by addition of FBS, the solution was pipetted off and allowed to stand for 1 min in a centrifugation tube. Large fragments at the bottom of tube were discarded, and the dissociated cells suspended in the supernatant were collected by brief centrifugation. The cells were resuspended in MEM containing 10% FBS and cultivated in a plastic culture flask. After 2 h, most non-neuronal cells attached to the culture flask, while neurons did not. The non-adherent cells, mostly neurons, were collected by centrifugation, resuspended in MEM supplemented with 2.5% FBS, filtered through sterile lens paper, and seeded onto poly-L-ornithine-coated plastic dishes at a density to $1\text{-}2 \times 10^4$ cells/cm². On the next day, cultures were fed with fresh medium and the fractionated KT-5 conditioned medium was added. The neurons were further cultivated for 2-5 days with daily medium change, and the length of neurites was measured on the photographs of cultures.

Results and Discussion

Characteristics of KT-5 in culture

Figure 1 shows the growth curve of KT-5 cells. The mean doubling time of this cell

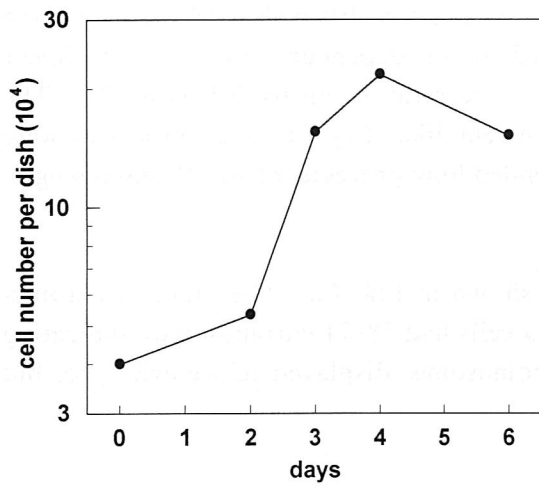


Fig. 1. Growth curve of KT-5. 4×10^4 cells were seeded onto a 35-mm plastic dish. Cells were counted with a Coulter counter.

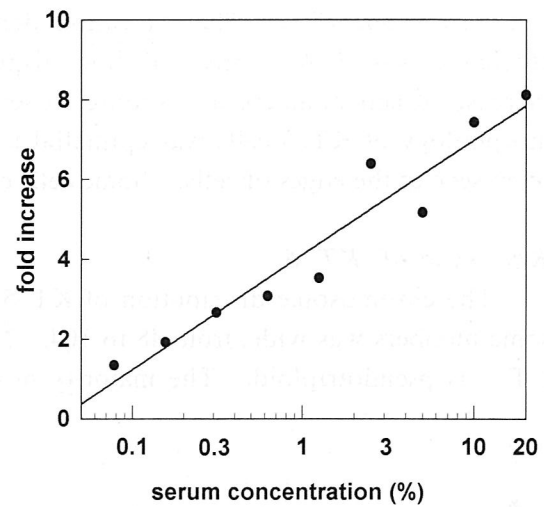


Fig. 2. Effect of serum concentration on the proliferation of KT-5. 3.3×10^4 cells were plated on a 35-mm plastic dish. Cell number was determined 8 days after plating with a Coulter counter. Values are normalized to the initial cell number as 1.

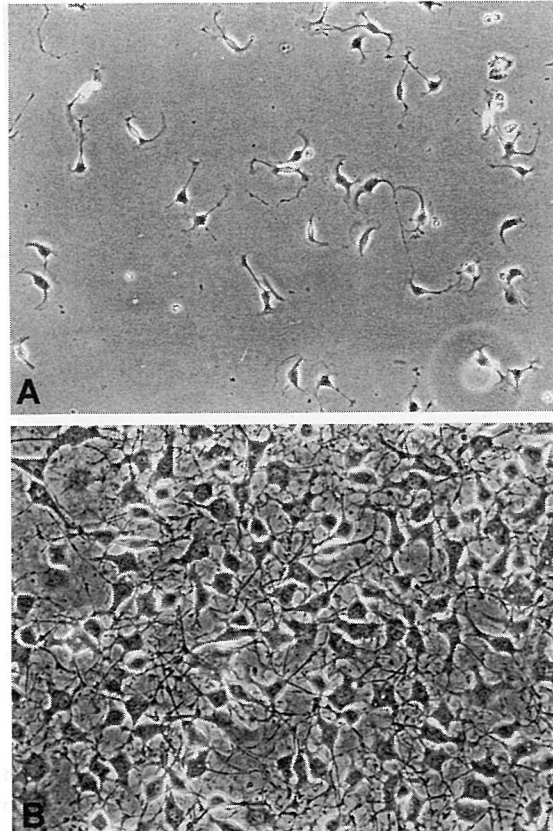


Fig. 3. Phase-contrast photomicrographs of KT-5. KT-5 cells were cultured for 1 day (A) and 5 days (B).

line was about 17 h. The saturation density was 2.1×10^4 cells/cm². The plating efficiency was 3.5%. This cell line displayed serum-dependent growth with linear increase of cell numbers in response to serum concentration up to 20% (Fig. 2). The morphology of KT-5 cells was epithelial to crescent-like (Fig. 3). Cell processes were often seen at the edges of cells. Some cells extended long processes of 10–20 μ m in length.

Karyotype of KT-5

The chromosome distribution of KT-5 is shown in Fig. 4a. The range of chromosome numbers was wide, from 48 to 104. Most cells had 58–71 chromosomes, indicating KT-5 is pseudotriploid. The majority of chromosomes displayed telocentric type, but

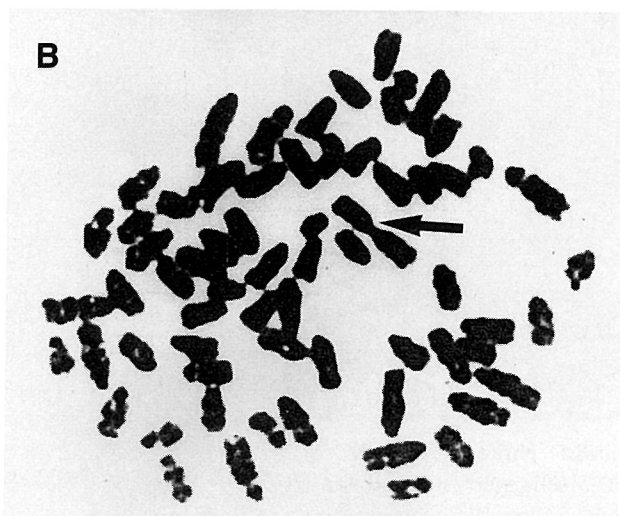
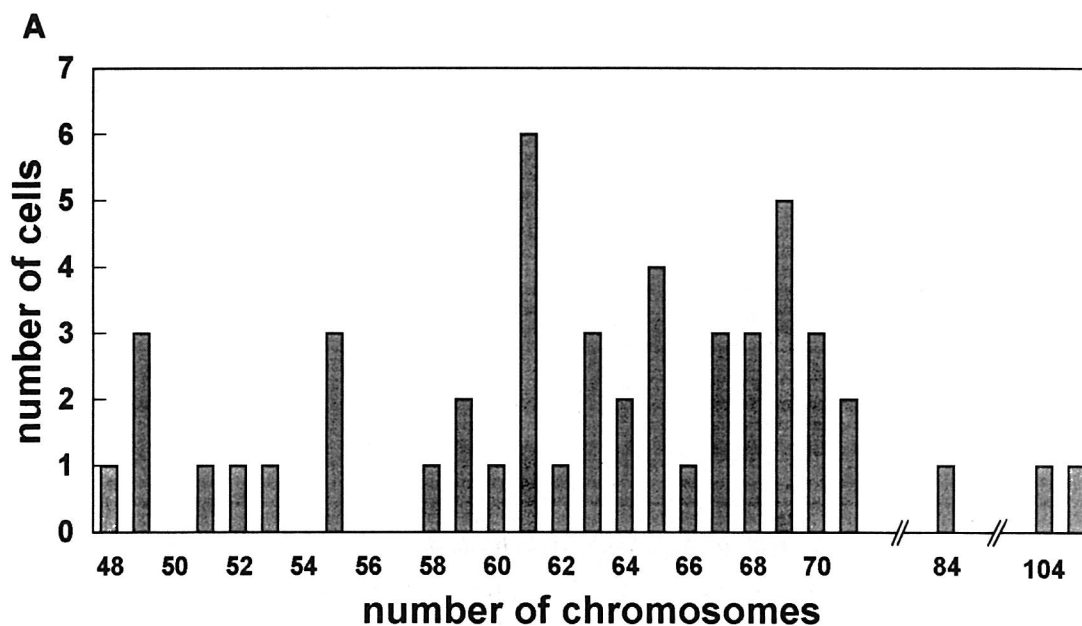


Fig. 4. Chromosome analysis. (A). Histogram of number of chromosomes for 50 cells. (B). A Giemsa-stained specimen of chromosome. Arrow, meta-centric chromosome.

Table 1. Tumorigenicity of KT-5 cells

Exp. No.	Injected specimens		Route	No. of mice injected	No. of tumor-bearing mice (%)
	Source	Quantity/mouse			
I	KT-5	5×10^5 cells	i.p	5	4 (80)
	Fragment	8 mm ³	i.p	5	5 (100)
II	KT-5	10^5 cells	s.c	6	6 (100)
	KT-5	10^4 cells	s.c	6	1 (17)
	KT-5	10^3 cells	s.c	5	1 (20)
	KT-5	10^2 cells	s.c	5	0 (0)

Mice were injected intraperitoneally (i.p.) or subcutaneously (s.c.) with cultured KT-5 cells, or tumor fragments stored frozen. After 5 weeks the mice were examined for tumor development.

some cells contained metacentric-submetacentric chromosomes (Fig 4b).

Tumorigenicity of KT-5

Table 1 shows the tumorigenicity analysis of KT-5 cells. The tumorigenicity was 80% when 6×10^5 cells were intraperitoneally injected to C3H mouse, and 100% in the case of subcutaneous transplantation of 10^5 cells. When an 8 mm³ piece of original tumor was intraperitoneally transplanted, all mice developed tumors. These results show that KT-5 is tumorigenic and confirm that its origin is C3H mouse.

Authentication of cell type

Isoenzyme analysis (6) confirmed that KT-5 is a mouse-derived cell line. Tests for mycoplasmas, bacteria, and fungi (6) were negative. The cell types constituting CNS can be distinguished by several brain-specific antigens. For instance, astrocytes contain an intermediate filament protein, GFAP, and S-100 protein (7). Neurons have another intermediate filament called neurofilament (14). Oligodendrocytes are characterized by a cell-surface antigen, myelin basic protein (4). Thy-1 is a marker of fibroblasts (12) that exist in meninges of the brain. To identify the type of KT-5 cells, they were immunostained for these antigens. KT-5 was immunopositive for GFAP and S-100 protein (Fig. 5), while other markers were negative. GFAP was also detected as a 45-kDa band in Western blotting analysis (not shown). These results demonstrate that KT-5 derives from murine astrocyte.

Biological activity of KT-5 conditioned medium

Astrocytes are a primary component of brain mesenchyme and play a role in the differentiation, growth, and survival of neurons (9). These functions are thought to be mediated in part by soluble factors (9). To date many growth factors and cytokines have been reported that are produced from astrocytes (13). As a preliminary study, we tested the biological activities of KT-5-conditioned medium (fraction 1-3) on the neurite-

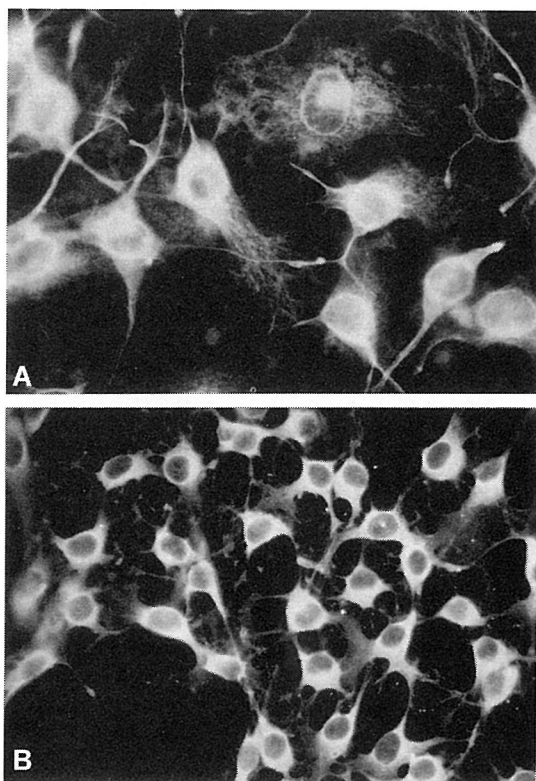


Fig. 5. Immunofluorescence micrographs of KT-5 cells. Indirect immunofluorescence for GFAP (A) and S-100 protein (B).

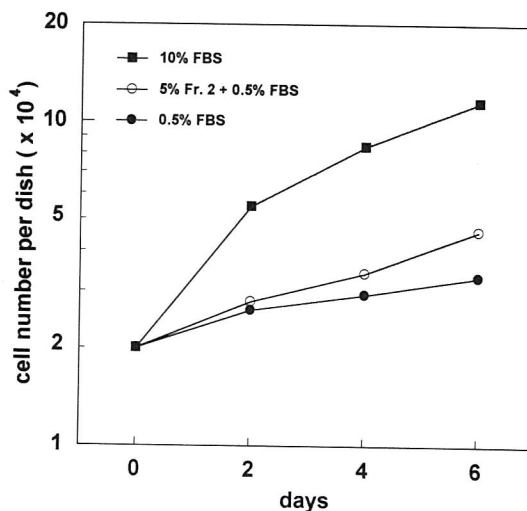


Fig. 6. Effect of fraction 2 on the proliferation of KT-5 cells. Cells were seeded onto 35-mm plastic dish at 2×10^5 cells/dish and cultivated in Ham's F12 supplemented with 0.5% FBS (●), 5% fraction 2 plus 0.5% FBS (○), or 10% FBS (■). Cell number was determined every second day.

outgrowth of chick forebrain neurons. The neurite length measured at day 4 *in vitro* is shown in Table 2. Neurite-promoting activity was found in a concentrate fraction with molecular weight >10 kDa (fraction 2) but not in the pass-through fraction (fraction 3). Astrocytes are known to produce growth factors that affect their own growth (5, 10). To examine whether KT-5 produces such an autocrine factor, fraction 2 was added to KT-5 cultures (Fig. 6). The proliferation of KT-5 cells was promoted by fraction 2. In addition, KT-5-conditioned medium promotes the proliferation of glial cells prepared from embryonic chick brain (not shown). These findings suggest KT-5 produces biologically active factors that affect the differentiation and growth of neural cells.

In conclusion, we have described a murine cell line from *N*-nitrosomethyl urea-induced brain tumor. This cell line possesses astrocytic markers together with biological activities similar to those of astrocytes. Therefore, KT-5 will be a useful tool to examine astrocyte functions. KT-5 is deposited at the Institute for Fermentation, Osaka.

Table 2. Effect of KT-5-conditioned medium on neurite outgrowth of chick forebrain neurons

Fraction	No. of cells examined	Total length of neurites (cm)	Mean (cm/cell)
Fr. 1	19	35.0	1.8
	25	53.0	2.1
Fr. 2	21	69.5	3.3
	45	148.6	3.3
Fr. 3	11	11.2	1.0
	11	15.5	1.4
none	24	34.0	1.4
	42	65.2	1.5

Number of cells and total length of neurites were measured from photographs (x 400) of chick forebrain neuronal cultures.

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A group I intron in the nuclear 18S rRNA gene of the yeast-like fungus, *Arxula terrestris*

Kumiko UEDA and Kozaburo MIKATA

Summary

Group I intron was detected in the 18S rRNA gene (18S rDNA) of a yeast-like fungus, *Arxula terrestris*. It was identified as an insertion of 352 nucleotides (AT1199) at position 1199 of the 18S rDNA. The predicted secondary structure of AT1199 has a simple P5 stem and a large P9 region; therefore, it does not resemble those of any subgroups listed by Michel and Westhof in 1990 (*J. Mol. Biol.* 216: 585-610). The results of BLAST search showed that AT1199 was similar to only three group I introns, found in 18S rDNA of *Cryphonectria parasitica*, *Cryphonectria parasitica*, and *Nadosoniella nigra*.

Keywords: *Arxula terrestris*, 18S rRNA gene, group I intron.

Group I introns are characterized by conserved internal sequence elements and a potential RNA secondary structure (2, 16). They have been found in the nuclei of eukaryotes (3, 21), mitochondria (14), chloroplasts (8, 18), eukaryotic viruses (24), cyanobacteria (13, 23), eubacteria (17), and bacteriophages (20). In the nuclear genome of the yeast-like fungus, *Arxula adenivorans*, group I introns have been reported in 25S ribosomal DNA (rDNA) sequences (19).

In the present paper, we describe the cloning of the 18S rDNA from *Arxula terrestris* and its characterization, including identification of a group I intron. The structure and properties of this intron resemble those found in *Cryphonectria parasitica* and *Cryphonectria radicalis*.

Materials and methods

Strains. *A. terrestris* IFO 10828 and IFO 10859, and *A. adenivorans* IFO 10857 and IFO 10858 were used as sources for the amplified 18S rDNA.

DNA and RNA preparation. The isolation procedures of DNA and RNA were as described by Holm et al. (12) and Yamada and Kawasaki (25).

Reverse transcriptase reaction and PCR. The reverse transcriptase reaction mixture

(20 μ l) consisted of 1–5 μ g RNA, 10 pmol primer 18-R (5'-GATCCTTCCGCAGGTT-CACC-3'), 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 0.5 mM dNTP (each) and reverse transcriptase. Samples were incubated at 37 °C for 1 h to produce the cDNA and at 90 °C for 10 min to denature enzyme. PCR was performed for 30 cycles of denaturation at 94 °C for 0.5 min, annealing at 55 °C for 0.5 min, and extension at 72 °C for 2.5 min with TaKaRa *Taq*TM DNA polymerase (Takara Shuzo Co. Ltd.) using the primer pair 18-F (5'-ATCTGGTTGATCCTGCCAGT-3') and 18-R.

Direct cycle sequencing. DNA sequences were determined using a Thermo SequenaseTM fluorescent-labeled cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech) following the supplier's protocol. Diluted PCR products were used as a template for sequencing, and 5' FITC-labeled primers were 400F (5'-TCCGGAGAGGGAGCCTGAGAAACG-3'), 550F (5'-GCAGCCGCGGTAATTC-CAGC-3'), 950F (5'-TCAAGAACGAAAGTTAGGGG-3'), 1200F (5'-AAAG-GAATTGACGGAAGGGC-3'), 1300F (5'-TTGGTGGAGTGATTTGTCTG-3'), 1500F (5'-GATGCCCTTAGACGTTCTGG-3'), and 18-F for the sense strand, and 400R (5'-TTCTCAGGCTCCCTCTCCGG-3'), 550R (5'-GAATTACCGCGGCTGCT-GGC-3'), 950R (5'-TCCCCTAACTTTCGTTCTTG-3'), 1200R (5'-GGTGCCCTTCCGTCAATTCC-3'), 1300R (5'-AGACAAATCACTCCACCAAC-3'), 1500R (5'-CCAGAACGTCTAAGGGCATCACAG-3'), and 18-R for the reverse strand.

Alignment, secondary structure modeling, and similarity searches. Intron secondary structure was drawn following the model of Cech et al. (4). GENETYX-MAC var. 8 (Software Development Co. Ltd. Japan) was used to help predict stem structures in the less conserved portions of the model. A BLAST search (1) was conducted using DDBJ, GenBank, and EMBL databases.

Results

Detection of group I intron within 18S rDNA.

The 18S rDNAs from PCR product of *A. terrestris* IFO 10828 and IFO 10859 were longer than the 1800 nucleotides predicted from *A. adenivorans* IFO 10857 and IFO 10858 (Fig. 1). On the other hand, the cDNAs from 18S rRNA of *A. terrestris* strains were equal to 18S rDNAs from *A. adenivorans*. These findings indicate the existence of an intron within the 18S rDNA of *A. terrestris*, which would be removed during maturation of rRNA transcripts.

Sequencing of group I intron in 18S rDNA.

Sequences of 18S rDNA from *A. terrestris* IFO 10828 and IFO 10859 were determined and found to be identical. They are deposited in DDBJ with accession number AB000663. On sequencing of the cDNA from the 18S rRNA of *A. terrestris* and comparison their sequences, an insertion of 352 nucleotides was identified at position 1199 (numbering according to Gargas et al. (9)) of the 18S rDNA. The intron (AT1199) was identified as belonging to group I based upon the conserved splice-site nucleotides, U at the 3' end of the 5' exon and G at the 3' end of the intron (Fig. 2).

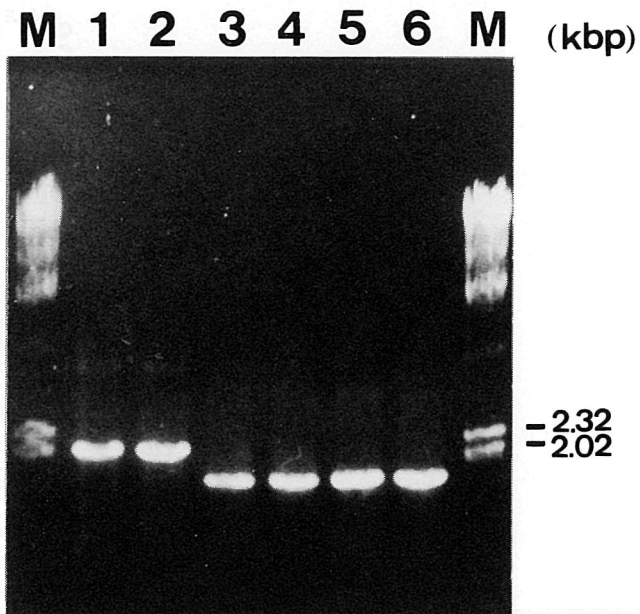


Fig. 1. Detection of splicing group I intron. PCR products from 18S rDNA of *Arxula terrestris* IFO 10828 (lane 1) and IFO 10859 (lane 2), cDNA from 18S rRNA of *A. terrestris* IFO 10828 (lane 3) and IFO 10859 (lane 4), and PCR products from 18S rDNA of *Arxula adenivorans* IFO 10857 (lane 5) and IFO 10858 (lane 6). Lane M, λ -HindIII molecular size markers.

Table 1. Comparison of conserved elements of Group I introns from *A. terrestris* (AT1199), *Cryphonectria parasitica* (A), *Cryphonectria radicalis* (B), and *Nadsoniella nigera* (C), which were found by BLAST search, and *Cladonia merochlorophaea* (D).

Intron	Position	Size	P	Q	R	S
AT1199	1199	352	GGU <u>ACAGGGAAC</u>	GAUC-CUGUGG	UCGCA <u>ACGCACGCU</u>	AAGGU <u>ACGUGCU</u>
A, B	1199	547	GGU <u>ACAGGGGAC</u>	<u>AAUC</u> -CUGUGG	UCGCA <u>ACGCGCGCU</u>	AAGGU <u>ACGUGCU</u>
C	1210	428	GGU <u>ACAGGAGAC</u>	GAUC-CUGUGG	UCGCA <u>ACGCGCGCA</u>	AAGGU <u>ACGUGCU</u>
D	1199	228	GGU <u>ACAGAGGAT</u>	GAU <u>U</u> -CUGUGG	UCU <u>CAACGCAUGCA</u>	CAGGU <u>ACAUGCU</u>

Nucleotides involved in the P4 and P7 pairing are underlined. Nucleotides differing from those of AT1199 are shaded. Position and size indicate the location of the intron in 18S rDNA and its base number, respectively.

Predicted secondary structure of group I intron.

The P, Q, R, and S conserved regions are shown with related sequences found by BLAST search in Table 1. The entire group I intron sequences of *Cryphonectria parasitica* (5) and *Cryphonectria radicalis* (6) were completely identical. The Q and S regions of AT1199 are identical to those of the black yeast *Nadosoniella nigera* group I

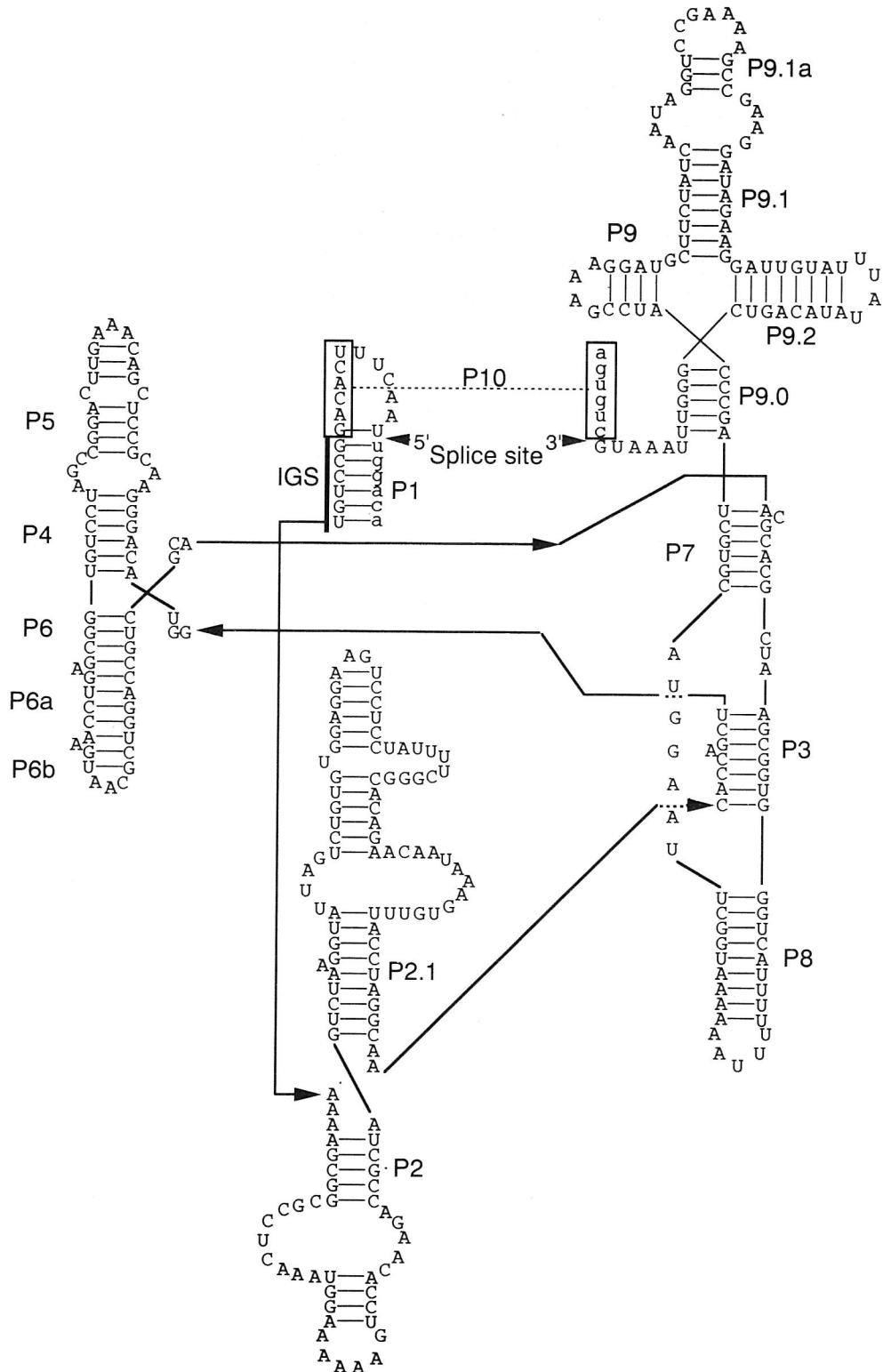


Fig. 2. Predicted secondary structure of the AT1199 intron. Lower-case letters are exon sequences and capital letters are intron sequences. IGS marks the internal guide sequence. Lines show continuity of the strands, from which no nucleotides have been omitted, and arrowheads indicate 5'→3' polarity.

intron at position 1210 of 18S rDNA (11), and the remaining parts of the intron are not similar.

The catalytic core sequences corresponding to P, Q, R, and S interact to form stems P4 and P7, which are parts of the conserved secondary structure core (3). The internal guide sequence (IGS) located just downstream of the 5' splice site and the upstream part of the 5' splice site interact to form stem P1. Using these sequences and structures as guides, the remaining parts of the sequence could be folded to fit the secondary structure model of Cech et al. (4) for group I introns. The complex P9 was predicted by use of the RNA secondary-structure prediction program in GENETYX software. The predicted secondary structure (Fig. 2) has a simple P5 stem and large P9 complex. BLAST search showed that the sequences of P, Q, R, and S (Table 1) were not similar to those of any subgroups listed by Michel and Westhof (15).

Discussion

PCR products of multi-copy 18S rDNA were detected as single band on agarose gel electrophoresis (Fig. 1), and the 18S rDNA sequence determined by the direct method was unique. These findings suggest that the group I intron AT1199 is distributed in all of the 18S rDNAs in the genome of *A. terrestris* strain. In general, within a genus or species, introns in the same location tend to be more similar than introns in different locations (10). It was reported that splicing of the *Tetrahymena* rRNA group I intron was fully reversible in vitro (22). 18S rDNAs of *A. adenivorans* and related species lack a group I intron at position 1199 (data not shown). Therefore, the prototype of AT1199 might have been inserted into an intron-lacking rDNA of *A. terrestris* after the species development, and AT1199 might have been distributed across all multi-copy 18S rDNAs by reversal transcription or homologous recombination.

Seventeen insertion positions have been reported for fungi 18S rDNA, and group I introns were found at 14 of the positions (9). At position 1199, two strains having insertions and three strains having a group I intron, *Cladonia chlorophaea* (9), *Cryphonectria parasitica* (5) and *Cryphonectria radicalis* (6), have been reported. Of the various types of group I intron, AT1199 resembled that of *C. parasticta* and *C. radicalis* at position 1199 of 18S rDNA, and that of *Nadosoniella nigra* (11) at position 1210 of 18S rDNA found by BLAST search (Table 1). Thus, the distribution of AT1199 type intron is more limited than that of the frequently reported subgroup IC1 (7). This suggests that the mobility of AT1199-type introns followed by horizontal transfer and stability might be lower than that of subgroup IC1 introns. As more organisms are sequenced, more insertions similar to AT1199 will probably be found in rDNA and other genes.

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Geographical and Seasonal Distribution of Arenicolous Marine Fungi along the Pacific Coast of the Bousou Peninsula

Akira NAKAGIRI, Izumi OKANE and Tadayoshi ITO

Summary

Sea foam on sandy beaches was examined for trapped spores of arenicolous marine fungi at seven sites along the Pacific coast of the Bousou Peninsula (central Japan) in four seasons, and 26 species in 12 genera of arenicolous marine fungi were recorded. Their occurrence according to locality and season were investigated, and three geographical patterns were recognized: species occurring over the whole region, species occurring mainly at southern sites, and species occurring mainly at northern sites. Some of the species showing the latter two patterns changed their distribution according to the season. Geographical and seasonal distribution of the fungi was influenced by seawater temperature, which in turn is influenced by the distribution of the coastal waters derived from the warm and cold Pacific Ocean currents.

Keywords: arenicolous marine fungi, biogeography, distribution.

The Bousou Peninsula (Chiba Pref., central Honshu Island, Japan) faces the Pacific Ocean where the Kuroshio (the Japan Current, a warm current) meets the Oyashio (the Kurile Current, a cold current). The two ocean currents affect the climate of the Pacific coast of the peninsula (the Sotobou coast). It is well known that the marine flora and fauna, that is, biodiversities of seaweed, fish, invertebrates, etc. are determined by the effects of the currents, and that the warm and cold sea marine organisms that inhabit the Sotobou coast are adapted to its unique coastal environments (8, 14). However, no study has been done on the flora of marine fungi along the coast.

Geographical distributions of marine fungi have been studied mainly by plotting the records of fungi on a global map divided into climate zones, including tropical, subtropical, temperate, arctic, and antarctic (1, 2, 6). According to the distribution pattern, species of marine fungi are recognized as cosmopolitan, tropical to subtropical, temperate, etc., though accumulation of more data is still necessary for a precise understanding of the fungal distribution. In Japan, Nakagiri (10) and Tokura (13) reported the occurrence of arenicolous marine fungi from Japanese coasts and recognized three types of distribution: wide distribution throughout Japan, distribution mainly in northern Japan, and distribu-

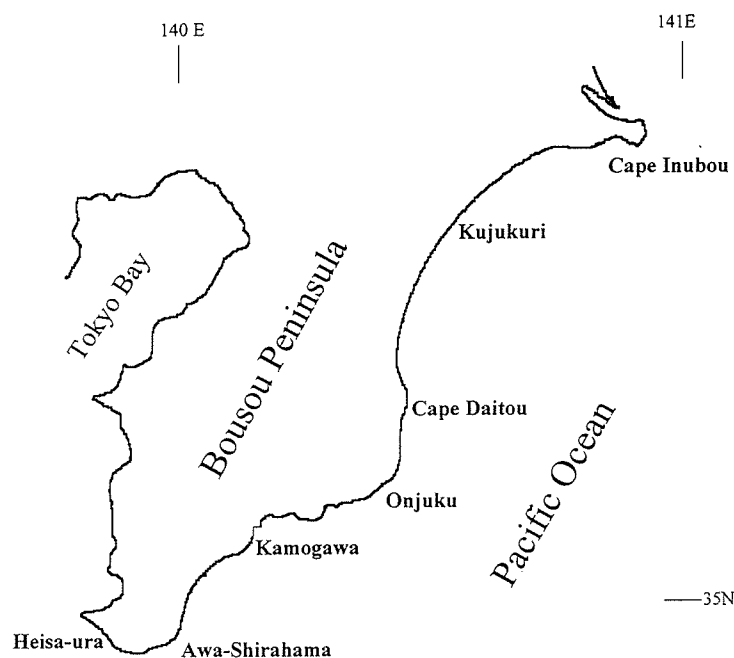


Fig. 1. Seven collection sites of sea foam along the Sotobou coast.

tion mainly in southern Japan. Although the seasonal distribution of marine fungi has been studied (4), no studies have focused on the relationship between fungal distribution and ocean currents, or on the seasonal change of fungal distribution along coasts where water temperature changes according to season and the influence of the currents.

The Sotobou coast is characterized by the fact that seasonal changes in the temperature of the seawater depend mainly on the influences of two ocean currents. Thus, we have investigated fungal occurrence on the Sotobou coast over the course of the year by examining sea foam samples. It is well known that spores of arenicolous marine fungi are trapped and accumulated in sea foam on sandy beaches, and that sea foam is a useful source of information on the mycoflora of the beach (3, 5, 7, 10). The purpose of this study is to estimate the geographical distribution of marine fungi along a stretch of ca. 150-km of the Sotobou coast and to examine how their distribution is influenced by the seasons and the ocean currents.

Material and Methods

Sea foam samples were collected from seven sites along the Pacific coast of the Bousou Peninsula (the Sotobou coast) (Fig. 1) in four months: March, September, December 1995, and June 1996. Because the temperature of seawater is lowest in February–March and highest in August–September, these months can be considered to correspond to winter (March), spring (June), summer (September), and autumn (December). The seven sites are, from north to south of the Sotobou coast, Cape Inubou, Kujukuri, Cape Daitou, Onjuku, Kamogawa, Awa-Shirahama, and Heisa-ura. Sea foam formed on sandy

Table 1. Occurrence of spores of marine fungi in sea foam from seven sites along the Sotobou coast in March 1995.

	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
<i>Arenariomyces parvulus</i>	+	+	+	++		+	
<i>A. trifurcatus</i>	+	++	++	+++	++	++	++
<i>Carbosphaerella leptosphaerioides</i>			+		++		
<i>Ceriosporopsis halima</i>		+		+	+	+	+
<i>Corollospora angusta</i>	+	+++	+	+++			
<i>C. colossa</i>			+				+
<i>C. fusca</i>	+			+	+	+	+
<i>C. gracilis</i>	+	++	++	++			
<i>C. intermedia</i>		+		++			+
<i>C. maritima</i>	++	+	++	++	++	++	++
<i>C. pseudopulchella</i>		+	+	++			
<i>C. pulchella</i>	+						
<i>C. quinqueseptata</i>		+++		+++			
<i>Halosphaeria salina</i>			+				
<i>Halosphaeriopsis mediosetigera</i>					+		
<i>Lulworthia crassa</i>	+		+				
<i>Lulworthia</i> sp.							+
<i>Marinospora calyptrata</i>					+		
<i>Ondiniella torquata</i>					+	+	
<i>Torpedospora radiata</i>		+				+	+
<i>Trailia ascophylli</i>							+
<i>Nia vibrissa</i>		+	+	+	+	+	
<i>Asteromyces cruciatus</i>		+		+			
<i>Sigmoidea marina</i>						+	
Species number	24	8	12	11	12	9	9

Data from sea foam samples collected on 13 March 1995. Relative abundance of spores is indicated as +, ++ and +++.

beaches at these sites was collected with a ladle and transported to the laboratory in bottles, which were cooled to prevent spore germination. The foam samples were examined under a microscope to identify spores of marine fungi, and some of spores were isolated and cultured to confirm their identification. The species identified from each site and in each season were compared to clarify the geographical and seasonal distribution of the arenicolous marine fungi of the Sotobou coast.

Results and Discussion

Occurrence of fungal spores in sea foam samples

The occurrence and abundance of marine fungi in foam samples collected from the seven sites in four seasons are listed in Tables 1-4. Thirty-three species of marine fungi including 12 genera and 26 species of arenicolous fungi were found in the foam samples. Though the presence or absence of spores in foam samples does not indicate directly the fungal distribution, these data probably give useful information on the relative activity of the arenicolous marine fungi in their natural habitats.

Table 2. Occurrence of spores of marine fungi in sea foam from seven sites along the Sotobou coast in June 1996.

	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inbou
<i>Arenariomyces parvulus</i>	+	+	++	+	+	+	
<i>A. trifurcatus</i>	+	+++	+++	+	++	+	
<i>Carbosphaerella leptosphaerioides</i>	+	+	+		+		
<i>Ceriosporopsis halima</i>			+	+	+		
<i>Corollospora angusta</i>	+	+	+	+			
<i>C. colossa</i>	+	+	+				
<i>C. fusca</i>	++		+	+	+		
<i>C. gracilis</i>	+++	+++	+++	+++	++	+++	
<i>C. intermedia</i>		+					
<i>C. maritima</i>	++	++	++	+	+++	+++	++
<i>C. pseudopulchella</i>		+		+		+	
<i>C. pulchella</i>		+	+	+			
<i>C. quinqueseptata</i>	+	+++		++			
<i>Halosphaeria appendiculata</i>					+		
<i>Halosphaeriopsis mediosetigera</i>	+		+	+			
<i>Lindra obtusa</i>				+	+		+++
<i>Lin. thalassiae</i>	+	++					
<i>Lulworthia crassa</i>	+	+	+		+		
<i>Lul. lignoarenaria</i>				+			
<i>Lulworthia</i> sp.	+	+	+	+	++		
<i>Marinospora calyptata</i>					+		
<i>Ondineilla torquata</i>					+		
<i>Torpedospora radiata</i>					+		
<i>Trailia ascophylli</i>	+						++
<i>Nia vibrissa</i>			+	+	+		+
<i>Asteromyces cruciatus</i>	+	+	+	++			
<i>Varicosporina ramulosa</i>	+	+++	+++	+			
<i>Sigmoidia luteola</i>			+	++	+		+
<i>S. marina</i>				++	+		+
Species number	28	16	16	17	17	5	6

Data from sea foam samples collected on 18-19 June 1996. Relative abundance of spores is indicated as +, ++ and +++.

The marine fungi show three patterns of occurrence: 1) species occurring at all the seven sites throughout the year, 2) species occurring mainly at sites north of Onjuku, and 3) species occurring mainly at sites south of Onjuku. As an example, Table 5 summarizes the three patterns of occurrence found in data of December 1995. The first pattern was shown by *Corollospora maritima* Werdermann and *Arenariomyces trifurcatus* Höhnk (Table 6a, b), whose ascospores occurred abundantly in the foam samples collected from the seven sites in all seasons. Both species are known as globally cosmopolitan marine fungi (1). The second pattern was shown by *Lindra obtusa* Nakagiri & Tubaki, *Ceriosporopsis halima* Linder, and *Nia vibrissa* Moore & Meyers (Table 7a, b, c). They occurred mainly to the north of Onjuku, but in March and December, the low temperature seasons, their distributions extended southward. This phenomenon suggests that they prefer lower temperature. In contrast, *C. quinqueseptata* Nakagiri, *Varicosporina ramulosa* Meyers & Kohlmeyer and *C. gracilis* Nakagiri & Tokura appeared mainly in the

Table 3. Occurrence of spores of marine fungi in sea foam from seven sites along the Sotobou coast in September 1995.

	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inbou	
<i>Arenariomyces parvulus</i>	+	+	++	+		++	++	
<i>A. trifurcatus</i>	++		++	++	+	+++	+++	
<i>Carbosphaerella leptosphaerioides</i>	++		+		+	+	++	
<i>Ceriosporopsis halima</i>			+		+	+	++	
<i>Corollospora angusta</i>	+	+	+	+				
<i>C. colossa</i>	++		++	+			+	
<i>C. fusca</i>			+	+		+	+	
<i>C. gracilis</i>	+++	++	++	+++	+	+	++	
<i>C. intermedia</i>	+	+		++	+		+	
<i>C. lacera</i>	+		+		+		+	
<i>C. luteola</i>				+				
<i>C. maritima</i>	+++	+	+++	++	+	+++	+++	
<i>C. pseudopulchella</i>	+	+	++	+	+		+	
<i>C. pulchella</i>	+			+				
<i>C. quinqueseptata</i>	++	+++		+++				
<i>Halosphaeria appendiculata</i>						+	+	
<i>Halosphaeriopsis mediosetigera</i>	+		+	+		+	++	
<i>Lindra obtusa</i>		+		+			+	
<i>Lin. thalassiae</i>	+	+		+		+		
<i>Lulworthia crassa</i>	+	+	++				++	
<i>Lul. lignoarenaria</i>	+				+	+	++	
<i>Lulworthia</i> sp.			+					
<i>Marinospora calyptorata</i>					+		+	
<i>Torpedospora radiata</i>					+	+	++	
<i>Nia vibrissa</i>			++			++	++	
<i>Anguillospora marina</i>		+						
<i>Asteromyces cruciatus</i>	+	+		+				
<i>Clavatospora bulbosa</i>			+		+	+		
<i>Varicosporina prolifera</i>	++	+					+	
<i>V. ramulosa</i>	++	+	+	+			+	
<i>Sigmoidia marina</i>							+	
Species number	31	19	14	17	17	12	14	22

Data from sea foam samples collected on 18-19 September 1995. Relative abundance of spores is indicated as +, ++ and +++.

southern regions, showing the third pattern of occurrence (Table 8a, b, c). In the low temperature seasons, especially in March, their spores appeared only at southern sites. However, their distributions extended northward in the higher temperature seasons. This indicates their preference for higher temperature.

Occurrence of teleomorph and anamorph states of *C. pulchella* Koblmeier, I. Schmidt & Nair and *C. intermedia* I. Schmidt were also examined (Table 9). Though no clear pattern of the occurrence was observed, their anamorphs, *Clavatospora bulbosa* (Anastasiou) Nakagiri & Tubaki and *V. prolifera* Nakagiri, appeared frequently in the higher temperate season, though the teleomorphs were prevalent in cooler seasons and at northern sites. This accords with a phenomenon that anamorphs are prevalent in seasons and regions of higher temperature, and teleomorphs are prevalent in seasons and regions of lower temperature (11).

Table 4. Occurrence of spores of marine fungi in sea foam from seven sites along the Sotobou coast in December 1995.

	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou	
<i>Arenariomyces parvulus</i>	+	+	+	++	++	+		
<i>A. trifurcatus</i>	++	++	++	++	+++	+++	+++	
<i>Carbosphaerella leptosphaerioides</i>	+				++		+	
<i>Ceriosporopsis halima</i>					++		+	
<i>Corollospora angusta</i>	+++	+++	++	+++	+			
<i>C. colossa</i>					+	+	+	
<i>C. fusca</i>	+		+			+	+	
<i>C. gracilis</i>	++	+	++	++	+	++	++	
<i>C. intermedia</i>	+	++						
<i>C. lacera</i>	+							
<i>C. luteola</i>	+			++				
<i>C. maritima</i>	+++	++	+++	++	++	+++	+++	
<i>C. pseudopulchella</i>				+			+	
<i>C. pulchella</i>		+						
<i>C. quinqueseptata</i>	+	+++						
<i>Halosphaeriopsis mediosetigera</i>	+				+			
<i>Lindra obtusa</i>		+		+			+	
<i>Lulworthia crassa</i>	+				+			
<i>Lulworthia</i> sp.	++	+			++	+		
<i>Ondiniella torquata</i>					+			
<i>Torpedospora radiata</i>						+		
<i>Trailia ascophylli</i>							+	
<i>Nia vibrissa</i>		+		+	++			
<i>Asteromyces cruciatus</i>	+							
<i>Clavatospora bulbosa</i>					+			
<i>Varicosporina ramulosa</i>		+		+				
<i>Sigmoidia luteola</i>							+	
<i>S. marina</i>				+				
Species number	28	15	12	6	11	14	8	11

Data from sea foam samples collected on 10–12 December 1995. Relative abundance of spores is indicated as +, ++ and +++.

Numbers of species found at the seven sites are summarized in Table 10. The total number of species was highest in September and lowest in March. This is attributed mainly to the fluctuating species number of deuteromycetes, which decrease in winter and increase in summer (see right column of Table 10). The annual total number of species was greatest at Onjuku, which indicates that the distribution areas of fungi preferring higher temperature and those preferring lower temperature may overlap at Onjuku. Similarity of the species profile between each site and Heisa-ura (the most southern site) or Cape Inubou (the most northern site) was calculated (see the lower column of Table 10). Higher similarity values to Heisa-ura are seen at the southern sites, Onjuku, Kamogawa and Awa-Shirahama, while the species profiles of the sites north of Onjuku are more similar to that of Cape Inubou than that of Heisa-ura. This strengthens the suggestion that there is a boundary between the distributions of particular species of marine fungi around Onjuku. The seasonal change of occurrence in particular species (see Tables 7 and 8) indicates that this boundary moves northward in summer and

Table 5. Three occurrence patterns of marine fungi observed in data of December 1995.

Species	Heisaura	Awa-Shirahama	Kamo-gawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
1) Occurring at all sites							
<i>Arenariomyces trifurcatus</i>	++	++	++	++	+++	+++	+++
<i>Corollospora gracilis</i>	++	+	++	++	+	++	++
<i>C. maritima</i>	+++	++	+++	++	++	+++	+++
2) Occurring mainly at north of Onjuku							
<i>Ceriosporopsis halima</i>					++		+
<i>Corollospora colossa</i>					+	+	+
<i>C. pseudopulchella</i>				+			+
3) Occurring mainly at south of Onjuku							
<i>Corollospora angusta</i>	+++	+++	++	+++	+		
<i>C. intermedia</i>	+	++					
<i>C. quinqueseptata</i>	+	+++					
<i>C. luteola</i>	+			++			

Table 6. Occurrence pattern of marine fungi appearing at all sites in all seasons.

a. <i>Corollospora maritima</i>							
	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
Mar.	+	+	+	+	+	+	+
Jun.	+	+	+	+	+	+	+
Sep.	+	+	+	+	+	+	+
Dec.	+	+	+	+	+	+	+
b. <i>Arenariomyces trifurcatus</i>							
	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
Mar.	+	+	+	+	+	+	+
Jun.	+	+	+	+	+	+	-
Sep.	+	-	+	+	+	+	+
Dec.	+	+	+	+	+	+	+

southward in winter.

Cultural properties and distribution of the marine fungi

Hyphal growth and reproduction of the fungi found show a close correlation to their occurrence patterns. *Corollospora maritima*, one of the fungi showing wide distribution from Cape Inubou to Heisa-ura throughout the year, is known to show good hyphal growth and ascomata formation over a wide range of temperature (10–35°C, optimum 30°C) (9). In contrast, *L. obtusa*, which appeared mainly in the region north of Onjuku,

Table 7. Occurrence pattern of marine fungi appearing mainly north of Onjuku.

a. <i>Lindra obtusa</i>							
	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
Mar.	—	—	—	—	—	—	—
Jun.	—	—	—	+	+	—	+
Sep.	—	—	—	+	—	—	+
Dec.	—	+	—	+	—	—	+

b. <i>Ceriosporopsis halima</i>							
	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
Mar.	—	+	—	+	+	+	+
Jun.	—	—	+	+	+	—	—
Sep.	—	—	+	—	+	+	+
Dec.	—	—	—	—	—	—	+

c. <i>Nia vibrissa</i>							
	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
Mar.	—	+	+	+	+	+	—
Jun.	—	—	+	+	+	—	+
Sep.	—	—	+	—	—	+	+
Dec.	—	+	—	+	+	—	—

Table 8. Occurrence pattern of marine fungi appearing mainly south of Onjuku.

a. <i>Corollospora quinqueseptata</i>							
	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
Mar.	—	+	—	+	—	—	—
Jun.	+	+	—	+	—	—	—
Sep.	+	+	—	+	—	—	—
Dec.	+	+	—	—	—	—	—

b. <i>Varicosporina ramulosa</i>							
	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
Mar.	—	—	—	—	—	—	—
Jun.	+	+	+	+	—	—	—
Sep.	+	+	+	+	—	—	+
Dec.	—	+	—	+	—	—	—

c. <i>Corollospora gracilis</i>							
	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
Mar.	+	+	+	+	—	—	—
Jun.	+	+	+	+	+	+	—
Sep.	+	+	+	+	+	+	+
Dec.	+	+	+	+	+	+	+

shows its optimum growth at 20°C and conidia are produced well at under 20°C. Hyphal growth of this species is depressed at above 25°C (9). On the contrary, *V. ramulosa*, which appeared mainly at the southern sites, shows a preference for higher temperature (above 25°C, growth optimum at 35°C) in culture. Conidium production and sclerocarp (degenerated ascoma) formation occur from 20°C to 40°C (9). Thus, the cultural prop-

Table 9. Occurrence pattern of teleomorph-anamorph species.

a. <i>Corollospora pulchella</i> (T)- <i>Clavatospora bulbosa</i> (A)							
	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
Mar.	T	-	-	-	-	-	-
Jun.	-	T	T	T	-	-	-
Sep.	T	-	A	T	A	A	-
Dec.	-	T	-	-	A	-	-

b. <i>Corollospora intermedia</i> (T)- <i>Varicosporina prolifera</i> (A)							
	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou
Mar.	-	T	-	T	-	-	-
Jun.	-	T	-	-	-	-	-
Sep.	A	A	-	T	T	-	A
Dec.	T	T	-	-	-	-	-

Table 10. Number of species of marine fungi appearing in sea foam collected at seven sites on the Sotobou coast.

	Heisa-ura	Awa-Shirahama	Kamogawa	Onjuku	Cape Daitou	Kujukuri	Cape Inubou	Total	Asco.	Deutero.	Basidio.
Mar.	8	12	11	12	9	9	9	24	21	2	1
Jun.	16	16	17	19	17	5	6	28	23	4	1
Sep.	19	14	17	17	12	14	22	31	24	6	1
Dec.	15	12	6	11	14	8	11	28	22	5	1
Total species in four seasons	58	54	51	59	52	36	48				
Total different species in year	23	22	21	23	25	19	24	33			
Species similarity* to Heisa-ura	-	76	73	78	63	57	72				
Species similarity to Cape Inubou	72	70	71	72	86	78	-				

*Species similarity (%) = no. of common species between A and B X 2 / species no. of A + species no. of B X 100

erties of the marine fungi accord with their appearance in the natural habitats.

Environmental factors and fungal appearance

Seawater temperature

Seawater temperature of the Sotobou coast is influenced by the climate and the ocean currents. Figure 2 is a graph of the monthly average temperature of seawater at four locations along the Sotobou coast [data from Shimizu (12)]. It shows that seawater temperature at Choushi (close to Cape Inubou) is lower than at other southern locations, especially in winter. The difference in temperature between Choushi and other places is about 2°C in summer, but more than 4°C in winter. Thus, the seawater temperature becomes similar in summer along the Sotobou coast, but in winter it differs greatly

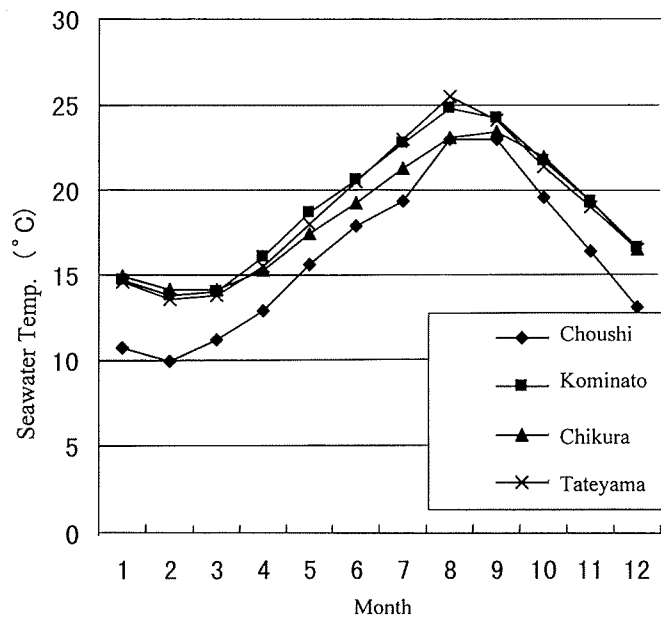


Fig. 2. Monthly average temperature of coastal seawater at four locations, Choushi (close to Cape Inubou), Kominato (close to Onjuku), Chikura (close to Awa-Shirahama) and Tateyama (close to Heisa-ura). [Data from Shimizu (12).]

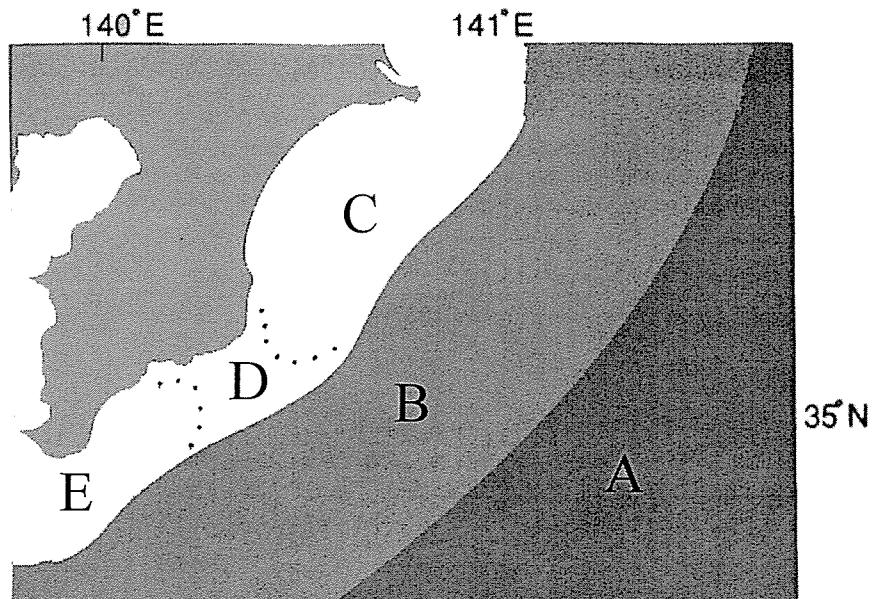


Fig. 3. Schematic illustration of the distribution of coastal waters off the Bousou Peninsula (A, Kuroshio water; B, Mixed water; C, Kujukuri coastal water; D, Sotobou-Kujukuri mixed coastal water; E, Sotobou coastal water).

between the north and the south. This may explain why the number of species found at the northern sites is similar to that at the southern sites in summer, but less in other seasons (Table 10).

Distribution of seawater area

Figure 3 schematically shows distribution of offshore coastal waters of the Bousou Peninsula. The northern coast of the Sotobou is washed by Kujukuri coastal water, which is derived from the Oyashio and has lower temperature and salinity. On the other hand, the southern coast is washed by Sotobou coastal water, which is derived from Kuroshio and has higher temperature and salinity. The coast around Onjuku is influenced by Sotobou-Kujukuri mixed coastal water (12). These coastal waters influence on the seawater temperature of the Sotobou coast and their distribution shifts northward in summer and southward in winter according to the strength of the original ocean currents. Thus, the distribution of the coastal waters influences the geographical and seasonal distribution of marine fungi along the coast.

In conclusion, the above observations indicate that: 1) there are three patterns of occurrence of marine fungi inhabiting the Sotobou coast, namely, the whole region, the area north of Onjuku, and the area south of Onjuku; 2) certain marine fungi have their distribution boundaries around Onjuku, where a boundary of the coastal waters is located; 3) the properties of hyphal growth and reproduction of the marine fungi accord well with their occurrence pattern; 4) complex coastal environments due to the influence of the different types of the coastal water produce a high diversity of marine fungi along the Sotobou coast; and 5) these investigations on the geographical and seasonal change of occurrence of marine fungi give useful information on the distribution of each species of marine fungi inhabiting the coasts.

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Mycoflora of the rhizosphere of *Salicornia europaea* L., a halophytic plant

Tadayoshi ITO, Izumi OKANE and Akira NAKAGIRI

Summary

To compare the mycoflora of its rhizosphere with that of mangrove, *Salicornia europaea* L., a halophytic plant like mangrove, was collected from Hokkaido, Japan. After washing the collected *S. europaea* roots and surrounding soil samples, their root- and soil-inhabiting fungi were isolated by washing and dilution plate methods, respectively. Population density of the rhizosphere soil showed 7.8×10^3 average colony-forming units (cfu)/g of dry weight. *Acremonium strictum* W. Gams, *Alternaria alternata* (Fr.) Keissler, and *Cladosporium cladosporioides* (Fres.) de Vries were dominant species on the rhizoplane of *S. europaea*, and these species were considered to be the main components of the rhizoplane mycoflora. The genera *Acremonium* Link, *Cladosporium* Link, *Penicillium* Link, *Phoma* Sacc., and *Trichoderma* Pers., were predominantly isolated from the washed root soils. Consequently, the mycoflora of the *S. europaea* rhizoplane differed from that of its surrounding soil. The mycoflora of the *S. europaea* rhizoplane also differed from that of mangrove, but the soil mycoflora of its rhizosphere was almost the same as that of mangrove. Tolerance to sodium chloride was tested for some isolates.

Keywords: Mycoflora, *Salicornia europaea* rhizosphere, halophytic plant, root-and soil inhabiting fungi.

Terrestrial microorganisms in the rhizoplane of higher plants are known to grow by using dead root tissue or secretions of root tissue, and to establish a micro-community in the roots and surrounding soil (1, 14, 15). The mycoflora of rice fields is affected by inundation with water (9, 14) and that of the mangrove rhizosphere by semi-anaerobic conditions and a high content of heavy metals (7, 8). *S. europaea* plant are also adapted to these conditions as a halophytic plant. To compare the mycoflora of *S. europaea* rhizosphere with that of mangrove, *S. europaea* samples were collected and surveyed. This is a preliminary study as we are studying for clarification of ecological and species diversity of endophytic fungi.

S. europaea is a halophytic plant that is distributed in the temperate region of the Northern Hemisphere. In Japan, it is found mainly in Hokkaido, growing in soils that

are semi-aerobic, low in nutrient, and have higher salinity than terrestrial soils, like soils of the mangrove habitat. The mycoflora of the *S. europaea* rhizosphere where such adverse conditions prevail has not been studied.

The present study compares the mycoflora of the *S. europaea* rhizosphere with the results of mangrove rhizosphere obtained in 1996 (7, 8).

Materials and Methods

Sampling sites. Fifteen healthy roots of *S. europaea* were collected from the side of Lake Notoro (4 samples) and Lake Saroma (11 samples), Abashiri and Monbetu-gun, Hokkaido, Japan on 1 and 2 August 1997. Five samples were collected from pasture, vegetable, and wheat fields near the lakes as root-free soil. This region has a so-called Okhotsk-type climate, with annual rainfall of 845 mm and average annual temperature of 5.9°C. The locality is at longitude 139°40'E and latitude 37°40'N. Soil type is sandy loam and soil pH is 7.2. Content of NaCl is 3.0%.

Isolation method. The same isolation method was adopted as in the previous report (8). Eight out of 15 root samples collected were used. Slender root were cut into sections of ca 5 cm in length and washed with physiological water (0.85%). Five sections of each sample were incubated on two plates at 15°C for one month. All fungi appearing during the incubation were isolated at 2, 3 and 4 wk under the dissecting microscope. Soil fungi were isolated from 15 samples after the first washing by x 50 and x 100 dilution plate methods.

Isolation medium. Cornmeal agar containing 50 µg/ml of tetracycline antibiotics was used for the slender root samples. Malt-yeast extract agar containing the same concentration and type of antibiotics was used.

Identification of isolated fungi. Isolates were inoculated on the plates of potato carrot, malt extract, potato sucrose, and oatmeal agar, and were also incubated at 24°C for appropriate periods. To identify the isolates, one representative strain of each species was used.

For species identification the following references and strains of IFO Culture Collection were consulted: *Acremonium* (6), *Penicillium* (10), *Fusarium* Link (2), Dematiaceous Hyphomycetes (4, 5), general taxonomy (3).

Results and Discussion

Fungal population density

The number of fungi present in each sample by the dilution plate method was counted under the dissecting microscope.

The average number of fungi for each site of *S. europaea* was 7.8×10^3 cfu/g of dry soil, whereas that of root-free soils of *S. europaea*, namely, pasture, vegetable, and wheat field soils, was 9.4×10^3 cfu/g. No significant differences were found in the average number of fungi between the *S. europaea* rhizosphere and root-free sample. The average number of fungi in the *S. europaea* rhizosphere was higher than that of mangrove soil

Table 1. Fungi isolated from *S. europaea* and mangrove roots, and their frequency of occurrence.

Species name	<i>S. europaea</i> frequency ^a	Species name	Mangrove frequency ^b
<i>Acremonium strictum</i> W.Gams	37.5	<i>Acremonium</i> sp.	3.6
<i>Acremonium</i> sp.	12.5	<i>Cylindrocarpon destractans</i>	
<i>Alternaria alternata</i> (Fr.) Keissler		(Zins.) Sholten	13.6
<i>Chrysosporium</i> sp.	75.0	Coelomycetes	13.6
<i>Cladosporium cladosporioides</i>	12.5	<i>Pestalotiopsis</i> sp.-1	27.3
(Fres.) de Vries	50.0	<i>Penicillium citrinum</i> Thom	13.6
<i>Cladosporium herbarum</i> (Per.)	12.5	<i>Penicillium</i> sp.-1	13.6
Link ex S.F.Gray		<i>Trichoderma harzianum</i> Rifai	50.0
Sterile mycelium	12.5		
Number of samples	8		22

^a: Number of positive samples / total number of samples x 100 (%).

^b: Data are cited from the IFO Res. Commun. 18: 40-44 (1997).

(1.2×10^3 cfu/g) (7). The reason is unclear, but the strongly anaerobic state and high content of heavy metals of the mangrove soil may be involved.

Mycoflora of rhizoplane of S. europaea

Table 1 shows the frequency of detection of fungi isolated from *S. europaea* root samples. Only six species were detected. *Alternaria alternata* (75.0% frequency), *Cladosporium cladosporioides* (50.0%), and *Acremonium strictum* (37.5%) were dominantly detected. The dominant species in roots of mangrove trees were *Trichoderma harzianum* Rifai (50.0%) and *Pestalotiopsis* sp.-1 (27.3%) (8). This difference is considered to be due to the host specificity.

Mycoflora of rhizosphere soil

Table 2 lists all the species of fungi isolated from 15 rhizosphere and 5 root-free samples.

Isolated species mainly belong to the taxon Deuteromycotina. The dominantly detected fungi from soil of *S. europaea* rhizosphere were *Acremonium* sp.-1 (66.7%), *Penicillium* spp. (33.3%), *Phoma* spp. (46.7%), *Trichoderma harzianum* (33.3%), and *T. koningii* Oudemans (33.3%), followed by *Acremonium strictum* (26.7%), *Alternaria alternata* (26.7%), *Cladosporium herbarum* (26.7%), and *Mucor hiemalis* Wehmer (26.7%). In root-free soil of *S. europaea*, *Apiospora montagnei* Sacc. (60.0%), *Mortierella nana* Linnemann (60.0%), *Mucor* spp. (80.0%) and *Phoma* spp. (60.0%) were detected. These fungi are typical soil fungi worldwide (3). It is considered that some of these fungi are psychrophilic or are adapted to low temperature.

Most of the fungi detected in the *S. europaea* rhizosphere resembled the mangrove rhizosphere fungi (7, 8, 11, 12, 13). Notable exceptions not found in the mangrove

Table 2. Fungi detected in *S. europaea* rhizosphere and root-free soil samples by the dilution plate method.

Rhizosphere soil		Root-free soil	
Species name	Frequency ^a	Species name	Frequency
<i>Acremonium murorum</i> (Corda) W.Gams	13.3	<i>Acremonium strictum</i> W.Gams	20.0
<i>Acremonium strictum</i> W.Gams	26.7	<i>Acremonium</i> spp.	40.0
<i>Acremonium</i> sp.1	66.7	<i>Apiospora montagnei</i> Sacc.	60.0
<i>Acremonium</i> spp.	20.0	<i>Chrysosporium mendartium</i> (Link:Fr.)	
<i>Alternaria alternata</i> (Fr.:Fr.) Keissler	26.7	Carmichael	20.0
<i>Apiospora montagnei</i> Sacc.	6.7	<i>Cladosporium cladosporioides</i> (Fres.) de Vries	40.0
<i>Arthrinium phaeospermum</i> (Corda) M.B.Ellis	6.7	<i>Cladosporium herbarum</i> (Pers.:Fr.) Link	20.0
<i>Aureobasidium pullulans</i> (de Bary) Arnaud	20.0	<i>Cladosporium</i> sp.	20.0
<i>Beauveria bassiana</i> (Balsamo) Vuill.	6.7	<i>Coniothyrium</i> sp.	20.0
<i>Beauveria brongniartii</i> (Sacc.) Petch	6.7	<i>Fusarium</i> spp.	40.0
<i>Bipolaris</i> sp.	6.7	<i>Gliocladium virens</i> Miller et al.	20.0
<i>Chrysosporium</i> sp.	6.7	<i>Gliocladium</i> sp.	20.0
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	20.0	<i>Gongronella butleri</i> (Lendner) Peyronel	
<i>Cladosporium herbarum</i> (Pers.:Fr.) Link	26.7	& Dal Vesco	20.0
<i>Cladosporium</i> sp.	6.7	<i>Mortierella alpina</i> Peyronel	20.0
<i>Coniothyrium</i> sp.	6.7	<i>Mortierella minutissima</i> van Tieghem	40.0
<i>Curvularia lunata</i> (Wakker) Boedijn	6.7	<i>Mortierella nana</i> Linnemann	60.0
<i>Cylindrocarpon</i> sp.	6.7	<i>Mortierella ramanniana</i> (Moller)	
<i>Eurotium amstelodami</i> Mangin	6.7	Linnemann var. <i>angulisporea</i>	
Eurotiales	6.7	(Naumov) Linnemann	20.0
<i>Fusarium graminearum</i> Schwabe	20.0	<i>Mortierella</i> sp.	20.0
<i>Fusarium oxysporum</i> Schlecht.:Fr.	6.7	<i>Mucor hiemalis</i> Wehmer	40.0
<i>Fusarium</i> spp.	13.3	<i>Mucor racemosus</i> Fres.	20.0
<i>Metarhizium anisopliae</i> (Metschn.) Sorokin	6.7	<i>Mucor</i> spp.	80.0
<i>Mortierella alpina</i> Linnemann	6.7	<i>Penicillium islandicum</i> Sopp	20.0
<i>Mortierella minutissima</i> van Tieghem	6.7	<i>Penicillium spinulosum</i> Thom	20.0

Table 2. (continued)

Rhizosphere soil		Root-free soil	
Species name	Frequency ^a	Species name	Frequency
<i>Mortierella</i> spp.	13.3	<i>Penicillium viridicatum</i> Westling (?)	20.0
<i>Mucor hiemalis</i> Wehmer	26.7	<i>Penicillium</i> spp.	40.0
<i>Mucor</i> sp.	6.7	<i>Phialophora cyclaminis</i> van Beyma	20.0
<i>Paecilomyces marquandi</i> (Masse) Hughes	13.3	<i>Phoma</i> spp.	60.0
<i>Paecilomyces</i> sp.	6.7	<i>Rhizopus</i> sp.	20.0
<i>Penicillium citrinum</i> Thom	20.0	<i>Scopulariopsis</i> sp.	20.0
<i>Penicillium aurantiogriseum</i> Dierckx	13.3	<i>Trichoderma aureoviride</i> Rifai	20.0
<i>Penicillium chrysogenum</i> Thom	6.7	<i>Trichoderma harzianum</i> Rifai	20.0
<i>Penicillium roquefortii</i> Thom	6.7	<i>Trichoderma polysporum</i> (Link:Fr.) Rifai	20.0
<i>Penicillium waksmanii</i> Zaleski	6.7	<i>Trichoderma viride</i> Per.:Fr.	20.0
<i>Penicillium</i> spp.	33.3	<i>Trichoderma</i> sp.	20.0
<i>Phialophora</i> sp.	6.7	Unidentified strains	40.0
<i>Phoma</i> spp.	46.7	Sterile mycelium	80.0
<i>Rhinoctadiella</i> sp.	6.7		
<i>Scopulariopsis</i> spp.	20.0		
<i>Talaromyces helicus</i> C.R.Benjamin var. <i>major</i> Stolk & Samson	6.7		
<i>Trichoderma harzianum</i> Rifai	33.3		
<i>Trichoderma koningii</i> Oudem.	33.3		
<i>Verticillium fungicola</i> (Preuss) Hassebr.	6.7		
Unidentified strain	6.7		
Sterile mycelium	6.7		
Total number of samples	15		5

^a: Number of positive samples / total number of samples x100 (%).

rhizosphere were *Alternaria alternata*, *Cladosporium herbarum* (Per.: Fr.) Link, and *Fusarium graminearum* Schwabe. The optimal growth temperature of these fungi are at 15–28°C and the minimum temperature are in the range 5–8°C. It is assumed that they grow in low temperature.

The genera *Mortierella* Coemans and *Mucor* Fresenius, which belong to the taxon Zygomycotina, were often detected in root-free soils. These fungi are known to be found dominantly in tomato field and forest soil (1, 3), but they were scarcely detected in mangrove root-free samples in Okinawa (7). It is considered that these fungi like to cold temperature and the optimum temperature is low. Consequently, low temperature is assumed to be one of the factors determining their geographical distribution.

Tolerance to sodium chloride

Tolerance to sodium chloride was tested for some isolates, namely, *Acremonium* sp.-1 (H9-6-7), *Mortierella ramanniana* (Möler) Linnemann var. *angulispora* (Naumov) Linnemann (H9-18-2), *Mucor hiemalis* (H9-18-1), *Penicillium aurantiogriseum* (H9-4-3), *Phoma* sp. (H9-3-3), and *Trichoderma harzianum* (H9-16Na-2). The fungi other than *M. ramanniana* var. *angulispora* grew at the concentration of NaCl from 7.0% to 15.0%. These fungi have been isolated from natural substrates and sugar, salted food products, and concentrated fruit juice (3). The results show that these fungi can withstand the high osmotic pressure if the soil in which *S. europaea* is standing dries up at high temperature or if they are carried into the salty water of a lake. *M. ramanniana* var. *angulispora* tolerated NaCl only up to the concentration of 2.0%. This means that this species will not survive in lake water of 3.0% NaCl when it is carried into the lake.

It was considered that the mycoflora of the *S. europaea* rhizosphere differs from that of the mangrove rhizosphere, but soil mycofloras of the two habitats are almost the same in spite of the differences in host plants and geographical distribution. Further investigations on ecology and species diversity are necessary.

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The Ubiquinone System in *Hasegawaea japonica* (Yukawa et Maki) Yamada et Banno: A New Method for Identifying Ubiquinone Homologs from Yeast Cells

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Summary

The ubiquinone homologs of the type strains of the two varieties of *Schizosaccharomyces japonicus* (\equiv *Hasegawaea japonica*), *Schizosaccharomyces japonicus* var. *japonicus* and *Schizosaccharomyces japonicus* var. *versatilis*, were determined to be Q-10 as major and Q-8 as minor by extraction with a chloroform/methanol mixture and by purification on a thin-layer plate developed with a solvent system composed of hexane/diethyl ether, followed by high performance liquid chromatography. However, the quantities of the ubiquinone homologs were extremely low (only one one-hundredth) compared with that of the type strain of *Schizosaccharomyces pombe*. The taxonomic position of *Hasegawaea japonica* (\equiv *S. japonicus*) was discussed chemotaxonomically and phylogenetically based on the ubiquinone systems and the 18S rRNA partial and complete sequence analyses. The conclusion is that the genus *Hasegawaea* should be retained along with the genus *Schizosaccharomyces*.

Keywords: ubiquinone homologs, *Hasegawaea japonica*, *Schizosaccharomyces*.

Three species are presently recognized in the genus *Schizosaccharomyces* Lindner (12): *Schizosaccharomyces pombe* Lindner as the type species, *Schizosaccharomyces octosporus* Beijerinck, and *Schizosaccharomyces japonicus* Yukawa et Maki [synonym: *S. japonicus* var. *versatilis* (Wickerham et Duprat) Slooff (11, 18)].

In 1960, Kudriavzev (4) introduced the genus *Octosporomyces* Kudriavzev for *S. octosporus* and *S. japonicus*. However, the name *Octosporomyces* has not been accepted

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[†]type strain; Q-n, ubiquinone homologs with a specified number (n) of isoprene units in a side chain, e.g., Q-9 and Q-10.

(11, 12, 18).

Mikata and Banno (7) studied ascospore morphology of strains including the type strains of the three species and two varieties of *Schizosaccharomyces* by scanning electron microscopy. The species accommodated in the genus *Schizosaccharomyces* were divided into three groups based on their ascospore morphology: warty in *S. pombe*, smooth with papillae in *S. octosporus*, and smooth without papillae in *S. japonicus* var. *japonicus* and *S. japonicus* var. *versatilis*.

Yamada and Banno (13) distinguished strains of the genus *Schizosaccharomyces* into three groups at the generic level by combining their ascospore morphology reported by Mikata and Banno (7), their ubiquinone systems reported by Yamada et al. (15), and their fatty acid composition reported by Kock and van der Walt (3): *Schizosaccharomyces* Lindner for strains of *S. pombe*, *Octosporomyces* Kudriavzev for strains of *S. octosporus* as *Octosporomyces octosporus* (Beijerinck) Kudriavzev, and *Hasegawaea* Yamada et Banno for strains of *S. japonicus* var. *japonicus* and *S. japonicus* var. *versatilis* as *Hasegawaea japonica* (Yukawa et Maki) Yamada et Banno var. *japonica* and *H. japonica* var. *versatilis* (Wickerham et Duprat) Slooff.

Kurtzman and Robnett (6) rejected the division of the genus *Schizosaccharomyces* into three genera based on their 18S and 25S (or 26S) rRNA partial base sequence analysis, since *S. octosporus*, *S. japonicus* var. *japonicus* and *S. japonicus* var. *versatilis* were separated from *S. pombe* by a distance only 25% greater than found outlying *Saccharomyces* species including *Saccharomyces kluyveri* Phaff, Miller et Shifrine and *Saccharomyces unisporus* Jörgensen.

Yamada et al. (17) stated that the genus *Hasegawaea* should be retained along with the genus *Schizosaccharomyces*, since the type strains of *H. japonica* var. *japonica* and *H. japonica* var. *versatilis* were phylogenetically separated from those of *S. pombe* and *S. octosporus*. In *The Yeasts: A Taxonomic Study*, 4th ed., however, Vaughan-Martini and Martini (12) did not accept the genus *Hasegawaea*.

This paper deals with the reexamination of the ubiquinone systems in the fission yeasts, especially in those classified in the two varieties of *S. japonicus* (11, 18) (\equiv *H. japonica*), and discusses the recognition of the genus *Hasegawaea*.

Materials and Methods

Yeast strains. The type strains of *S. pombe* (IFO 1628^T), *S. octosporus* (IFO 10373^T), *S. japonicus* var. *japonicus* (IFO 1609^T), and *S. japonicus* var. *versatilis* (IFO 1607^T) were analyzed for ubiquinone homologs. *Saccharomyces cerevisiae* Rees ex Hansen IFO 10217^T, *Pichia membranifaciens* (Hansen) Hansen IFO 10215^T, and *Debaromyces hansenii* (Zopf) Lodder et Kreger-van Rij IFO 0083^T were used as reference standards for extraction and identification of ubiquinone homologs.

Cultivation of yeast strains. All strains were precultured on YPD medium composed of 1.0% yeast extract, 2.0% peptone and 2.0% glucose in test tubes for one day with shaking. Precultures were inoculated into 400 ml of YPD medium in a 1-liter Erlenmeyer flask and incubated on a rotary shaker for 16–30 h at 28°C. For each strain, 800

ml of YPD medium was used in total.

Extraction, purification and identification of ubiquinone homologs from yeast cells. About 3 g of dried yeast cells was suspended in 100 ml of a chloroform/methanol mixture (2 : 1, by volume), and the suspension was stirred continuously for 16 h to extract the ubiquinone homologs. After removal of yeast cells by filtration, the extract was evaporated to dryness on a rotary evaporator. The residue was dissolved in about 1 ml of acetone. The acetone solution was spotted on a silica gel plate (0.5 mm thickness; 60F₂₅₄, E. Merck, U.S.A.) and the ubiquinone homologs on the plate were developed with a solvent system composed of hexane/diethyl ether (85 : 15, by volume) for purification. The presence of ubiquinone homologs on the silica gel plate was detected under ultraviolet light (model UVG-54 Minerlight lamp, UVP, U.S.A.). The yellow silica gel containing ubiquinone homologs was scraped from the plate, and the ubiquinone homologs were eluted from the silica gel with a small volume of acetone. The acetone solution was evaporated to dryness again, and the residue was dissolved with about 0.3 ml of ethanol. The ethanol solution was preserved at -20°C until the analysis of the ubiquinone system.

Analysis of ubiquinone system. The ubiquinone system was analyzed by high performance liquid chromatography on a Shimadzu model LC-6AD liquid chromatograph equipped with a Zorbax column (4.6 mm \times 15 cm). Ubiquinone homologs were eluted with a mixture of methanol/isopropyl ether (7 : 1, by volume) at the flow rate of 1.0 ml/min at 30°C and detected by their absorbance at 275 nm with a Shimadzu model SPD-6A spectrophotometric detector. The elution times and the areas of the elution peaks of ubiquinone homologs were calculated with a Shimadzu Chromatopac model C-R6A. Authentic Q-6, Q-7, Q-8, Q-9, and Q-10 were used as reference standards.

Mass spectroscopy. Mass spectroscopic analyses of the ubiquinone homologs purified by thin-layer chromatography were performed with a JEOL model JMS-AX505W single focusing mass spectrometer at a chamber temperature of 338°C . The samples were vaporized at the ion source with a heated direct inlet system operating at 170°C .

Reagents and chemicals. The reagents and chemicals used in this experiment were obtained commercially. Authentic Q-6 and Q-10 were purchased from Nakarai, Kyoto, Japan. Authentic Q-7, Q-8, and Q-9 were prepared from cells of *Pichia anomala* (\equiv *Hansenula anomala*) IFO 10213^T, *Pichia capsulata* (\equiv *Kuraishia capsulata*) IFO 0721^T, and *Rhodospiridium toruloides* IFO 0559^T, respectively.

Results and Discussion

In the type strains of *S. japonicus* var. *japonicus*, *S. japonicus* var. *versatilis*, and *S. pombe*, the ubiquinone systems were mainly comprised of Q-10 (Table 1). Q-8, but not Q-9, was found as a minor component in both varieties, the calculated percentage being 20.8 and 9.2, respectively. In the type strain of *S. pombe*, the minor components were 0.2% Q-7, 1.4% Q-8, and 3.8% Q-9. The type strain of *S. octosporus* had the Q-9 system (93.3%) with very small amounts of Q-7, Q-8, and Q-10. The percentages of the minor components were calculated to be 2.1, 1.9 and 2.7, respectively.

Mass spectroscopic analyses of the ubiquinone homologs in the type strains of *S.*

japonicus var. *japonicus*, *S. japonicus* var. *versatilis*, and *S. pombe* confirmed the presence of Q-10, with the parent peak at m/z 862 and an intense fragment ion peak at m/z 235. However, the fragment ion peak at m/z 197 was hardly found. The mass spectroscopic data obtained here indicated that the isolated ubiquinone homologs are mostly in an oxidized form, i.e., in a quinone but not a quinol form, unlike in a previous study (14).

The ubiquinone systems of the type strains of *Saccharomyces cerevisiae* (IFO 10217^T), *D. hansenii* (IFO 0083^T), and *P. membranifaciens* (IFO 10215^T) were Q-6 (97.6%), Q-9 (95.1%), and Q-7 (94.7%), respectively (Table 1). The minor components were 1.1% Q-7, 1.2% Q-8, and 0.1% Q-9 in the type strain of *Saccharomyces cerevisiae*, 1.0% Q-7, 2.8% Q-8, and 1.1% Q-10 in the type strain of *D. hansenii*; and 3.6% Q-6, 1.6% Q-8, and 0.1% Q-9 in the type strain of *P. membranifaciens*.

The relative quantities of the total ubiquinone homologs were estimated to be 27.6% in *S. pombe* IFO 1628^T, 2.1% in *S. octosporus* IFO 10373^T, only 0.2% in *S. japonicus* var. *japonicus* IFO 1609^T and 0.3% in *S. japonicus* var. *versatilis* IFO 1607^T, when compared with the type strain of *P. membranifaciens* (IFO 10215^T) (Table 1).

The isoprenoid quinone homologs are now used widely as one of the most important chemotaxonomic criteria for classifying and identifying bacteria, yeasts, and fungi at the generic level (1, 2, 5, 14-16).

Yamada et al. (15) and Yamada and Banno (13) reported that no ubiquinone homologs were found in the type strains of *H. japonica* var. *japonica* (\equiv *S. japonicus* var. *japonicus*) and *H. japonica* var. *versatilis* (\equiv *S. japonicus* var. *versatilis*). Billon-Grand (personal communication) also stated that ubiquinone homologs were not detected in the type strains of the two varieties of *H. japonica*. In the present experiment, which differed from earlier experiments in not using sodium hydroxide and pyrogallol for extraction of ubiquinone homologs (1, 14-16), it was obvious that the type strains of *S. japonicus* var. *japonicus* and *S. japonicus* var. *versatilis* have the Q-10 system, as reported in *S. pombe*

Table 1. The ubiquinone homolog composition in the type strains of species of the genera *Schizosaccharomyces*, *Saccharomyces*, *Debaryomyces*, and *Pichia*.

Species and type strain	Relative composition ^a (%) of					Total ^b
	Q-6	Q-7	Q-8	Q-9	Q-10	
<i>S. japonicus</i> var. <i>japonicus</i> IFO 1609	0	0	20.8	0	79.2	0.2
<i>S. japonicus</i> var. <i>versatilis</i> IFO 1607	0	0	9.2	0	90.8	0.3
<i>S. pombe</i> IFO 1628	0	0.2	1.4	3.8	94.6	27.6
<i>S. octosporus</i> IFO 10373	0	2.1	1.9	93.3	2.7	2.1
<i>Sac. cerevisiae</i> IFO 10217	97.6	1.1	1.2	0.1	0	31.3
<i>D. hansenii</i> IFO 0083	0	1.0	2.8	95.1	1.1	5.7
<i>P. membranifaciens</i> IFO 10215	3.6	94.7	1.6	0.1	0	100

^aThe retention times of ubiquinone homologs were: 4.0 min for Q-6, 5.0 min for Q-7, 6.5 min for Q-8, 8.5 min for Q-9, and 11.3 min for Q-10.

^bIn the estimation of the relative quantities of the total ubiquinone homologs, the type strain of *P. membranifaciens* (IFO 10215) was designated as a reference standard (total of quinone peak areas 14832772; relative quantity, 100%).

S., *Schizosaccharomyces*; *Sac.*, *Saccharomyces*; *D.*, *Debaryomyces*; *P.*, *Pichia*.

(15). However, the quantities of the ubiquinone homologs in the two varieties of *S. japonicus* were greatly different from that of *S. pombe*: the total quantities were one one-hundredth or less than that of the type strain of *S. pombe* (Table 1).

Yamada et al. (17) argued that the genus *Hasegawaea* should be retained, based on partial 18S rRNA sequences at positions 1451 through 1618, 168 bases, the differences in the phenotypic features of the fission yeasts. However, Vaughan-Martini and Martini (12) rejected their opinion. The three species *S. pombe*, *S. octosporus*, and *S. japonicus* (\equiv *H. japonica*) were accommodated in the single genus *Schizosaccharomyces* in The Yeasts: A Taxonomic Study, 4th ed. (12).

Naehring et al. (8) determined the complete sequences of 18S, 25S (or 26S), and 5.8S rRNAs of the type strain of *S. japonicus* var. *versatilis* (\equiv *H. japonica* var. *versatilis*). They concluded that there is no need to introduce the genus *Hasegawaea* into the group of fission yeasts, since *S. japonicus* var. *versatilis* and *S. pombe* are more closely related than are *Saccharomyces cerevisiae* and *Kluyveromyces lactis* (Dombrowski) van der Walt. They calculated the similarity between the 18S rRNA sequences of *S. japonicus* var. *versatilis* and *S. pombe* to be 95.7%, however, and this value is low enough to distinguish the two taxa, *S. japonicus* and *S. pombe*, at the generic level.

In the family Acetobacteraceae Gillis and De Ley, for example, a nearly equal similarity value (95.6%) was calculated by Sievers et al. (9) between the 16S rRNA sequences of the type strains of *Acetobacter aceti* (Pasteur) Beijerinck, the type species of the genus *Acetobacter* Beijerinck and *Gluconobacter oxydans* (Henneberg) De Ley, the type species of the genus *Gluconobacter* Asai.

Sjamsuridzal et al. (10) determined the full sequence of 18S rRNA of *S. japonicus* var. *japonicus* IFO 1609^T. Their conclusion is identical with that of Naehring et al. (8). However, it is obvious in their phylogenetic trees that the distance between *S. japonicus* var. *japonicus* (\equiv *H. japonica* var. *japonica*) and *S. pombe* is much longer than those between *Saitoella* and *Neolecta* species as well as between *Taphrina* and *Protomyces* species (10). In addition, the phylogenetic distance between *S. japonicus* var. *japonicus* and *S. japonicus* var. *versatilis* is not close (10), differing from the sequence data of Kurtzman and Robnett (6) and Yamada et al. (17).

The phylogenetic and chemotaxonomic data obtained previously and presently indicate that the genus *Hasegawaea* should be accepted, contrary to the opinions of Kurtzman and Robnett (6), Naehring et al. (8), and Sjamsuridzal et al. (10).

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Phylogenetic analysis of actinobacteria in the mangrove rhizosphere

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Summary

Twenty-five actinobacteria isolated from the mangrove rhizosphere were characterized taxonomically. On the basis of phylogenetic and chemotaxonomic characteristics, the taxonomic positions of 24 of the strains were clarified: 13 yellow or orange strains were located in the genus *Cellulomonas*, 4 yellow to pale yellow strains in the genus *Agromyces*, 2 yellow strains in the genus *Microbacterium*, 2 pale brown strains in the genus *Mycobacterium*, and 1 strain each in the genera *Micrococcus*, *Gordonia* and *Rhodococcus*. Some strains were considered to be new species in their respective genera. However, we could not determine the taxonomic position of the remaining one strain containing LL-diaminopimelic acid in the cell wall. These results indicate that the mangrove rhizosphere is a good source for isolation of new and diverse actinobacteria.

Keywords: diversity of actinobacteria, mangrove rhizosphere

The diversity and population of microorganisms in the mangrove rhizosphere have been studied in the Institute for Fermentation, Osaka (IFO) for several years. Mangrove forests in Japan are mainly located in the subtropical and tropical regions of Okinawa. Hatano examined the actinomycete populations in the rhizosphere soils of seven kinds of mangrove forest in the Iriomote and Ishigaki islands in Japan and suggested that the mangrove rhizosphere is a good source for isolating new and diverse actinomycetes (7). Recently, we determined the taxonomic positions of actinobacteria isolated from the mangrove rhizosphere in the Iriomote island and found that 4 strains out of 9 isolated actinobacteria were new species in the genera *Gordonia* and *Microbacterium*: *Gordonia rhizosphaera* (31), *Microbacterium halophilum*, and *Microbacterium thalassium* (30, 32). However, the taxonomic positions of the remaining 5 isolates were not determined. In addition, 20 actinobacteria were isolated from the mangrove rhizosphere at the same sampling sites in different seasons.

In this study, we determined the almost complete 16S rRNA gene sequences of 25 strains isolated from the mangrove rhizosphere and compared them with the sequences of representative validated gram-positive bacteria, to clarify the phylogenetic diversity of

actinobacteria in the mangrove rhizosphere.

Material and Methods

Soil samples and treatments. Soil samples were taken from the mangrove rhizosphere at four sampling sites in the estuaries of the Maira, Shiira, and Urauchi rivers in Iriomote island (7) in October 1995 and July 1997 (Table 1). All soil samples were stored at 5~8°C, then dried in air at 17°C for six days. Soils were passed through sieves of 20 and 60 mesh, and soil and fine roots were separated.

Isolation and cultivation. Selective isolation of soil actinomycetes was described by

Table 1. Bacterial strains investigated and their 16S rDNA accession numbers

Isolation ¹⁾	Color of colony	Strain No.	IFO No.	Isolation site ²⁾	Source	DDBJ Accession No.
I-1	Pale yellow	No. 2	16063	No. 1	fine root	AB004731
I-2	Pale yellow	Y-1	16066	No. 1	soil	AB004733
I-3	Pale yellow	Y-2	16067	No. 1	soil	
I-4	Pale brown	No. 105	16064	No. 4	soil	AB004732
I-5	Pale brown	No. 151	16065	No. 4	fine root	
II-1	Rose	No. 60	16247	No. 4	soil	AB023368
II-2	Orange	No. 61	16244	No. 4	soil	AB023365
II-3	Orange	No. 66	16245	No. 4	soil	AB023366
II-4	Orange	No. 24	16242	No. 3	root	AB023363
II-5	Pale orange	No. 10	16239	No. 3	soil	AB023360
II-6	Pale orange	No. 27	16243	No. 3	soil	AB023364
II-7	Pale orange	No. 77		No. 1	root	
II-8	Yellow	No. 58 (5)	16237	No. 4	soil	AB023358
II-9	Yellow	No. 12 a	16248	No. 3	root	AB023369
II-10	Yellow	No. 13	16249	No. 3	root	AB023370
II-11	Yellow	No. 73	16250	No. 5	root	AB023371
II-12	Pale yellow	No. 8	16235	No. 3	soil	AB023356
II-13	Pale yellow	No. 65	16238	No. 4	soil	AB023359
II-14	Pale yellow	No. 19	16241	No. 4	root	AB023362
II-15	Pale yellow	No. 68	16246	No. 4	soil	AB023367
II-16	Pale yellow	No. 14	16236	No. 3	root	AB023357
II-17	Pale yellow	No. 16	16240	No. 4	root	AB023361
II-18	Pale brown	No. 58	16252	No. 4	soil	AB023373
II-19	Pale brown	No. 74	16253	No. 5	root	AB023374
II-20	Pale brown	No. 48	16251	No. 4	soil	AB023372

1) I, isolated in October 1995; II, isolated in July 1997

2) No. 1, Maira River, rhizosphere of *Avicennia marina*; No. 3, Shiira River, rhizosphere of *Sonneratia alba*; No. 4, Shiira River, rhizosphere of *Bruguera gymnorhiza*; No. 5, Urauchi River, rhizosphere of *Kandelia candel*

Hayakawa & Nonomura (8, 9). Dried soil (1 g) was suspended in 9 ml of YE-SDS solution (7) and treated with an Ultrasonic Cleaner (38 kHz, Kaijou Denki, Tokyo, Japan). A diluted aliquot of soil suspension was spread on HV agar plates (8) and incubated for 2 to 3 weeks at 28°C. Among colonies appearing on the plates, bacteria-like colonies that were yellow, orange, red, or white in color were picked up and transferred to HV and PY agar plates (1% peptone, 0.2% yeast extract, 0.2% NaCl, 0.2% D-glucose, and 15g of agar, pH 7.2) as actinobacteria. Cells used for biochemical tests were cultured in PY medium, harvested by centrifugation during the stationary phase, washed with water, and lyophilized.

Strains investigated. Five strains isolated from the soils of the four sampling sites in Iriomote island in October 1995, and 20 strains isolated from the same sampling sites in July 1997 were used in this study (Table 1).

Analysis of 16SrDNA. Extraction of genomic DNA and amplification of the 16S rDNA were carried out as described previously (30). PCR products were sequenced directly by using a Thermo Sequenase Fluorescent Labelled Primer Cycle Sequencing Kit with 7-deaza-dGTP (Amersham) and a Pharmacia Model ALF DNA sequencer following the manufacturer's protocol.

Phylogenetic analysis. Nucleotide substitution rates (K_{nuc}) (13) were calculated by using the CLUSTAL V software package (10), and phylogenetic trees were constructed by the neighbour-joining method (20). The topology of the phylogenetic trees was evaluated by the bootstrap resampling method of Felsenstein (3) with 1000 replicates.

Nucleotide sequence accession numbers. The accession numbers for the 16S rDNA sequences determined in this study are AB023356 to AB023374 (Table 1) and those of *Promicromonospora sukumoe* IFO 14650^T and *Cellulomonas cellulans* (formerly *Oerskovia xanthineolytica*) IFO 16148 are AB023375 and AB023355. The DDBJ, GenBank, and EMBL accession numbers for the 16S rDNA sequences of the reference strains used in the phylogenetic analyses are shown in each figure.

Chemotaxonomic analysis. Isomers of diaminopimelic acid (A_2pm) in the cell wall were examined by the method of Hasegawa et al. (6). Cell walls were prepared from about 500 mg (dry wt.) of bacterial cells as described by Schleifer & Kandler (21), and amino acids in the acid hydrolysate of the cell walls were identified by HPLC as described by Yokota et al. (34). Fatty acids were extracted from dried cells, purified, and analysed by GC-MS with a GCMS-QP5000 MS Workstation computer system as described previously (30). The G+C content of the DNA was determined by the method of Mesbach et al. (16) after treatment with P1 nuclease and alkaline phosphatase.

DNA-DNA hybridization. DNA-DNA hybridization was performed fluorometrically in microdilution wells by using biotinylated DNA (2).

RESULTS AND DISCUSSION

We selected 9 colonies from the soil samples collected from the mangrove rhizosphere in October 1995. Among them, four strains were identified as new species in the genera *Gordonia* (31) and *Microbacterium* (30, 32), while the taxonomic positions of the remain-

ing five strains, No. 2, 105, 151, Y-1, and Y-2, were not determined. Additionally, 20 colonies were isolated from soil samples collected at the same sampling sites in the mangrove forests in July 1997 (Table 1).

Before comparing the 16S rDNA sequences of isolated actinobacteria with those of related microorganisms, we first examined the isomers of diaminopimelic (A_2pm) in the cell wall. Among the 25 strains, 4 strains, No. 48, 58, 60, and 74, contained *meso*- A_2pm ; strain No. 77 contained *LL*- A_2pm ; and the remainder did not contain *meso*- or *LL*- A_2pm (data not shown). We next determined the almost complete 16S rDNA sequences of the 25 strains, which consisted of between 1468 and 1511 nucleotides, and aligned the sequences with the published sequences from DDBJ, GenBank, and EMBL.

The 16S rDNA sequences of the 20 strains which did not contain *meso*- or *LL*- A_2pm in the cell wall were compared with representative members in the genera of suborder *Micrococccineae* (25) (Fig. 1), and the 16SrDNA sequences of 5 strains which contained *meso*- or *LL*- A_2pm in the cell wall were compared with representative members in the genera of suborder *Corynebacterineae* (25) (Fig. 2), respectively. The 16S rDNA similarity values were determined (data not shown).

As shown in Fig. 1, 13 strains, comprising 6 pale yellow strains, No. 2, 16, 19, 68, Y-1, and Y-2, 2 pale brown strains, No. 105 and 151, and 5 orange to pale orange strains, No. 10, 24, 27, 61, and 66, were all located in the large cluster of family *Cellulomonadaceae* or family *Promicromonosporaceae* (19, 24). Four yellow to pale yellow strains, No. 8, 14, 58 (5), and 65, were in the cluster of the genus *Agromyces* (28, 35) and 2 yellow strains, No. 12a and 13, were located in the genus *Microbacterium* (30). Yellow No. 73 strain was included in the genus *Micrococcus* (14, 23), and the closest related species to strain No. 73 was *Micrococcus luteus*.

Of the *meso*- A_2pm containing actinobacteria, as shown in Fig. 2, strain No. 60, strain No. 74, and strains No. 48 and 58 formed a coherent cluster with species of the genera *Gordonia* (27), *Rhodococcus* (18) and *Mycobacterium* (33), respectively. The sequence of the 16S rDNA of strain No. 77, which contained *LL*- A_2pm in the cell wall, was aligned with some members of actinomycetes, but its position in the bacterial phylogenetic tree remained uncertain.

Taxonomic positions of actinobacteria lacking meso-A₂pm.

Cellulomonas species: Thirteen strains, No. 2, 10, 16, 19, 24, 27, 61, 66, 68, 105, 151, Y-1, and Y-2, had lysine as cell wall diamino acid, G+C contents of DNA ranging from 67.2 to 73.0 mol%, and cellular fatty acids composed mainly of *anteiso*-15:0, *iso*-15:0, *iso*-16:0 and/or *iso*-17:0. Their major menaquinone was MK-9 (H_4), and some strains also contained MK-10 (H_2). These chemotaxonomic features indicate that they may belong to the genera *Cellulomonas* (24, 26), *Promicromonospora* (12) or *Sanguibacter* (4) (Table 2). However, as shown in Fig. 3, phylogenetic analyses suggested that all 13 strains belong to the genera *Cellulomonas* or *Promicromonospora*, both of which exhibit very similar profiles in chemotaxonomic properties (Table 2).

Nine of the 13 strains, No. 10, 24, 27, 61, 66, 105, 151, Y-1 and Y-2, formed a phylogenetic coherent cluster, and this cluster was attached to two *Promicromonospora*

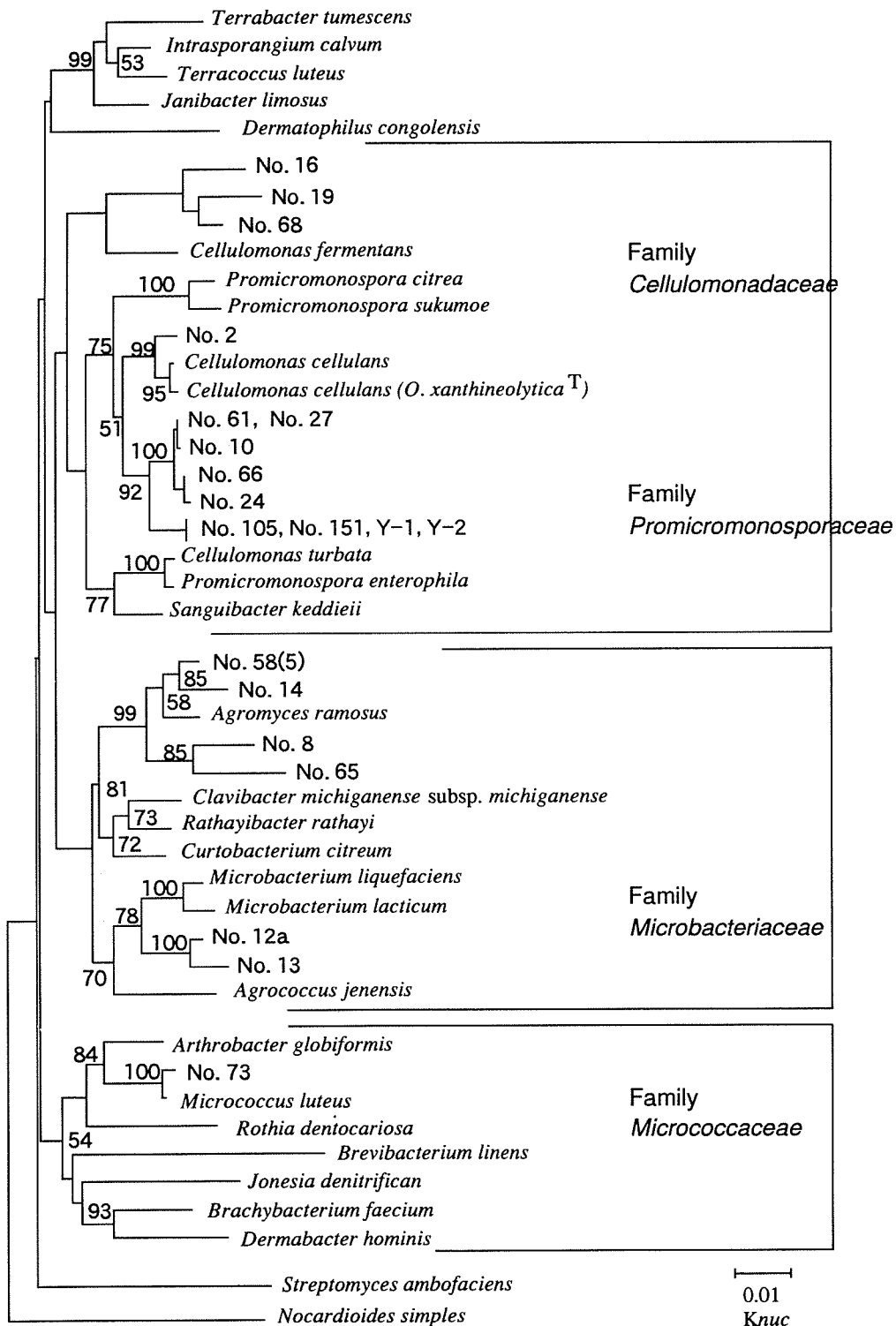


Fig. 1. Unrooted phylogenetic tree displaying the relationships to reference organisms of actinobacteria which do not contain *meso*-A₂pm. The numbers on the tree indicate bootstrap values (percent) derived from 1,000 samples, for the branch point (3). Bar=0.01 K_{nuc} unit

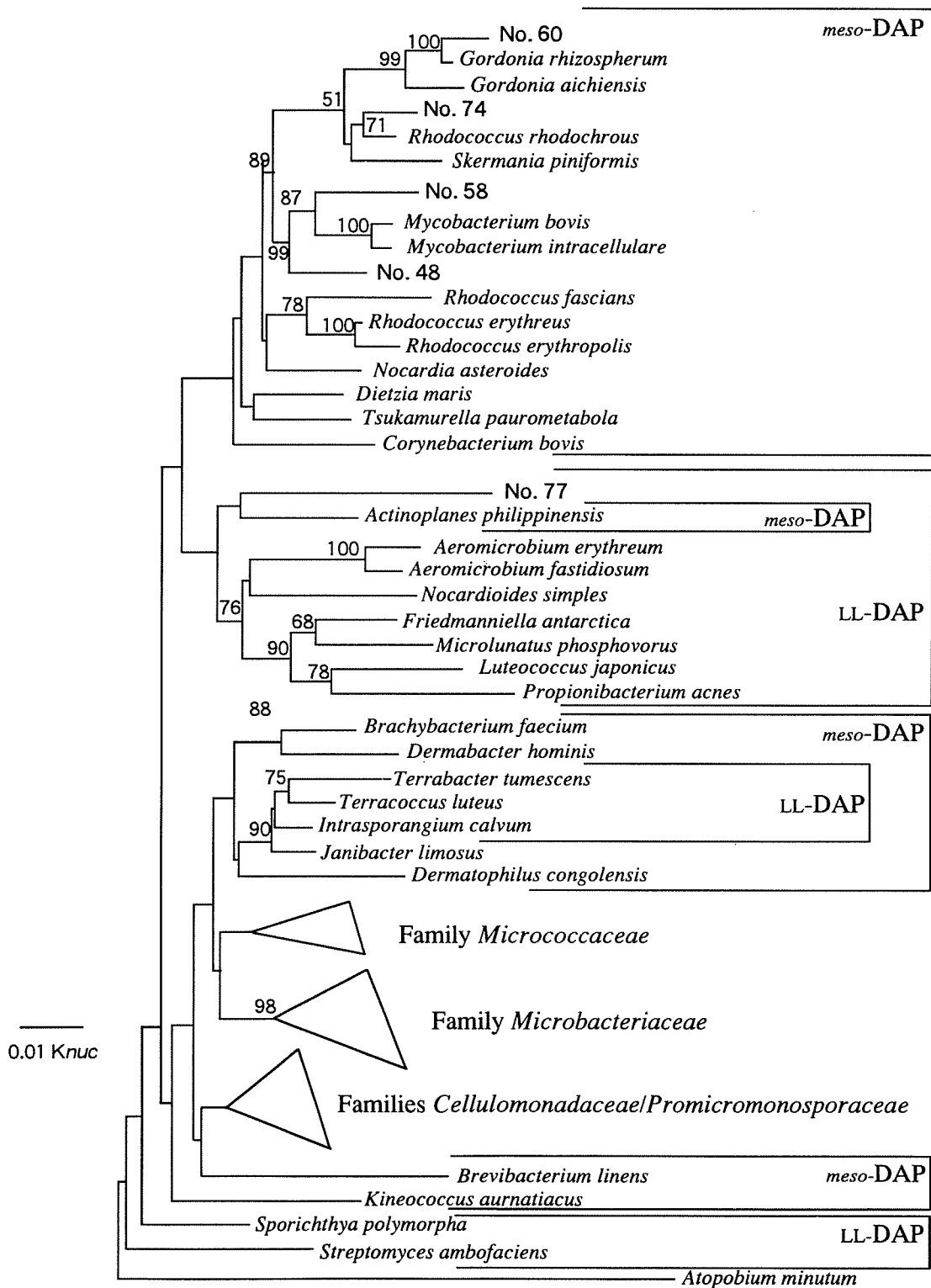


Fig. 2. Unrooted phylogenetic tree displaying the relationships to reference organisms of actinobacteria which contain *meso-A₂pm*. The numbers on the tree indicate bootstrap values (percent) derived from 1,000 samples, for the branch point (3). Bar = 0.01 K_{nuc} unit

Table 2. Differential characteristics of actinobacteria

Genus	G+C content (mol%)	Diamino acid	Major menaquinone
<i>Arthrobacter</i>	61-66	Lys	MK-8, 9/MK-9 (H ₂)
<i>Nestenkononia</i>	70-72	Lys	MK-8, 9
<i>Kytococcus</i>	68-71	Lys	MK-8, 9, 10
<i>Kocuria</i>	66-75	Lys	MK-7 (H ₂), MK-8 (H ₂)
<i>Micrococcus</i>	70-76	Lys	MK-8, MK-8 (H ₂)
[No. 73]	72.5	Lys	MK-8 (H ₂)
<i>Microbacterium</i>	65-72	Lys/Orn	MK-11, 12, 13, 14
[No. 12a]	72.5	Orn	MK-10, 11
[No. 13]	70.3	Orn	MK-12, 11
<i>Promicromonospora</i>	70-75	Lys	MK-9 (H ₄)
<i>Sanguibacter</i>	69-70	Lys	MK-9 (H ₄)
<i>Cellulomonas</i>	71-76	Lys/Orn	MK-9 (H ₄)
[No. 2,10, 16, 19, 24, 27, 61, 66, 68, 105, 151, Y-1, Y-2,]	67.2-73.0	Lys	MK-9 (H ₄), MK10 (H ₂)
<i>Rarobacter</i>	65-66	Orn	MK-9
<i>Clavibacter</i>	67-78	DAB	MK-9, 10
<i>Crybacterium</i>	65	DAB	MK-10
<i>Rathayibacter</i>	63-72	DAB	MK-10
<i>Leucobacter</i>	66.2	DAB	MK-11
<i>Agrococcus</i>	74	DAB	MK-11, 12
<i>Agromyces</i>	71-76	DAB	MK-11, 12
[No. 8, 65, 58 (5), 14]	70.0-73.3	DAB	MK-11, 12

species, *Promicromonospora citrea* (12) and *Promicromonospora sukumoe* (29), at a significant distance (Fig. 3). The remaining one species of the genus, *Promicromonospora enterophila* (11), was more closely related to *Cellulomonas turbata* (26) than to the other two species. Though the species in the genera *Cellulomonas* and *Promicromonospora* are phylogenetically intermixed (5, 19), we determined that these 9 strains should be placed the species in the genus *Cellulomonas*, based on their morphological characteristics, namely, that neither aerial mycelia nor single spores are observed.

Three of the 13 strains, No. 16, 19 and 68, also formed a phylogenetic coherence cluster, which constituted the deepest branch in the genus *Cellulomonas*, and *Cellulomonas fermentans* (1) attached to this cluster at some distance. The remaining strain, No. 2, formed a sister lineage to the *Cellulomonas cellulans* (19, 24, 26) group.

Microbacterium species: Strains No. 12a and 13 had ornithine as cell wall diamino acid, G+C contents of DNA ranging from 70.0 to 74.1 mol%, and cellular fatty acids composed mainly of *anteiso*-15:0, *iso*-16:0, and *anteiso*-17:0. Their major menaquinones

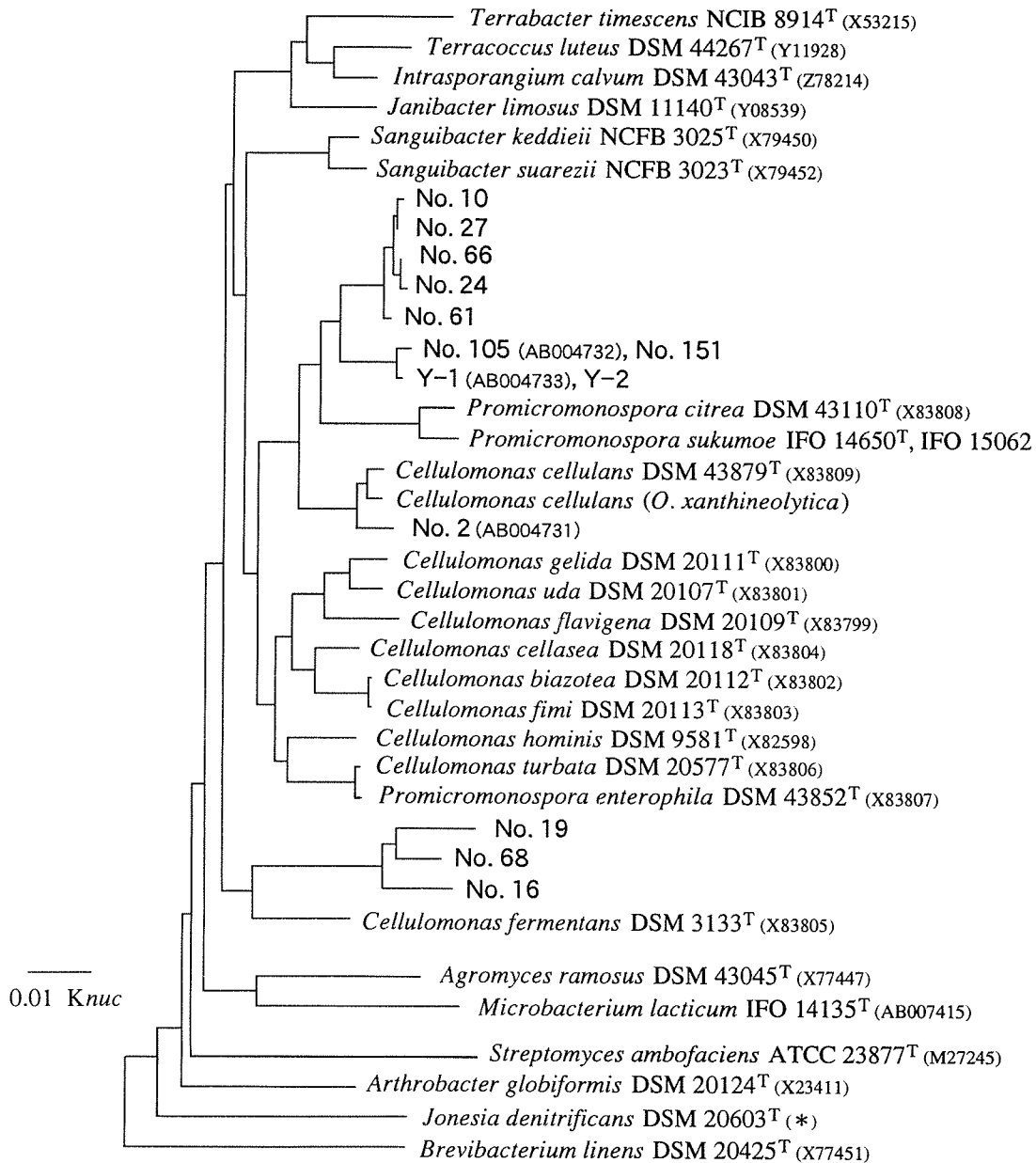


Fig. 3. Unrooted phylogenetic tree displaying the relationships of *Cellulomonas* species to reference organisms. Bar=0.01 K_{nuc} unit

*Sequence data obtained from the Ribosomal Database Project (15).

were MK-10, 11, and 12. These chemotaxonomic features indicate that they belong to the genus *Microbacterium* (30) (Table 2). We therefore compared the almost complete the 16S rDNA sequences of the two strains with the published sequences of the 26 known species in the genus *Microbacterium* (32). As shown in Fig. 4, strains No. 12a and No. 13 appear to be two new species in the genus *Microbacterium*. It is interesting that the closest related species to these two isolates, *Microbacterium halophilum* (32), was also

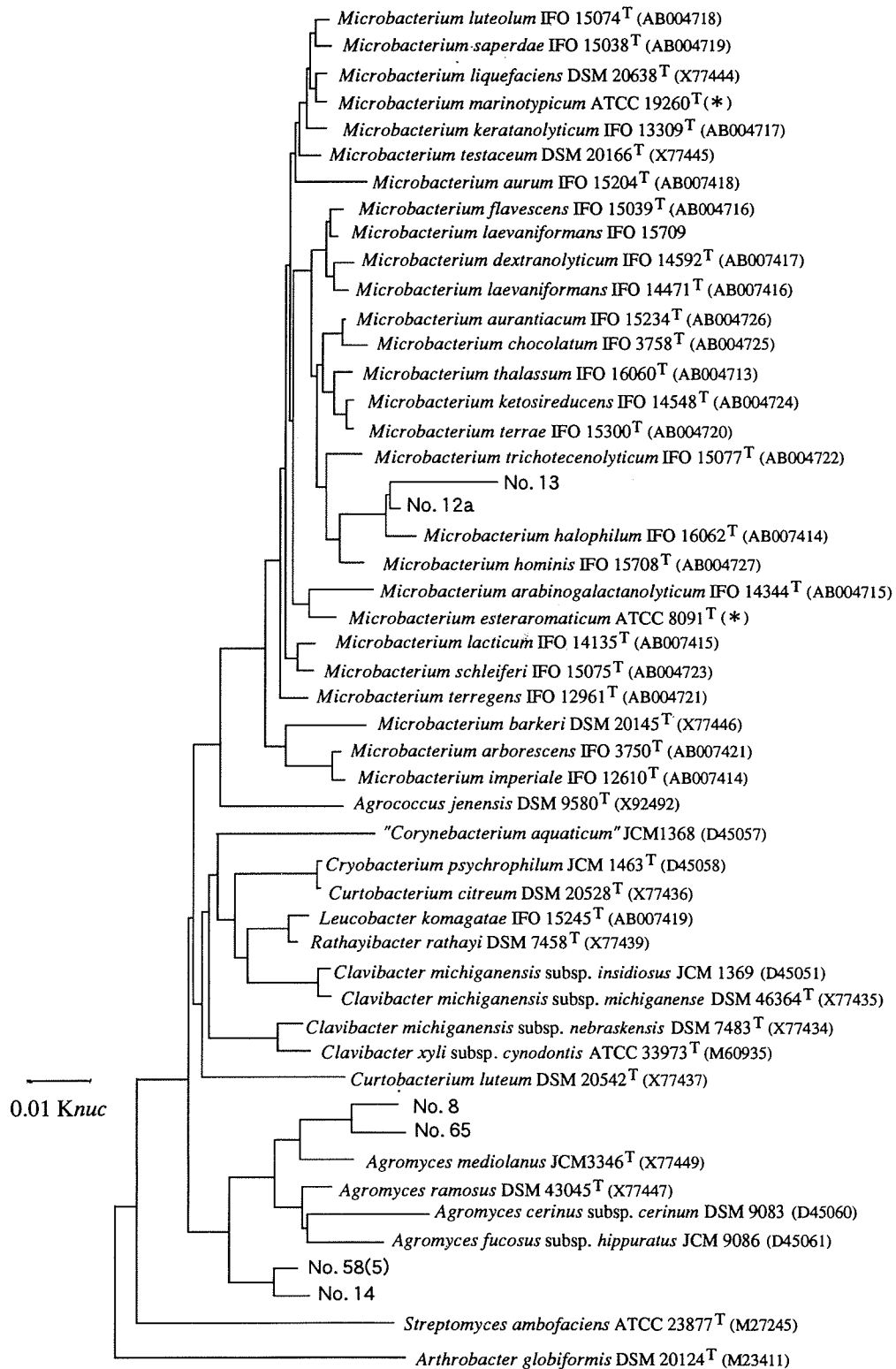


Fig. 4. Unrooted phylogenetic tree displaying the relationships of *Microbacterium* species to reference organisms. Bar=0.01 K_{nuc} unit

*Sequence data obtained from the Ribosomal Database Project (15).

isolated from the mangrove rhizosphere.

Agromyces species: Four strains, No. 8, 14, 58 (5), and 65, had 2,4-diaminobutyric acid as cell wall diamino acid, G+C contents of DNA ranging from 70.0 to 73.3 mol%, and cellular fatty acids composed mainly of *anteiso*-15:0, *iso*-16:0, *anteiso*-17:0, and/or *iso*-15:0. The major menaquinones of these strains were MK-12 with smaller amounts of MK-10 and MK-11. These chemotaxonomic features indicate that they belong to the genus *Agromyces* (28, 35) (Table 2), of which six species have been described previously. As shown in Fig. 4, the four strains appear to be new species in the genus *Agromyces*.

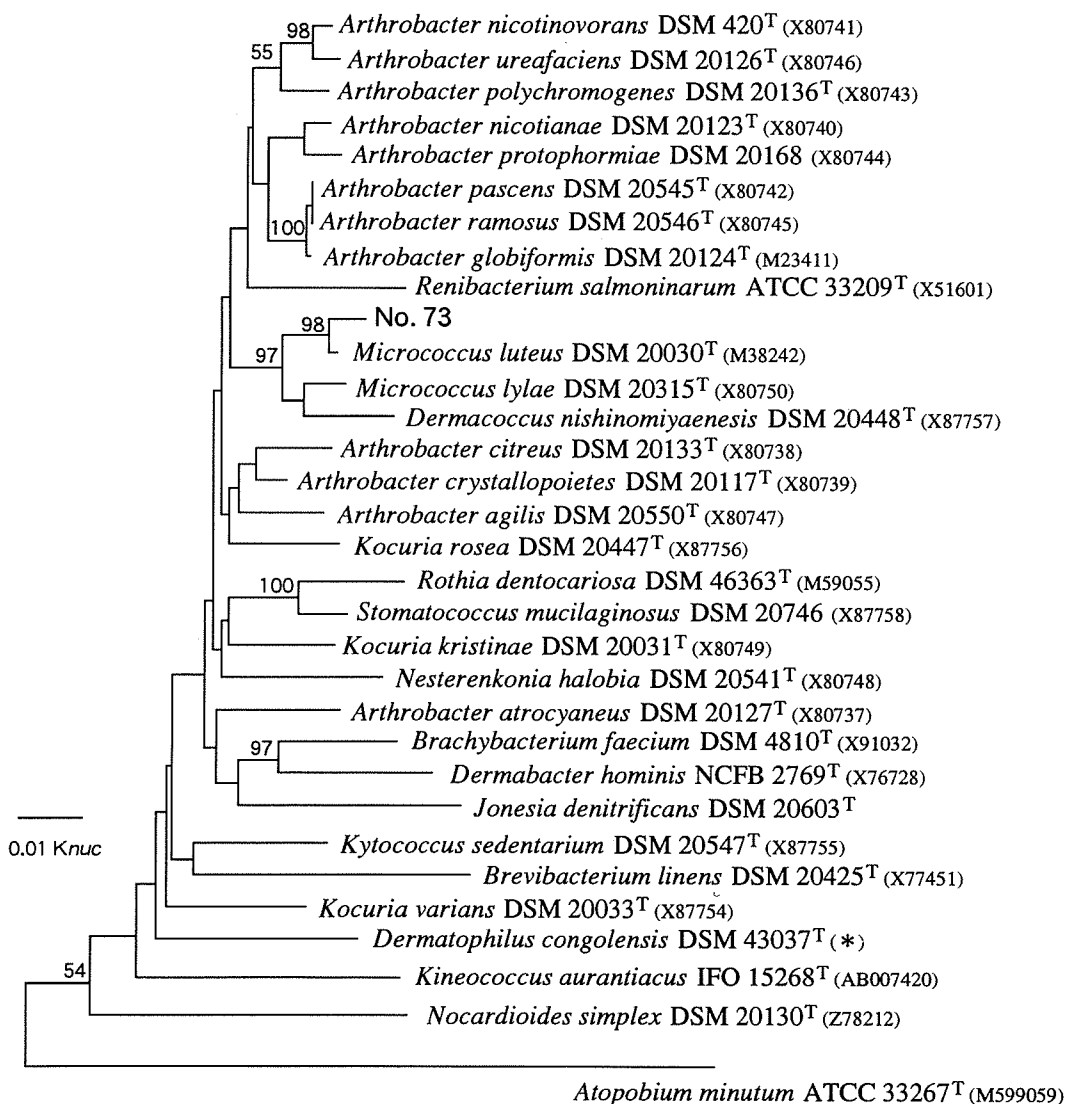


Fig. 5. Unrooted phylogenetic tree displaying the relationships of *Micrococcus* species to reference organisms. The numbers on the tree indicate bootstrap values (percent) derived from 1,000 samples, for the branch point (3). Bar=0.01 K_{nuc} unit

*Sequence data obtained from the Ribosomal Database Project (15).

Micrococcus species: The diamino acid in the cell wall of the strain No. 73 was lysine, the G+C content of the DNA was 72.5 mol%, and cellular fatty acids were composed mainly of 16:0 and *iso*-16:0. The major menaquinone was MK-8 (H₂). These chemotaxonomic features indicate that this strain belongs to the genus *Micrococcus* (Table 2), in which only two species are recognized, *Micrococcus luteus* and *Micrococcus lylae* (14, 23). As shown in Fig. 5, yellow strain No. 73 was closely related to *Micrococcus luteus*.

Taxonomic positions of actinobacteria containing meso-A₂pm

Gordonia species: The diamino acid in the cell wall of strain No. 60 was *meso*-A₂pm, G+C content of the DNA was 65.8 mol%, and the cellular fatty acids were composed mainly of 16:0 and 10-methyl 18:0 (tuberculostreic acid, TBSA). The major menaquinone was MK-9 (H₂). These chemotaxonomic features indicate that this strain belongs to the genus *Gordonia* (Table 3), of which nine species have previously been described (31). As shown in Fig. 6, rose-colored strain No. 60 was included in the genus *Gordonia* and was closely related to *Gordonia rhizosphaera*, which was also isolated from the mangrove rhizosphere. The level of DNA-DNA relatedness between strain No. 60 and *G. rhizosphaera* IFO 16068^T was 75.8–85.3%, and we determined that strain No. 60 should be *G. rhizosphaera*.

Rhodococcus species: The diamino acid in the cell wall of strain No. 74 was *meso*-A₂pm, G+C content of the DNA was 64.5 mol%, and the cellular fatty acids were composed mainly of 16:0, and 10-methyl 18:0 (TBSA). The major menaquinones were MK-8 (H₂) and MK-7 (H₂). These chemotaxonomic features indicate that this strain belongs to the genus *Rhodococcus* (Table 2). As shown in Fig. 6, pale brown strain No. 74 was included in the genus *Rhodococcus* (18).

Mycobacterium species: The diamino acid in the cell wall of strains No. 48 and 58

Table 3. Differential characteristics of actinobacteria containing *meso*-A₂pm

Genus	G+C content (mol%)	Diamino acid	Mycolic acids	Major menaquinone
<i>Trukamurella</i>	67–68	<i>meso</i> -A ₂ pm	+	MK-9
<i>Dietzia</i>	73	<i>meso</i> -A ₂ pm	+	MK-8 (H ₂)
<i>Rhodococcus</i>	63–73	<i>meso</i> -A ₂ pm	+	MK-8 (H ₂)
[No. 74]	64.5	<i>meso</i> -A ₂ pm	+	MK-8 (H ₂)
<i>Corynebacterium</i>	51–67	<i>meso</i> -A ₂ pm	+	MK-9 (H ₂), MK-8 (H ₂)
<i>Gordonia</i>	63–69	<i>meso</i> -A ₂ pm	+	MK-9 (H ₂)
[No. 60]	65.8	<i>meso</i> -A ₂ pm	+	MK-9 (H ₂)
<i>Mycobacterium</i>	61–71	<i>meso</i> -A ₂ pm	+	MK-9 (H ₂)
[No. 48, 58]	68.2–68.5	<i>meso</i> -A ₂ pm	+	MK-9 (H ₂)
<i>Nocardia</i>	64–72	<i>meso</i> -A ₂ pm	+	MK-8 (H ₄ , ω-cycl)

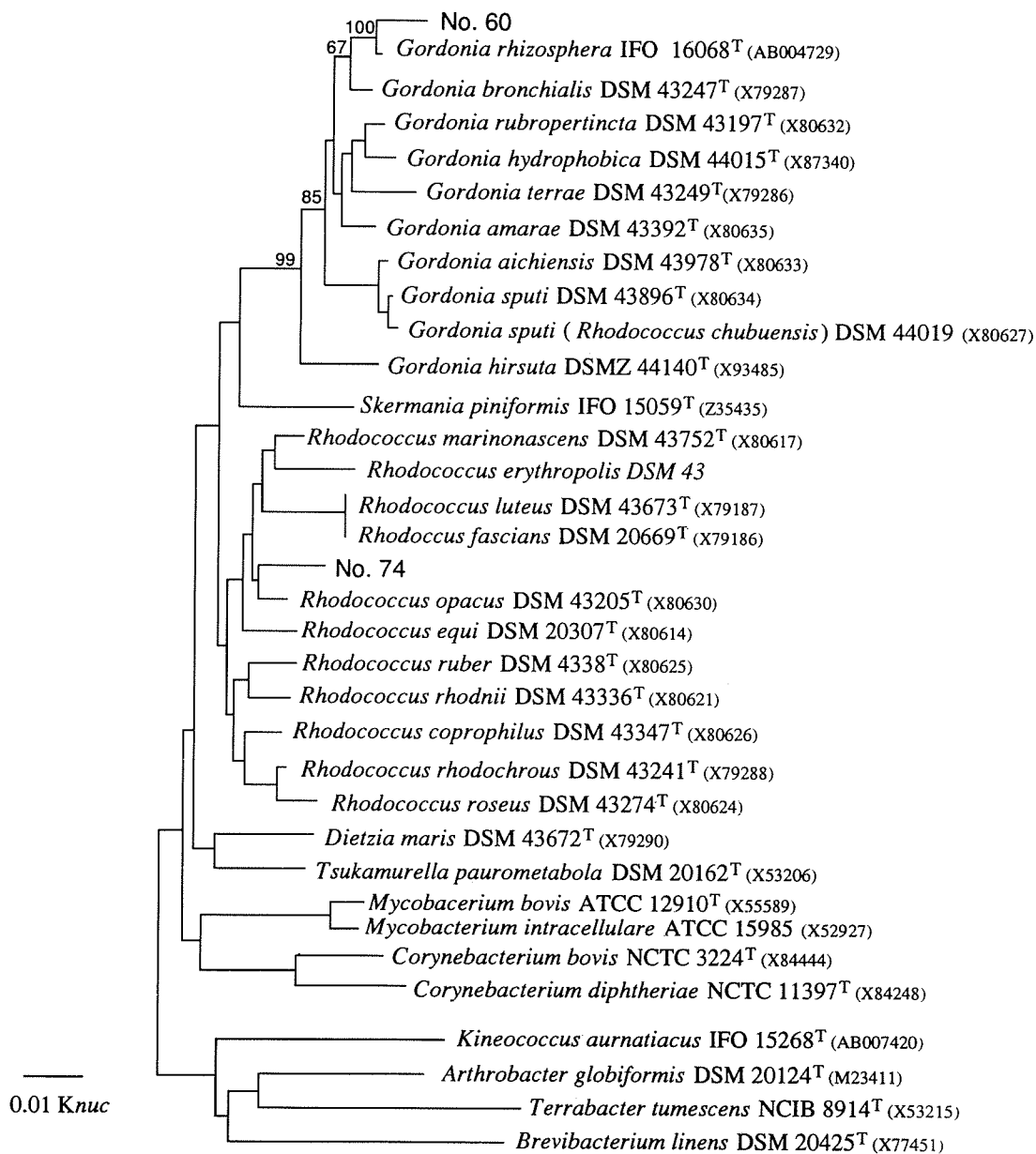


Fig. 6. Unrooted phylogenetic tree displaying the relationships of *Gordonia* species and *Rhodococcus* species to reference organisms. The numbers on the tree indicate bootstrap values (percent) derived from 1,000 samples, for the branch point (3). Bar=0.01 K_{nuc} unit

was *meso*-A₂pm, G+C contents of DNA ranged from 68.2 to 68.5 mol%, and the cellular fatty acids were composed mainly of 16:0, 16:1, 18:1, and 10-methyl 18:0 (TBSA). The major menaquinone of these strains was MK-9 (H₂). These chemotaxonomic features indicate that these strains belong to the genus *Mycobacterium* (Table 2). This genus contains more than 80 reported species, which for convenience have been divided into three groups: slow growers (grossly visible colonies appear only after 7 or more days);

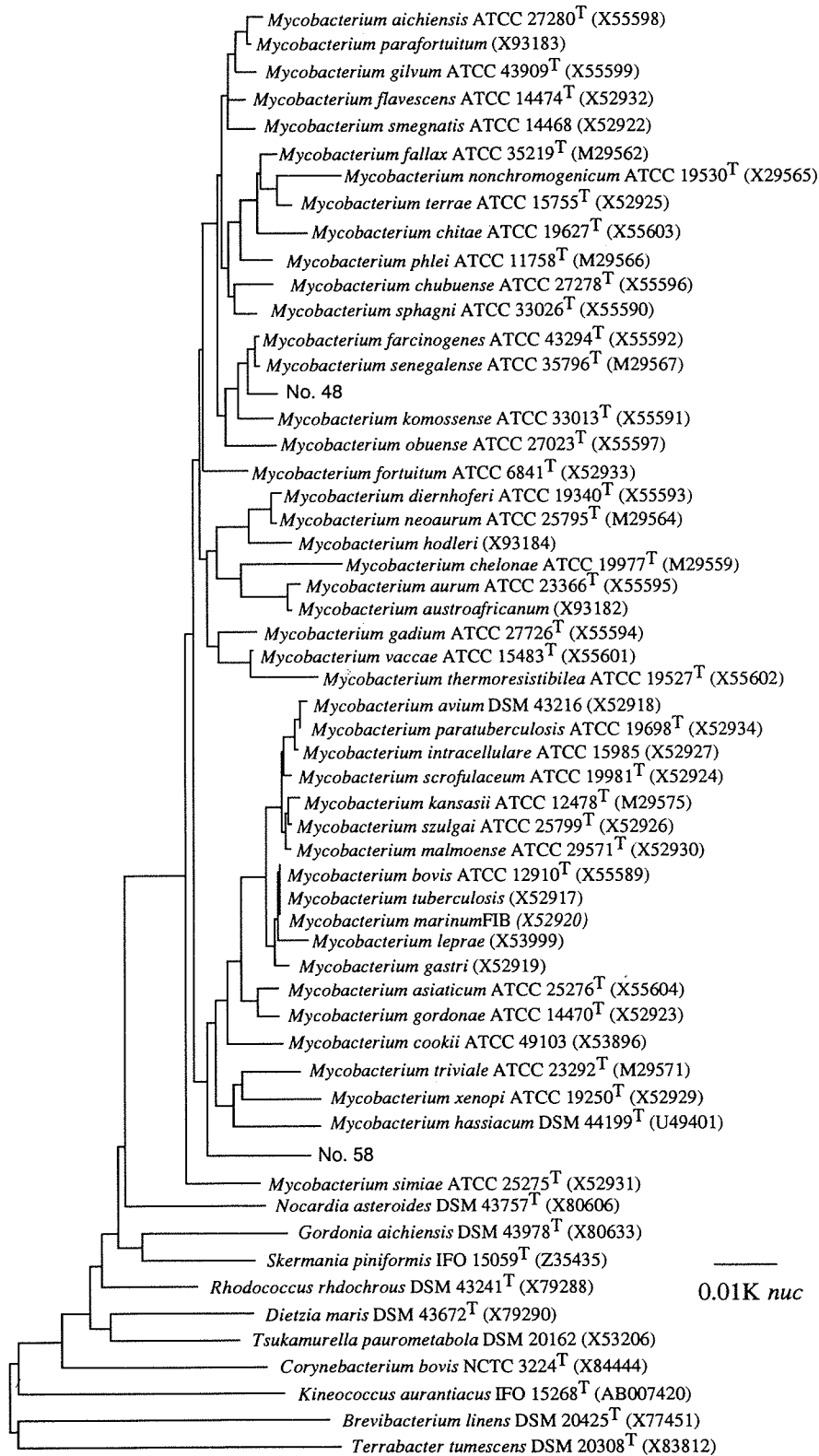


Fig. 7. Unrooted phylogenetic tree displaying the relationships of *Mycobacterium* species to reference organisms. Bar=0.01 K_{nuc} unit

rapid growers (grossly visible colonies in less than 7 days) (17, 22); and organisms which have special growth requirements or have not been cultivated *in vitro* (33). As the two isolated actinobacteria, No. 48 and 58, grew well within 3 days, we compared their 16S rDNA sequences with those of 45 rapid-growing mycobacteria (17, 22). As shown in Fig. 7, strain 48 and strain 58 appear to be new species in the genus *Mycobacterium*.

In this study, we determined the almost complete 16S rRNA gene sequences of 25 strains isolated from the mangrove rhizosphere and compared them with the sequences of representative validated gram-positive microorganisms, to clarify the phylogenetic diversity of actinobacteria in the mangrove rhizosphere. On the basis of phylogenetic and chemotaxonomic characteristics, the taxonomic positions of 24 of the strains were clarified, but that of the remaining one strain which contained LL-A₂pm in the cell wall was not clarified.

Actinobacteria in a diversity of genera, *Agromyces*, *Cellulomonas*, *Gordonia*, *Micrococcus*, *Microbacterium*, *Mycobacterium*, and *Rhodococcus* species, were isolated from the mangrove rhizosphere, and some isolates were considered to be new species in their respective genera. It is especially interesting that *Mycobacteria* species, which had mainly been isolated from clinical environments such as phlegm and sputum, and from soils, and which are considered to be etiologic agents of pulmonary disease, were isolated from the mangrove rhizosphere. This is the first report of the appearance of *Mycobacterium* species in the mangrove rhizosphere. We confirmed that mangrove rhizosphere is a good source for isolating new and diverse actinomycetes, as suggested by Hatano (7).

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Phylogenetic Reassessment of Taxonomic Position of Five *Pseudomonas* strains

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The taxonomic positions of four strains of *Pseudomonas ovalis* isolated from silkworm larvae, IFO 12950, IFO 12951, IFO 12952, and IFO 12953, and one strain of *Pseudomonas caryophylli*, IFO 13694, were re-evaluated phylogenetically. From the results of 16S rRNA sequence analysis and DNA-DNA hybridization, two strains, IFO 12951 and IFO 13694, were reidentified as *Ochrobactrum intermedium*. IFO 12951 is the first *Ochrobactrum* strain isolated from insect. IFO 12950 exhibited a species level of DNA relatedness with IFO 12952 and IFO 12953 and occupies an independent phylogenetic position within the *Brucella-Ochrobactrum* cluster. The close relationship between the genera *Brucella* and *Ochrobactrum* is also discussed.

Keywords: *Ochrobactrum*, *Brucella*, phylogeny, 16S rRNA

Four *Pseudomonas ovalis* strains, I-2106 (= IFO 12950), I-1201 (= IFO 12951), K-101 (= IFO 12952), A-210 (= IFO 12953), were isolated from silkworm larvae by Nakasuji et al. (12). *Pseudomonas caryophylli* IFO 13694, which produces D-threo-aldose dehydrogenase (16), was isolated originally by Fewkes (5). We investigated the chemotaxonomic characteristics of *Pseudomonas* strains in IFO and found that they had ubiquinone-10 (Q-10) as the major quinone. Since typical *Pseudomonas* species are characterized by Q-9 (22), these strains were judged not to belong to the genus *Pseudomonas*. In this study, we determined the taxonomic positions of these strains phylogenetically.

Materials and Methods

Bacterial strains and cultivation. The strains examined were IFO 12950, IFO 12951, IFO 12952, and IFO 12953, which were isolated from silkworm larvae (12) and deposited as *Pseudomonas ovalis* in IFO; IFO 13694, which was deposited as *Pseudomonas caryo-*

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phylli; *Ochrobactrum anthropi* LMG 3331^T (^T=type strain) (= IFO 15819^T), and *Ochrobactrum intermedium* LMG 3301^T (= IFO 15820^T). The organisms were cultivated aerobically at 28°C in the medium containing (per liter) 10.0 g of Polypepton (Wako Pure Chemical Industries, Osaka, Japan), 2.0 g of yeast extract (Difco Laboratories, Detroit, Mich., U.S.A.), 1.0 g of MgSO₄ · 7H₂O, and 15 g of agar, if needed (pH 7.0). Cells were harvested by centrifugation at the stationary phase.

Preparation of DNA. Total genomic DNA was extracted and purified by the methods of Marmur (8) and Saito and Miura (14) with some modifications (10). DNA solutions with an absorbance ratio at 260 nm/280 nm of above 1.8 were used in DNA-DNA hybridization experiments.

PCR amplification, cloning and sequencing of 16S rDNA. The 16S rRNA gene was amplified by PCR (13) by using TaKaRa *Taq* (Takara Shuzo, Kyoto, Japan) and primers 9F and 1541R (11). The 1.5-kb amplified 16S rDNA fragment was purified by agarose gel electrophoresis and a Gene Clean II kit (BIO 101, Vista, Calif., USA). The methods for cloning and sequencing of the purified fragment were previously described in detail (11).

Phylogenetic analysis. The 16S rRNA sequences of the strains examined and the sequences of reference organisms derived from databases (for accession numbers, see below) were aligned against the *Escherichia coli* sequence (1). The CLUSTAL W ver. 1.7 software package (19) was used to generate the evolutionary distances (K_{nuc} values [7]) and the similarity values, and to reconstruct the phylogenetic tree by using the neighbor-joining method (15) and the K_{nuc} values. Positions at which secondary structures varied between strains (66–103, 179–220, 447–487, 841–845, 1004–1036, 1134–1140, 1247–1290, and 1446–1456 [*E. coli* numbering system]) and positions after 1483, for which sequences were not determined in any reference organisms were excluded from the analysis. The total number of nucleotides compared was 1,186 after we had eliminated all sites at which sequences were not determined in any organism. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (4) with 1,000 replicates.

DNA-DNA hybridization. Levels of DNA relatedness were determined by the photobiotin-microplate method of Ezaki et al. (3). Hybridization was performed at 50°C in 2×SSC buffer (0.3 M NaCl and 0.03 M sodium citrate, pH unadjusted) containing 50% (v/v) formamide.

Nucleotide sequence accession number. The 16S rDNA sequence data of IFO 12950 and IFO 13694 were deposited in the DDBJ database and appear in the DDBJ, EMBL, GSDB, and NCBI nucleotide sequence databases under accession number D63836 and D63837, respectively. For the reference, the following sequences were used: *Agrobacterium tumefaciens*, M11223; *Azorhizobium caulinodans*, D11342; *Azospirillum lipoferum*, M59061; *Bartonella quintana*, M11927; *Bradyrhizobium japonicum*, D11345; *Brevundimonas diminuta*, M59064; *Brucella abortus*, X13695; *Brucella canis*, L37584; *Brucella melitensis*, L26166; *Brucella neotomae*, L26167; *Brucella ovis*, L26168; *Brucella suis*, L26169; *Devosia riboflavina*, D49423; *Erythrobacter longus*, M96744; *Legionella pneumophila* subsp. *pneumophila*, M59157; *Magnetospirillum magnetotacticum*, M58171; *Mesorh-*

izobium loti, D14514; *Mycoplana dimorpha*, D12786; *Ochrobactrum anthropi*, D12794; *Paracoccus denitrificans*, X69159; *Phyllobacterium myrsinacearum*, D12789; *Phyllobacterium rubiacearum*, D12790; *Rhizobium galegae*, D11343; *Rhizobium leguminosarum*, D12782; *Sinorhizobium meliloti*, D01265; *Sphingomonas paucimobilis*, D16144.

Results

16S rDNA sequence analysis. In this study, we analyzed two independent clones each of PCR-amplified 16S rDNA from IFO 12950 and IFO 13694. The DNA sequences of clones originating from the same strain were identical. The determined 16S rDNA sequences of both strains ranged from position 29 to position 1524. The initial phylogenetic analysis, including representatives of the different subclasses of the *Proteobacteria*, showed that IFO 12950 and IFO 13694 belonged to the α -2 subclass (data not shown). Their precise positions were investigated by including representative species of the α -2 subclass. The phylogenetic tree reconstructed by the neighbor-joining method and the K_{nuc} values shows that IFO 12950 and IFO 13694 clustered with the genera *Brucella* and *Ochrobactrum* (Fig. 1). This grouping was supported by a high bootstrap confidence value of 98.4%. IFO 13694 was closely related to *O. anthropi* LMG 3331^T, with similarity values of 99.9% (Fig. 1 and Table 1), whereas, IFO 12950 occupied an independent position in the *Brucella*-*Ochrobactrum* cluster. The close relationship between the genera *Brucella* and *Ochrobactrum* was also recognized.

DNA-DNA hybridization. The results of DNA-DNA hybridization experiments divided the strains into three groups; the first group consisted of IFO 12950, IFO 12952 and IFO 12953; the second, IFO 12951, IFO 13694, and *O. intermedium* LMG 3301^T; and the third, *O. anthropi* LMG 3331^T (Table 2). The levels of DNA relatedness were comparatively high among IFO 12951, IFO 13694, *O. anthropi* LMG 3331^T, and *O. intermedium* LMG 3301^T. IFO 13694 exhibited the species level of DNA relatedness (i. e., > 70% relatedness) with *O. intermedium* LMG 3301^T and IFO 12951. However, IFO 12950 showed the low hybridization levels (below 37% relatedness) with IFO 13694, *O. anthropi* LMG 3331^T, and *O. intermedium* LMG 3301^T.

Discussion

The genus *Ochrobactrum*, of which *O. anthropi* is the type and sole species, was established for the strains of CDC group Vd by Holmes et al. (6). It has been suggested that *O. anthropi* is heterogeneous and separated into two groups of strains (referred to as hybridization groups 1 and 2) by DNA-DNA hybridization studies (6). The classical morphological and physiological tests (6, 20) could not distinguish between the hybridization groups 1 and 2. Recently, Velasco et al. (20) found that hybridization group 2 could be discriminated by PCR with specific oligonucleotide primers and proposed *O. intermedium* for this group. Phylogenetic analysis based on 16S rRNA sequences showed that IFO 13694 was closely related to the genus *Ochrobactrum* (Fig. 1). The results of DNA-

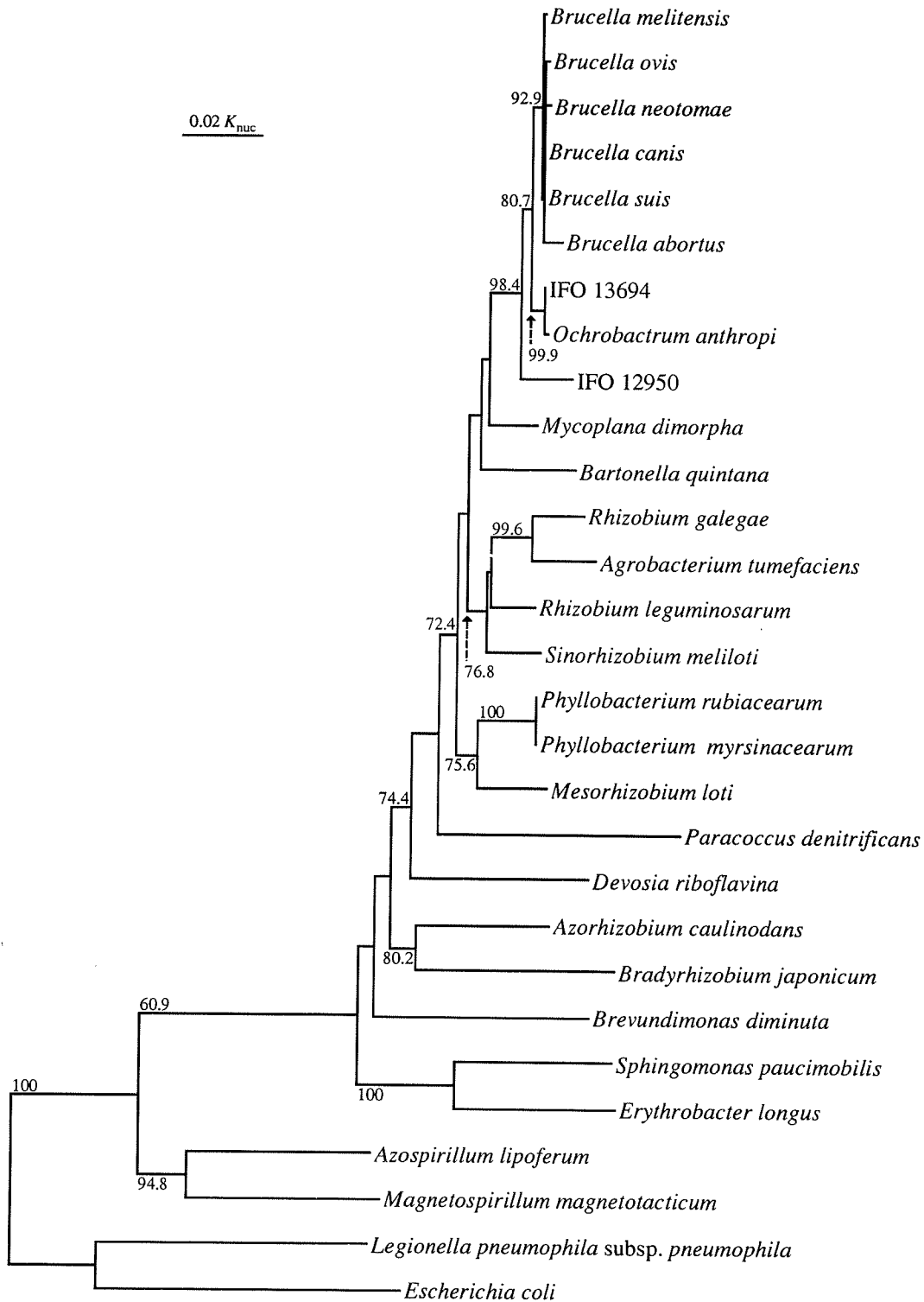


Fig. 1. Phylogenetic tree derived from 16S rRNA sequences for IFO 12950, IFO 13694, and related organisms. *Escherichia coli* and *Legionella pneumophila* subsp. *pneumophila* were used as the root organisms. Scale bar = 0.02 K_{nuc} in nucleotide sequences. The lengths of the vertical lines are not significant. The numbers on the branches refer to the confidence limit (expressed as a percentage) estimated by bootstrap analysis with 1000 replicates. Confidence limits less than 60% are not shown.

Table 1. Similarity matrix for 16S rRNA sequences of IFO 12950, IFO 13694, and related organisms.

Organism	% Similarity														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. IFO 12950															
2. IFO 13694	98.2														
3. <i>Ochrobactrum anthropi</i>	98.1	99.9													
4. <i>Brucella melitensis</i>	98.2	99.2	99.2												
5. <i>Brucella ovis</i>	98.1	99.2	99.2	99.8											
6. <i>Brucella neotomae</i>	98.0	99.2	99.1	99.8	99.8										
7. <i>Brucella canis</i>	98.2	99.3	99.2	99.9	99.9	99.8									
8. <i>Brucella suis</i>	98.2	99.3	99.2	99.9	99.9	99.8	100								
9. <i>Brucella abortus</i>	97.6	98.8	98.7	99.4	99.4	99.3	99.5	99.5							
10. <i>Agrobacterium tumefaciens</i>	95.1	94.8	94.7	94.9	94.8	94.7	94.9	94.9	94.6						
11. <i>Mesorhizobium loti</i>	95.0	95.4	95.3	95.5	95.4	95.5	95.5	95.5	95.0	94.4					
12. <i>Devosia riboflavina</i>	92.4	92.4	92.3	92.4	92.3	92.2	92.3	92.3	92.1	92.2	93.0				
13. <i>Bradyrhizobium japonicum</i>	90.4	91.7	91.7	91.3	91.3	91.2	91.4	91.4	91.2	89.7	90.0	89.6			
14. <i>Erythrobacter longus</i>	89.5	89.5	89.5	89.7	89.5	89.5	89.6	89.6	89.4	89.6	89.1	88.6	88.2		
15. <i>Magnetospirillum magnetotacticum</i>	89.6	90.0	89.9	89.9	89.8	89.7	89.9	89.9	89.5	88.7	89.1	89.5	88.4	87.6	
16. <i>Escherichia coli</i>	83.9	84.2	84.1	84.2	84.2	84.0	84.2	84.2	83.7	83.1	83.6	83.2	81.3	82.4	83.6

Table 2. Levels of DNA relatedness.

Strain	% Reassociation with DNA from:						
	IFO 12950	IFO 12951	IFO 12952	IFO 12953	IFO 13694	LMG 3301	LMG 3331
IFO 12950	100	8	70	65	29	32	37
IFO 12951	16	100	18	15	101	97	78
IFO 12952	92	13	100	82	29	34	35
IFO 12953	92	14	85	100	31	30	32
IFO 13694	8	86	8	11	100	96	65
<i>Ochrobactrum intermedium</i> LMG 3301 ^T					93	100	62
<i>Ochrobactrum anthroopi</i> LMG 3331 ^T					62	67	100

DNA hybridization (Table 2) and API 20 NE tests (BioMérieux S.A., Marcy-l'Étoile, France) (data not shown) indicated that IFO 12951 and IFO 13694 were strains of *O. intermedium*. The strains hitherto included in the genus *Ochrobactrum* were isolated predominantly from human clinical specimens (6, 17, 18), whereas IFO 12951 was isolated from silkworm larvae (12). This is the first isolation of a *Ochrobactrum* strain from insect.

IFO 12950 occupied an independent position within the *Brucella*-*Ochrobactrum* cluster, equidistant from both *Brucella* and *Ochrobactrum* (Fig. 1, Table 1). IFO 12950 exhibited the species level of DNA relatedness with IFO 12952 and IFO 12953, but not with other species examined (Table 2). Therefore, the taxonomic status of IFO 12950, IFO 12952, and IFO 12953 remains unknown and will be discussed elsewhere in future. These three strains are distributed by IFO under the tentative identity of *Ochrobactrum* sp.

As shown in Fig. 1 and Table 1, the genus *Ochrobactrum* is closely related to the genus *Brucella*. The 16S rRNA sequence similarities among the genera *Brucella* and *Ochrobactrum* were higher than 98.7 % (after exclusion of hypervariable regions, see Materials and Methods). Their close relationship has previously been indicated by DNA: rRNA hybridization studies (2) and 16S rRNA sequence analysis (21). From the phylogenetic point of view, the genera *Brucella* and *Ochrobactrum* may be combined. However, the genus *Brucella* is a well-known organism that needs biosafety level 3 precaution and causes infectious disease of the genitourinary tract in domestic and other animals and of the reticuloendothelial system in humans, as known brucellosis, febrile illness (9). Therefore, the genera *Brucella* and *Ochrobactrum* should not be combined in one genus until the taxonomic merit of so doing is clear, because it may cause practical confusion.

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Preservation of Yeast Cultures by L-drying: Viability after 15 years of Storage at 5°C

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Summary

All yeasts kept at the Institute for Fermentation, Osaka (IFO) are processed by L-drying for long-term preservation. In this study, 100 strains for which cell survival had been counted after preservation for 5 years at 5°C, were examined after preservation for 14-18 years at 5°C to determine whether the rate of decrease in cell numbers observed in the first 5 years was maintained thereafter.

The numbers of surviving cells observed after preservation for 14-18 years at 5°C were compared with those of three tests: an accelerated test of 30 days at 37°C immediately after preparation, an accelerated test of 60 days at 37°C, and a test after 5 years of storage at 5°C. The coefficient of correlation for each test was between them were respectively 0.922, 0.880, and 0.951. The low correlation coefficient for the accelerated storage test of 60 days at 37°C showed that its conditions are excessively severe compared with those of the accelerated test of 30 days at 37°C. The high correlation with the value after 5 years of storage at 5°C indicated that the death rate during 14-18 years of preservation was lower than that for the 5 years immediately after drying. The number of years required for surviving cell numbers to reach the value of 10⁴ CFU/amp., which is estimated to be the safest number for the renaturation of surviving cells, was predicted based on the assumption that surviving cells would decrease at the same rate as in the first 5 years at 5°C. Twenty-six of the 100 stocks were calculated to reach 10⁴ CFU/amp. in less than 15 years. But the number of stocks which actually realized a value below that was less than expected, namely, 4 strains, *Ambrosiozyma cicatricosa* IFO 1846, *Arthroascus schoenii* IFO 1579, *Leucosporidium antarcticum* IFO 1917, and *Sporobolomyces holsaticus* IFO 1109. The results revealed that these samples preserved at 5°C underwent only a minor decrease in the number of surviving cells during the periods of 5 and 14-18 years. It was suggested that stable numbers of surviving cells could be maintained provided no changes occurred in the preservation conditions.

Keywords: long-term storage of yeast, liquid-drying, accelerated storage test

General methods of long-term storage of microorganisms are freeze-drying, liquid-drying (L-drying), and freezing at -80°C or -196°C, all of which enjoy satisfactory

records of preserving effects.

L-drying involves vacuum-drying of samples from the liquid state without freezing. It differs from freeze-drying in achieving drying through evaporation, while freeze-drying is drying through sublimation. Cell deaths caused by drying, however, are supposed to be affected not by dehydration, but mainly by the degree in which residual water is dehydrated (2).

Samples of surviving bacterial cells after 5 years and 20 years of preservation at 5°C have been examined. It is reported that a preservation for 5 to 20 years is possible with only a slight decrease in surviving cell numbers (8).

The method of L-drying has been successfully applied to the long-term preservation of yeasts (1, 3, 5 and 7). All the yeasts maintained in the IFO yeast collection were L-dried and have been preserved in a cold room at 5°C. In this study, viabilities of the dried cultures of strains which had been preserved for 14–18 years at 5°C were examined and compared with those predicted by the accelerated storage test reported in the previous paper (5).

Materials and Methods

One hundred strains belonging to 54 species in 23 genera, shown in Table 1, were L-dried by the standard method described previously (1) and stored in a cold room at 5°C. Two of 18 ampules of the dried cultures which had been preserved for 14–18 years were rehydrated. Viability was determined as reported previously (1).

Viable counts per ampule were determined and survival values were expressed as percentages of surviving cells relative to the viable counts after preservation for 14–18 years.

Results and Discussion

The survival rates of 100 dried yeast cultures after preservation for 14–18 years at 5°C are shown in Table 1. The table also shows the survival rates immediately after drying, in the accelerated test, and after preservation for 14–18 years.

Variation in the number of surviving cells made it possible to classify the specimens into three groups. Group A consisted of 76 strains which had good resistance to drying and excellent preservability: the viability immediately after drying and after storage for 15 years of at 5°C, and the viable counts after 5 years and 15 years at 5°C were practically unchanged. Group B consisted of 20 strains which had a weak resistance to drying but good preservability: the viability was less than 10% in the above periods, and the viable counts after 5 years and 15 years at 5°C were practically unchanged. Group C consisted of 4 strains which needed to be rejuvenated: the viable count after five years at 5°C or 15 years at 5°C being lower than 10⁴ CFU/amp., the safe value for renaturation.

The viable counts after storage for 15 years at 5°C were compared with results of other three tests, i.e., an accelerated test of 30 days at 37°C immediately after preparation, an accelerated test of 60 days at 37°C, and a test after 5 years of storage at 5°C. The

Table 1. Survival rates of L-dried yeast cultures after preservation

Species	IFO No.	Strage years	Log CFU per ampule (survival value %)					Group	
			before drying	after drying	30 days	60 days	preservation at 5°C		
<i>Ambrosiozyma cicatricosa</i>	1846	18	6.99	5.35 (2.3)	3.89 (0.08)	3.69 (0.05)	4.29 (0.2)	3.69 (0.05)	C
<i>Ambrosiozyma monospora</i>	1965	16	7.77	6.71 (8.8)	5.07 (0.2)	4.07 (0.02)	5.07 (0.2)	4.96 (0.15)	B
<i>Arthroascus javanensis</i>	1848 ^T	18	7.52	6.76 (17.3)	6.36 (6.9)	6.09 (3.7)	6.40 (7.6)	6.05 (3.4)	A
<i>Arthroascus schoenii</i>	1579	14	7.08	5.16 (1.2)	3.68 (0.04)	2.92 (0.007)	2.08 (0.001)	2.08 (0.001)	C
<i>Arxiozyma telluris</i>	1897	17	7.30	5.97 (4.7)	5.38 (1.2)	4.00 (0.05)	5.66 (2.3)	5.00 (0.5)	A
<i>Brettanomyces bruxellensis</i>	0628	16	7.90	7.14 (18.4)	NT	5.67 (0.6)	5.49 (0.4)	5.99 (1.2)	B
<i>Brettanomyces custersianus</i>	1585 ^T	16	7.71	6.32 (4.1)	NT	5.00 (0.2)	4.71 (0.1)	4.88 (0.15)	B
<i>Candida albicans</i>	1974	17	8.11	7.61 (31.6)	7.49 (23.8)	7.31 (15.8)	7.51 (25.1)	7.59 (30.0)	A
<i>Candida boidinii</i>	1967	17	7.97	6.99 (10.5)	6.55 (3.8)	5.67 (0.5)	6.76 (6.1)	6.88 (8.2)	A
<i>Candida boidinii</i>	10035	16	7.54	7.03 (31.3)	6.78 (17.6)	6.50 (9.2)	6.73 (15.5)	6.69 (14.3)	A
<i>Candida buinensis</i>	1642 ^T	14	8.33	7.43 (13.6)	NT	6.08 (0.6)	6.34 (1.1)	6.15 (0.7)	B
<i>Candida conglobata</i>	0959 ^T	17	7.86	7.30 (27.4)	7.37 (32.7)	7.14 (19.2)	7.10 (17.5)	7.08 (16.6)	A
<i>Candida diversa</i>	1091 ^T	14	8.51	7.75 (18.6)	NT	6.32 (0.7)	7.14 (4.8)	5.25 (0.6)	B
<i>Candida etchellsii</i>	1942	16	8.08	7.80 (52.8)	7.20 (13.4)	6.98 (8.0)	6.78 (5.1)	6.91 (6.8)	A
<i>Candida guilliermondii</i>	1913	17	8.38	8.21 (68.2)	8.23 (71.8)	8.06 (48.1)	8.22 (69.0)	8.21 (67.2)	A
<i>Candida kefir</i>	0432	15	7.66	6.77 (12.0)	NT	6.20 (3.3)	6.23 (3.5)	5.91 (1.7)	A
<i>Candida mesenterica</i>	0969	17	7.18	6.50 (21.3)	NT	4.48 (0.2)	5.45 (1.9)	5.51 (2.1)	A
<i>Candida mesenterica</i>	1211 ^T	14	8.08	6.72 (4.8)	NT	6.73 (4.9)	6.52 (3.0)	6.62 (3.7)	A
<i>Candida pintoopesii</i>	0729 ^T	17	7.07	5.96 (7.8)	3.67 (0.04)	3.67 (0.04)	4.67 (0.4)	4.40 (0.2)	B
<i>Candida pinus</i>	1327 ^T	14	7.83	6.95 (13.1)	NT	5.68 (0.7)	6.68 (7.3)	6.74 (8.2)	A
<i>Candida santamariae</i>	1982 ^T	17	7.61	6.83 (16.6)	6.71 (12.6)	6.58 (9.3)	6.52 (8.0)	6.54 (8.5)	A
<i>Candida sorboxyloxa</i>	1578 ^T	17	7.93	7.65 (52.1)	7.52 (38.8)	7.57 (42.9)	7.56 (42.6)	7.61 (47.7)	A
<i>Candida versatilis</i>	1941	16	7.90	7.60 (50.0)	7.30 (25.0)	7.10 (15.9)	7.31 (25.4)	7.13 (17.3)	A
<i>Candida vinaria</i>	1092 ^T	17	8.19	7.72 (33.8)	NT	6.73 (3.5)	7.38 (15.7)	7.09 (7.9)	A
<i>Cryptococcus albidus</i> var. <i>aerius</i>	1322	15	6.88	4.95 (1.2)	NT	4.78 (0.8)	4.78 (0.8)	4.23 (0.2)	A
<i>Cryptococcus ater</i>	1862 ^T	16	7.08	6.70 (41.6)	6.55 (29.5)	6.60 (32.6)	6.43 (22.1)	6.70 (41.4)	A

<i>Cryptococcus curvatus</i>	0732	14	7.71	6.71 (10.1)	NT	5.71 (1.0)	5.91 (1.6)	6.01 (2.0)	A
<i>Cryptococcus curvatus</i>	1159 ^T	14	7.38	6.04 (4.6)	NT	5.14 (0.6)	4.68 (0.2)	5.09 (0.5)	A
<i>Cryptococcus dimenae</i>	1863 ^T	16	6.86	5.11 (1.8)	4.64 (0.6)	4.34 (0.3)	4.14 (0.3)	4.68 (0.7)	A
<i>Cryptococcus humicolus</i>	0760 ^T	16	7.85	7.14 (20.2)	6.63 (6.0)	6.28 (2.7)	6.36 (3.3)	6.11 (1.8)	B
<i>Cryptococcus marinus</i>	1979 ^T	17	8.04	7.48 (27.7)	7.04 (10.2)	7.23 (15.7)	7.14 (13.2)	6.73 (4.9)	A
<i>Cystoflobasidium inferno-miniatum</i>	1933 ^T	17	7.89	7.38 (31.1)	7.35 (29.3)	7.17 (19.7)	7.14 (17.9)	7.25 (23.0)	A
<i>Debaryomyces castellii</i>	1359 ^T	16	7.38	6.11 (5.4)	NT	5.70 (2.1)	5.88 (3.2)	5.66 (1.9)	A
<i>Debaryomyces coudertii</i>	1817 ^T	18	8.05	7.71 (46.3)	6.44 (2.5)	4.04 (0.01)	7.04 (10.3)	7.13 (12.2)	A
<i>Debaryomyces hansenii</i>	1751	18	8.34	8.14 (65.7)	7.21 (7.4)	6.66 (2.1)	7.86 (32.6)	7.54 (15.9)	A
<i>Debaryomyces hansenii</i>	1752	18	8.25	7.97 (52.2)	7.18 (8.5)	7.14 (7.8)	7.48 (16.8)	7.73 (30.2)	A
<i>Debaryomyces polymorphus</i>	1363	16	7.11	6.17 (11.5)	NT	5.65 (3.5)	5.74 (4.2)	5.88 (5.9)	A
<i>Dekkera anomala</i>	1591	16	7.73	7.00 (19.4)	NT	6.36 (4.3)	6.55 (6.7)	6.44 (5.1)	A
<i>Endomycopsisella vini</i>	1748 ^T	16	7.46	7.14 (47.6)	7.05 (39.6)	4.11 (0.3)	7.15 (48.1)	7.13 (47.1)	A
<i>Endomycopsisella vini</i>	1750	14	6.65	5.78 (13.3)	NT	7.00 (35.3)	5.03 (2.4)	5.19 (3.4)	A
<i>Hanseniopsis guilliermondii</i>	1411 ^T	17	7.59	7.00 (26.4)	6.81 (16.4)	6.58 (9.6)	6.82 (16.9)	6.76 (14.9)	A
<i>Hasegawae japonica</i>	1609 ^T	14	6.70	5.90 (16.0)	NT	5.40 (5.0)	5.50 (6.4)	5.11 (2.6)	A
<i>Hasegawae japonica</i>	1713	17	6.36	5.04 (4.9)	4.25 (0.8)	4.25 (0.8)	4.63 (1.9)	4.21 (0.7)	A
<i>Hormoascus philentomus</i>	1847 ^T	18	7.49	5.57 (1.2)	4.49 (0.1)	4.17 (0.05)	4.38 (0.08)	4.75 (0.2)	A
<i>Issatchenkia occidentalis</i>	1904 ^T	17	8.01	7.69 (47.3)	7.47 (28.7)	7.30 (19.1)	7.40 (24.2)	7.30 (19.2)	A
<i>Issatchenkia scutulata</i> var. <i>exigua</i>	1896 ^T	17	7.71	6.94 (16.9)	6.73 (10.4)	6.60 (7.7)	6.54 (6.9)	6.20 (3.1)	A
<i>Issatchenkia scutulata</i> var. <i>exigua</i>	10051	14	7.69	6.68 (9.8)	6.37 (4.8)	5.53 (0.7)	5.28 (0.4)	5.89 (1.6)	A
<i>Kloeckera japonica</i>	0151	14	7.81	6.84 (10.6)	NT	5.59 (0.6)	6.33 (3.3)	5.82 (1.0)	B
<i>Kluyveromyces lactis</i>	1902	17	7.74	7.38 (42.7)	7.20 (29.1)	7.28 (34.3)	7.52 (59.2)	7.48 (55.0)	A
<i>Kluyveromyces phaffii</i>	1883	16	7.83	6.40 (3.8)	5.83 (1.0)	4.80 (0.1)	4.82 (0.1)	4.83 (0.1)	B
<i>Kluyveromyces phaffii</i>	1884	16	7.80	6.87 (12.0)	6.32 (3.4)	5.84 (1.1)	6.41 (1.2)	5.81 (1.0)	A
<i>Kluyveromyces polysporus</i>	0996	15	8.08	6.20 (1.3)	NT	5.08 (0.1)	4.99 (0.08)	4.64 (0.04)	B
<i>Kodamaea ohmeri</i>	0202	17	7.73	6.88 (14.2)	6.78 (11.2)	6.68 (9.0)	6.85 (13.1)	6.26 (3.4)	A
<i>Kondoa malvinella</i>	1936	17	7.92	7.54 (41.5)	7.20 (19.0)	7.00 (12.0)	7.47 (35.5)	7.52 (39.8)	A
<i>Laucosporidium antarcticum</i>	1917 ^T	17	6.82	4.30 (0.3)	3.12 (0.02)	1.00 (0.0001)	2.30 (0.003)	1.88 (0.001)	C

<i>Laucosporidium scottii</i>	1923 ^T	17	7.58	6.80 (16.9)	6.71 (13.4)	6.59 (10.2)	6.65 (11.7)	6.68 (12.5)	A
<i>Lipomyces starkeyi</i>	1289 ^T	16	7.28	5.78 (3.2)	NT	4.98 (0.5)	3.98 (0.05)	4.01 (0.05)	B
<i>Mrakia frigida</i>	1920 ^T	17	7.20	5.08 (0.8)	4.67 (0.3)	4.49 (0.2)	4.49 (0.2)	4.86 (0.5)	A
<i>Mrakia gelida</i>	1921 ^T	17	7.05	4.34 (0.2)	4.05 (0.1)	3.83 (0.09)	4.53 (0.3)	4.68 (0.4)	A
<i>Mrakia stokesii</i>	1926	17	7.77	5.80 (1.1)	5.67 (0.8)	5.36 (0.4)	5.36 (0.4)	5.50 (0.5)	A
<i>Myxozyma melibiosii</i>	1871 ^T	16	7.93	7.60 (47.0)	7.46 (34.1)	7.55 (41.9)	7.54 (41.5)	7.68 (56.4)	A
<i>Ogataea pini</i>	1342	14	7.66	6.14 (3.1)	3.56 (0.008)	3.28 (0.004)	4.14 (0.03)	4.29 (0.04)	B
<i>Ogataea pini</i>	1793	18	7.98	7.70 (52.9)	7.43 (28.1)	7.28 (20.2)	7.49 (32.1)	7.53 (35.1)	A
<i>Pichia angophorae</i>	10016 ^T	16	7.42	5.90 (3.0)	5.60 (1.5)	4.90 (0.3)	5.57 (1.4)	5.81 (2.4)	A
<i>Pichia chamberlainii</i>	1029	14	7.30	6.59 (19.1)	6.08 (6.1)	5.14 (0.7)	5.27 (0.9)	5.59 (1.9)	B
<i>Pichia ciferrii</i>	0793 ^T	17	7.58	6.99 (25.3)	6.75 (14.9)	6.69 (12.7)	6.88 (19.7)	6.80 (16.7)	A
<i>Pichia ciferrii</i>	0905	18	6.98	6.04 (11.6)	NT	4.88 (0.8)	5.60 (4.2)	5.35 (2.4)	A
<i>Pichia fluxuum</i>	0773 ^T	14	7.76	6.91 (14.2)	6.38 (4.2)	5.34 (0.4)	6.38 (4.2)	6.36 (4.0)	A
<i>Pichia fluxuum</i>	1784	18	7.96	7.59 (42.2)	7.43 (29.5)	7.30 (21.5)	7.25 (19.5)	7.44 (30.3)	A
<i>Pichia membranaefaciens</i>	0460	14	7.72	7.00 (19.7)	6.64 (8.8)	5.49 (0.6)	5.95 (1.7)	5.16 (0.5)	B
<i>Pichia subpelliculosa</i>	0808 ^T	14	7.89	7.14 (18.0)	6.74 (7.2)	5.59 (0.5)	6.57 (4.8)	7.00 (12.9)	A
<i>Rhodosporeidium sphaerocarpum</i>	1937 ^T	17	7.73	7.48 (55.9)	7.47 (55.6)	7.31 (37.8)	7.29 (36.4)	7.36 (42.6)	A
<i>Rhodosporeidium toruloideis</i>	0559	16	8.02	7.74 (52.7)	5.72 (0.5)	5.32 (0.2)	5.81 (0.6)	5.57 (0.4)	B
<i>Rhodosporeidium toruloideis</i>	10032	17	7.97	7.11 (14.2)	6.99 (10.4)	6.82 (7.0)	7.04 (18.7)	6.94 (9.2)	A
<i>Rhodotorula acuta</i>	1912 ^T	17	7.96	7.89 (86.1)	7.86 (80.5)	7.80 (69.1)	7.91 (89.5)	7.95 (98.7)	A
<i>Rhodotorula diffluens</i>	1522	16	7.40	6.75 (22.4)	NT	5.00 (0.4)	6.25 (7.3)	5.96 (3.6)	A
<i>Rhodotorula ingenuosa</i>	10002 ^T	17	7.46	6.11 (4.6)	5.31 (0.7)	5.30 (0.7)	5.04 (0.4)	4.86 (0.3)	B
<i>Saccharomyces cerevisiae</i>	1950	17	7.36	6.62 (18.1)	6.58 (16.5)	6.73 (23.6)	6.48 (12.8)	6.59 (17.0)	A
<i>Saccharomyces servazzii</i>	1838 ^T	18	7.80	7.66 (72.0)	7.55 (55.5)	7.40 (39.5)	7.28 (29.9)	7.51 (51.5)	A
<i>Saccharomycopsis capsularis</i>	0672	16	6.40	5.32 (8.9)	4.16 (0.6)	4.23 (0.8)	4.84 (2.9)	4.95 (3.6)	A
<i>Sakaguchia dacryoides</i>	1930 ^T	17	7.80	7.57 (57.9)	7.49 (49.3)	7.41 (41.0)	7.54 (54.6)	7.53 (53.9)	A
<i>Schizosaccharomyces octosporus</i>	0353	14	7.62	6.63 (10.2)	NT	5.52 (0.8)	5.32 (0.5)	5.29 (0.5)	B
<i>Schwannomyces occidentalis</i>	1839	18	7.84	7.20 (22.9)	6.96 (13.1)	6.78 (8.8)	6.91 (11.6)	7.07 (16.8)	A
<i>Schwannomyces occidentalis</i>	1841	18	8.25	6.49 (1.6)	5.84 (0.4)	5.23 (0.1)	6.00 (0.6)	5.55 (0.2)	A

<i>Sporidiobolus salmonicolor</i>	1845	18	7.31	6.40 (12.0)	6.12 (6.4)	5.75 (2.8)	5.69 (2.4)	5.36 (1.1)	B
<i>Sporobolomyces holsaticus</i>	1034 ^T	14	6.78	4.25 (0.3)	NT	4.25 (0.3)	4.38 (0.4)	4.60 (0.7)	A
<i>Sporobolomyces holsaticus</i>	1109	14	5.48	4.17 (5.2)	NT	4.00 (3.4)	3.71 (1.7)	3.18 (0.4)	C
<i>Sporobolomyces pararoseus</i>	0471	17	6.98	5.84 (7.4)	NT	3.98 (0.1)	4.67 (0.5)	4.22 (0.2)	B
<i>Sporobolomyces roseus</i>	1037	18	6.70	5.90 (16.0)	NT	5.30 (4.0)	5.48 (6.0)	5.19 (3.1)	A
<i>Torulasporea delbrueckii</i>	1739	17	8.03	6.71 (47.4)	6.69 (46.2)	6.55 (33.9)	6.28 (17.1)	6.37 (21.6)	A
<i>Trichosporon brassicae</i>	1584 ^T	17	7.74	7.34 (39.0)	7.13 (24.9)	7.00 (18.1)	6.86 (13.1)	6.75 (10.1)	A
<i>Williopsis saturnus</i>	1762	18	7.93	7.52 (38.0)	7.16 (16.7)	7.28 (22.4)	7.20 (18.0)	7.51 (18.5)	A
<i>Yamadazyma media</i>	1824 ^T	18	7.45	7.08 (43.2)	7.13 (48.8)	7.08 (42.9)	6.92 (30.5)	6.95 (31.5)	A
<i>Yamadazyma stipitis</i>	1720	18	8.20	6.61 (2.6)	6.24 (1.1)	6.04 (0.7)	6.55 (2.3)	6.60 (2.5)	A
<i>Yamadazyma stipitis</i>	10063	14	8.01	6.78 (5.9)	6.39 (2.4)	6.32 (2.0)	6.61 (4.0)	6.37 (2.3)	A
<i>Zygosaccharomyces bailii</i>	1098 ^T	16	7.70	6.82 (13.4)	NT	6.30 (4.0)	5.40 (0.5)	5.30 (0.4)	B
<i>Zygosaccharomyces mellis</i>	1732	17	7.16	7.11 (88.8)	5.92 (5.8)	5.23 (1.2)	6.52 (22.8)	6.67 (32.6)	A
<i>Zygosaccharomyces rouxii</i>	0320	16	6.98	6.45 (30.5)	NT	4.88 (0.8)	6.04 (11.7)	5.68 (5.0)	A
<i>Zygosaccharomyces rouxii</i>	1813	18	7.65	6.65 (10.0)	6.44 (6.1)	6.25 (4.0)	6.74 (16.2)	6.96 (20.6)	A
<i>Zygowilliopsis californica</i>	1771	18	7.93	7.38 (28.9)	6.85 (8.4)	6.87 (9.0)	6.94 (10.5)	6.99 (11.5)	A

correlation between viability after 15 years and that indicated by each test was respectively 0.922, 0.880, and 0.951. The low correlation value for the accelerated test of 60 days at 37°C showed that its condition are excessively severe compared with the test of 30 days. The viable count of accelerated testing for 30 days at 37°C was similar to that of storage for more than 15 years at 5°C. The high correlation with 5 years of storage at 5°C showed lower death rates in 5–15 years than in the 5 years immediately after drying.

Four strains, i.e., *Ambrosiozyma cicatricosa* IFO 1846, *Arthroascus schoenii* IFO 1579, *Leucosporidium antarcticum* IFO 1917, and *Sporobolomyces holsaticus* IFO 1109, showed viable counts lower than 10⁴ CFU/amp. However, these strains could be preserved for another 15 years if they were recultivated. As for the remaining 96 strains, their death rates were found to be equal or lower during 5–15 years at 5°C, compared with that in the first 5 years immediately after drying. It was suggested that they could maintain stable viable counts if no changes occurred in preservation conditions. As a criterion for recultivation, those strains with 0.1–1.0% viability in accelerated storage tests should be reprepared after 15 years, and those with rates of less than 0.1% which required special attention, should be reprepared in less than 10 years.

Although stable viable counts were obtained in the above cases, strains less tolerant of L-drying were investigated with regard to a method to improve viable counts. For basidiomycetous yeasts, growth media were examined. And for ascospore-forming ascomycetous yeasts, L-dried samples of were prepared and their viable counts were measured.

Examination of growth media for cells: When cells of basidiomycetous yeasts on YM agar culture media (glucose 1%, peptone 0.5%, yeast extract 0.3%, malt extract 0.3%, agar 1.5%) were L-dried, viability in accelerated storage tests was less than 0.2%, which implied poor prospects for stable long-term storage. Therefore, viable counts were examined for L-dried cells that had been grown on PDA agar media (potato 20%, glucose 2%, agar 1.5%). The results were compared with those of YM media (Table 2).

Among the eight basidiomycetous yeasts in accelerated storage tests, three *Holtermannia corniformis* strains and one *Udeniomyces megalospora* strain showed a strikingly improved viability by adopting cells grown on PDA media. Four other strains, *Bullera globospora*, *B. sinensis*, *B. variabilis*, and *Rhodotorula bacarum*, were able to increase their survival value by 1.6–16.6-fold, which indicated they could be preserved for a long time.

Examinations of ascospore L-drying: Among ascomycetous yeasts, two types were L-dried and their survival rates were examined. One was vegetative cells which were grown on YM agar media cells *Kluyveromyces*, which had a weak resistance to L-drying. The other was which had ascospores grown sufficiently for 14 days after transferring the cells grown on YM agar medium onto ascospore-forming medium (potassium acetate 1%, glucose 0.1%, yeast extract 0.05%, agar 1.5%) (Table 3).

Two strains of *Kluyveromyces blattae* IFO 10599 and IFO 10600, and *K. yarrowii* IFO 10608 had their survival value raised by 5–46.9-fold through forming ascospores. *K. lodderae* IFO 10605 and IFO 10606 did not show much difference between the YM medium and ascospore-forming medium because ascospores formed sufficiently on YM

Table 2. Effect of preculture medium on viability of the basidiomycetous yeasts.

Species	IFO no.	Medium	Log CFU per ampule (survival value %)			Rate
			before drying	after drying	preservation at 37°C, 30 days	
<i>Bullera globispora</i>	10753 ^T	YM	5.78	4.31 (3.4)	2.98 (0.16)	7.5
		PDA	7.00	6.04 (11.0)	5.08 (1.2)	
<i>Bullera sinensis</i>	10757	YM	7.26	6.17 (8.3)	4.88 (0.42)	16.6
		PDA	6.90	5.98 (12.0)	5.83 (8.6)	
<i>Bullera variabilis</i>	10758 ^T	YM	7.20	5.83 (4.2)	3.98 (0.06)	1.6
		PDA	6.93	4.77 (0.7)	3.93 (0.1)	
<i>Holtermannia corniformis</i>	10742	YM	7.30	5.56 (1.8)	3.78 (0.03)	86.6
		PDA	7.85	6.72 (7.5)	6.26 (2.6)	
<i>Holtermannia corniformis</i>	10744	YM	7.53	6.21 (4.8)	4.00 (0.03)	266.6
		PDA	7.91	6.96 (11.0)	6.82 (8.0)	
<i>Holtermannia corniformis</i>	10745	YM	7.40	5.63 (1.7)	3.40 (0.01)	1000
		PDA	7.64	6.76 (13.0)	6.64 (10.0)	
<i>Rhodotorula bacarum</i>	10768 ^T	YM	7.67	6.51 (6.9)	4.45 (0.06)	15.3
		PDA	7.53	6.21 (4.8)	5.50 (0.92)	
<i>Udeniomyces megalospora</i>	10754 ^T	YM	6.11	3.41 (0.2)	1.11 (0.0001)	800
		PDA	5.78	3.08 (0.2)	2.68 (0.08)	

Table 3. Viability of vegetative cells and ascospores of *Kluyveromyces*.

Species	IFO no.	Medium	Spore	Log CFU per ampule (survival value %)			Rate
				before drying	after drying	preservation at 37°C, 30 days	
<i>Kluyveromyces blattae</i>	10599 ^T	YM	+w	7.67	5.75 (1.2)	5.29 (0.4)	8.5
		PA	++	6.99	5.61 (4.1)	5.52 (3.4)	
<i>Kluyveromyces blattae</i>	10600	YM	-	7.27	4.26 (0.1)	3.44 (0.02)	5.0
		PA	++	6.43	4.45 (1.0)	3.51 (0.1)	
<i>Kluyveromyces lodderae</i>	10605	YM	++	7.98	7.72 (54.3)	7.51 (33.9)	1.6
		PA	+++	7.33	7.08 (55.3)	7.08 (55.4)	
<i>Kluyveromyces lodderae</i>	10606 ^T	YM	++	7.93	7.62 (48.2)	7.42 (30.6)	1.8
		PA	+++	7.03	6.85 (66.2)	6.77 (54.4)	
<i>Kluyveromyces yarrowii</i>	10607	YM	-	8.34	6.91 (3.7)	6.19 (0.7)	-
		PA	-	7.05	4.89 (0.7)	4.20 (0.1)	
<i>Kluyveromyces yarrowii</i>	10608 ^T	YM	-	8.03	6.78 (5.6)	6.02 (1.0)	46.9
		PA	+	6.79	6.62 (68.6)	6.45 (46.9)	

medium. *K. yarrowii* IFO 10607 did not form ascospores due to being a haploid strain, but rather had a decrease in its viability because it was left on ascospore-forming medium for a long time. These facts showed that ascospores are resistant to drying. It became

Table 4. Viability after L-drying and freezing.

Species	IFO no.	Viability, log CFU per ampule (survival value %)			
		L-drying		-80°C freezing	
		before drying	preservation at 37°C, 60 days	before drying	preservation at -80°C, 2 years
<i>Ambrosiozyma cicatricosa</i>	1864 ^T	6.99	3.69 (0.05)	6.53	6.10 (36.8)
<i>Ambrosiozyma monospora</i>	1965	7.77	4.07 (0.02)	6.49	6.00 (32.3)
<i>Ambrosiozyma monospora</i>	4841	6.61	2.91 (0.02)	6.62	5.99 (22.4)
<i>Arthroascus javanensis</i>	1579 ^T	7.78	3.62 (0.007)	7.26	6.76 (31.5)
<i>Ashbya gossypii</i>	0560	6.78	0	5.00	4.68 (48.0)
<i>Ashbya gossypii</i>	1355	6.93	0	5.00	4.88 (75.0)
<i>Brettanomyces bruxellensis</i>	0628	7.90	5.68 (0.6)	8.01	7.74 (53.7)
<i>Brettanomyces bruxellensis</i>	0629	7.73	4.73 (0.1)	7.80	7.35 (35.4)
<i>Brettanomyces bruxellensis</i>	0677	7.85	4.85 (0.1)	7.90	7.58 (47.3)
<i>Brettanomyces bruxellensis</i>	1587	7.33	4.63 (0.2)	6.94	6.52 (38.6)
<i>Brettanomyces custersianus</i>	1585	7.70	5.00 (0.2)	7.45	7.22 (58.8)
<i>Candida boidinii</i>	1967	7.97	5.67 (0.5)	7.83	7.44 (45.4)
<i>Candida buinensis</i>	1642 ^T	8.33	6.11 (0.6)	8.12	7.58 (28.7)
<i>Candida diversa</i>	0861	7.93	5.83 (0.8)	8.12	7.54 (26.4)
<i>Candida diversa</i>	1085	7.91	5.76 (0.7)	8.05	7.53 (30.3)
<i>Candida diversa</i>	1091 ^T	8.51	6.35 (0.7)	8.26	7.91 (44.8)
<i>Candida etchelisii</i>	1229	7.68	5.98 (0.2)	8.14	7.83 (48.8)
<i>Candida etchelisii</i>	1592 ^T	7.67	5.52 (0.7)	8.00	7.50 (31.7)
<i>Candida etchelisii</i>	1595	7.98	0	6.61	6.60 (97.6)
<i>Candida fructus</i>	1581 ^T	8.14	5.14 (0.1)	7.30	7.17 (74.3)
<i>Candida holmii</i>	0660	7.54	4.54 (0.1)	7.44	5.39 (0.9)
<i>Candida holmii</i>	1629 ^T	7.50	4.80 (0.2)	7.80	6.25 (2.8)
<i>Candida mesenterica</i>	0969	7.17	4.47 (0.2)	6.97	6.32 (22.6)
<i>Candida mesenterica</i>	1123 ^T	7.45	0	6.97	6.84 (73.4)
<i>Candida pintolopsii</i>	1313	7.35	0	7.21	6.68 (29.8)
<i>Candida pintolopsii</i>	1315	7.06	0	6.64	6.57 (84.9)
<i>Candida pinus</i>	1327	8.27	6.11 (0.7)	7.95	7.78 (67.5)
<i>Candida psychrophila</i>	1532	7.29	0	7.57	7.31 (55.1)
<i>Candida psychrophila</i>	1533	7.55	0	6.28	4.48 (1.6)
<i>Candida sake</i>	1515	7.74	4.74 (0.1)	7.72	7.22 (31.7)
<i>Candida sake</i>	1516	7.80	5.58 (0.6)	7.72	7.48 (51.5)
<i>Candida sake</i>	1517	7.70	5.48 (0.6)	7.50	7.13 (43.3)
<i>Candida sake</i>	1518	7.69	5.39 (0.5)	7.58	7.31 (35.3)
<i>Candida sake</i>	1519	7.88	5.36 (0.3)	7.76	7.32 (36.2)
<i>Cryptococcus albidus</i>	1322	6.78	4.77 (0.8)	7.43	6.38 (8.9)
<i>Cryptococcus curvatus</i>	1159 ^T	7.38	5.16 (0.6)	7.34	6.57 (17.1)
<i>Cryptococcus dimennae</i>	1863 ^T	6.86	4.34 (0.3)	7.27	6.26 (9.7)
<i>Cryptococcus humicolus</i>	1527	8.31	4.61 (0.02)	7.40	6.93 (33.5)
<i>Cryptococcus hungaricus</i>	1052 ^T	7.49	4.49 (0.1)	6.63	4.93 (2.0)
<i>Debaryomyces coudertii</i>	1381	7.67	0	7.45	7.20 (55.9)

<i>Debaryomyces coudertii</i>	1817 ^T	8.04	4.04 (0.01)	7.61	7.35 (54.6)
<i>Debaryomyces polymorphus</i>	1166 ^T	7.20	4.90 (0.5)	7.83	6.76 (8.6)
<i>Debaryomyces polymorphus</i>	1357	7.04	4.34 (0.2)	7.44	6.66 (16.6)
<i>Dekkera bruxellensis</i>	1590 ^T	7.79	5.63 (0.7)	7.78	7.26 (30.2)
<i>Dipodascus ovetensis</i>	1201	7.28	0	6.52	5.81 (19.6)
<i>Hormoascus philentoma</i>	1847 ^T	7.49	4.19 (0.05)	7.15	6.74 (39.2)
<i>Kluyveromyces phaffii</i>	1883	7.83	4.83 (0.1)	8.12	7.63 (32.4)
<i>Kluyveromyces polysporus</i>	0996	8.08	5.08 (0.1)	7.91	7.24 (21.5)
<i>Leucosporidium antarcticum</i>	1917 ^T	6.82	0.82 (0.0001)	6.90	5.70 (6.3)
<i>Leucosporidium antarcticum</i>	1918	7.73	1.73 (0.0001)	7.06	6.38 (21.1)
<i>Leucosporidium antarcticum</i>	1919	7.48	4.96 (0.3)	7.33	6.78 (28.3)
<i>Leucosporidium scottii</i>	1287	7.00	4.00 (0.1)	6.77	5.95 (15.3)
<i>Leucosporidium scottii</i>	1304	8.25	5.25 (0.1)	7.97	7.66 (49.5)
<i>Lipomyces starkeyi</i>	0678	6.70	3.70 (0.1)	7.27	5.38 (1.3)
<i>Lipomyces starkeyi</i>	1289	7.28	4.98 (0.5)	7.89	6.00 (0.8)
<i>Mrakia frigidum</i>	1851	7.49	4.49 (0.1)	6.86	6.67 (64.4)
<i>Mrakia frigidum</i>	1920 ^T	7.20	4.50 (0.2)	7.50	6.71 (16.1)
<i>Mrakia gelida</i>	1921 ^T	7.04	3.82 (0.06)	7.23	6.29 (11.4)
<i>Mrakia nivalis</i>	1852	6.17	3.47 (0.2)	6.08	3.98 (0.8)
<i>Mrakia nivalis</i>	1922 ^T	7.34	5.29 (0.9)	7.40	7.07 (47.1)
<i>Mrakia stokesii</i>	1926	7.76	5.36 (0.4)	7.84	7.48 (43.7)
<i>Nematospora coryli</i>	0658	6.65	2.65 (0.01)	6.86	6.40 (34.4)
<i>Nematospora coryli</i>	1220 ^T	6.65	3.95 (0.2)	7.26	6.76 (31.5)
<i>Ogatae pini</i>	1342 ^T	7.66	3.26 (0.004)	7.55	7.10 (35.2)
<i>Pichia angophorae</i>	10016 ^T	7.43	5.38 (0.9)	7.76	7.43 (47.0)
<i>Pichia chambardii</i>	1029 ^T	7.30	5.15 (0.7)	7.75	6.64 (7.8)
<i>Pichia fermentans</i>	0815	7.83	5.13 (0.2)	7.78	7.24 (29.0)
<i>Pichia fluxuum</i>	0773 ^T	7.75	5.35 (0.4)	7.93	7.50 (37.3)
<i>Pichia membranaefaciens</i>	0457	7.80	5.40 (0.4)	7.93	7.49 (35.9)
<i>Pichia membranaefaciens</i>	0460	7.72	5.50 (0.6)	7.83	7.50 (47.2)
<i>Pichia membranaefaciens</i>	0814	7.77	5.67 (0.8)	7.77	7.28 (32.3)
<i>Rhodotorula acheniorum</i>	10052 ^T	6.78	4.08 (0.2)	5.90	5.37 (29.0)
<i>Rhodotorula auriculariae</i>	10054 ^T	7.47	5.31 (0.7)	6.88	5.50 (4.2)
<i>Rhodotorula bogoriensis</i>	1966 ^T	4.95	0	6.59	4.44 (0.7)
<i>Rhodotorula glutinis</i>	0391	6.00	3.60 (0.4)	7.18	6.95 (59.5)
<i>Rhodotorula ingeniosa</i>	10002 ^T	7.46	5.30 (0.7)	5.78	4.90 (13.2)
<i>Waltmyces lipofer</i>	0673	7.02	4.47 (0.2)	7.41	5.82 (2.6)
<i>Waltmyces lipofer</i>	1288 ^T	6.70	3.40 (0.05)	6.81	5.22 (2.2)
<i>Yamadazyma stipitis</i>	1720	8.20	6.04 (0.7)	7.92	7.21 (19.7)
<i>Yamadazyma stipitis</i>	10006	8.06	5.54 (0.3)	8.18	7.74 (36.4)
<i>Yamadazyma stipitis</i>	10007	7.98	5.98 (1.0)	8.12	7.61 (31.0)
<i>Zygosaccharomyces mellis</i>	1055	7.93	3.23 (0.002)	7.41	7.21 (62.5)

clear that those among ascospore yeasts, of which vegetative cells were poorly resistant to L-drying could be maintained for a long period by producing and preparing sufficient ascospores.

Among the strains of which viable counts had previously been examined in L-dried samples, 81 strains from 51 species in 23 genera were chosen for comparison of survival rates, because they were poorly tolerant of L-drying in accelerated tests, with the rates of less than 1%. Viable counts were compared between L-dried cells (3 and 5) and those which were suspended in 10% glycerol, poured into a 2 ml serum tube (Sumitomo Bakelite Co., Ltd.) in a volume of 1 ml, and stored frozen at -80°C (4 and 6) (Table 4).

It is shown that the viable counts of the accelerated test of 60 days at 37°C were equivalent to those of 5 years at 5°C (5). Their numbers were found to decrease when cells were repeatedly processed by freezing and thawing (4). The data of survival tests of 2 years' freezing, which were obtained from preparations by repeating freezing and thawing three times, showed four stocks with viability of less than 1%, namely, *Candida holmii* IFO 0660, *Lipomyces starkeyi* IFO 1289, *Mrakia nivalis* IFO 1852, and *Rhodotula bogoriensis* IFO 1966. But they were found to have survival values of 2.5–21.7% when thawed 5 years later for the first time (6). This fact revealed that even strains which had a weak resistance to L-drying could be preserved for a long period through frozen storage at -80°C .

It was possible to preserve almost all yeasts for a long period by L-drying. Basidiomycetous yeasts, which were poorly resistant to L-drying, showed increased viable counts after pre-cultivation on PDA medium. Ascomycetous yeasts, which were also poorly resistant to L-drying, showed increased viable counts through sufficient formation of ascospores. Even yeasts in which no increase in viable counts was observed with the above modifications were found to become suitable for long-term storage through freezing at -80°C .

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A History of Microbial Culture Collections in Japan **日本の微生物株保存事業**

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長谷川 武治

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日本の微生物株保存事業

1. 揺籃時代

長谷川武治

学名既知の微生物株を集めて培養し保存するという仕事は、大学であれ、会社であれ、およそ微生物を研究している機関なら、どこでもやっていることである。高等動物や高等植物を見つけたり、種類を決めたりという仕事は肉眼でも容易にできるが、微生物の場合は、いわゆるきのこ類を除くとそれほど簡単ではない。大部分のものは顕微鏡によらなければ存在さえ認めることができないばかりか、それらが入り混った状態で存在している自然界から分離し培養するのでもなければ、種類を判定することは困難である。このような微生物は、いってみれば、研究室の培養物コレクションにおかれることで初めて存在を顕示することができる。このことは、学名の生体標本の供給源であるカルチャーコレクション (culture collection) の存在意義と国際性を保証する理由になっている。保存事業を営む保存機関では、そのコレクションをカタログの出版などの方法で公開し、利用しやすいように一般研究者への便宜を計る。

国際的に活動している微生物株保存機関はいずれも20世紀に誕生した。そのなかで、最も早かったのは、菌類コレクションとして著名なオランダのCentraalbureau voor Schimmelcultures (CBS) で、その最初のカタログとして、80株を掲載したものを1906年に公表した。また細菌のコレクションを主に始めたAmerican Type Culture Collection (ATCC) は1911年、英国のNational Collection of Type Cultures (NCTC) は1922年以降の歴史を誇っている。

それでは、日本における微生物株保存事業はいつごろ始まったのであろうか。この事業を事業目的として掲げた微生物学研究機関、財団法人長尾研究所が1941年(昭和16年)、東京に設立され、糸状菌374株、酵母179株、バクテリア41株を掲載したカタログを1942年に出版したが、第2次世界大戦のさなかの労作で、その内容はきわめて不十分なものであった。この研究所から、本格的なカタログが出たのは1950年(昭和25年)である。また、カタログの公表をもって事業の表徴とするならば、さらにさかのぼり、1927年(昭和2年)、その当時、日本が中国から租借して関東州と

呼んでいた現在の東北地区遼寧省遼東半島南部の大連(ターリエン、当時はダイレン)にあった南満州鉄道株式会社中央試験所から、“Catalogue of Cultures of Fungi”が出版されたことがある。これには糸状菌および酵母436株が載った。このカタログの新版が1930年に出たが、その後の出版はみられなかった。1945年(昭和20年)を境にして考えると、それ以前に出版された保存微生物のカタログは上記以外にはない。このようにカタログなどによる公開が珍しかった理由としては、コレクションの造成が国立機関で行われたために、有償配布の対象物となることがなく、専門家の間以外では、自由に分譲や供与を受けることができないといった事情もあった。また、応用微生物学者の片手間仕事であったから、保存技術や施設も今日からみると、幼ないものであった。事実、微生物株保存事業の将来への展望が開けるのには、長尾研究所のような民間専門機関の出現を待たなければならなかったのである。

I. 揺籃時代を導いた応用微生物学

前記の保存微生物株カタログに載った菌名は、発酵学特に醸造学と深いかかわりをもったものが大部分で、戦後における日本の微生物株保存事業の発展についても、その影響を無視しては語ることができない。この点から日本の保存事業を眺めると、医学、農学の病理学分野からの要請に端を発した欧米の場合といささか趣きを異にしているようにみえる。その理由として、医学における伝染病予防法を例にとればわかるように、日本ではきびしい規制が早くから行われたことも考えられるが、別の見方をすれば、明治初期、欧米の学者たちによって導入された応用微生物学を初めとする微生物科学の分野が、この国に根づいて順調に発展した証拠でもある。明治から大正にかけて活躍した微生物学者の人文地図が、本学会の名誉会員であった故坂口謹一郎東京大学名誉教授(1897-1994)によって作られている(図1)(9)。カルチャーコレクションの造成とのかかわりに留意しながらこれを眺めると、日本における造成の歴史的背景が浮び上がってくる。

1877年(明治10年)は、日本における最初の総合大

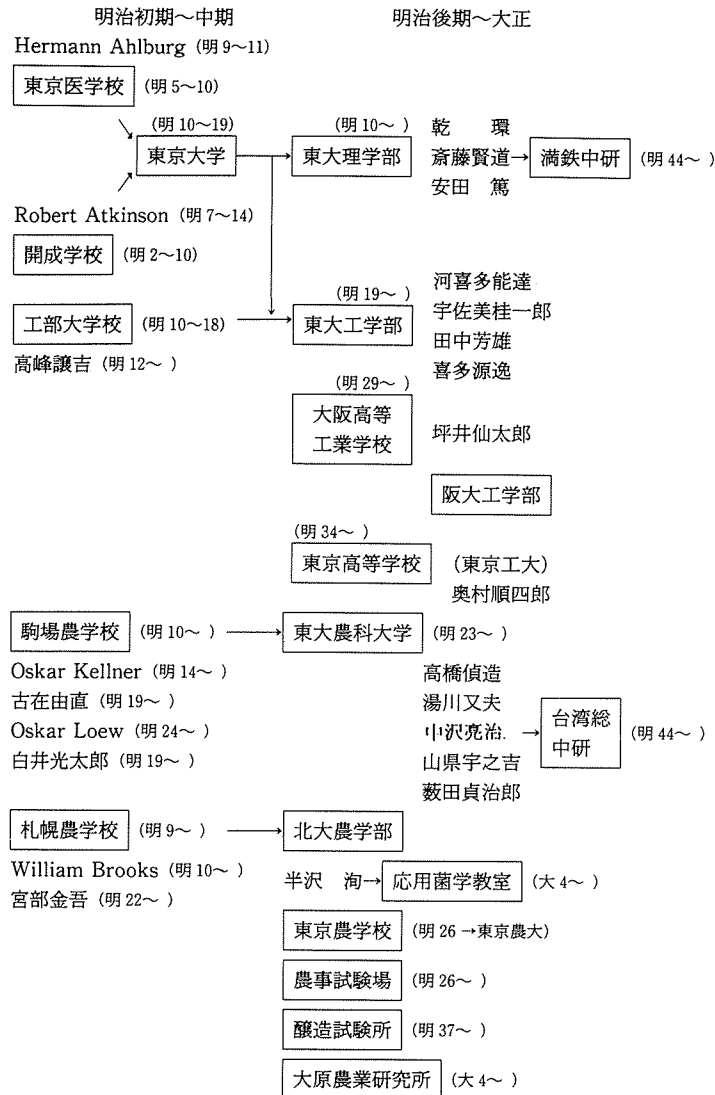


図1 揺籃時代を導いた応用微生物学の系譜

学であった東京大学が創られた年である。当時の名称も「東京大学」で、設立の基礎になったのは、明治の新政府が徳川幕府から引き継いだ学術啓蒙の諸機関であった(図2)。創立時の東京大学には、医学部、理学部、法学部、文学部と大学予備門は設けられたが、農学および工学関係の学部がなかったから、同年、内務省によって駒場農学校が、また工部省によって工部大学校が設けられた。その後、駒場農学校は明治14年に新設の農商務省へ移管された。ほかにも、1876年(明

治9年)、開拓使(北海道庁の前身)によって設立された札幌農学校が存在した。明治18年の工部省廃止によって工部大学校が文部省へ移管されたのを機に、翌19年、東京大学は帝国大学と改称され、各学部はそれぞれ自立性を強めた分科大学制を採用することになり、この体制は、1919年(大正8年)、官制改正によって分科大学制が元の学部制に戻るまで続いた。駒場農学校は東京山林学校と合併(1886年)、東京高等農林学校になったが、1890年(明治23年)、文部省へ移管されて

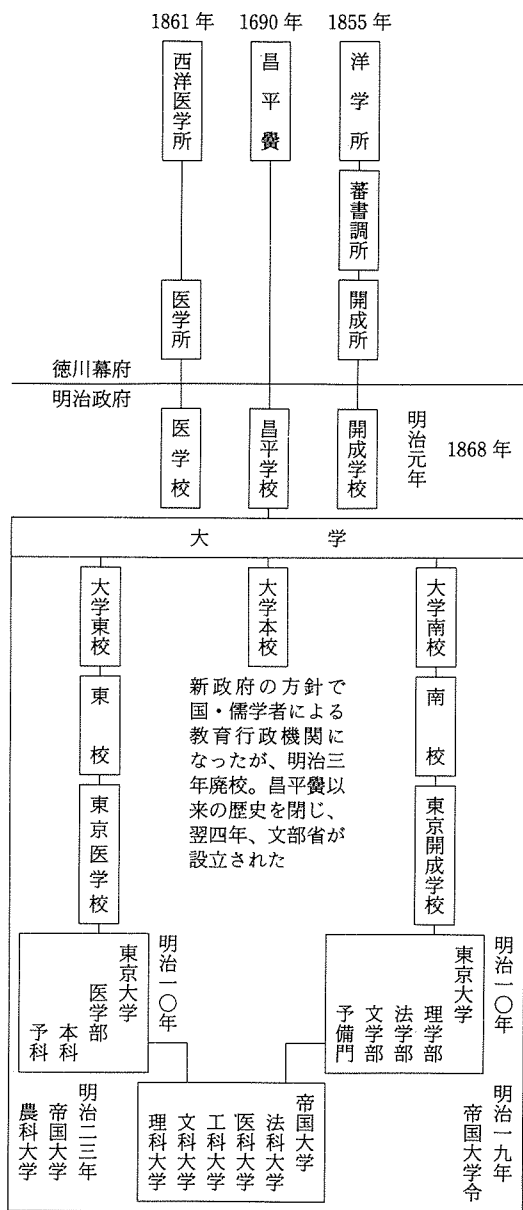


図2 東京大学の起源

校名を帝国大学農科大学に改めた。1897年(明治30年)に京都帝国大学が創られ、それ以後の帝国大学は東京帝国大学と名称を変更した。札幌農学校は、1907年(明治40年)、仙台に大学本部を置く東北帝国大学の創設時に文部省所管の同大学農科大学になり、1918年(大正7年)、北海道帝国大学が設立されると、同大

学農科大学となって東北帝国大学を離れることになる。

明治の初め、政府はオランダに頼ってきた医学教育の体制をイギリス医学へ切り換えようとしたが、医学はドイツが最も進歩しているというオランダ人宣教師の進言を容れてドイツ医学を導入する方針を決定し、ドイツ政府に対して医学教授の派遣を要請した。1871年(明治4年)、2名のドイツ人医師が来日し、そのひとり、Leopold B. Müller博士が東校(東京医学学校)の教頭となり、ドイツ学派による指導体制を作りあげた。

さて日本には古来、酒、味噌、醤油、味醂などの醸造製品があり、それらの製造方法は伝統の過程で洗練され、品質の優れたものが生み出されてきた。特に日本酒の場合、醸造酒では世界でも比類のない高濃度のアルコール含有に至る、独特の技術があったのである。この日本の醸造技術に近代科学の光があたるきっかけを作ったのは、Müller教授に同行したもうひとりの医師 Theodor E. Hoffmann 教授であった。教授は、醸造の過程で起こる糖化作用に与かるこうじ菌に強い関心を抱いた。その理由は、欧州では穀類糖化にもっぱら麦芽が用いられてきたからである。当時、横浜で印刷出版されていた独文誌、Mitteilungen der Deutschen Gesellschaft für Natur- und Völkerkunde Ostasiens(東アジア・ドイツ博物学民族学協会報告)に醤油、酒、味醂の醸造法に関する紹介論文を載せたのは1874年(明治7年)であった(3,7)。教授にとって専門分野ではなかったから単なる紹介に終わったけれども、これを引き継いで本格的研究に発展させたのは、翌年、東京医学学校の予科で製薬化学を教えるために来日した Oskar Korschelt 教授であった。Korschelt 教授の興味はこうじ菌だけでなく、高率にアルコールを生成する日本酒の製造技術にも向けられた。彼は醸造工程を(1)「こうじ」の製造、(2)「もと」の製造、(3)「仕込み」と発酵、(4)圧搾と清澄の4段階に分けて詳しく調査を行い、糖化とアルコール発酵に関与する微生物について研究するなどして、それ以前は「杜氏」の経験だけに委せてきた清酒の製造技術に初めて近代科学のメスを入れた。1876年(明治9年)、博物学教授として東京医学学校へ着任した植物学者 Hermann Ahlburg 博士は Korschelt 教授からこの研究への協力を求められ、これに応じて「こうじ」の菌学的研究を行い、最初の学名 *Eurotium oryzae* を命名した。ゼラチンを用いた固形培地による菌類純粋培養法は、ドイツで Koch より10年も早く、Brefeld が1870年ごろから試

みており、それ以前でもパレイシヨの切片などを用いる方法がすでにあつた。Ahlburg 教授がどういふ方法によつたかは明らかにされていないが、その他の種類も含めたレポートを引用した Korschelt 教授の研究論文は、1876 年、前記の独文誌に発表された(4, 7)。Ahlburg 教授のレポートは、当時、その助教(助教授)であつた松原新之助氏によって翻訳され、『麴(こうじ)の説』と題して発表された(5, 6)。醸造学最初の日本語論文であつたが、これを書いた松原氏は、発表後もなく教授の急逝に遭い、翌年から内務省御用掛り兼務となつて水産学へ移つていった(2)。

R. W. Atkinson 教授(1850-1929)は1874年(明治7年)に来日以来、南校(東京開成学校)で教鞭を取つていたイギリス人化学者であつた。東京大学では理学部化学科教授となり、1881年(明治14年)、“Chemistry of Sake-Brewing”という70ページを越える大論文を『東京大学理学部紀要』に発表した(1)。この年、ドイツから来日して駒場農学校教授となつた O. Kellner 博士が農産製造学を講義、応用微生物学分野はこれら外国人教授たちに導かれて、醸造学を中心に発展の途につくことになる(9)。そしてこれを引き継いだ最初の日本人教授が古在由直博士(1864-1933)であつた。古在教授の事績については、坂口博士執筆の『古在由直先生』から、その一部を以下に引用する(10)。「ケルネル博士の育つた当時のドイツは、リービッヒの感化によつて物理化学、農芸化学、工業化学、微生物化学などの研究室をつくつて、化学を第一級の学問のうちに加えるようになったころのことで、博士はこの新しい教育を受けた最先端の若い学徒として、来日直前にはホーヘンハイム農事試験場で家畜栄養に関する研究に従事してゐた。日本に来て一番はじめになされた講義は植物生理化学で、次いで、土壌学と肥料学、家畜飼養学、農産製造学である。博士の着任の年に入学された古在先生はもちろん、これらの講義をきかれたわけである。それのみでなく、明治19年(1886年)、駒場農学校を卒業後も助教授としてケルネル博士と数多く共著を発表された。先生の研究で大きな影響をひき起したのは、わが国の土壌ではリン酸肥料の肥効が特に大切なことを証明された研究であらう。これによつて、肥料会社の創立が刺激され、またその他の肥料の施用を一般化して、明治以来のわが国の米産額が倍増する原因となつたといわれている。先生が後年まで御自身の研究として続けられた発酵学の研究は、明治24年(1891年)にケルネル博士が帰国され、新たにロエブ(Oskar Loew)教授がドイツから来られ、博士と共

著のプロジジオス菌の生理に関する研究発表以後のことである。もっともそれより先にも、在来の清酒醸造において酵母の純粹培養の応用の必要なことを初めて指摘されたが、酒酵母の研究を通じて日本酒製造の実態を広く世界に知らせたり、麴菌酵素の酒製造での意義を明らかにせられるなど、多くの醸造上の研究を発表されたのは、もっぱらそれ以後のことである。ことに先生がドイツ留学中になされた牛乳の自然発酵における各種乳酸菌の研究は、当時、世界の学界から高く評価されてその後の乳酸発酵や乳酸菌の分類の研究に対して大きな影響を与えた。先生は明治28年(1895年)、外国留学を命ぜられ、はじめはベルギーに、後にドイツに移られ、ベルリン醸造試験所などで研究を続け、明治32年(1899年)、帰朝と同時に東京帝国大学教授を命じられて当時新たに発足した農産製造学講座を担当され、明治36年(1903年)には農事試験場長をも兼務されることになった。また当時、わが国には家畜飼養のための飼料消化の研究がほとんどなかつたのであるが、家畜飼養の問題の研究を開始させて、家畜試験場(後の畜産試験場)創立の気運をつくり出された。農事試験場ばかりでなく、専門研究者の立場から、明治34年(1901年)、大蔵省醸造試験所の創立委員として研究上の重要方針を定め、明治38年、同所の商議員となられ、日本酒に技術上の指針を与えられたのも先生の大きな功績のひとつである。」

明治の初期に応用微生物学が醸造学分野から始まつた経緯は、ほぼ上記のようなものであつた。東京帝国大学農科大学では、醸造学は農産製造学に含まれてゐたが、外部では1896年(明治29年)、大阪高等工業学校に醸造科が設けられたり、酒税法の制定に伴う、1904年(明治37年)の大蔵省醸造試験所の設立などが醸造学の発展を加速させた。さらに大正時代に入ると、各帝国大学に応用微生物学に関する講座が設けられ、各地における研究所活動とあひまつて、基礎、応用の分野にわたり微生物学の発展がみられた。その詳細は各学会出版物に譲るほかはないが、これらの発展に伴つて微生物株保存事業を支えるカルチャーコレクションの造成が導かれた過程については、その一例として大蔵省醸造試験所の場合を取り上げてみよう。

醸造試験所は設立されて以後、大蔵省の一部局となつたが、建設を担当したのは農商務省で、設立事務取扱所も同省商工局内におかれた。「酒類および醸造物中、特に清酒の品質およびその醸造方法を改良し、酒造家をしてその実績を挙げしむる」を目的とする同試験所は、地下水の佳良豊富、土地の高燥、交通の便な

どを条件として敷地が選定され、また、環境の微生物学的調査も行われた。これらの技術的調査には、設立事務嘱託となった古在由直博士や臨時調査嘱託の東大理学部大学院生、斎藤賢道氏らがあたった。試験所は当初、研究科、酒類醸造科、醤油醸造科、機械科などに分かれたが、後には研究科と醸造科だけになった。昭和49年(1974年)に出版された『醸造試験所70年史』にある「微生物株の保管」の項を以下に要約する。

「醸造試験所の設立に当り、その設立委員として活躍した東京帝国大学農科大学教授古在由直、設立事務臨時調査嘱託斎藤賢道の2氏は日本における著名な微生物学者であるが、その当時、中沢亮治氏と共に多数の微生物株を分離して醸造試験所の保管に委ね、また海外に留学した大学教授その他多くの人が帰国した折に持ち帰った珍しい微生物株の寄託を受け、当時の醸造試験所はわが国唯一の微生物利用総合研究機関の偉容を誇った。その後、職員の研究の進捗に伴って、こうじ菌、酵母、火落菌その他の細菌など、醸造に関与する多数の微生物株が分離収集され、国内外の試験研究機関との微生物株交換などによって貴重なコレクションができたが、1945年(昭和20年)、戦災のためほとんど焼失した。その後、昭和25年ごろから糸状菌、酵母の分離収集を始め、こうじ菌の分類整理、酵母優良菌株の育種、乳酸菌・火落菌など細菌の分類を行って今日に至り、試験所の微生物株保管業務は、設立委員のひとりで清酒酵母の発見者(明治28年)であった矢部規矩治氏が担当して以来、わが国で最も永い伝統をもつものである。」

上記のなかで、醸造試験所の最初のコレクションは戦災で失われたと記されているが、1924年(大正13年)、東京帝国大学に新しく醸造学などの講座が設けられたとき、それらの微生物株の多くが研究資料として同教室へ移され、東大農学部(ATU)および同大学分子細胞生物学研究所(IAM)コレクションに発展して今日に至っている。

II. 揺籃時代を築いた人々

1. 斎藤賢道(1878-1960)

金沢市に生まれ、第四高等学校を経て、1900年(明治33年)、東京帝国大学理科大学植物学科を卒業、大学院へ進んだ。その当時のことを博士は次のように述べている(8)。

「初め、植物生理学という専攻題目をもって大学院へ入学し、三好先生の指導を受けた。大学在学中から一般菌類に興味があり、先生から大気菌子に関する研

究をするようにいわれた。筆者が行った大気中の菌子の研究は明治37年にかび類、同40年に細菌類について発表した(酵母は大正年代になってから行った)。これは大気中の菌子の季節的消長を知ると共に、分離した種類を決定するのが目的であった。大気菌子を研究していた関係から、明治34年に醸造試験所設立の際、敷地の大気菌子の調査を命ぜられたりして以来、わが国の醸造物に関する発酵菌を研究する機会と便宜とを得た。特に醤油醸造に関する微生物は、筆者が初めて系統的に調べたもので、麴(こうじ)・醪(もろみ)のかび、酵母菌、細菌がわかったが、その後、この方面の研究は、高橋偵造、湯川又夫、松本憲次、石丸義夫氏らによって、ますます精細に行われた。」

1904年(明治37年)、大学院を卒業、在学時代に引き続き、東京税務監督局酒類鑑定事務取扱嘱託になり、1909年(明治42年)、学位取得の後、農商務省海外実業練習生としてドイツに留学し、主にベルリン醸造試験所で醸造学を研修した。帰国後1911年(明治44年)、南満州鉄道株式会社中央試験所(満鉄中試, Central Laboratory, South Manchuria Railway Co.)に入所、1922年(大正11年)、同研究所長に就任した。ドイツ留学当時の欧州では、オランダのユトレヒト大学で1904年に初めてCentralstelle für Pilzkulturenが設けられ、1907年, Centraalbureau voor Schimmcultures(CBS)と所名を変え、菌類保存ならびに菌学研究機関として事業活動を始めたばかりのころで、博士に強い関心を抱かせたことであろう。満鉄中試では醸造学上の貢献に加えて菌類研究者としても声名を高め、CLMRコレクションは、1927年のカタログ出版でその知名度を高めた。1927年(昭和2年)、退社して大阪高等工業学校講師、1929年、同校の昇格による大阪工業大学(1933年、大阪帝国大学工学部)の教授に就任した。CLMRコレクションの複製が同大学へ移ったのはいうまでもない。1940年(昭和15年)、定年を迎え、同年、財団法人長尾研究所の設立に参画して、1948年(昭和23年)まで理事兼主任研究員を続けた。1942年(昭和17年)に出版された『財団法人長尾研究所菌類目録』の編集は博士が担当した。研究所は、経営上の事情から1971年に閉鎖された。

2. 中沢亮治(1878-1974)

岡山市の生まれで、第一高等学校中退後、東京帝国大学農学部農芸化学科専科を終了し、1905年(明治38年)、醸造試験所に入所して技手に任じられた。1907年(明治40年)、農商務省海外実業練習生としてドイツに留学、ガイセンハイム王立園芸学校、ミュンヘン醸造

試験所およびハンノーヴァ高等工業学校において醸造微生物学を研修した。帰国後、醸造試験所、税務監督局などに勤務し、1911年、台湾総督府へ技師となって赴任、1916年(大正5年)、同研究所醸造学部長となる。その後、官制改正によって台湾総督府中央研究所工業部醸酵工業科長と職名の変更があり、1937年(昭和12年)、同中央研究所工業部長に就任した。1921年(大正10年)に学位を取得し、1930年(昭和5年)以降、台北帝国大学農学部教授を兼任した。1939年(昭和14年)、定年退官、同時に兼任も解かれて大阪に移り、武田薬品工業株式会社顧問となった。在台中に作った微生物株コレクションは、菌類を主とし、CLMRコレクションに匹敵する内容で、国内および欧州の専門機関には、GRIFコレクション(GRIFはGovernment Research Institute of Formosaの略称)として知られたが、カタログの出版はなかった。1944年(昭和19年)、戦時下における科学技術統制機関であった内閣技術院が、武田薬品との共同出資で財団法人航空醸酵研究所を設立、中澤博士は初代所長となり、1946年(昭和21年)まで在任した。同研究所は応用微生物学の研究と共に、有用微生物株の収集保存ならびに一般研究者への配布を事業目的とする民間機関で、開設時に保存していたカルチャーコレクションは、台湾総督府から分譲されたGRIFコレクション(複製)ならびに、東京帝国大学農学部(坂口謹一郎教授)から戦時下の保全疎開の目的で武田薬品が保管を委託されたATUコレクション(複製)であった。ただしカタログによる公開は、1953年(昭和28年)の第1版以降である。戦争が終結した1945年、内閣技術院は解散したが、上記の研究所は文部省の所管となって存続することが決まり、所名を醸酵研究所(Institute for Fermentation, Osaka, IFO)に変更、さらに1961年(昭和36年)、応用研究部門を廃止し、基礎微生物学研究部門と保存事業部門だけを残して名称を現在の発酵研究所に改めた。中澤博士の台湾総督府在任中の事績について、1987年(昭和62年)に出版された『農芸化学の100年』所載の記事(清水俊秀他:戦前・戦中の台湾における農芸化学)から関連部分を要約して引用する(11)。

「中澤先生は、まず現地在来法による各種の醸造製品由来の発酵菌類の分離、検索から始め、発酵学的研究を精力的に進められた。その結果、台湾におけるアルコールや各種酒類の製造法は飛躍的進歩を遂げた。特筆すべきはアミロ法の完成であった。台湾では古くから甘蔗を原料として砂糖が製造されており、その糖蜜を用いて糖蜜酒、アルコールが作られていた。先生は、

在来法の製造工程から分離した数多くの酵母株のなかから、有名な396号菌(*Saccharomyces formosensis* Nak.)を選抜し、その純粋培養をアルコール発酵に使用することによって発酵時間の短縮、発酵歩合の向上をもたらされた。その後*Saccharomyces robustus* Nak. et Simoなどさらに強力なものも発見された。そのほか、沖縄の泡盛こうじ菌として得られた*Aspergillus awamori* Nak.によるクエン酸製造研究や当時としては画期的なラジウム放射線による変異株の生成(1936年)などの研究もあって、先生は台湾における発酵工業の父として大きな足跡を残された。」

3. 長西廣輔 (1889-1979)

満鉄中央試験所において、斎藤賢道博士の指導の下に、CLMRカルチャーコレクションの保存管理と、本邦最初のカタログの出版を担当したのは長西廣輔博士であった。博士は山口県徳山のひと、1910年(明治43年)、大阪高等工業学校醸造科を卒業、兵役を終えて1912年、満鉄中央試験所に入所した。1923年(大正12年)から1925年まで海外へ出て欧米を視察、ロンドンのLister研究所生物化学課に1年間留学した。満鉄中試では、高粱によるアルコール製造法で発明表彰(1927年)を受けるなどの貢献もあった。斎藤博士退社のあと、1930年(昭和5年)、CLMRコレクションのカタログの新版を出し、その表題を『Catalogue of the Collection of Type Cultures』としたことに保存事業への期待が表わされたが、博士の満鉄中試への願望はついに実らなかった。まもなく始まる戦争の時代が、当時の満州ではすでに兆しを現わしていたからであろう。同年、満鉄を退社、広島高等工業学校(のちに広島工業専門学校、昭和25年以降、広島大学工学部)教授に就任した。満鉄の退社にあたって、CLMRコレクション(複製)の分譲を受け、これを同校へ移した。1945年(昭和20年)、広島市の中心部に原子爆弾が投下され、同校に直接の被害はなかったものの、研究室に必要な実験機能が停止したため、その翌年、醸酵研究所に協力を求めてコレクションの修復に努力した。博士は子囊菌酵母、特に*Debaryomyces*の研究で学位を取得している。

4. 高橋偵造 (1875-1952)

長野県飯山の生まれで、第一高等学校を経て1900年(明治33年)、東京帝国大学農科大学農芸化学科を卒業した。卒業後、古在教授について醸造学を専攻、1906年(明治39年)、助教授、1923年(大正12年)、教授に就任した。1917年(大正6年)米国学留学、Samuel C. Prescott教授に師事したが、翌年病のため帰国した。

1924年(大正13年),農産製造学から分かれて醸造学および微生物生理学の新しい講座(後に醸酵学と命名)を創設した。1929年(昭和4年),大蔵省醸造試験所の商議員となって醸造技術行政の指導に当たり,1950年(昭和25年),日本学士院会員となった。高橋博士の研究業績については,坂口博士執筆による『高橋偵造先生』のなかから抜粋して以下に引用する(10)。

「高橋先生の御生涯の研究を大別して二つの大きな時期に分割できる。前期は大正10年ごろまでの約25年間,この間にあげられた業績はわが国の醸造の各分野における微生物学的研究に先鞭をつけるものであった。先生は,広く酒,味噌,醤油そのほかの醸造品製造に使われているこうじ菌をできるだけ多く分離,収集して研究された結果,こうじ菌は決して単一の菌株ではなくて無数の変種から成り立っているものであるという事実を,菌の形態と生理との面における数多くの研究によって明らかにされた。このことは,ひとり醸造菌学上の問題であるばかりでなく,酵素学および酵素工業に大きな貢献をされたことにもなった。また先生は多年にわたって「火落ち」の研究に取り組み,この腐敗現象の原因が「火落ち菌」という一種の乳酸菌であることを確かめて,形態生理ばかりでなく,火落ちの予知,防止などの方法について研究し,醸造技術の実際についても功績を立てられた。そのほか,醸造業に関係した微生物の研究としては,清酒酵母,醤油酵母,中国の紹興酒酵母,日本の酢の酢酸菌などについて,それぞれ膨大な研究をなしとげられた。これらの研究で,先生の行き方の特徴は,こうじ菌の場合と同様に,それに関係した微生物のあらゆる変種を分離して,それらの性質を総合的に観察するという方法をとられたことである。先生の生化学的研究は,はじめはこうじ菌酵素に関するものが多かったが,後には米のタンパク質,酒,味噌,醤油など醸造物中のアミノ酸を主とした各成分の組成やその醸造中の変化,したがってフーゼル油やメタノールの研究などにも入られ,清酒の「うま味」の主要成分としての「コハク酸」の発見,高級アルコールがバニリンと濃硫酸とで発色する「バニリン硫酸反応」の発見などがあり,後者は今に至るまでフーゼル油の検出,定量に使われている。

以上は先生の研究生活の前期の業績の大略である。大正10年から12年にかけて,先生は *Rhizopus* 属の発酵生産物など,もっぱらかびの有機酸発酵の問題に興味の中心を移され,その研究は,昭和11年,大学を退職されるまで続けられた。そしてその途中,すなわち昭和5年前後には,かび類のほか,有名な酢酸発酵

に関する研究も開始された。これら両研究は,主に朝井勇宣博士が先生の助手として多年にわたり,中心になって遂行されたのであるが,筆者も先生の「くものすかび」研究の初期にはお手伝いさせていただいた。先生がこれらの研究を始められた当時の世界の学界は,ソビエトのコスチチェフ,チェコのベルンハウエル,ウィーンのプトケウィチらが,かびやバクテリアの有機酸発酵の研究を始めたばかりのころであって,後年,これらの発酵が糖類のメタボリズムの機構を解明する鍵となろうとは,夢にも考えられなかったことである。先生は微生物の生産物としては初めての有機酸の数々を発見され,彼らに先立って発表された。」

東京大学農学部コレクションは高橋教授の時代に集められたものが多く,有用菌株を主とする点に特色があった。坂口教授に引き継がれてさらに充実,1953年(昭和28年)における東京大学応用微生物研究所の設立に大きな貢献を果した。1994年(平成6年),この研究所は名称を東京大学分子細胞生物学研究所に変更した。

5. 半澤 洵 (1879-1972)

北海道札幌市に生まれ,1892年(明治25年),札幌農学校に入学,1901年(明治34年),同校を卒業した。本科では,宮部金吾教授(農業生物学科)に師事して植物病理学を専攻,卒業後,農芸化学科へ移ってその研究生となり,1907年(明治40年),同校が東北帝国大学農科大学になると共に助教授に任官して応用菌学を講義,1915年(大正4年),応用菌学講座の新設によって教授に就任した。その間,1911年(明治44年),欧米各国へ出張,ドイツでは,ハンノーヴァ高等工業学校で発酵菌類,ライプチヒ大学で土壤微生物学,またフランスでは,パストール研究所で生物化学を研修して,1914年(大正3年)に帰国した。1918年(大正7年),北海道帝国大学の新設によって同大学へ所属の変更はあったが,教授は同大学でも応用菌学講座の担当を続け,1941年(昭和16年),定年退官を迎えた。半澤教授の事績については,『米寿記念半澤洵先生業績目録』(1966)からその一部を借りて以下に引用する。

「半澤先生は,わが国では未だ行なわれていなかった応用菌学の講義を開講され,その発展に尽された功績は大きい。土壌および肥料の微生物に関しては先生のドイツ留学中の有名な F. Loehnis 教授との共同研究以来,数多くの研究を発表されたが,その中でも *Azotobacter* に関する研究は重要である。食品および肥料の微生物は特に先生の意を注がれたところで,果実貯蔵,牛乳および乳製品,蜂蜜の変敗,水産物の缶

詰や乾物、さらに埋蔵飼料（エンシレージ）などに関して数多くの研究があり、なかでも納豆菌と納豆製造に関する研究は有名である。製造工業では、アミロ法によるアルコール製造に用いられる *Rhizopus delemar* は先生の研究により1912年、その菌学的性質が明らかになり、新種として発表された。先生はこの属の分類学的研究で世界の菌学界に大きな貢献を果たされた。水産業では養魚用水、孵化魚卵、各種水産製造品に関する広汎な研究があって、わが国の水産微生物学界に先導的役割を果たされた。」

今日の北海道大学農学部(AHU)のコレクションは、半澤教授によってその基礎が固められた。

6. まとめ

以上、現代の日本における微生物株保存事業の基礎になったカルチャーコレクションを造った人々の事績をまとめたが、これらのコレクションは、当時の日本における微生物学者の研究努力の集大成というべきものである。その造成にかかわった各分野のなかで菌類については、日本菌学会がまだ存在しなかったころのことなので、植物病理学の進歩の貢献が大きく、揺籃期を脱した後の保存事業にその成果が加えられた。植物病理学が日本に導入された時期は、応用微生物学とあまり変わらない。坂口博士によると、東京医学校の H. Ahlburg 教授（前出）が1877年（明治10年）、神田の開農義会（大日本農会の前身）が経営する混々会という私塾で植物病理学を開講したが(9)、この年、札幌農学校でも米国人 William P. Brooks 博士（農学、植物学）の講義が始められた。Brooks 教授の講義を聞いた学生のなかにいたのが、日本における植物病理学の開祖として知られる宮部金吾博士（1860-1951）で、札幌農学校を卒業してさらに東京大学理学部植物学科へ進学、米国ハーバード大学へ留学後、1889年（明治22年）、札幌農学校教授となった。宮部教授の東大卒業は1886年（明治19年）であったが、同じ年、同理学部植物学科を卒業した白井光太郎博士（1863-1932）もまた植物病理学の開拓者として知られる人で、卒業後直ちに東京高等農林学校に赴任、1899年（明治32年）、ドイツ留学から帰って東京帝国大学農科大学教授に就任した。両教授の門下から著名な植物病理学者が輩出したが、それらの人々の事績については、他日、適任者の筆に待ちたい。

III. 揺籃時代のカタログに現れた微生物名

1. 菌類

Absidia butleri, *A. coerulea*, *A. cylindrospora*, *A.*

glauca, *A. hyalospora*, *A. lichtheimi*, *A. orchidis*,
A. ramosa, *A. septata*, *A. spinosa*

Acrostalagmus cinnabarius

Actinomucor repens

Allescheria boydii

Alternaria circinans, *A. tenuis*

Armillaria mucida

Arthrotrichum oligospora

Ashbya gossypii

Aspergillus albus, *A. atropurpureus*, *A. aureus*, *A. auricomus*, *A. awamori*, *A. batatae*, *A. caesiellus*,
A. candidus, *A. carbonarius*, *A. cellulosa*, *A. cinnamomeus*, *A. citrosporus*, *A. clavatus*, *A. conicus*, *A. elegans*, *A. fischeri*, *A. ferrugineus*,
A. flavus, *A. fumigatus*, *A. fuscus*, *A. giganteus*,
A. glaucus, *A. gymnosardae*, *A. japonicus*, *A. luchuensis*,
A. medius, *A. melleus*, *A. minimus*, *A. mollis*,
A. nidulans, *A. niger*, *A. ochraceus*, *A. okazakii*,
A. oryzae, *A. ostianus*, *A. parasiticus*, *A. penicillopsis*,
A. pseudoflavus, *A. pulverulentus*, *A. rehmi*, *A. repens*,
A. sulfuratus, *A. sydowi*, *A. tamarii*, *A. terricola* var.
americana, *A. varians*, *A. versicolor*, *A. wentii*

Atelosaccharomyces sp.

Basidiobolus ranarum

Basisporium sp.

Blastomyces luteus

Botryococcus japonica

Botryosphaeria ribis chromogena

Botryosporium pulchrum

Botrytis cinerea, *B. dichotoma*, *B. stephanoderis*

Byssoclamis nivea

Catenularia fuliginea

Cephaloscypha fragrans

Cephalothecium asteris, *C. roseum*

Chaetocladium johnesii

Chaetomidium japonicum

Chaetomium sp.

Circinella aspera, *C. minor*, *C. mucoroides*, *C. sydowi*

Citromyces glaber, *C. pfefferianus*

Cladosporium album, *C. herbarum*

Coniospora cerebella

Cortinellus shiitake

Cunninghamella blakesleeana, *C. echinulata*, *C.*

- elegans*
Debaryomyces fabryi, *D. globosus*, *D. kloeckeri*, *D. mandshuricus*, *D. matruchoti*, *D. membranaceifaciens*, *D. sake*, *D. tyrocola*
Dematium chodati, *D. pullulans*
Dendriophium penicillatum
Endoconidiophora fimbriata
Endomyces albicans, *E. bisporus*, *E. capsularis*, *E. decipiens*, *E. fibuliger*, *E. hordei*, *E. javanicus*, *E. lindneri*, *E. magnusii*, *E. vernalis*
Endomycopsis monosporus
Eremoascus fertilis
Fumago sp.
Fusarium sp.
Glomerella sp.
Gymnoascus candidus, *G. setosus*, *G. umbrinus*
Hansenia apiculata
Hanseniaspora valbyensis
Hansenula anomala, *H. schneegii*
Helicostylum piriforme
Helminthosporium sp.
Hemispora stellata
Humicola grisea
Kloekera japonica, *K. muelleri*
Merulius lacrymans
Monascus albus, *M. anka*, *M. purpureus*
Monilia candida, *M. variabilis*
Monosporium silvaticum var. *macrosporum*
Mortierella candellabrum
Mucor adventitus var. *aurantiacus*, *M. alpinus*, *M. alternans*, *M. botryoides* var. *minor*, *M. brevipes*, *M. christianensis*, *M. circinelloides*, *M. corticolus*, *M. dimorphosporus*, *M. dispersus*, *M. dubius*, *M. erectus*, *M. fuscus*, *M. genevensis*, *M. griseocyanus*, *M. hiemalis*, *M. hygrophilus*, *M. janssenii*, *M. jauchae*, *M. javanicus*, *M. lamprosporus*, *M. lusitanicus*, *M. mandshuricus*, *M. plumbeus*, *M. prainii*, *M. pusillus*, *M. pyriformis*, *M. racemosus*, *M. ramannianus*, *M. rouxii*, *M. saturnus*, *M. sphaerosporus*, *M. spinescens*, *M. strictus*, *M. vallesiacus*
Mycoderma bordetii, *M. chevalieri*, *M. mandshuricus*, *M. tannica*, *M. vini*
Nadsonia elongata, *N. fluvescens*, *N. richteri*
Nematospora coryli
Neurospora sitophila (= *Oidium lupuli*)
Oidium lactis, *O. ludwigii*, *O. pullulans*, *O. suaveolens*
Penicillium brevicaulis, *P. divaricatum*, *P. echinosporum*, *P. fluitans*, *P. funiculosum*, *P. fuscoglaucum*, *P. glaucum*, *P. humicola*, *P. jensenii*, *P. lividum*, *P. luteoviride*, *P. luteum*, *P. mandshuricum*, *P. notatum*, *P. olivaceum*, *P. oxalicum*, *P. purpurogenum*, *P. raciborskii*, *P. roquefortii*, *P. roseopurpureum*, *P. spiculisporum*, *P. spinulosum*, *P. vinaceum*, *P. wortmannii*
Periconia felina
Pestalozzia sp.
Phycomyces blakesleeanus, *P. nitens*, *P. theobromatus*
Pichia alcoholophila, *P. farinosa*, *P. hyalospora*, *P. mandshurica*, *P. membranaceifaciens*, *P. rosa*
Polyporus destructor
Prototheca zopfii
Pseudomonilia mesenterica
Pseudosaccharomyces apiculatus, *P. muelleri*, *P. santacruzensis*
Pyronema sp.
Racodium cellare
Rhizoctonia mucoroides
Rhizopus acidus, *R. artocarpi*, *R. batatas*, *R. chinensis*, *R. chiuniang*, *R. delemar*, *R. formosensis*, *R. formosensis* var. *chlamidosporus*, *R. hangchao*, *R. japonicus*, *R. liquefaciens*, *R. nigricans*, *R. niveus*, *R. nodosus*, *R. oryzae*, *R. peka*, *R. pseudochinensis*, *R. reflexus*, *R. salebrosus* var. *instratus*, *R. tonkinensis*, *R. tritici*, *R. usamii*
Saccharomyces agglutinans, *S. anamensis*, *S. awamori*, *S. bailii*, *S. bayanus*, *S. carlsbergensis*, *S. cartilaginosus*, *S. cerevisiae*, *S. chevalieri*, *S. dairensis*, *S. ellipsoideus*, *S. exiguus*, *S. fragilis*, *S. hansenii*, *S. intermedius*, *S. lindneri*, *S. logos*, *S. mandshuricus*, *S. marxianus*, *S. monacensis*, *S. mongolicus*, *S. muntzii*, *S. paradoxus*, *S. sake*, *S. thermantitonum*, *S. turbidans*, *S. unisporus*, *S. validus*, *S. vordermannii*, *S. willianus*
Saccharomycodes ludwigii
Sachsia suaveolens
Schizosaccharomyces asporus, *S. formosensis*, *S.*

formosensis var. *akoensis*, *S. formosensis* var. *tapaniensis*, *S. mellacei*, *S. octosporus*, *S. pombe*, *S. santawensis*, *S. taito*, *S. vordermanii*
Schwanniomyces occidentalis
Sclerotinia demissa
Scopulariopsis rufulus
Sordalia fimicola
Spicaria aphodii
Sporobolomyces roseus, *S. salmonicolor*, *S. tenuis*
Stachybotris alternans
Syncephalastrum cinereum, *S. racemosum*
Thamnidium chaetocladioides, *T. elegans*
Thermoascus aurantiacus
Thielavia basicola
Torula aerius, *T. albida*, *T. aurea*, *T. candida*, *T. colliculosa*, *T. corallina*, *T. fermentati*, *T. flava*, *T. flavescens*, *T. galactosa*, *T. gelatinosa*, *T. luteola*, *T. minuta*, *T. rubescens*, *T. rubra*, *T. rubra* var. *α*, *T. rufula*, *T. sanguinea*, *T. utilis*
Torulasporea delbrueckii, *T. fermentati*, *T. rosei*
Torulopsis albus, *T. liquefaciens*, *T. nadaensis*
Trichosporium terrestre
Umbellomyces tetrasporus
Verticillium glaucum
Willia anomala, *W. belgica*, *W. javanica*, *W. odesa*, *W. productiva*, *W. saturnus*, *W. schneegii*
Zygopecthia chevalieri var. *fermenti*, *Z. farinosa*
Zygorhynchus dangeardi, *Z. moelleri*, *Z. vuilleminii*
Zygosaccharomyces barkeri, *Z. bisporus*, *Z. chevalieri*, *Z. dairensis*, *Z. fermentati*, *Z. major*, *Z. mandshuricus*, *Z. mellis*, *Z. mongolicus*, *Z. nadsonii*, *Z. naniwaensis*, *Z. nukamiso*, *Z. pastorianus*, *Z. priorianus*, *Z. sake*, *Z. salsus*, *Z. soya*, *Z. vini*
Zyosaccharomycodes japonicus

2. 細菌・放線菌

Achromobacter aceris, *A. aquatilis*, *A. dendriticum*, *A. stutzeri*
Actinomyces thermophilus
Bacillus aerogenes, *B. aerosporus*, *B. batatas*, *B. coli*, *B. fluorescens*, *B. lactis immobilis*, *B. mesentericus*, *B. natto*, *B. prodigiosus*, *B. subtilis*, *B. vulgatus*
Bacterium aceti, *B. acetigenum*, *B. acetosum*, *B.*

xylum
Caseococcus sp.
Enterococcus sp.
Flavobacterium nadaense
Gastrococcus sp.
Micrococcus lutescens, *M. percitreus*, *M. perflavus*
Sarcina sp.
Streptothrix chromogena, *S. odorifera*

1. 学名は“Catalogue of the Collection of Type Cultures, 1930, The Central Laboratory, South Manchuria Railway Co.”ならびに「財団法人長尾研究所菌類目録, 昭和17年」の菌名を当時の学名のまま記載した。表記法は現代式に統一し、著者名および来歴の記載は省略した。
2. 欧米産ワイン酵母, ビール酵母, パン酵母など醸造用酵母の名が多数含まれていたが, それらの記名は省略した。

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日本の微生物株保存事業 2. 国内連盟とその活動 (上)

長谷川武治

1940年前後、すでに戦時下の日本では、物資生産手段として微生物工業への期待は大きく、カルチャーコレクションはその基盤として重要視された。しかしながら、これを備えるほどの研究施設は、いずれも生産研究の主要拠点であったから、コレクションだけの管理部門を設ける余裕などはなく、研究者は自分たちの研究テーマに必要な微生物株の収集管理と平行して、研究所のカルチャーコレクションの分担保存を義務づけられた。戦局がきびしくなり、研究関係者の軍務に召集される数が増えると、そのような管理態勢をとることさえ困難になった。これが民間に微生物株保存を事業目的とする研究機関設立の機運を生じた。1941年、東京に財団法人長尾研究所が、1944年、大阪に財団法人航空醸酵研究所ができて活動を始めたのは、いずれもそうした時代の要請によるものであった。しかしながら、これらの民間機関も、たとえば碧素（ペニシリン）研究会に参加して菌株の探索に当たるなどといったような生産協力研究を要求されたから、それらが保有していたカルチャーコレクションの維持管理が容易でなかったのは、他の諸機関と大差はなく、保存事業活動を進めるためには戦争の終結を待つほかはなかったのである。

I. 終戦直後の保存事業

1945年8月、太平洋戦争を最後にして第二次世界大戦は終結した。日本は連合国軍隊によって占領され、全土が東京丸の内の連合軍総司令部（GHQ）の間接統治下に置かれて、政府の各省局はその下部機関かのような体制になった。海外からの学術情報は文部省が窓口機関になってGHQからのニュースを流し、また国際的な対応も文部省を通じて行われた。戦争中の日本では入手することが困難を極めていた新しい科学情報は、こうした経路を中心に溢れんばかりに押しよせ、各国立研究機関はその対応に追われる毎日であった。一方、民間研究機関の方は、戦時体制の転換や整理という問題は抱えていたものの、比較的自由的な立場にあって本来の業務への復帰に努力していた。

長尾研究所（Nagao Institute, NI）は、理事長兼所

長の長尾欽弥氏が戦時下の政府の要請に対処してきたが、戦後は小南 清主任研究員が中心になってカルチャーコレクションの整備を進め、1950年に『財団法人長尾研究所培養保存菌株目録』を出版した。この年、のちに日本菌学会へと発展する「菌類談話会」が国立科学博物館にあった小林義雄博士の研究室で呱呱の声をあげた。1952年、長尾研究所では小南 清氏の所長就任と同時に機関誌“Nagaoa”を発刊、小林博士を主任研究員（兼任）に招いて菌類談話会と連携を深めながら、保存事業の発展に努めた。1950年代における同研究所の活動にはめざましいものがあったが、その後は経営が順調に伸びず、1971年に閉鎖した。日本における微生物株保存事業の先駆者として、長尾研究所の実績は惜しみても余りあるものがある。

航空醸酵研究所は、戦時中、内閣技術院が研究費を負担して研究業務を行ってきたから、終戦になって内閣技術院が解散すると、その存廃が問題になった。しかし武田長兵衛理事長は存続させることを決断し、所名を醸酵研究所（Institute for Fermentation, Osaka, IFO）に変更して文部省所管の財団法人になった。創立の時から、研究所は応用研究部門が主体で、保存事業部門は併設であった。所長は1946年に財団専務理事、佐藤喜吉博士が中澤亮治初代所長と交代して経営は順調であった。しかしながら、応用研究部門の発展が急で、公益法人としては主体であるべき微生物株保存事業部門を圧迫する傾向が強まった。1961年、研究所は応用研究部門を切離し、微生物株保存事業と基礎研究部門だけになり、所長を筆者が引き継いで、所名を醸酵研究所に改めた。IFO Research Communications（英文機関誌）の隔年発行は1963年に始まる。

終戦直後の1946年、研究所のカルチャーコレクションの整備担当を命じられた筆者は、保存微生物株の分類学的再検討に掛った。海外の保存機関から標準株を取り寄せて比較研究を行うなどということは、当時の内外の事情から到底できそうになかった。その多くは、前報に記した種類で、国内の各所に同じ株から出た系統株が存在していることはわかっていたので、それらができるかぎり集めて専門雑誌などの原著記事を標準

にして比較研究する方法を採り、1953年、ようやく“IFO List of Cultures”の第1版を出した。

こうした作業は、長尾研究所が『菌株目録』を出版した際にも行われたことは確かで、骨の折れる仕事であった。民間機関のこれらの作業の結果が、後に述べる日本微生物株保存機関連盟 (IFCC) の初期の活動方針に図らずも強い影響力を発揮することになったのは、当時、文部省が『国内微生物株総目録』(1953年)を出版したのとたまたまその時期が一致したからであって、作業を担当していたころの筆者には予想もつかないなりゆきであった。

II. 国際微生物株保存機関連盟始末記

この国際連盟は、戦争によって機能の低下を招いた各国の微生物株保存施設の回復と発展を支援する目的で、ユネスコやWHOなどとほぼ同じ時期に設立されたが、連盟の内部事情が災いし、活動が始まったばかりで解散した短命な機関であった。大戦末期、エジプトで発生したコレラなどの伝染病予防のために、戦後設立されたばかりの国際連合(国連)から協力を求められたスイス医学アカデミーは、病原微生物株の培養保存と分譲を任務とする Centre de Collections de Types Microbiens (CCTM) を1946年にローザンヌの衛生研究所内に設け、P. Hauduroy 教授をその責任者に任命した。この年、国連は国際保健会議を召集、「保健は世界平和と安全の基礎」との理念に基づく世界保健機関憲章を採択し、1948年、憲章の発効とともに世界保健機関 World Health Organization (WHO) を設立した。その本部はジュネーブに置かれたから、CCTM の業務に対し強力な支援組織が生まれたわけである。一方、国際科学連合会議 International Council of Scientific Unions (非政府間組織 NGO) のメンバーとして、国際的学術活動支援を担う国際生物科学連合 International Union of Biological Sciences 所属の国際微生物学者協会 International Association of Microbiologists (IAMB) は、戦禍を被った各国のカルチャーコレクションの機能回復のために、経済復興の支援と保健対策を目的に掲げてユネスコなどからの援助を受け、国際微生物株保存機関連盟 International Federation of Culture Collections of Microorganisms (IFCC) を創設した。IFCC の設立には第4回国際微生物学会議(コペンハーゲン、1947年)でのIAMB総会の承認が必要だったから、活動を開始したのはローザンヌにCCTM ができて1年経ったころになる。その運営を担当する常任委員会は、IAMB の命名

委員会によって指名された次のような陣容であった。

Prof. A. J. Kluyver (President), Dr. H. Aragão, Dr. R. E. Buchanan, Sir A. Fleming, Prof. P. Hauduroy, Prof. Th. Madsen, Prof. S. Mudd, Prof. E. G. D. Murray, Dr. R. St. John-Brooks (Secretary).

IFCC の加盟機関は、30カ国60機関に及び、そのなかには、米国のATCC、オランダのCBS、英国のNCTC など著名なカルチャーコレクションの名もみられた。事務局はHauduroy教授の申し出によってスイスのCCTMにおかれ、ユネスコおよびWHOからの助成金の配分額や各機関の活動状況を告知する年報の発行も1949年から始まった。このころから、連盟とCCTMとの複雑な関係が活動に影響しはじめ、常任委員会では運営方針がしばしば混乱してトラブルが絶えず、ついに英国のSt. John-Brooks博士(NCTC)が1951年にSecretaryを辞任した。連盟は事務局を米国ニューヨーク州衛生局付属研究所へ移し、同所のJ. M. Coffey 女史がSecretaryを代行したが、事態の改善は望むべくもなく、IFCCは1954年を最後に活動を停止してしまった。

ローザンヌのCCTMもユネスコから援助を受けて従来どおりの活動を続けることが困難になったので、Hauduroy教授はCCTMの任務を一般の保存微生物株に関する情報の提供と分譲の斡旋に切り換えた。機関の名称も同時に変更してInternational Center for Information on and Distribution of Type Culturesと称し、当面、スイス医学アカデミーとWHOの援助で活動を続けたが、Hauduroy教授の没後、1967年、後任者の任務辞退によって解散が決定した。

III. 日本微生物株保存機関連盟の誕生

先に述べたとおり、終戦直後の日本は連合軍総司令部(GHQ)の管理下に置かれていたから、IFCCの発足とその活動の状況はGHQから文部省へ伝えられ、それと同時にIFCCの年報が保存機関に届けられるようになり、これによって日本の微生物株保存事業は、急速に活性化の時代を迎えることになった。文部省の主導による国内連盟結成の動きは、1951年の早春、1通の文部省通達(写真1)によって始まった。以下引用の文章は1951年3月9日、文部省で開かれた「培養微生物株の保存に関する懇談会」における稲田清助大学学術局長の開会の挨拶を記録したものである。

「生物学・医学・農学・工学を通じて研究関係者が最も頭を悩ます問題の一つは研究の対象または材料としての微生物の株の培養保存の問題である。研究者が必

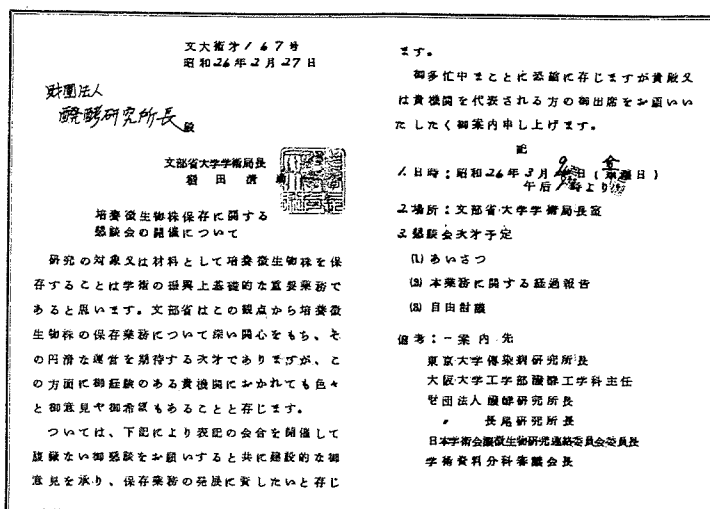


写真1

要とする研究材料を自由に得るためには、各自が必要とする微生物の株を培養保存しておればよいわけであるが、これは人員・施設・資材などの負担からとうてい不可能に近く、現在自分で研究している最小限度の株の保存においてすらこと欠くのがわが国の現状である。まして、いつ必要が起こるともわからない微生物株全部を研究者各自が培養保存していくのは及びもつかないことであるし、またいづれから見ても全く無駄なことである。

しかし、研究者各自が求める研究材料をその必要に応じて自由に得ることは、ぜひとも必要なことである。このことが研究を促進し学術を発達させる所以でもある。この役目を果たす機能は国内研究機関にとっても海外との学術交流の見地からも必要であると考えられる。微生物の株を培養保存するという自体は、きわめて地味な、いわば縁の下の力持ち的な仕事である。また相手が生物であり微細であるだけに、その培養管理に当っては、専門的で細心な技術と研究が必要である。さらに国内研究者へはいうまでもなく、海外の機関に対しても広く求めに応じて供給していくことは並たいていの仕事ではない。しかしながら研究者のこれに期待または依存するところはきわめて大きく、学術の振興に計り知れない明るい基盤を与えることは疑う余地はないと考える。

以上十分に意を尽し得ないが、要するに微生物の株を培養保存するという仕事は学術の基礎として重要な業務であり、さらに国家的見地からこの体制を整える

ことは、文化の国としても意義のあることと思う。文部省はこの視点から、各機関における微生物の株の培養保存の業務に深い関心を寄せると共に、発展を期待してやまない。さらに国家的見地から、その体制を整えるためには学界方面の事情も伺っておきたい。保存業務に経験のある機関として、東京大学伝染病研究所、大阪大学工学部醸酵工学科、財団法人長尾研究所、財団法人醸酵研究所に、また学界事情を伺う意味で日本学術会議微生物学研究連絡委員会にお集りをお願いし、文部省にはこのような問題を調査審議するために学術資料分科審議会が設置されているので、その会長に御足労をいただいた。どうか腹藏のない御懇談をお願いし、建設的な御意見を承りたい。」

この会合で微生物株保存連盟準備委員会が発足し、委員長に長谷川秀治東大伝染病研究所長が選ばれた。設立に必要な連盟規約案と役員人事については伝染病研究所と文部省双方から担当者を出して作業部会を編成、審議を重ねて連盟規約案を作成し、その名称を日本微生物株保存機関連盟とすることを決定した。1951年4月28日、関係者全員が伝染病研究所に集まって連盟規約案を承認し、下記の理事会役員を選出した。この日、連盟は誕生を迎えたのである。

i. 設立時の役員名簿

(以下、敬称略、五十音順)

理事長：田宮猛雄（日本学術会議微生物学研究連絡委員会代表）

常務理事：安東洪次（東大伝染病研究所代表）

理事：朝比奈泰彦（文部省学術資料分科審議会長），稲田清助（文部省大学学術局長），北村包彦（東大医学部代表），小南 清（長尾研究所代表），坂口謹一郎（東大農学部代表），佐藤喜吉（醸酵研究所代表），照井堯造（阪大工学部代表），藤野恒三郎（阪大微生物病研究所代表）

注，東京大学伝染病研究所は，1967年，同大学医科学研究所と所名を変更した。

ii. 日本微生物株保存機関連盟規約

1951年4月28日制定

1953年5月20日改正

（組織，名称，事務所）

第1条 本連盟は，日本国内で微生物株の収集および保存業務を行っている機関をもって組織し，日本微生物株保存機関連盟 Japanese Federation of Culture Collections of Microorganisms (JFCC) と称する。本連盟の事務所は，東京大学伝染病研究所内に置く。

（目的）

第2条 本連盟は，加盟機関相互の協力によって，微生物株の収集および保存業務の円滑な遂行をはかり，その発達に寄与することを目的とする。

（事業）

第3条 本連盟は，前条の目的を達成するために，次の事業を行う。

- (1) 微生物株の収集および保存に関する連絡。
- (2) 微生物株の保存および同定に関する技術上の連絡
- (3) 微生物株の交換および頒布上の連絡
- (4) 微生物株に関する講演会および研究会の開催
- (5) その他本連盟の目的を達成するために必要な事項

（評議員および評議員会）

第4条 本連盟に評議員をおく。評議員は，各加盟機関を代表できる者，日本学術会議微生物学研究連絡委員会委員長および文部省大学学術局長をもってこれに当てる。評議員は評議員会を組織する。理事長は評議員会を年1回，定時に招集する。評議員会は本連盟の意志決定の任に当る。

（理事および理事会）

第5条 本連盟に理事5名以上7名以内をおく。理事は評議員会で互選する。理事の任期は2年とする。ただし再選を妨げない。理事は理事会を組織する。理事会は，評議員会の決定に従い，本連盟の運営の任に当る。

（理事長および常務理事）

第6条 本連盟に各1名の理事長および常務理事をおく。理事長および常務理事は，理事の互選によってこれを定める。理事長は会務を統括し，本連盟を代表する。常務理事は，理事長を補佐し，理事長に事故があるときはその職務を行う。理事長および常務理事の任期は2年とし，重任を妨げない。

（幹事）

第7条 本連盟に若干名の幹事をおく。幹事は理事長がこれを依嘱する。幹事は理事長の命を受けて会務を処理する。

（専門委員および専門委員会）

第8条 本連盟に専門委員をおくことができる。専門委員は，理事長が加盟機関の職員中適当な者より委嘱する。専門委員は専門委員会を組織する。専門委員会は，第3条にかかげる事業のうち，専門的事項について調査審議する。

（顧問）

第9条 本連盟に顧問若干名をおく。顧問は，学識経験のある者のうちから，評議員会が選定し，理事長がこれを委嘱する。

（経営）

第10条 本連盟の経費は，加盟機関の分担金，寄付金その他の収入をもってこれに当てる。加盟機関の分担金は評議員会がこれを決定する。

（会計年度）

第11条 本連盟の会計年度は，毎年4月1日より翌年3月31日までとする。

付 則

第12条 新しく加盟を希望する機関で，第1条の規定に該当するものは，評議員会の承認を経て加盟することができる。

iii. 加盟機関と規約改正後の役員その他

1955年5月11日現在（敬称略，五十音順）

A. 加盟機関（略称記号）

大阪大学工学部 (OUT)・同微生物病研究所 (RIMD)・東京大学医学部 (MTU)，同農学部 (ATU)・同応用微生物研究所 (IAM)・同伝染病研究所 (IID)・財団法人長尾研究所 (NI)・農林省家畜衛生試験場 (NIAH)，財団法人醸酵研究所 (IFO)・広島大学工学部 (HUT)・北海道大学農学部 (AHU)

B. 顧問

朝比奈泰彦・安東洪次・斎藤賢道・中澤亮治・長西廣輔

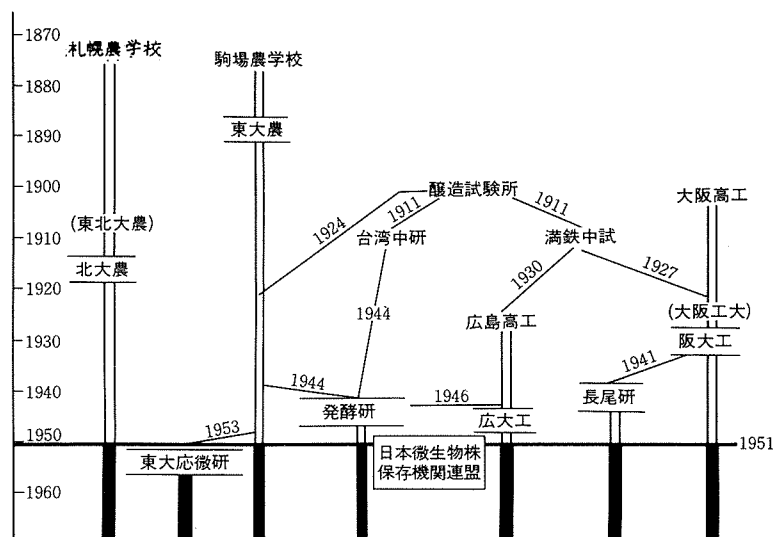


図1 非病原関係加盟機関の系譜

C. 役員

理事長：小南 清 (NI)

常務理事：工藤正四郎 (IID)

理事：坂口謹一郎 (ATU)・佐藤喜吉 (IFO)・照井堯造 (OUT)・藤野恒三郎 (RIMD)

評議員：朝井勇宣 (IAM)・石井 進 (NIAH)・稲田清助 (文部省大学学術局長)・北村包彦 (MTU)・佐々木西二 (AHU)・田宮猛雄 (日本学術会議微生物学研究連絡委員長)・馬場為二 (HUT)

D. 幹事

飯塚 廣 (IAM)・小田雅夫 (OUT)・小林義郎 (IID)・常松之典 (IID)・長谷川武治 (IFO)・百瀬静男 (文部省大学学術局)

E. 専門委員

飯塚 廣・小田雅夫・小南 清・工藤正四郎・佐藤和男 (IID)・高橋吉定 (MTU)・根平武雄 (HUT)・長谷川武治・藤野恒三郎・村瀬信雄 (NIAH)

注1. 東京大学応用微生物研究所(1953年設立)は1993年, 分子細胞生物学研究所となった. 前報(p. 7)の1994年は誤りにつき訂正する.

注2. 非病原関係の加盟機関については前報ならびに図1を参照のこと.

IV. 保存微生物株の調査と整理

文部省は JFCC の設立と同時に, 各大学を初め全国

の微生物学関係研究機関が保存する既知微生物株の所在について調査し, 144 機関, 251 研究室から 22,300 余株の回答を得た. この調査結果は財団法人長尾研究所の小南 清所長が担当して, 学名, 保存番号, 来歴など必要な記載事項を整理し, 1953 年, 『国内微生物株総目録』と題して文部省が出版した. 編者であった小南所長は, その序文のなかで次のようにことわっている.

「これらの伝えられ, 保存された微生物株は必ずしも正しいもののみとはいえない場合がある. すなわち, 微生物自体が雑菌の汚染によって, 全く異なった種類となってしまうたり, 移植をたび重ねるにしたがって胞子形成能や代謝物質生成能に変化を及ぼすことが往々あるからである.」

前述の民間機関による調査結果から, 戦争の時代を通過した日本の保存微生物株は管理担当者が軍務に召集され, また培養資材の入手難などが原因したために, 検討すべき点を多く含む資料であることが明らかになっていた. 小南発言はその事実と結果の具体的な指摘であった. 同年の JFCC 総会は, 次のような活動方針を決議として議事録にとどめている.

「文部省より今般『国内微生物株総目録』が刊行されたのを機に, 本年度より菌株保存責任分担の範囲を定め, その整備を図っていく. 専門委員は病原関係 (第1部) および非病原関係 (第2部) の小委員会を編成し, 逐次責任分担を決めていく. 小委員会は適宜連絡をとり, 必要に応じて合同の専門委員会を開催する.」

この決議に基づき、小南理事長、安東常務理事を中心に、『国内微生物株総目録』に記載された微生物株の分類学的再評価と整備のための研究班編成の準備委員会は速やかに発足した。こうして文部省科学研究費総合研究「国内保存微生物株の分類および整備に関する研究」が、坂口謹一郎東大農学部教授を代表者とし、JFCCの内外から広く研究者の参加を得て1954年度から開始された。総合研究は6年間継続して行われ、これに参加した研究者の数は50名を超えた(7)。研究費申請は2期に分けられ、後期3カ年は朝井勇宣東大応用微生物研究所教授が代表者になった。総合研究の成果は『JFCC Catalogue of Cultures 1962』として飯塚幹事が連盟から出版した。なお、このカタログのadditional editionが1966年と1968年に出版された。

V. 連盟組織の変革 (その1)

1951年9月、サンフランシスコにおいて、連合国側諸国と日本との間で講和条約が締結され、日本は連合国軍の管理下から解放されることとなった。これは同時に、日本政府の各省局の執務体制が大きく変わることの意味した。1956年、文部省福田局長からJFCCの役員を辞退する旨の申し出があり、翌年の総会で承認された。同総会で改選されて理事長に就任した坂口謹一郎教授の提案によって連盟は理事、評議員を廃止し、加盟機関による会員制を採用することになった。理事長、常務理事に替って会長、副会長が置かれることになり、任期などは第6条に従い、坂口教授と工藤教授がそれぞれ会長と副会長に選ばれた。幹事の定数は4名になり、飯塚 廣、常松之典、百瀬静男の各博士と筆者が指名された。専門委員は廃止された。なお元理事長田宮猛雄東大名誉教授が新たに顧問に加わった。

VI. 坂口謹一郎博士の国際活動

JFCC会長の任期は、規約によって2年であったから、坂口会長は1959年に退任、後任の会長には朝井教授が選ばれ、工藤教授の副会長再任が決まった。坂口教授は1958年、定年を迎えて東京大学を退職、同大学名誉教授になるとともに、日本ユネスコ国内委員会自然科学小委員会委員(微生物分科会主査)に就任した。1962年、保存微生物株整備の総合研究の完了を見とどけた坂口博士は、「微生物の研究の促進」を日本政府の提案としてユネスコ自然科学事業への採用を求め、手続きをとった。この提案の内容をユネスコ国内委員会議事録から要約して次に掲げる。

「最近におけるライフサイエンスの研究のなかで、微

生物はきわめて重要な役割を果たしており、その研究成果の各種産業への応用は、人類福祉の増進、特に新興国の社会経済的発展に寄与するところが大きいから、ユネスコが次の事業を実施して微生物の研究を促進することを要請する。

(1) 微生物株の保存と交換ならびに研究情報の交換のための国際組織を育成すること。

世界の各地域における調査研究の目的に従って、課題別または地域別に各種の微生物株が集められ保存されて、研究従事者の要請に対し、即応的に提供されるのでなくてはならない。

(2) 微生物資源の開発と微生物研究者の養成を促進すること。

乾燥地帯や湿性熱帯なども含め、開発途上国に対してユネスコが支援してきた諸事業および産業の振興に貢献する点を考慮に入れ、各地域に専門家または調査団を派遣して微生物資源の調査研究を行うとともに、加盟国の調査研究を技術的および財政的に援助する。また各地域の微生物研究者を養成する目的で専門家を派遣し、あるいは地域の研究者にフェローシップを提供して海外で研究させる。」

日本政府の提案は、第12回ユネスコ総会で採択され、1965年度から実施される国際連合の「開発の10年」計画に組み入れられることとなり、“Microorganisms Decade”と命名された(日本ユネスコ国内委員会議事録)。

1962年はモントリオールで第8回国際微生物学会議が開催された年である。開催母体は国際微生物学会連合 International Association of Microbiological Societies (IAMS)で、元は国際微生物学者協会(前出)と呼ばれた国際生物科学連合の下部組織であった。会議を主催したのは National Research Council of Canada (NRC)で、その Post Congress として、NRCに所属する Canadian Committee on Culture Collections がオタワにおいて Specialists' Conference on Culture Collections を開催した。この会議では、226名(28カ国)の専門家が出席、微生物学分野における culture collection の役割と国際的重要性を中心テーマとして論議が進められ、その結論として、IAMS がその下部組織に、Bacteriology, Mycology, Virology の各 Section と並ぶ Section on Culture Collections を設ける勧告案を議決した。新しい IAMS セクションは1963年に発足し、President にはオーストラリアの V.B.D. Skerman 教授 (Medical School, University of Queensland, ブリスベン) が就任した。

1965年, “Microorganisms Decade” が開始されて間もないころ, Skerman 教授は来日して坂口博士を訪ね, 微生物株保存事業の国際会議を日本で開くよう要請した。これに応じて坂口博士は, 当時の JFCC 会長, 飯塚 廣東大応用微生物研究所教授を引き合せ, 日本開催への協力を約束した。1966年, パリのユネスコ本部において, IAMS セクションとユネスコの合同会議が Skerman 議長, 飯塚副議長の下に行われ, 国際微生物株保存会議の日本開催が承認された。この決議を受けて翌1967年1月, 坂口博士(日本ユネスコ国内委員会自然科学小委員会代表), 越智勇一麻布獣医科大学学長(日本学術会議微生物学研究連絡委員会代表), 柳田友道東大応用微生物研究所教授(ICRO/UNESCO Panel on Microbiology 代表, ICRO: International Cell Research Organization, ユネスコ特命委託機関), 飯塚教授(JFCC 代表)その他の関係者が合同会議を開き, 日本ユネスコ国内委員会と JFCC の共同主催によって1968年の夏に国際微生物株保存会議を, ユネスコの援助と IAMS の協力で開催することを決定, 次いで組織委員会の編成に入り, 委員長に越智教授が指名され, 委員28名の入選が行われた。その結果, 副委員長に日本学術会議微生物学研究連絡委員会から植村貞治郎東大応用微生物研究所教授が, 事務局長に飯塚教授がそれぞれ就任した。

VII. 微生物株保存事業と日本学術会議

組織委員会の編成に当たって, 日本学術会議が主導する形になったのには, それなりの理由があった。日本学術会議では, 遺伝学研究連絡委員会が1965年に策定した長期計画案のなかで共同施設としての gene bank 設立の必要性が指摘され, 微生物学研究連絡委員会その他の生物学関係委員会がこれに賛意を表して系統保存懇談会が翌年発足した。これと同時に「研究用生物系統株保存機構の整備について」という勧告が朝永振一郎日本学術会議会長から政府へ提出された(6)。朝永会長は, 勧告のなかでユネスコの「微生物の研究の促進」事業を引用し, 研究用微生物株保存利用機構として「微生物株センター」(仮称)設立の要望切なるを訴えた。勧告の基礎となった「微生物株センター」(仮称)案は, 飯塚 廣 JFCC 会長らが作成を担当し, 1967年には微生物学研究連絡委員会名でその第4次案が発表されていたのである。

VIII. 第1回国際微生物株保存会議

国際微生物株保存会議は, 1968年10月7~12日,

東京の高輪プリンスホテルにおいて行われた。52カ国から526名が参加, 海外からの出席者数は223名(国内から303名);世界の主要な微生物株保存機関の実務者と微生物学者が一堂に会して, カルチャーコレクションに関する技術上, 運営上の諸問題に焦点を合わせた熱心な討論が連日繰り広げられたのは, おそらくこれが最初であっただろう。閉会式の挨拶の中で, ユネスコ代表の A. Burgers 博士, ICRO 代表の J.C. Sénez 博士, IAMS セクション代表の Skerman 教授から JFCC 飯塚会長に対して謝辞が述べられた(4)。会議の経過と概要については Proceedings(4)のほか, 学術月報21巻12号(1969)に坂口博士の感想文と越智組織委員長以下関係者の報告が出ている(1)。決議(勧告)の主要な内容を以下に記す。

(1) カルチャーコレクションに関する国際連盟の設立のために準備委員会を組織し, 委員として飯塚教授(chairman), Sénez 博士, Skerman 教授および S. M. Martin 博士を指名する。

(2) 国際微生物株保存会議 International Conference on Culture Collections (ICCC) を今後も定時の間隔で継続開催する。

(3) IAMS Section on Culture Collections へ Special Training Course に関する要望調査を付託し, また ICRO に協力を求めて1970年に保存技術に関する Special Training Course を行う。

(4) 開催途上国の研究助成を担当する研究機関のためにユネスコ, WHO, FAO(国際食糧農業機関)へ援助を求める。

(5) 国際情報センターの設立を検討する。

(6) 培養株保存法標準化への共同研究その他。

以上の決議によって, 以後, この東京会議は第1回国際微生物株保存会議(ICCC-1)と呼ばれることになった。ただし, この点について全く問題がなかったわけではない。決議要旨の(1)にその名が出ている S.M. Martin 博士は, 1962年, モントリオールで第8回国際微生物学会が開かれたとき, 会議の主催者であった National Research Council of Canada (NRC) に所属する Canadian Committee on Culture Collections の Chairman として, Post Congress の1つ, Specialists' Conference on Culture Collections のオタワ開催を担当した。博士はオタワ会議をこそ ICCC-1 と呼ぶべきであると主張した。しかしながら, オタワ会議の開催母体は IAMS であったが, ユネスコの関与は全くなく, 今回の東京会議のようなユネスコ自然科学事業という性格は存在しなかった。ICCC の運営面で今後

ユネスコとの関係を大切にしていける必要を考慮すれば、東京会議を第1回とするのがよいという結論になった。

このICCC-1は、次に述べる世界微生物株保存連盟の設立の基礎となったきわめて重要な会議であるが、日本の微生物学分野にとっては初めての国際会議であった。この点から、実際に主催したのはJFCCであったにもかかわらず、予算などの都合でユネスコ国内委員会が主催者に加わり、また日本学術会議主導の組織委員会が設けられた。会議の主体はユネスコ自然科学事業であるのに、開催を主導したのはIAMS Section on Culture Collectionsであった。これらのことは、ICCCという国際会議がもつ多面性をそのまま現していた。組織委員会のなかでは、どちらかといえば客観的な立場で会議のなりゆきを眺めることができた植村教授(副委員長)は、その感想を次のように述べている(1)。

「1967年以来、ICCCの開催のための準備がなされたが、この会議の性格が十分に明確でないために越智微研連委員長をはじめ、関係者が悩まされた。特に『微生物の研究の促進』事業の内容が、i) 微生物株の保存と分譲の強化、ii) 微生物資源の開発、iii) Training Courseの開設とされているが、i) ii) はそれぞれ事業遂行のためのConferenceをもつことがおこなった仕事であり、遂行の担任にはIAMSの関係分科会が当たっているものの、ユネスコとの関係からICRO(国際細胞研究機関)に新しくPanel on Microbiologyを設けて、このユネスコ自然科学事業の実際の企画を担当させ、ユネスコに対してはこのPanelが実際の責任者である。しかし、ICROは、その組織機構が個人参加で、組織としては参加していないから、会議開催という事業を、もちろんし得ない。このような組織機構上だけでなく、この会議の内容も一半は事業的であり、他半は学術的であることや、会議の根本の目標が発展途上国あるいはその地域の文化向上にある点などがさらに問題を複雑にした。このような事情にもかかわらず、内外からの支援を得て非常な成功のうちに会議を終えることができたことは、私ども関係者としては何よりも喜びとしたい。この会議の成功で、わが国から提案された『微生物の研究の促進』事業も初めてその内容がいずれも実際の緒につきえたわけで、今後IAMSにとっても代表的な会議となると期待される。」

IX. 世界微生物株保存連盟の設立

ICCC-1の決議に基づき、微生物株保存事業に関す

る連盟設立準備作業部会がIAMSセクションの臨時委員会 *ad hoc* committee of the Section on Culture Collections of IAMSとして1969年に東京で開かれ、Skerman教授、飯塚教授のほかS. M. Martin博士(NRC, カナダ)、S.P. Lapage博士(NCTC, 英国)が出席した。この会議において新連盟をWorld Federation for Culture Collections (WFCC)と命名し、IAMS総会へ提出する連盟の規約案を作成した。

新しく発足する連盟の名称にWorld Federation for Culture Collectionsを選んだのは、この連盟が過去に存在したInternational Federation of Culture Collections of Microorganisms (IFCC)と無関係であることを表明する必要があったからである。この委員会では設立時の役員分担も協議され、次のとおり決定した(5)。

President : Dr. S. M. Martin
 Vice-President : Prof. H. Iizuka
 Treasurer : Prof. V. B. D. Skerman
 Secretary : Dr. S. P. Lapage

第10回国際微生物学会議は、1970年8月、メキシコシティで開かれ、会期中のIAMS総会に規約案が提出され、承認された。WFCCが新しくIAMSの下部組織に加わり、Section on Culture Collectionsはその業務のすべてをWFCCに託して解散した(5)。WFCCの日本語名は「世界微生物株保存連盟」が一般に用いられている。WFCCの会員は、次の3種類すなわちordinary member(通常会員)、affiliated member(機関会員)、sustaining member(維持会員)からなる。まず通常会員はカルチャーコレクションに関心を示したすべての個人に与えられる資格である。機関会員は連盟総会が定めた基準によって入会を許可された保存機関、維持会員は連盟設立の趣旨に賛同してその活動を支援する個人または団体を遇する資格である。機関会員は保存機関の責任者またはこれに準ずる個人によって代表される。維持会員は投票権を除いてあらゆる権利と特典を連盟から与えられる。

WFCCのもう1つの特徴はWorld Data Center (WDC)の併設であった。保存微生物株の所在を国際的規模で明らかにしておくことは、研究者にとって計り知れない利益を生むものである。1966年のパリ会議でもこの問題が取りあげられ、IAMS Section on Culture Collections (Skerman教授)は“World Directory of Collections of Cultures of Microorganisms”作成のために調査を行うことを承諾した。必要経費はユネスコその他が負担し、カナダのNRC (Martin博士)

とオーストラリアの Queensland 大学 (Skerman 教授) が実務を担当することなどが決まった。翌年、ローザンヌにあった International Center for Information on and Distribution of Type Cultures の責任者から閉鎖の申し出を受けた IAMS は、Skerman 教授と Martin 博士を同所へ派遣した。引継ぎの事務処理を済ませた両氏はジュネーブ WHO 本部で IAMS を代表して合同会議を開き、“World Directory”の出版など時代の要請に対処できる新しい情報センター構想を提案して承認を得た。その結果、ユネスコおよび WHO の資金提供によって World Data Center が Queensland 大学医学部に設けられ、1972 年に“World Directory of Collections of Cultures of Microorganisms”第 1 版が出版された。World Data Center は WFCC の部局と考えられていたので、1975 年以後、同センターが事務局を兼ねることになり、会費の徴収とともに WFCC Newsletter の発行も行われるようになったが、1986 年、同センターの日本移転を機に事務局は分離することになる。

X. ICC-1 と JFCC

WFCC の設立など ICC-1 の開催から得られたものは決して小さくはないが、JFCC の側に立ってみることも必要であろう。1970 年の国際微生物学会議では総会に先立ち、連盟規約案説明のために開かれた Section on Culture Collections 分科会の席上で、ICC-1 および連盟設立準備委員会の開催、さらに連盟への要望調査に関する援助も含め、JFCC の果たした貢献について Skerman 教授から報告があり、JFCC に対する感謝の決議が満場一致で可決された(5)。財政上の苦しみはすべてのカルチャーコレクションに共通の悩みであるから、JFCC に対する関心が高まったのは当然のことであった。JFCC にならって国内連盟を結成する動きが生じ、1973 年には英国で United Kingdom Federation for Culture Collections (UKFCC) の発足があり、1975 年、米国にも United States Federation for Culture Collections (USFCC) が誕生した。共同体組織が与える微生物株保存事業発展への効果を JFCC に認めたということであろうか。

会議中の Special Meeting において、事業の共通の問題が、さまざまな視点で捕えられたのは大きな収穫であった。たとえば保存機関が抱える問題では、保存微生物株の正当性とこれを支える保存技術の問題点、保存担当者が保存機関の経済的条件の下にあってどのようにその責任を果たすべきであるかという課題、あ

るいはまた、研究者からの専門的なコレクション供託の依頼に対してどのような対応をするべきであるかなどの点について論議が交わされた。なお関連する問題として有償配布も取りあげられた。保存株の有償配布は保存機関の運営上からばかりでなく、株に対する責任保証の点からも当然のことであるが、当時の日本ではあいまいな状態で放置されていた。戦後の一時期、長尾研究所が有料で配布を行ったことがあり、また JFCC の 1952 年度総会の議事録にも「頒布は有料とし、実費を徴収するものとする」との申し合わせ事項が記されている。しかしながら、実状はどうであったかということ、総合研究が終わって『JFCC Catalogue of Cultures, 1962』が出版された後でも、保存株分譲に当たって料金の徴収は行われなかった。その理由は、長尾研究所、発酵研究所を除けば、加盟機関はいずれも国立機関なので、分譲料金収入を処理する経理面での手続きのための規定がなく、実施が困難だったからである。したがって民間機関としても、連盟の一員である以上、有料化には慎重にならざるを得なかった。

Special Meeting (最終日) にカルチャーコレクションの管理と経営に関する panel discussion があって、有償配布が話題に上った。S.P. Lapage 博士 (NCTC) から日本はどうなっているかと質問され、筆者は苦しい回答を強いられた。(proceedings(4), p. 236)。その当時、保存機関から出ていたカタログに記された 1 株当たりの価格は、ATCC (米国) で標準が 25 ドル、教育機関には 15 ドル、ただし特定の教材セットにはさらに割引がある (1 ドル=360 円)。CBS では 40 ギルダ、ただし学校など公共機関には 15 ギルダ (1 ギルダ=100 円)、英国では 3.3 ポンド、外国機関には 5 ポンド、教育機関などには割引料金 (1 ポンド=850 円) などとなっていた。有償配布が国際会議の話題になった以上、国内事情にとらわれてあいまいなまま放置すれば、国際交流にも支障を生じる恐れがあるので、発酵研究所は独自の判断で CBS の価格に合わせることにし、1969 年度から原則的な有料化を実施、1972 年発行の IFO List of Cultures (第 5 版) に初めて価格を明記した。

以上、JFCC の側に立って ICC-1 の東京開催が内外に与えた影響をまとめてみた。以上のほかには、JFCC への問題提起という点で特に重要なことはなかったが、会議のなかにいろいろと示唆に富む内容があったことをつけ加えておく(1, 3)。

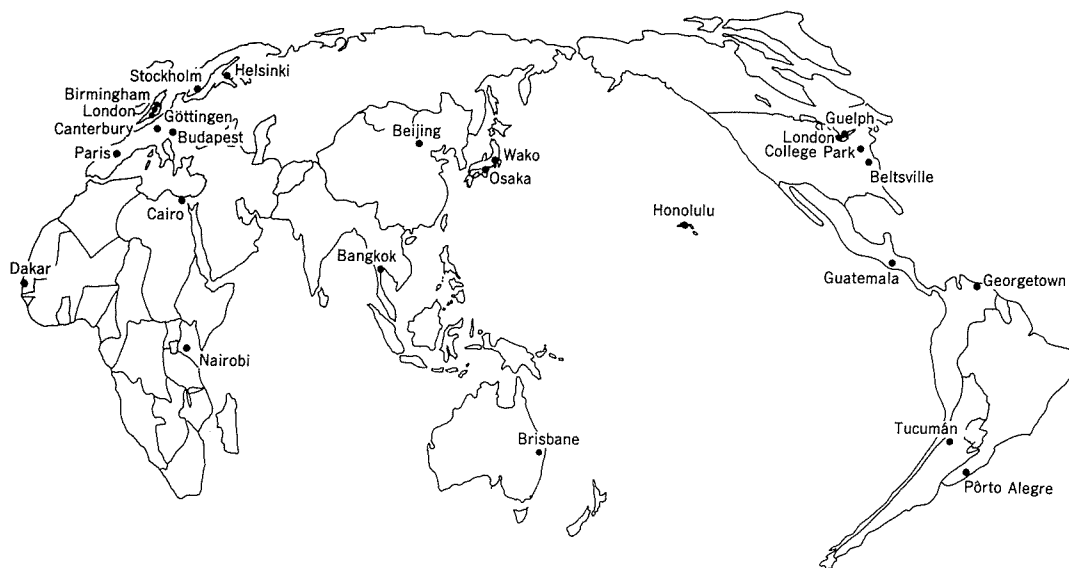


図2 MIRCENs (Microbiological Resources Centres)

XI. ユネスコ自然科学事業のゆくえ

国際微生物株保存会議ならびにこれに続く世界微生物株保存連盟の設立は、1965年に始まったユネスコ自然科学事業の一部をなすものであったが、これらに対してJFCCの果たした貢献が小さなものでなかったのは、この事業の提案国が日本であったことを考えれば、むしろ当然であったというべきであろう。以下、事業の進展について簡単に述べておく。

開発途上国における微生物の利用はそれぞれの国に与える経済的効果が期待できるから、先進国の技術協力による各地域での研究の振興と地域ごとのカルチャーコレクションの育成が、この自然科学事業の次の段階であった。開発途上国研究者の教育、その研究活動に必要なカルチャーコレクションの育成、バイオテクノロジーの分野でこれらに応える先進国の指導などを支援するために、ユネスコは世界の各地域にMicrobiological Resources Centre (MIRCEN) を設けて、先進国と途上国をそのネットワークで結ぶことを計画し、最初、そのモデル地域として東南アジアを選び、その地域センターにはバンコクを予定、指導は日本の担当とする方針を立てた。日本では、大阪大学工学部に微生物学国際大学院研修講座が設けられ、照井堯造教授を中心に、国内各大学の協力態勢を整えて、1972年度から東南アジア諸国を対象に研究生の募集を毎年行うことになった。その教科項目のなかで、微

生物分類学とカルチャーコレクションは東京大学応用微生物研究所(駒形和男教授)と財団法人発酵研究所(椿啓介博士、坂野勲博士、筆者)が担当した。

ユネスコ自然科学事業の拠点であるMIRCENは、東南アジアにつづいて、アフリカ、南アメリカへとその開発協力地域が拡大し、今日では先進諸国の協力によって世界の各地域に置かれている(図2)。日本では、大阪大学生物工学国際交流センター(大阪大学工学部)とWFCC World Data Center(理化学研究所)がMIRCENの指定を受けた。MIRCENの活動は生活関連分野で行われるが、食糧分野の1例として、生物学的窒素固定(BNF)による土壌改良支援のためのBNF-MIRCENの活動状況を表1に示す(2)。ユネスコのMIRCENはいずれもWFCCの機関会員になっている。

XII. まとめ

前報において、明治の初期に欧米から入ってきた微生物科学の発展に伴ってカルチャーコレクションが造成された経緯について説明した。本報では短命に終わった国際微生物株保存機関連盟(IFCC)の呼び掛けに応じ、文部省の主導でこれらのカルチャーコレクションを組織して作られた日本微生物株保存機関連盟(JFCC)が、その初期のめざましい活動と発展の結果、1970年の世界微生物株保存連盟(WFCC)の設立に重

表1 BNF-MIRCENの活動

A. *Rhizobium* Culture Collections at MIRCENS

MIRCENS	Number of strains
Bambey (Dakar)	50
Beltsville	938
Hawaii	2,000
Nairobi	208
Porto Alegre	650
	3,846

B. Distribution of Cultures of *Rhizobium*

MIRCEN	Number of Cultures	Recipient institutions in
Bambey (Dakar)	8	Gambia, Mali, Yemen
Beltsville	508	Zimbabwe, Nigeria, Yugoslavia, India, Spain, Vietnam, Ireland, Malaysia, England, Italy, Canada, Brazil, Mexico, Colombia, South Africa, Senegal, Egypt, Poland, Argentina, Turkey, W. Germany, Austria, Australia and New Zealand
Hawaii	200	Global
Nairobi	95	Uganda, Malawi, Tanzania, Mauritius, Sudan, Congo, Zaire, Rwanda
Porto Alegre	943	Argentina, Chile, Bolivia, Uruguay, Peru, Ecuador, Colombia, Venezuela, El Salvador, Dominican Republic, Mexico, U.S.A., Trinidad, Brazil

要な役割を果たすまでの過程をまとめた。

東京にあった連合軍総司令部による支配体制が図らずも文部省を動かしたことで、保存微生物株の国内における所在調査から、それら保存株の整理と再検討への速やかな展開に好ましい影響があったことは確かである。JFCCが戦後の不自由な学術事情のなかで誕生し、順調かつ円滑に活動してその基盤を固めることができたのは、願えば設立の初期に優れた指導者に恵まれた点も含めて、まことに幸運であったというべきであろう。

日本の提案によるユネスコ自然科学事業「微生物の研究の促進」と、その一環として東京で開催されたICCC-1によって、JFCCの名は国際的にも知られ、WFCCの設立に際して果たした貢献は高い評価を受けた。

WFCCの設立準備はIAMSが担当し、組織上は

IAMSに所属するが、その設立の動機はユネスコ自然科学事業にあったから、設立後もユネスコその他国連諸機関からの積極的な援助の下に活動するのが普通になった。特にユネスコのMIRCEN活動とは強い絆で結ばれることになる。

JFCCの会長として、ICCC-1の開催を担当した飯塚 廣教授は、1973年、Vice-Presidentを辞任後、WFCCからHonorary Life Memberの称号を贈られて、1992年(平成4年)3月18日、69年の生涯を終えた。また、日本政府へユネスコ自然科学事業、「微生物の研究の促進」の提案を建言して、開発途上国の応用微生物学的進歩、および世界と日本の微生物株保存事業に多大の貢献を果たした坂口謹一郎博士は1994年(平成6年)12月9日、97歳の天寿を全うして帰らぬ人となった。会誌第8巻第1号と第10巻第2号に師弟それぞれへの追悼の辞と略歴紹介がある。

表2 JFCC 会長・副会長一覧表 (その1)

1. 理事長	田宮猛雄
常務理事	安東洪次
	1951年(昭和26年)―1953年(昭和28年)
2. 理事長	小南 清
常務理事	安東洪次(1955年から工藤正四郎)
	1953年(昭和28年)―1957年(昭和32年)
3. 会長	坂口謹一郎
副会長	工藤正四郎
	1957年(昭和32年)―1959年(昭和34年)
4. 会長	朝井勇宣(としのぶ)
副会長	工藤正四郎
	1959年(昭和34年)―1963年(昭和38年)
5. 会長	工藤正四郎
副会長	飯塚 廣
	1963年(昭和38年)―1966年(昭和41年)
6. 会長	飯塚 廣
副会長	常松之典
	1966年(昭和41年)―1971年(昭和46年)

最後に、日本学術会議が政府に勧告した「微生物株センター」(仮称)案のその後であるが、予算その他の事情から、単なる試案にとどまって実施には至らなかった。しかしながら、内閣の科学技術会議の進言によって、政府は1970年代の国策として「ライフサイエンス推進事業」の開始を決定し、その支援部門の1つとして、1980年、「微生物系統保存施設」を理化学研究所に設置し、保存事業に参加させた。これに関する説明は次報へ譲る。1966年に日本学術会議会長が提出した勧告には、ほかに研究用高等動植物生殖質保存利用機構として「遺伝子センター」(仮称)案などが含まれていたが、これらもまた形を変えて「ライフサイエンス推進事業」のなかに生かされている。

本報の記述に当る期間のJFCC会長(理事長)、副会長(常務理事)の氏名および在任期間を表2に掲げた。

本報に述べたMIRCENに関する情報は駒形和男東京農業大学教授から頂いた。ここに記して厚く御礼申しあげる。

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日本の微生物株保存事業

3. 国内連盟とその活動 (下)

長谷川武治

第1回国際微生物株保存会議 (ICCC-1, 1968年)の東京開催とそれに続く世界微生物株保存連盟 (WFCC)の設立に関する, 日本微生物株保存機関連盟 (JFCC)の協力活動はJFCC創設以来の歴史的な大事件であった(3)。いうまでもなく, この国際的貢献は, 日本ユネスコ国内委員会および日本学術会議との強力なチームワークによるものではあったが, JFCCの将来性への期待を膨らませる効果があったことは, 昭和46年(1971年)度JFCC総会において将来構想が初めて議題として取りあげられた点に端的に表われていた。本報では, まずこの総会議事録を取りあげ, 当時の状況をかいまみることから稿を起すことにする。

I. 昭和46年度JFCC総会議事録

日時: 昭和46年5月26日

場所: 学士会館本郷分館

出席者(敬称略, 五十音順): 赤松 弘[○](東大応微研, IAM)・岩田和夫[○](東大医, MTU)・小寺健一(阪大微研, RIMD)・高田信男[○](阪大工, OUT)・田村俊秀 (RIMD)・常松之典(東大医科研, IID)・長谷川武治[○](発酵研, IFO)・堀三津夫[○](RIMD)・本間 遜[○](IID)・箕浦久兵衛[○](廣大工, HUT)・安田純一 (RIMD)・山里一英[○](IAM)・吉田 忠[○](北大農, AHU) [○]印は機関代表。

招待者: 宮山平八郎(文部省科学官)

議長: 飯塚 廣会長欠席のため, 常松副会長が議長を代行した。

本部報告(抜粋)

(1) 昭和45年8月, メキシコ市での国際微生物学会は, Section on Culture Collectionsの解散とWorld Federation for Culture Collections (WFCC)の設立を承認, WFCC役員として会長(President)にS.M. Martin, 副会長(Vice-President)に飯塚 廣, 庶務理事(Secretary)にS.P. Lapage, 会計理事(Treasurer)にV.B.D. Skermanの4氏を選んだ。なお, WFCC設立までのJFCCの援助に対して感謝の意が表された。

(2) 昭和45年10月13日の幹事会では,

WFCCへの加盟問題について, JFCCとしていかに対処すべきかが論議され, 加盟は各保存機関の自由意志に任せ, JFCCとしては加盟しないことになった。

議事(協議事項抜粋)

〔1〕 菌株保存について

(1) 菌株保存では, 素性の正しい株の専門別コレクション(specialized collection)を重視するべきで, 将来のJFCCは専門別コレクションのネットワークを考慮において再組織することが望ましく, 現加盟機関もそのような線で努力が望まれる。

(2) そのためには現実の問題として, 各機関の人員, 予算, 施設の不足の点が解決されなければならない。

(3) 加盟機関のうち, 阪大工, 同微研, 北大農, 東大医科研では文部省に対し, 昭和47年度に微生物株保存施設の要求を, また東大応微研は既存の施設の充実, 東大医は人員の要求を出す予定で, 個々の機関の要求をJFCCとしても支持すべきである。

〔2〕 微生物株センター案について

(1) 個別機関の充実と微生物株センターの設立との関係が問題になる。学術会議提案の株センターは, 現在まだ学術審議会資料部会での審議にかけられるだけの機が熟していない。高等生物種の保存については, すでに個別機関の充実の方針が決った。実験動物の系統保存も近い将来, 資料部会で審議される段階に来ている。

(2) 微生物株センター問題を審議の軌道に乗せるには, JFCCとして効果はともかく, 日本学術会議(微生物株センター設立準備委員会), 関係学会, 関係官庁などへのはたらきかけをすることはできよう。JFCC加盟機関のなかには, 微生物株センター案に強く反対の向きもある。しかしながら, 多くの機関は基本的な態度として微生物株センターの将来の設立は好ましいと考えている。より重要なことは, まずセンターの下部機構となる

べき機関の整備と充実を早急に実現することである。個別機関の充実と株センターの設立は、二者択一ではないと理解する。

(3) 文部省は、恒久的な系統保存機関は施設として承認するが、一代かぎりの場合とは区別して特別事業費でまかなうことを一般方針とする。微生物株の保存については、将来計画の青写真、保存機関の優先順位、基準などが明確でないと各機関からの予算要求の取扱いが困難になるだろう。

[3] JFCC の将来計画について

(1) 社団法人の学会とする。個人会員、機関会員、賛助会員を設け、それぞれより会費を徴収し、理事会によって運営する。研究発表講演会と機関部会を開催する。現在は機関の加盟だけであるが、将来は個人加入を主体とし、WFCC の組織と類似した組織にする。

(2) 学会が困難であれば協会的なものにし、経費は協賛者の寄付によりまかなう。

(3) 株の有償配布は、国立大学学部の教室は事実上実施が困難で、関係財団あるいは(1)、(2)の中央的機関に有償配布を委託することは一層困難である。しかし大学付置研究所や省庁直属の研究所の有償配布はそれほど困難ではないだろう。

(4) 将来計画は、JFCC の内部で最初に原案をつくるとしても閉鎖的ではなく、関係諸機関、学会、学識経験者などと連絡をとり、それらの意見との調整をはかりつつ計画を固めていくべきである。

[4] 将来計画委員会について

上記の論議の後、結論として、従来の将来計画委員会を解散し、常松之典氏(東大医科研)を委員長に選び、非病原、病原関係を一本とした将来計画委員会をつくり、従来の JFCC の機構にとらわれないで前向きな方向で将来計画を検討し、かつそれらをふまえて各加盟機関の充実という当面の問題の対策を作成することになった。

[5] 会長、副会長の改選

常松氏が会長に推薦されたが、同氏より飯塚現会長欠席でもあり、国際関係のこともあるので、飯塚氏と相談の上決定したいと発言があり、了承された。

[6] WFCC の membership その他

安田氏より、藤野恒三郎氏(RIMD 前代表)からの質問状に対する Skerman 氏の返事の内容が参考意見として紹介された。

WFCC の通常会員と機関会員にはそれぞれ個人および保存機関が加入する。JFCC が参加するのであれば維持会員が適当であろう。保存機関の資格としては、個人的な意見であるが、①10年以上の実績、②経験5年以上の微生物学者の存在、③設備の程度、④リストの発行、⑤保存株交換の実績、⑥保存状況向上への努力、を条件としたい。

付表 1. 昭和45年度 JFCC 会計報告

2. 昭和45年度保存機関報告

以上が昭和46年度 JFCC 総会議事録の要点である。この当時、会員の大半は国立大学所属機関であったから、この総会には文部省科学官、宮山平八郎氏の出席を求め、微生物株保存事業について大学関係の保存機関がもつ問題点を会員が質問し、それに回答してもらった。その大要は議事録に記されているとおりで、大学だけでなく一般の国立機関も含め、保存機関の性格によって生じる保存事業の限界にも及んでおり、保存機関として考慮し対処すべき問題への示唆を含むものになった。会員から出た改善の要望に関する大学機関の状況について、文部省大学学術局、石川秀夫氏の「大学等における系統保存事業の現状と問題点—微生物株を中心に」と題する論文(5)のなかに具体的な説明があるから、これを抜粋して紹介する。まず最初に昭和47年(1972)年度微生物系統保存費・特別事業費の一覧表が同論文に出ているので、これを表1に掲げた。なおこの表には以下の説明が添えられていた。

「……現行の文部省系統保存事業費のありかたを再検討する必要がある。現在、学術審議会学術資料分科会において、系統保存事業全体に対するありかたを検討している。昭和48年半ばには改善策などが報告書にまとめられるであろう。」

国立大学での系統保存事業の問題点について、同氏は次のように述べている。

(1) 微生物株は培養をくり返しているうちに変種が現われやすく、ただ機械的に培養することも危険である。それに要する人件費、経費、場所などの面から困難があり、微生物の種類を一定時期に整理する必要がある。

(2) 系統保存費が少ないため、研究者の研究費からの持出しが多く、かつ労力の面からも系統保存活動に多くを割かれ、研究活動に支障をきたし、あるいは保存系統を制限せざるをえない状態に追い込まれている。

表1 昭和47年度微生物株保存に関する系統保存費ならびに特別事業費

		(千円)
1. 系統保存費		
北海道大学農学部	菌株保存	466
東北大学農学部	好気性細菌等保存	300
東京教育大学農学部	麦角菌株保存	300
東京大学農学部	菌株保存	620
〃	遊離窒素固定菌株保存	502
〃	レプトスピラ保存	846
東京大学医学部	病原菌株保存	1,019
信州大学繊維学部	フザリウム菌株保存	200
大阪大学工学部	菌株保存	374
広島大学工学部	菌株保存	316
小計		4,943
2. 特別事業費		
千葉大学腐敗研究所	腐敗菌株保存	413
東京大学医科学研究所	病原微生物菌株保存	1,481
東京大学応用微生物研究所	有用微生物菌株保存	1,370
〃	有用藻類保存	1,777
小計		5,041
3. その他		
国立遺伝学研究所	細菌突然変異株保存	889
計		10,873

(3) 国の内外の研究者との情報および資料の交換が急激に増えつつあり、かつ系統供与の希望者の増大のため、これに多くの時間と経費と労力を要するようになってきた。

(4) 微生物株の培養、保存それに分譲、交換という業務は激務なため、下働きをする人を探しても少なく、従事してもすぐやめていく人が多く、したがって研究者自身に負担が掛ってくる。またアルバイトを探しても、そのための人件費は系統保存費、特別事業費、または講座の研究費でまかなわれているため、最近のように物価高、人件費高の状態では、他の研究に要する費用などにまでしわ寄せが来るようになってきている。最近の最も深刻な問題は、せっかく貴重な微生物株を所有していても、この菌株を培養、保存し、研究を継続していく後継者がいなくなる場合があって、現研究者かぎりて事業を閉鎖せざるを得ないものもある。

以上で石川氏の論文の引用を終えるが、議事録に記された大学からの要望を理解するに足る当時の事情を

この論文からくみとることができる。微生物株保存機関としてその任務を果たすのに必要な人員、施設の充実を求める会員の要望は切実に受けとめるべきものであり、日本学術会議から出された微生物株センター案への賛否両論もその真意は要望実現の可能性の模索とみるべきではなかったか。要するに、議事録の菌株保存とその将来計画の項で述べられている「専門別コレクションのネットワークを考慮においた再組織」も「微生物株センターの下部組織の整備と充実」もそうした会員の要望を無視しては成り立たないものであった。また微生物株センター論議から、連盟の今後の方針として、保存事業に関心を寄せる研究機関へ会員としてJFCCに参加することを広く呼び掛けるべきであるとの認識が連盟のなかに定着した。こうして連盟が抱えるさまざまな課題がようやくその姿を現し、連盟は再び変革への道を歩み始めることになる。なお、東京大学医学部(MTU)は、教授の交代によって教室の方針が変り、1983年、連盟から離脱した。

II. 連盟組織の変革 (その2)

新会員の募集のために、1973年、総会の了承を経て常松会長名で出された加盟案内にこたえて、新たに以下の機関が会員として連盟に加わった(五十音順、これらの名称は機構改革などによって、その後、いろいろと変更された場合が少なくない。新しい名称とその呼称略号はJFCCカタログを参照のこと。代表者名は敬称略。)

1. 群馬大学医学部薬剤耐性菌保存センター (IMRG, 代表者: 三橋 進)
2. 京都大学ウイルス研究所 (IVR, 代表者: 植竹久雄)
3. 厚生省衛生試験所衛生微生物部 (NHL, 代表者: 倉田 浩)
4. 国税庁醸造試験所 (RIB, 代表者: 菅間誠之助)
5. 千葉大学生物活性研究所 (旧名: 腐敗研究所, IFM, 代表者: 新井 正)
6. 農林省農業技術研究所病理昆虫部病理科 (NIAS, 代表者: 富永時任)
7. 農林省林業試験場保護部 (FFPRI, 代表者: 青島清雄)
8. 理化学研究所動物薬理研究室 (IPCR, 代表者: 光岡知足)

昭和49年(1974年)度総会(常松会長)以後、名称は日本微生物株保存機関連盟から日本微生物株保存連盟に変更され、その英文名もWFCCにならって、Japan Federation for Culture Collections (JFCC)となった。名称の変更に合わせて連盟は個人会員制を採用、従来の会員を機関会員と呼ぶことにしたので、規約の改正が求められた。新規約は昭和52年度総会(長谷川武治会長)で審議承認された(表2)。従来の連盟を包んでいた保存機関の組分的色彩はなくなり、学会的性格を強めることになった。この年、発表された会員数は、個人会員104、機関会員18、なお、新規約に基づく依頼を受けて加盟した賛助会員は33社に及んだ。

III. 出版および学会活動

1977年、規約改正とともに連盟はニュースレターを年2回発行することを決めた。このニュースレターの名称は『JFCC Newsletter』と英文になっていたが、内容は必要な場合以外は和文で総会議事(報告、協議事項)、連盟の行事と活動の内容、関連する内外の学界事情などを主とし、編集事務局は、東京大学医科学研究所、同大学応用微生物研究所ならびに後述の理化学

研究所微生物系統保存施設が交代で担当した。ニュースレターは順調に発行が続き、1984年、初めて原著論文が掲載されたのを機に翌年からは会誌になり、誌名は『日本微生物株保存連盟会誌 Bulletin of the Japan Federation for Culture Collections』が採用された。新しい会誌の編集事務局は、日本微生物資源学会の発足時まで財団法人発酵研究所に置かれた。学会の発足によって1994年以降、会誌は誌名を『日本微生物資源学会誌(英文名, Microbiology and Culture Collections)』に変更された。ニュースレターの発行、会誌の刊行と続いて連盟の歴史は、1977年を境にようやく先史時代を脱して有史時代へ移ることになる。

『JFCC Catalogue of Cultures』は1968年に出るからその出版はしばらく途絶えたが、文部省科学研究費(B)の申請が認可になり、1977年度に「日本微生物保存株総目録刊行のための予備調査」(代表者: 池田庸之助東京大学応用微生物研究所教授)が始まり、1979年12月に第3版として刊行された。第3版となったのは、1966年と1968年に出た Additional edition を第2版に見立てたからである。この編集は池田教授を委員長として行われたが、その後は金子太吉博士(理化学研究所微生物系統保存施設室長)を委員長とし、1987年に第4版、1992年に第5版と刊行が続いた。なおこれらの編集内容について金子博士の報告がある(6)。

学会活動は他学会総会に参加して共催する形で始められた。1973年、日本細菌学会に設けられたカルチャーコレクション研究会(会長: 岩田和夫東大教授)に協力、学会の総会プログラムに掲げられた「第1回カルチャーコレクション研究会シンポジウム」の企画に参加し、以後も共催のサテライトシンポジウムとして例年継続して行うことになった。また、1977年度日本醸酵工学会(現・日本生物工学会)総会において、同学会と共催でシンポジウム「微生物の化学分類」を実施し、以後、総会時のシンポジウム共催が続いた(ニュースレター)。

JFCCは、会誌の刊行が始まった1985年以降、連盟の学会的行事を自主的に行うことになり、総会シンポジウム、化学分類セミナー、日本学術会議シンポジウム、ユネスコワークショップ、各種講習会など多彩な企画を発表実施した。これに関する説明は会誌各号に出ている。こうした活動はJFCCの学会への発展に一層の弾みをつけることになった。

IV. ライフサイエンス推進事業とJFCC

近年、バイオテクノロジーその他ライフサイエンス

表2 日本微生物株保存連盟規約

(1977年6月3日制定 1979年9月7日改正)

第1章 総 則	
第1条	本連盟は、日本微生物株保存連盟と称する。この名称の英訳は、Japan Federation for Culture Collections, その略号は JFCC とする。
第2条	本連盟は、日本国内で微生物株の収集、保存、交換もしくは配布などに関する事業に従事する機関または個人および本連盟の事業目的に賛同する個人または団体を以て組織する。
第3条	本連盟は、事務所を東京都内に置く。
第2章 目的及び事業	
第4条	本連盟は、会員相互の緊密な連絡と協力によって、微生物株の収集、保存、交換、配布などに関する事業の円滑な実施を図り、もって微生物学とその応用研究の進歩に寄与することを目的とする。
第5条	本連盟は、前条の目的にそって、次の事業を行う。 (1) 微生物株の収集及び保存に関する総合計画の樹立と推進 (2) 微生物株の収集、保存、交換、配布などに関する連絡及びあっせん (3) 微生物株の同定及び保存の方法などに関する研究上の連絡及びあっせん (4) 微生物に関する情報の収集、整理、保存、伝達 (5) 国外諸機関との連絡 (6) 微生物に関する研究会及び講演会の開催 (7) その他、目的を達成するために必要な事業
第3章 会 員	
第6条	本連盟の会員は機関会員、個人会員及び賛助会員の三種とする。
第7条	機関会員の入会は幹事会が推薦し、総会の議を経て決定される。 2. 機関会員は代表者1名を定め、これを連盟宛通知しなければならない。 3. 機関会員の代表者を変更した場合も同様とする。
第8条	個人会員として入会を希望するものは、所定の申込書を以て連盟宛申込みのものとする。
第9条	賛助会員は本連盟の趣旨に賛同しその事業を援助するため、所定の会費を納入した団体または個人とする。
第10条	会員が次の各号のいずれかに該当するときは、会員の資格を失う。 (1) 脱会の申出があったとき (2) 総会において会員の資格が失われたと認められたとき
第11条	個人会員および賛助会員は毎年会費を連盟に納めるものとする。会費の額は別にこれを定める。
第4章 役員及び顧問	
第12条	本連盟に、会長1名及び副会長1ないし2名
第13条	を置く。 会長及び副会長は、総会の議を経て決定される。
第14条	会長は本連盟の業務を総理し、この連盟を代表する。 2. 副会長は会長を補佐し、会長に事故があるときは、幹事会の議を経てその職務を代行する。
第15条	会長及び副会長の任期は2年とする。但し、再任を妨げない。 2. 補欠によって就任した会長及び副会長の任期は、前任者の残任期間とする。
第16条	本連盟に、幹事若干名を置く。
第17条	幹事は、会長が委嘱し総会の承認をえる。
第18条	幹事は、会長を補佐し、この連盟の会務を処理する。 2. 幹事会は会長、副会長及び幹事を以て構成する。
第19条	本連盟に監事1名をおく。 2. 監事は会長が委嘱し総会の承認をえる。 3. 監事は本連盟の業務及び財務を監査する。
第20条	本連盟に顧問を置くことができる。 2. 顧問は、会長が推薦し、総会の議を経て決定される。
第5章 総 会	
第21条	総会は、連盟の最高議決機関とする。
第22条	総会は、全会員の三分の一の出席によって成立する。但し、委任状を提出したものは出席とみなす。 2. 議決は、すべて出席会員の過半数の賛成による。
第23条	総会は、毎年1回定時に会長が招集する。会長が必要と認めたときは、臨時に総会を招集することができる。 2. 会長は、総会の議長となる。
第6章 特別委員会	
第24条	特別の事項を調査研究するため必要があるときは、この連盟に特別委員会を設けることができる。
第7章 会 計	
第25条	この連盟の経費は、会員の会費、寄附金その他の収入をもってあてる。
第26条	この連盟の会計年度は、毎月4月1日に始まり翌年3月31日に終る。
申し合せ事項	
1.	第17条の幹事は機関会員(代表者)、個人会員及び賛助会員のうちから選ぶものとする。
2.	第24条の特別委員会の一つとして、顧問、機関会員及び幹事よりなる保存機関連絡会議を設ける。
3.	事務所は東京大学医学研究所におく。

の発展とともに、生物資源ならびにそれに関連する情報の価値が欧米において広く認識されるようになったこともあって、わが国でも内閣総理大臣の諮問機関である科学技術会議にライフサイエンス部会が設けられ、1974年、新特殊法人としてライフサイエンス研究推進センターを設立する計画を発表、とりあえず理化学研究所にライフサイエンス推進部が設置されて実験動物や植物などの生物資源センターとその関連情報センターの整備の検討が始められた。その後、予算その他の事情から、新特殊法人設立の計画が「同センターの機能を果せるような組織を理化学研究所に置く」という方針が変わった1976年には、「理化学研究所ライフサイエンス研究情報室」が設置されることが決まった。微生物関連情報システムの整備については、ライフサイエンス推進部にJFCCが協力することになり、常松之典東大医科学研究所教授(JFCC副会長)を委員長とし、推進部が委嘱した5名の委員(理研側3名、その他2名)からなる専門部会が発足した(12)。

理化学研究所で行われるライフサイエンス推進事業において取り上げられた研究は、①老化制御の研究、②バイオリクターに関する研究、③人工臓器の研究、④知能機械に関する研究、⑤生物活性物質の探索の5課題であった。これらの研究の推進支援のために、理化学研究所は1966年に日本学術会議が政府へ提案した下記の3機構、

- (1) 研究用微生物株保存利用機構
—微生物センター(仮称)
- (2) 研究用高等動植物生殖質保存利用機構
—遺伝子センター(仮称)
- (3) 研究用純系動物保存利用機構
—実験動物センター(仮称)

を具体化することになり、1979年、「微生物系統保存施設」(和光市)とともに、「ライフサイエンス筑波研究センター」および「実験動物維持施設」(つくば市)の設立を決定した。これらの建設の進捗にあわせて、1981年、「ライフサイエンス研究支援業務運営委員会」が発足し、委員長に田村三郎理化学研究所招聘研究員(東京大学名誉教授)、委員には、理化学研究所ライフサイエンス研究顧問を委嘱された、田嶋嘉雄東大名誉教授(財団法人実験動物中央研究所長)と筆者(財団法人発酵研究所理事)、および光岡知理化学研究所動物薬理研究室主任研究員の3名の就任が決まった。微生物系統保存施設の構成主体として理化学研究所にライフサイエンス培養生物部が新設され、部長(兼任)に決定した駒形和男東大応用微生物研究所教授が施設長とな

り、同時にライフサイエンス研究情報室長を兼ねることになった。「理化学研究所微生物系統保存施設」はそのまま正式名となり、英文名は“Japan Collection of Microorganisms (JCM)”が採用され、1983年、『JCM Catalogue of Strains』の第1版が刊行された。

日本学術会議は微生物株センターをバンク的性格をもった公共事業体とし、国立大学その他、系統分類学などの基礎研究や人材養成を主目的とする系統保存部門と区別した。JFCCが保存事業発展の見地から、それを追認したことは、前掲の昭和46年度JFCC総会議事録に記されている。要するに微生物株を内外から幅広く集めて研究分野に提供することを主務とするサービスコレクションをセンターと位置づけ、系統保存事業を組織化する構想が基調となっていたことは明らかである。JFCCでは、従来から株の分譲配布を保存事業の一部として行ってきたが、各機関会員の間にこのような分業区分が存在したことはない。JCMも発足以来JFCCの機関会員となり、他の機関会員と同等の立場でサービスコレクションとして活動を続けているが、現在のところ、特にセンター的立場をとることはしていない。

V. WFCC への協力

さきに掲げた昭和46年度JFCC総会議事録のなかで、連盟としてはWFCCに参加しないという協議の結果を記述しているが、同じ議事録の末尾にWFCC会計理事、V.B.D. Skerman教授の意見を書き添えたために、この決議はあいまいなものになってしまった。発足5年目の1975年、WFCCが年間会費の徴収を開始した年にJFCCはその要請を受けて維持会員(sustaining member)になった。JFCC各会員のWFCCへの参加は、上記の議事録にあるとおり自由であった。したがって、機関会員の多くはWFCCの機関会員(affiliated member)を兼ね、また機関関係者その他は通常会員(ordinary member)として参加した。飯塚廣教授(1970~1973年)および筆者(1976~1981年)はWFCCの副会長(Vice-President)に選ばれ、理事(Board Member)として駒形和男教授(1981~1989年)、飯島貞二博士(1989~1992年)、中瀬崇博士(1992~1996年)、竹内昌男博士(1996年~)の順にWFCCの運営にかかわった。駒形教授は、一時期設けられた“Data Coding and Processing Committee”の議長(Chairman)に就任(1976~1981年)してから、情報部門などでの協力が続き、Skerman教授の退職を機として、1986年、World Data Centre

for Microorganisms (=World Data Center, WDCM, WDC) がブリスベン市 (オーストラリア) の Queensland 大学医学部から和光市の理化学研究所 ライフサイエンス研究情報室へ移されたとき、その Director を兼務することになった (7)。WDCM の Director は、Editor of Newsletter とともに、Member Exofficio (職責上の資格をもつ役員) として WFCC の理事会 (Executive Board) に加わることが定められている。1989 年、駒形教授のあとを受けてライフサイエンス研究情報室長となった菅原秀明博士は、同時に WDCM の Director の兼任とともに、“World Directory of Collections of Cultures of Microorganisms” (第 4 版, 1993 年) などの編集出版を担当した。同氏は 1996 年、国立遺伝学研究所生命情報研究センター分子分類研究室長に転出したが、WDCM のほうは Director を引きつづいて兼務することになったので、WDCM の業務も 1997 年 4 月以降、同センター (三島市) に移ることになった。

VI. 日本のサービスコレクション

JFCC の規約のなかで、微生物株の分譲業務について、創立時の規約第 3 条 (前報) には「交換および配布」とあるのに対し、新規約第 2 条に「交換もしくは配布」という表現を用いたのは、国立機関が大部分を占める機関会員に分譲配布を一律に義務づけるのは、機関それぞれの性格に基づく制度の上から必ずしも適切でないとの認識が生じたためであった。事実、病原微生物株には分譲規制があるし、民間会社などへの株の分譲は、特殊法人を除く国立機関の場合、経理面の処理に問題が起こる恐れがあるから、それぞれがもつ外部組織を通じて行っている機関もある。たとえば、系統保存を事業目的の一つにして 1953 年に設立された東京大学応用微生物研究所 (現・分子細胞生物学研究所, IAM) の場合であるが、保存事業部門として 1969 年に「有用菌株保存施設」が設けられた。この施設は 1989 年に「微生物微細藻類総合センター」とその名を変え、研究所名が改められた 1993 年には「同細胞・機能高分子総合センター」に含められた。医学分野を除けば、大学関係で最大の規模をもつこの研究所の微生物系統保存部門では、1969 年以後、研究部とは別に施設を設けて、基礎研究を任務とする研究部と保存事業を担当する施設が協力し、系統分類学の研究や系統保存に従事する人材の養成に励んだが、現在は両部門が一元化され、微細藻類部門を加えて杉山純多教授の指導下に置かれている。この研究所における微細藻類研

究の歴史は設立当初にさかのぼり、1950 年代からすでに分離収集や保存などが行われてきた。1993 年には、微生物株と微細藻類株の総合カタログとして『IAM Catalogue of Strains』(第 1 版) が刊行されている。微細藻類については国立環境研究所 (NIES) の微生物保存施設でもその系統保存が行われており、『NIES—Collection List of Strains (Microalgae and Protozoa)』の第 1 版を 1985 年に刊行、1997 年に第 5 版を重ねた。これら 2 つの国立機関の株の分譲は、国公立機関が主な配布先であるが、同時に、それぞれの外郭団体である財団法人応用微生物学研究奨励会、財団法人地球人間環境フォーラムを通じ、民間会社などへの有償配布も行っている。

微生物株カタログの自主出版を継続し、分譲料金を公示して保存株の配布分譲を主務としている保存機関に、財団法人発酵研究所 (IFO) と特殊法人理化学研究所 (微生物系統保存施設, JCM) がある。保存微生物株カタログ、『IFO List of Cultures』は 1996 年に第 10 版を重ね、『JCM Catalogue of Strains』は 1995 年に第 6 版を出した。いずれも菌類と細菌が主要な内容であるが、IFO のリストにバクテリオファージが加わっているのは特徴的である。そのほか、微生物ではないが、IFO には動物細胞株の系統保存部門があって 1994 年に『IFO List of Cultures (Animal Cell Lines)』(第 4 版) を別冊として出した。病原ウイルスそのほか、医学分野に関する系統保存については、他日適任者の筆を待ち、ここでは触れない。JFCC 会員以外では American Type Culture Collection が、住友商事株式会社を在日総代理店として広範囲の分野にわたる株の分譲配布を行っている。

この報告シリーズの冒頭でも触れたが、微生物株の大部分は単離培養された状態でなければ、微生物学の研究における株の取扱いは困難であり、学名の基準株や参考株を集めたカルチャーコレクションは研究を進める上でなくてはならない資料である。さらにまた、カルチャーコレクションが研究者の要望に応えるためには「多ますます弁ず」で、保存微生物株の種類内容が豊富かつ緻密であればあるほど、そのサービスコレクションとしての価値が高まって、利用者の研究に資するところは大きい。

サービスコレクションという用語には、何となく営利的な響きがあるが、それはまちがいである。自然界では消長激しく存在常ならぬ微生物、それらは単離培養する以外には種類を保存する方法がないのであるから、サービスコレクションに保存されている微生物株

は、種類の実在を保証する貴重な証拠資料であり、株がもつ公共性は問わずして明らかである。各国のサービスコレクションには、広範囲に種類を集めた総合型コレクション (general collection) と種類の範囲を特定した専門別コレクション (specialized collection) がある。その保存微生物株は、自然界から分離されて長期にわたりさまざまな目的の研究に使われたのち、研究者どうしの信頼と善意によって供託されるものであり、決して一保存機関の占有物ではない。サービスコレクションに加えらるまでの研究期間、微生物株は数え切れぬ頻度で移植が繰り返され、その間の研究目的に基づく培養条件など研究者の取扱い方が株に与える影響を免れることはできない。それに加えて、株自身が生来持っている適応性にはそれぞれの株によって強弱などの相違があり、移植の過程で突然変異を誘発することがある。こうした突然変異は、放射線などの誘導変異原を用いて人為的に作り出した突然変異に対して自然突然変異と呼ばれる。自然突然変異 (natural mutation, spontaneous mutation) の研究が始まったのは 1930 年代で、おからのペニシリン生産研究ともからんで、1940 年代から 1950 年代へかけて盛んに研究され、その結果、細胞増殖時における DNA 複製の誤りによって現われると理論づけられた。自然突然変異が出現するのは上述のように株自身の性質による場合が多いが、それを予知するのは容易ではない。したがって同一系統株であっても保存歴が異なれば、株どうしの間で性質に差異を生じることがある (2)。保存微生物株に自然変異を起こさせないためには、移植回数をできるかぎり少なくする必要があり、移植の間隔の長期化と同時に低温や乾燥処理などによって細胞を休眠状態へ導くことが求められる。真空凍結乾燥法その他の近代的保存技術が微生物株保存の常法になったのはそうした理由に基づいている。こうして保存技術が進歩し、保存設備が新しくなっても、それぞれの微生物株の過去における保存歴、これからの半永久的保存の過程で起こりうるさまざまなトラブルなどを考えるとき、微生物学分野の研究者にとってみれば、サービスコレクションが国内に 1カ所しか存在しないということは不便でもあり、不安でもある。しかしながら、財政上からみた場合、保存管理に要する莫大な費用の上に、所属研究員その他従事者の人件費および研究費が加わると、微生物株の分譲配布その他の諸収入を合わせてもバランスのとれない支出になり、各種の援助に頼りつつきびしい経営に耐えていかなければならないのは、世界の保存機関に共通する悩みである。

幸いにも日本には、民営機関と国営機関という資金系統の全く異なる複数のサービスコレクションが存在することで、利用者には選択や比較研究の余地を与えているのは重要な利点である。そればかりでなく、必要な場合は技術面あるいは経営面での相補的關係への期待が可能である点も留意されてよいであろう。

そのほかにも日本国内には、日本微生物資源学会所属の機関会員その他系統保存に関する研究施設は少なくないから、組織化によりサービスコレクションへの協力、支援の態勢をつくることも不可能ではない。さきに掲げた昭和 46 年度 JFCC 総会議事録はまさにそのことをうたっており、これからの微生物株保存事業の課題となることが望まれる。

ここに、微生物学関連学会とサービスコレクションの協力が成功を収めた事例がある。国際微生物学会連合 (IAMS, 前出, 1982 年に Association から Union に昇格し、略号は IUMS になった。) が第 7 回国際微生物学会議 (ストックホルム, 1958 年) での承認を受けて下部組織である国際細菌命名委員会 International Committee on Bacteriological Nomenclature (現在は、国際細菌分類命名委員会 International Committee on Systematic Bacteriology) のなかに、放線菌分類小委員会 Subcommittee on Taxonomy of Actinomycetes (STA, Chairman: Dr. D. Gottlieb) を設けたのは、抗生物質の生産で著名なこの菌群が、当時、分類体系の不備からしばしば特許上の紛争を起こしていたからであった。STA は各国研究者の協力を得て、各学名の標準株を集め、所定の分類学的性質についてそれぞれを比較再調査した上、その範囲の諸性質に基づく標準記載を発表して安定した分類体系の構築にも資する目的で、国際的規模をもった共同研究、International Streptomyces Project (ISP) を発足させた。18カ国の研究者が ISP に参加して 1964 年度から実施に入ったが、その方法は 3カ国からそれぞれ 1名の協力研究者を選んで 3名が 1グループを作り、計 6グループが分担して学名の標準株の性質を調べる。各グループ内で試験データが一致をみないときは、第四の協力研究者の再調査に委ねる。検討が終了した株については International Journal of Systematic Bacteriology 誌上に標準記載を発表、その数は 454 株に達した (1, 10, 11)。この共同研究は、その規模の大きさからいっても他に例のないもので、被験株とその学名の標準記載とはきわめて貴重な分類学上の研究資料であるから、それら学名の標準株は直ちに STA が国際センターに指定した次の 4 機関へ、それぞれ同一内容

の標準株コレクションとして供託された。

Centraalbureau voor Schimmelcultures (CBS, オランダ)

American Type Culture Collection (ATCC, 米国)
Research Institute for Antibiotics (RIA, ソ連)
Institute for Fermentation, Osaka (IFO, 日本)

上記の4機関に重複して同一内容のコレクションが配置されたのは、各地域の研究者の便宜ばかりではなく、将来、起こりうる株の変異や死滅などのトラブルに備えてのことであった。IFOでは、当時、所長であった筆者の判断で、ISP標準株供託を仲介した日本放線菌研究会(岡見吉郎博士)に協力を求め、ISP各標準株の定期的確認試験を実施することを計画した。計画の実施については岡見博士と合議の上、研究会会員のなかから実施チームを編成して行うチェックシステムを確立した(4)。サービスコレクション管理モデルとしての試みであったが、このチェックシステムは軌道に乗って発展し、今日では日本放線菌学会が主導する保存管理体制に成長した。これは微生物株保存事業の1つのあり方を示すばかりでなく、自然誌学上からみても重要な意義をもっている。

VII. ま と め

JFCCを学会にという将来構想が初めて語られたのは1971年であった(昭和46年度JFCC総会議事録)。その目標に向って連盟は着実な歩みを見せ、1992年には大多数の会員の支持によって、この構想は確定的な動きになった。1993年7月9日に開かれた平成5年度JFCC総会(山里一英会長)において連盟の解散が決定、同日、「日本微生物資源学会 Japan Society for Culture Collections」が設立された(8,9)。学会の初代会長には、中瀬 崇博士(理化学研究所微生物系統保存施設・部長)が選ばれた。発足時の会員数は、個人会員272、機関会員26、賛助会員63であった。

第1報から第3報に至るこの報告シリーズは、「日本の微生物株保存事業」という表題の下での記述であるが、日本微生物株保存連盟が設立された歴史的背景と設立後の活動状況、学会組織となるまでの過程の説明にとどまる内容で、微生物学の各分野からみた場合には、きわめて不十分な記述に終わったことを認めざるをえない。各分野における系統保存については、それぞれに執筆者を得て記述されることが望ましい。

日本のカルチャーコレクションは、明治・大正・昭和の3代にわたる微生物学研究の進歩に伴って蓄えられた保存微生物株を基礎に造成され、いわば研究の足

表3 JFCC会長・副会長一覧表(その2)

7.	会 長	常松之典
	副会長	飯塚 廣
		1971年(昭和46年)–1973年(昭和48年)
8.	会 長	常松之典
	副会長	長谷川武治 ¹⁾
		1973年(昭和48年)–1975年(昭和50年)
9.	会 長	長谷川武治
	副会長	常松之典
		1975年(昭和50年)–1979年(昭和54年)
10.	会 長	長谷川武治 ²⁾
	副会長	池田庸之助・本間 遜 ³⁾
		1979年(昭和54年)–1981年(昭和56年)
11.	会 長	駒形和男
	副会長	渡辺 貞 ⁴⁾
		1981年(昭和56年)–1983年(昭和58年)
12.	会 長	駒形和男
	副会長	飯島貞二 ⁵⁾ ・藪内英子 ⁶⁾
		1983年(昭和58年)–1985年(昭和60年)
13.	会 長	飯島貞二
	副会長	藪内英子・山里一英
		1985年(昭和60年)–1989年(平成元年)
14.	会 長	山里一英 ⁷⁾
	副会長	中瀬 崇・山口英世 ⁸⁾
		1989年(平成元年)–1993年(平成5年)

¹⁾ 財団法人発酵研究所長

²⁾ 同理事

³⁾ 東京大学医科学研究所教授

⁴⁾ 東京大学医科学研究所教授

⁵⁾ 財団法人発酵研究所長

⁶⁾ 岐阜大学医学部教授

⁷⁾ 東京大学応用微生物研究所教授

⁸⁾ 帝京大学医真菌研究センター教授

跡であるばかりでなく、研究資材として生物学の諸分野で活用されてきた歴史があり、平成の世になってますます発展を続けるライフサイエンス研究分野からの需要はさらに高まりをみせて、その種類の豊富さから、微生物学では世界で屈指の文化資産であることに異議を挿むものはない。これからも盛んに生みだされるであろう微生物学各分野における研究成果を背景に、ますます膨大化していく日本のカルチャーコレクションを保護育成し、それを通じて世界のコレクション、さらには微生物学の進歩に貢献することこそ、日本微生物資源学会の使命というべきであろう。

本報の執筆にあたってご助言をいただいた、日本微生物資源学会名誉会員岡見吉郎、椿 啓介両博士、ならびに日本微生物株保存連盟歴代会長駒形和男、飯島貞二、山里一英の各博士に厚く御礼申しあげる。

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Descriptive Catalogue of IFO Fungus Collection XVI.

In the routine identification work on fungi isolated in Japan, and in 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 authors of the descriptions of these fungal taxa are shown in parentheses.

104. *Acremonium furcatum* (F. & V. Moreau) ex W. Gams (Figs. 1-2) Hyphomycetes Nova Hedwigia 18: (1969) 3 (1970); W. Gams, *Cephalosporium*-artige Schimmelpilze (Hyphomycetes), p. 102 (1971), Gustav Verlag, Stuttgart.
Syn: *Cephalosporium furcatum* F. & V. Moreau, Revue Mycol. 6: 65 (1941). (nom. inval. Art. 36).

Colonies on oatmeal agar with a daily growth rate of 3.2-3.7 mm at 25 °C, creeping on agar surface, pale ochraceous shade, velvety, sometimes funiculose at the central area, thin at the margin. Sporulation abundant; reverse uncolored to pale ochraceous. On potato-carrot agar 3.2-3.6 mm/day growth, hyaline to pale ochraceous, thin, velvety. Sporulation abundant; reverse uncolored to pale ochraceous. On potato sucrose agar 3.4-3.8 mm/day growth, pale ochraceous to buff, velvety to lanose. Sporulation abundant; reverse uncolored to pale ochraceous. On half-strength malt extract agar 1.0-1.1 mm/day growth, pale ochraceous to buff, velvety, restricted, slower growth. Sporulation poor; reverse uncolored to pale ochraceous. Vegetative hyphae hyaline, thin-walled, 2.0-2.5 μm , plectonematogenous to synnematogenous at the central area. Conidiophores sometimes verticillate or consisting of simple phialides. Phialides arise short and singly or in whorls from the conidiophores, straight or slightly bent, 15-22 (-30) μm long, tapering from 2.1-2.5 μm near the base to 1.0-1.5 μm ; often proliferating sympodially and forming schizophialides in old cultures. Conidia form mucilaginous heads, ovoid to ellipsoid, broadly rounded at both ends, one-celled, hyaline, smooth-walled, 2.8-3.6 \times 1.8-2.3 μm , L/W 1.5-1.7; chlamydospores lacking. At 37 °C, growth is nil.

Hab.: garden soil, Minamidaito-mura, Shimajiri-gun, Okinawa, Japan, 25 Sept. 1996 (IFO 32986=Tad. Ito H8-3-11, IFO H-12223).

The species was originally described by F. & V. Moreau (1941) as *Cephalosporium furcatum* invalidly. *Acremonium furcatum* was validly published with the Latin diagnosis by W. Gams (1969).

This species is classified in sect. *Nectroidea* and is characterized in having schizophtialides. *A. hyalinulum* (Sacc.) W. Gams is the only known species with predominating schizophtialides, but the present fungus is distinct from it in lacking dry conidial chains.

The present fungus is commonly isolated from soil and plant remains, particularly agricultural soils and sandy soils worldwide. According to Domsch et al. (1980), it decomposes cellulose and oxidizes manganese in soil.

(Tad. Ito and A. Nakagiri)

105. *Cordyceps heteropoda* Y. Kobayasi (Figs. 3-13) Hypocreales
Bull. Biogeogr. Soc. Japan 9: 158 (1939); Kobayasi and Shimizu, Iconography of Vegetable Wasps and Plant Worms, p. 194 (1983).
Anamorph: *Tolypocladium* sp.

Stalk cylindrical, arising from cicada larva, 12-15 cm long, 3-4 mm in diam, white to dull yellow above ground, light brown in the ground. Head globose to obovate, 5-10 × 5-7 μm, greyish brown, finely mammillate with ostiola. Perithecia embedded in peridial layer of head showing palisade-like structure, elliptical to pyriform, with a short ostiole, 630-750 × 130-150 μm. Asci filiform, 480-550 × 4-5 μm, emerging from ostiole when ascoma becomes dry. Apical cap hemispherical, 4.5-6 μm, with an isthmus opening at the center of the apex. Ascospores filiform, 400-450 × 1-1.5 μm, fragmenting into secondary spores, cylindrical, 6-9 × 1-1.5 μm.

Colonies growing slowly on potato sucrose agar, 13-16 mm in diam at 25°C in 37 days, white to dull yellow, floccose, forming synnemata from the aerial hyphae. Reverse pale brown. Edge of the colony smooth without aerial hyphae and synnemata, brown. Yellow pigment secreted at the margin of the colony. On other media, such as malt extract agar and silkworm chrysalis extract agar, no synnema was produced, though the hyphal growth was improved, 10-12 mm/24 days, on the latter medium. Hyphae hyaline, smooth-walled, 2-2.5 μm wide. Synnemata composed of loosely gathering hyphae and conidiophores, white, 3-4 mm long, 120-160 μm wide. Conidiophores hyaline, branched, 60-160 × 2-6 μm, arising from the side and apex of the synnema. Phialides solitary or verticillate, 8-12 × 3-4 μm, swollen at the base (4-6 × 3-4 μm) and narrowing to a neck (4-8 × 0.5 μm). Conidia ovate to oblong-elliptical, one-celled, hyaline, 3-5 × 2-3 μm (\bar{x} =4.4 × 2.3 μm), forming a slimy conidial drop on the apex of the phialide.

Hab.: on larva of cicada (probably *Graptopsaltria nigrofuscata* Motschulsky; Aburazemi), Botanical Garden of the Faculty of Science, Kyoto University, Kitashirakawa, Kyoto, Japan, 18 April 1998 (Specimens were collected under the guidance of Prof. M. Tsuda, Faculty of Agriculture, Kyoto Univ.) [IFO 33060=AN-1634 (a single ascus isolate), IFO H-12226].

This is the first report of the anamorph of *Cordyceps heteropoda*. The anamorph is most similar to *Tolypocladium extinguens* Samson & Soares, which was reported as a pathogen on the glowworm, *Arachnocampa luminosa* (Diptera; Mycetophilidae) from New Zealand (Samson and Soares, 1984). However, this species has smaller phialides ($3.5\text{--}9 \times 1.5\text{--}2 \mu\text{m}$) and ellipsoidal conidia ($2\text{--}4 \times 1.5\text{--}3.5 \mu\text{m}$). This species also differs from the present fungus in having no synnema and no teleomorph. Thus, the anamorph is an unknown species of *Tolypocladium*. This is the second report of the *Tolypocladium* anamorph of *Cordyceps* species since *T. inflatum* W. Gams [= *T. niveum* (O. Rostrup) Bissett] was reported as an anamorph of *C. subsessilis* Petch (Hodge et al., 1996). The present fungus is different from *T. inflatum* in morphology, host and culture characteristics.

(A. Nakagiri and Tad. Ito)

106. *Monochaetia monochaeta* (Desmazieres) Allescher (Fig. 14–19) Coelomycetes in Rabenhorst, Kryptog. Fl. 7: 667 (1902); Sutton, The coelomycetes. CMI, England (1980); Nag Raj, Coelomycetous anamorphs with appendage-bearing conidia. Mycologue Publication, Waterloo, Ont. (1993).
Syn.: *Pestalotia monochaeta* Deamazieres, Ann. Sci. net., ser. 3, 10: 355 (1848)

A part of colony on potato sucrose agar initially pale orange or brown to dark brown, humectate; producing various-shaped secondary conidium-like structures, hyphae septate, hyaline, $2.5\text{--}4 \mu\text{m}$ in diam. Conidiogenous cells cylindrical to subcylindrical, colourless, smooth. Conidia fusiform, mainly 4-septate, wall not constricted at the septa, initially smooth, eventually minutely verruculose, $17\text{--}22 \times 5\text{--}6 \mu\text{m}$, bearing appendages; median cells doliiform to subcylindrical brown, together $12\text{--}15 \mu\text{m}$ long, appendages tubular, attenuated, unbranched; apical appendage single, $6\text{--}10 \mu\text{m}$ long; basal appendage, single, unbranched, centric, $3\text{--}6 \mu\text{m}$ long.

Hab.: on leaves of *Rhododendron pulchrum* var. *speciosum* Hara, Ikeda, Osaka Pref., 21 Mar. 1994. (IFO 33068=IOC 797, IFO H-12228)

This fungus was isolated from leaf-surface of *Rhododendron pulchrum* var. *speciosum* by hyphal isolation. Initially, a part of the colony of this fungus was pale orange or brown to dark brown, humectate, and yeast-like in appearance, and secondary conidium-like structures of various shapes, including spindle, cylindrical, forked, and irregular with septa, were observed under the microscope. These secondary conidium-like structures were produced directly on germ tubes from normal conidia, and also secondarily from other secondary conidium-like structures by blastic or arthric proliferation. These secondary conidium-like structures have the ability to germinate. Eventually, normal conidia were produced. This phenomenon was observed on several agar media, that is, potato sucrose agar, potato carrot agar, malt extract agar, corn meal agar, and oat meal agar. It is considered to be an ecological characteristic of this isolate. Producing such the secondary conidium-like structures before normal conidia is considered to be an

effective manner of dispersal for coelomycetous fungi producing conidiomata on host plants after invasion.

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

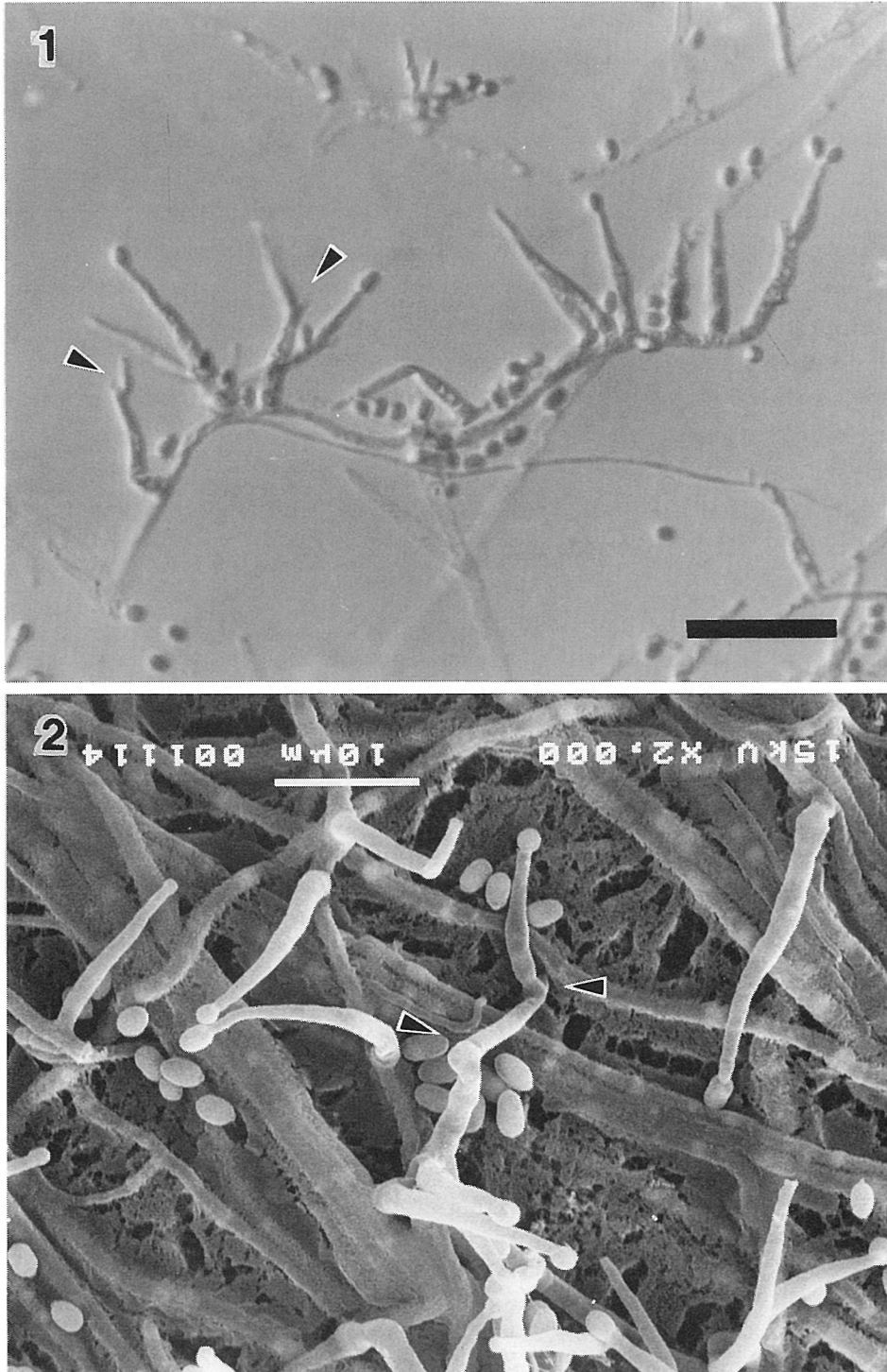
107. *Cirrenalia pygmaea* Kohlmeyer (Fig. 20-23) Hyphomycetes
Ber. Dtsch. Bot. Ges. 79, 35 (1966); Kohlmeyer and Kohlmeyer, Marine mycology, The higher fungi. Academic Press, New York (1979)

Colonies on oat meal agar olivaceous to black, growing slowly, hyphae septate, 2.5-5 μm in diam. Conidiophores obsolete. Conidia acrogenous, solitary, helicoid, contorted 1/2 or 1 time, three- to six-septate, mostly four- or five-septate, not constricted at the septa, fist-shaped or reniform, black or fuscous, smooth, lower cells light-colored, cells increasing in diameter from base to apex, distinctly dissimilar, spirals 25-30 \times 29-35 μm , terminal cell 14-22.5 μm in diameter, subglobose to reniform, basally flattened, basal cells 4-5.5 μm in diameter.

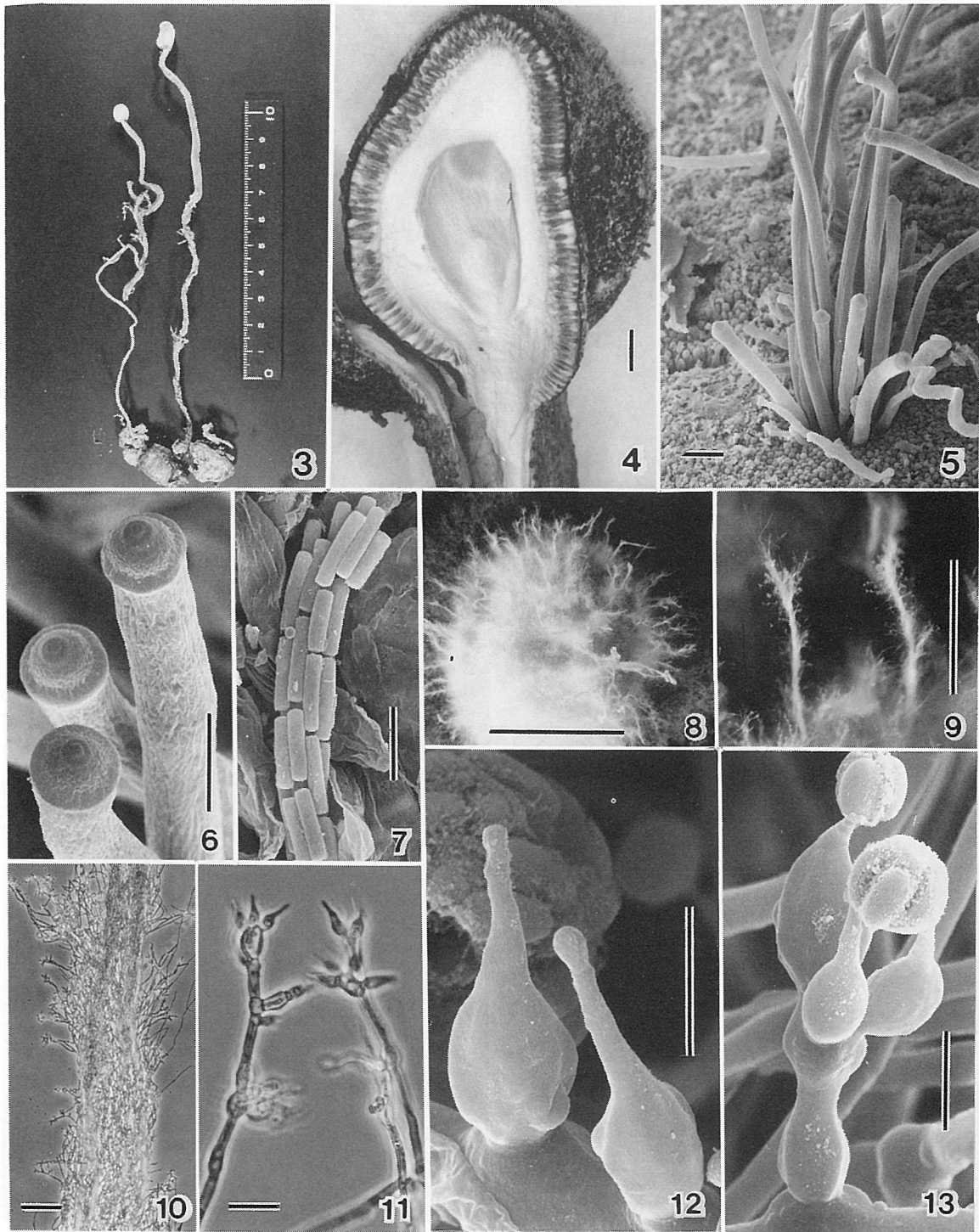
Hab.: on fallen leaves of *Bruguiera gymnorrhiza* Lamk., Shiira Riv., Iriomote Is., Okinawa Pref., 8 July, 1997. (IFO 33069=IOC 1237, IFO H-12229)

This fungus was isolated from fallen leaves of *Bruguiera gymnorrhiza*. Conidia in culture had up to six septa, while Kohlmeyer reported that the number is three or four. The number of septa might vary with culture medium. In cultural tests, better hyphal growth was observed on oat meal agar than on potato sucrose agar, potato carrot agar, malt extract agar, corn meal agar, or corn meal sea water agar.

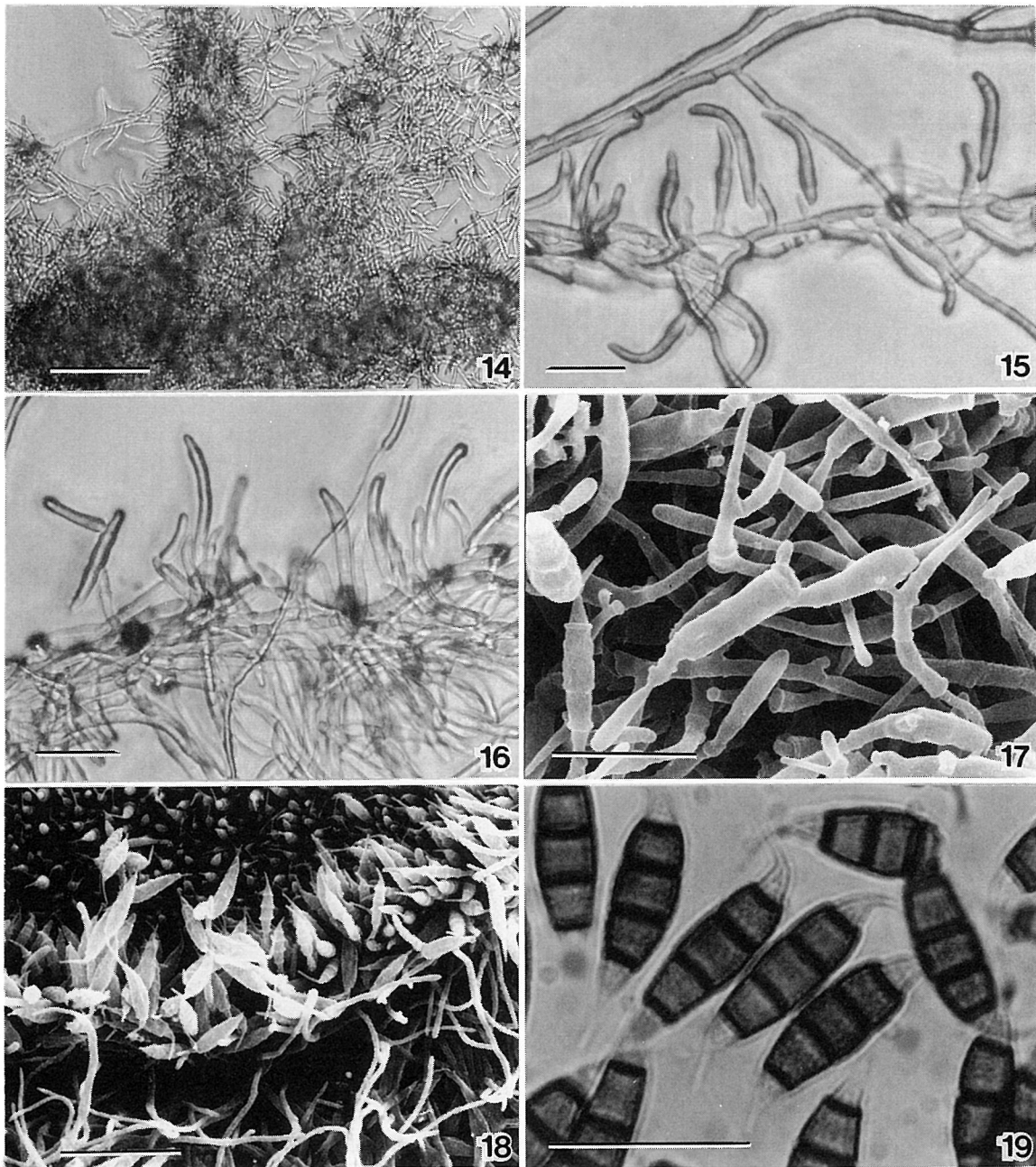
(I. Okane, A. Nakagiri and Tad. Ito)



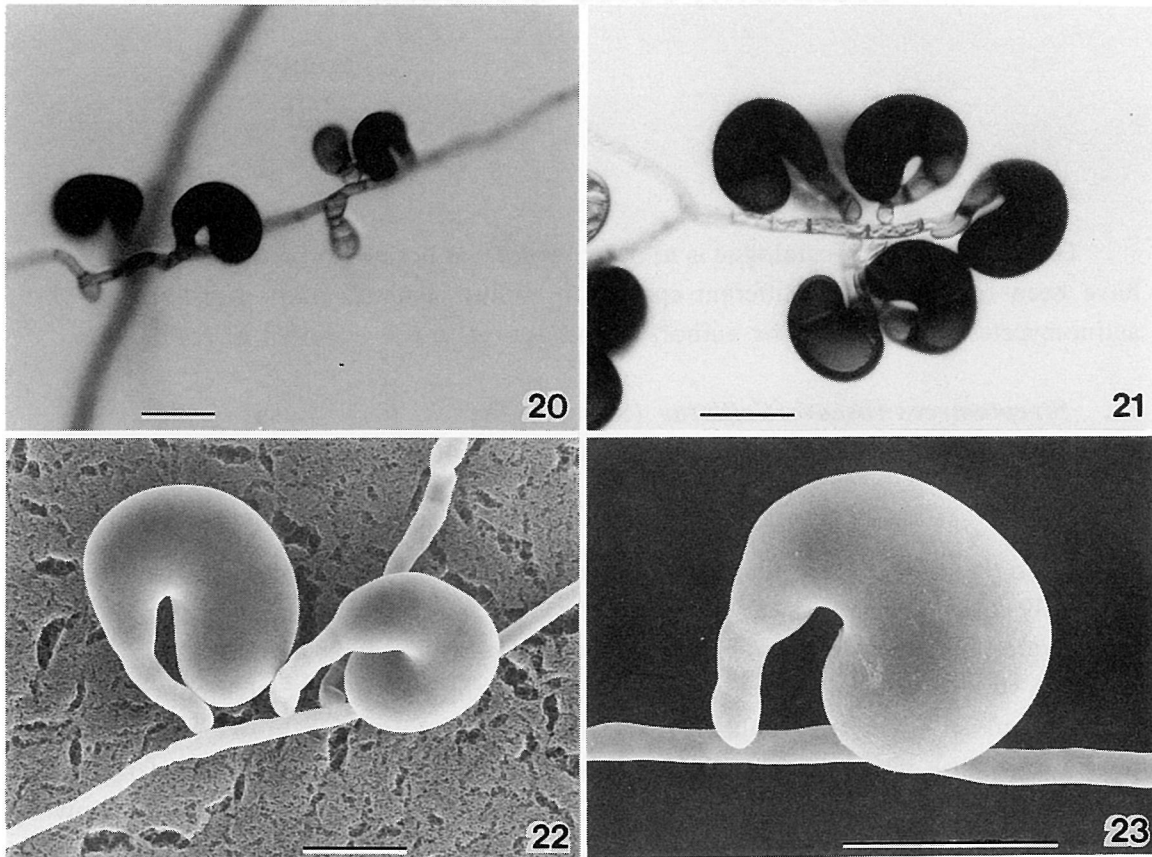
Figs. 1 & 2. *Acremonium furcatum*. Phialides and conidia. IFO 32828. Sympodial proliferation (arrowheads). 1. Light micrograph. (Bar=20 μ m.) 2. Scanning electron micrograph. (Bar=10 μ m.)



Figs. 3-13. Light and SEM micrograph of *Cordyceps heteropoda* (IFO 33060). 3. Stromata arising from larvae of cicada. 4. Longitudinal section of the head of stroma, showing perithecia at the peridial layer. 5. Asci emerging from ostiole opening at the surface of the stroma. 6. Apices of asci. 7. Ascospores fragmented into secondary spores. 8. Colony on PSA, showing *Tolypocladium* anamorphic state. 9. Synnemata. 10. Conidiophores arising from a synnema. 11. Conidiophores. 12. Phialides. 13. Phialides and a conidial drop. Scale bars: 3=10 cm; 4, 9=1 mm; 5, 11=10 μm ; 6, 7, 12, 13=5 μm ; 8=5 mm; 10=100 μm .



Figs. 14-19. *Monochaetia monochaeta*. 14. Proliferation of *M. monochaeta* on PSA. 15 and 16: secondary conidium-like structures. 17. Branched secondary conidium-like structures, by scanning electron microscopy. 18. Conidia, by scanning electron microscopy. 19. Conidia. Scale bar: Fig. 14 and 18 : 40 μm ; Fig. 15, 16, 17, 19 : 20 μm .



Figs. 20-23. *Cirrenalia pygmaea*. 20 and 21. Conidia produced on the hyphae. 22 and 23. Conidia, by scanning electron microscopy.
Scale bar: Fig. 20 and 21 : 20 μm ; Fig. 22 and 23 : 10 μm .

Descriptive Catalogue of IFO Actinomycetes Collection IV

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

- 12. *Streptomyces roseoverticillatus*** (Shinobu) Witt & Stackebrandt
Witt, D. and E. Stackebrandt, Int. J. Syst. Bacteriol., 41: 456 (1991)

IFO 14694

Strain IFO 14694 was deposited under the name of "*Streptomyces* sp.," having originally been designated as "*Streptoverticillium rubrochlorinum*" Locci et al. Its phenotypic characters closely resemble those of *Streptomyces roseoverticillatus* IFO 12817^T (T=type strain). Spore chains are verticillate and the spore surface is smooth. Aerial mass is pinkish white to purplish pink, substrate mycelium is reddish yellow, reddish brown to reddish purple, or dark red; and melanin formation is positive on International *Streptomyces* Project (ISP) media 1 and 6. Glucose and inositol are utilized for growth. The level of DNA relatedness between this strain and *Streptomyces roseoverticillatus* IFO 12817^T is 79%. From these results, IFO 14694 is identified as a strain of *Streptomyces roseoverticillatus*.

(Hatano, K. & T. Nishii)

- 13. *Streptomyces blastmyceticus*** (Watanabe et al.) Witt & Stackebrandt
Witt, D. and E. Stackebrandt, Int. J. Syst. Bacteriol., 41: 456 (1991)

IFO 13202

Strain IFO 13202 (ISP 5021) was deposited under the name of "*Streptomyces* sp.," having originally been designated as "*Streptomyces mediocidicus*" Okami et al. Its phenotypic characters closely resemble those of *Streptomyces blastmyceticus* 12747^T. Spore chains are verticillate and the spore surface is smooth. Aerial mass is white with yellowish tinge to gray, substrate mycelium is yellowish brown; and melanin formation is positive on ISP media 1 and 6. Glucose, fructose, and inositol are utilized for growth. The level of DNA relatedness between this strain and *Streptomyces blastmyceticus* 12747^T is 94-95%. From these results, IFO 13202 is identified as a strain of *Streptomyces blastmyceticus*.

(Hatano, K. & T. Nishii)

14. *Streptomyces ehimensis* (Shibata et al.) Witt & Stackebrandt
Witt, D. and E. Stackebrandt, Int. J. Syst. Bacteriol., 41: 456 (1991)

IFO 13082

Strain IFO 13082 (ISP 5464) was deposited under the name of "*Streptomyces* sp.," having originally been designated as "*Streptomyces rubrreticuli*" Waksman & Henrici. Its phenotypic characters closely resemble those of *Streptomyces ehimensis* IFO 12858^T. Spore chains are verticillate and the spore surface is smooth. Aerial mass is white to reddish brownish gray, substrate mycelium is yellowish brown to brown; and melanin formation is positive on ISP media 1 and 6. Glucose, fructose, inositol, and mannitol are utilized for growth. The level of DNA relatedness between this strain and *Streptomyces ehimensis* IFO 12858^T is 100%. From these results, IFO 13082 is identified as a strain of *Streptomyces ehimensis*.

(Hatano, K. & T. Nishii)

IFO 13470

Strain IFO 13470 (ISP 5576) was deposited under the name of "*Streptomyces takataensis*" anonymous. Its phenotypic characters closely resemble those of *Streptomyces ehimensis* IFO 12858^T. Spore chains are verticillate and the spore surface is smooth. Aerial mass is white to reddish brownish gray (bisque), substrate mycelium is yellowish brown to brown; and melanin formation is positive on ISP media 1 and 6. Glucose, fructose, inositol, and mannitol are utilized for growth. The level of DNA relatedness between this strain and *Streptomyces ehimensis* IFO 12858^T is 99%. From these results, IFO 13470 is identified as a strain of *Streptomyces ehimensis*.

(Hatano, K. & T. Nishii)

15. *Streptomyces griseocarneus* (Benedict et al.) Witt & Stackebrandt
Witt, D. and E. Stackebrandt, Int. J. Syst. Bacteriol., 41: 456 (1991)

IFO 13428

Strain IFO 13428 (ISP 5520) was deposited under the name of "*Streptomyces tropicalensis*" Gupta. Its phenotypic characters closely resemble those of *Streptomyces griseocarneus* IFO 12776^T. Spore chains form whorls and the spore surface is smooth. Aerial mass is reddish brownish gray to gray, substrate mycelium is yellowish brown to dark brown; and melanin formation is positive on ISP media 1 and 6. Glucose, fructose, and inositol are utilized for growth. The level of DNA relatedness between this strain and *Streptomyces griseocarneus* IFO 12776^T is 96%. From these results, "*Streptomyces tropicalensis*" IFO 13428 is identified as a strain of *Streptomyces griseocarneus*.

(Hatano, K. & T. Nishii)

16. *Streptomyces hachijoensis* (Hosoya et al.) Witt & Stackebrandt
Witt, D. and E. Stackebrandt, Int. J. Syst. Bacteriol., 41: 456 (1991)

IFO 12363

Strain IFO 12363 was deposited under the name of "*Streptomyces* sp.," having originally been designated as "*Streptomyces cinnamoneus* subsp. *azacoluta*" Pridham et al. Its phenotypic characters closely resemble those of *Streptomyces hachijoensis* IFO 12782^T. Spore chains form whorls and the spore surface is smooth. Aerial mass is reddish brownish gray (bisque) to grayish rose, substrate mycelium is yellowish brown to brown; and melanin formation is negative on ISP media 1, 6, and 7. Glucose and inositol are utilized for growth. The level of DNA relatedness between this strain and *Streptomyces hachijoensis* IFO 12782^T is 80–90%. From these results, IFO 12363 is identified as a strain of *Streptomyces hachijoensis*.

(Hatano, K. & T. Nishii)

IFO 13864

Strain IFO 13864 was deposited under the name of "*Streptomyces* sp.," having originally been designated as "*Streptoverticillium verticillum* subsp. *verticillum*" Okami. Its phenotypic characters closely resemble those of *Streptomyces hachijoensis* IFO 12782^T. Spore chains form whorls and the spore surface is smooth. Aerial mass is reddish brownish gray (bisque) to gray, substrate mycelium is brown to dark reddish brown; and melanin formation is negative on ISP media 1, 6, and 7. Glucose and inositol are utilized for growth. The level of DNA relatedness between this strain and *Streptomyces hachijoensis* IFO 12782^T is 94%. From these results, IFO 13864 is identified as a strain of *Streptomyces hachijoensis*.

17. *Streptomyces morookaensis* (Locci & Schofield) Witt & Stackebrandt
Witt, D. and E. Stackebrandt, Int. J. Syst. Bacteriol., 41: 456 (1991)

IFO 13461

Strain IFO 13461 (ISP 5565) was deposited under the name of "*Streptomyces aspergilloides*" Rao et al. Its phenotypic characters resemble those of *Streptomyces morookaensis* IFO 13416^T. Spore chains form whorls and the spore surface is smooth. Aerial mass is yellow to yellowish gray, substrate mycelium is yellowish brown to brown; and melanin formation is negative on ISP media 1, 6, and 7. Glucose, fructose, inositol, and mannitol are utilized for growth. The level of DNA relatedness between this strain and *Streptomyces morookaensis* IFO 13416^T is 100%. From these results, IFO 13461 is identified as a strain of *Streptomyces morookaensis*.

(Hatano, K. & T. Nishii)

18. *Streptomyces fameus* Shibata
Shibata, M. J. Antibiotics Ser. B, 12: 398 (1959)

IFO 13399

Strain IFO 13399 (ISP 5480) was deposited under the name of "*Streptomyces* sp.," having originally been designated as "*Streptomyces galbus*" Okami et al. Its phenotypic

characters closely resemble those of *Streptomyces rameus* IFO 3782^T (T=type strain). Spore chains form spirals and the spore surface is smooth. Aerial mass is brownish gray to gray, substrate mycelium is brownish yellow to dark brown; and melanin formation is positive on ISP media 1, 6, and 7. Glucose, arabinose, fructose, inositol, mannitol, and xylose are utilized for growth, sucrose is doubtful, and rhamnose is not utilized. The level of DNA relatedness between this strain and *Streptomyces rameus* IFO 3782^T is 90%. In addition, the similarity of *gyrB* and GyrB partial sequences of these strains is 99.8 and 100%, respectively. From these results, IFO 13399 is identified as a strain of *Streptomyces rameus*.

(Hatano, K. & T. Nishii)

19. *Streptomyces septatus* (Locci et al.) Witt & Stackebrandt
Witt, D. and E. Stackebrandt, Int. J. Syst. Bacteriol., 41: 456 (1991)

IFO 3354

Strain IFO 3354 was deposited under the name of "*Streptomyces reticuli*" (Waksman & Curtis) Waksman & Henrici. Its phenotypic characters closely resemble those of *Streptomyces septatus* IFO 13471^T. Spore chains form whorls and the spore surface is smooth. Aerial mass is white, reddish brownish gray (bisque) to gray, substrate mycelium is brown to dark brown; and melanin formation is positive on ISP media 1 and 6. Glucose and inositol are utilized for growth. The level of DNA relatedness between this strain and *Streptomyces septatus* IFO 13471^T is 96%. In addition, the similarity of *gyrB* sequences of these strains is 99.4%. From these results, IFO 3354 is identified as a strain of *Streptomyces septatus*.

(Hatano, K. & T. Nishii)

Phase-contrast Micrographs of the IFO Animal Cell Cultures

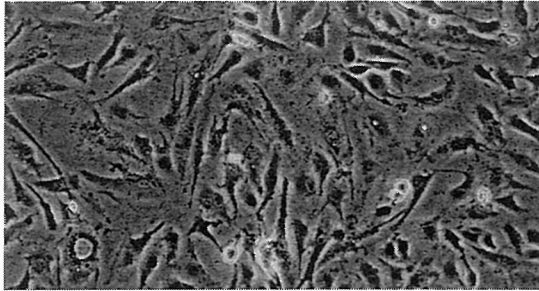
Kengo TSUJI, Motonobu SATOH, and Touho YOSHIDA

Here we show phase-contrast micrographs of the IFO animal cell cultures. The IFO cell bank has collected over 500 cell cultures originating from a variety of species since its beginning in 1984. After undergoing quality control and authentication procedures, 192 cell cultures have been registered as the IFO cell cultures that are available to investigators on request. These cells preserved in liquid nitrogen were thawed and re-cultivated in appropriate culture conditions described in the IFO List of Cultures (5th ed., 1998). Typical morphological features of the living cells were photographed by phase-contrast microscopy. The morphology of cells in culture provides the important informations to understand the properties of the cells. It is the simplest and most direct technique to identify cells. Culture conditions or contamination with microorganisms or other cells affect the cell morphology. Therefore, investigators should monitor the cell morphology during experiments. This monograph should be useful to know the morphology of the IFO cell cultures.

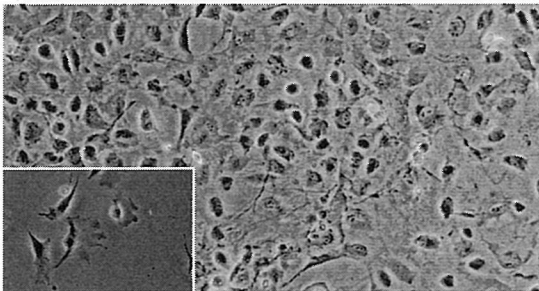
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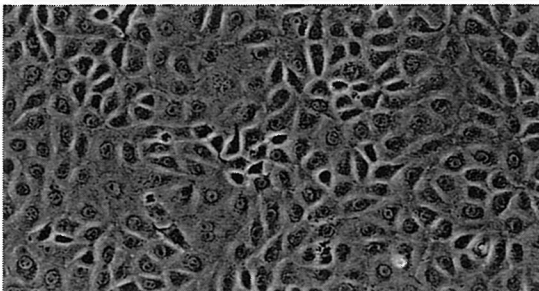
BOVINE



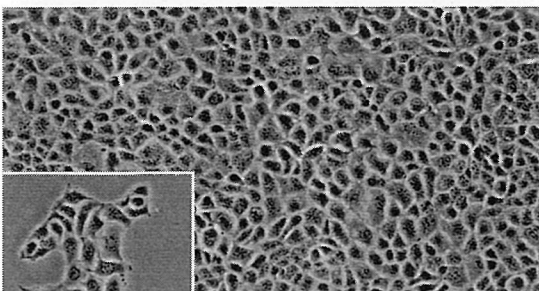
1. IFO 50154 FBHE Endothelial heart endothelial cell, FGF-dependent for survival



2. IFO 50272 CPA Endothelial pulmonary artery endothelial cell

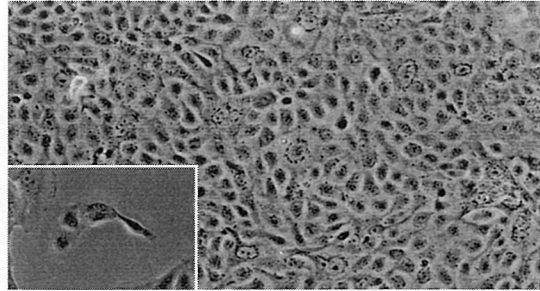


3. IFO 50014 MDBK (NBL-1) Kidney established from normal kidney



4. IFO 50003 CKT-1 Kidney useful to assay bovine adenovirus 3

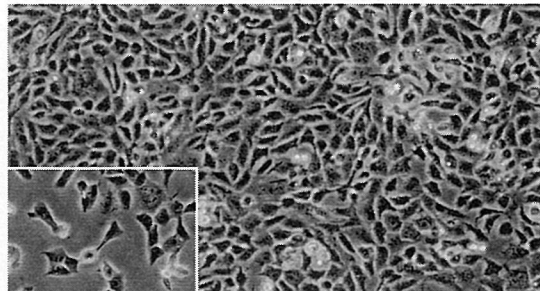
DOG



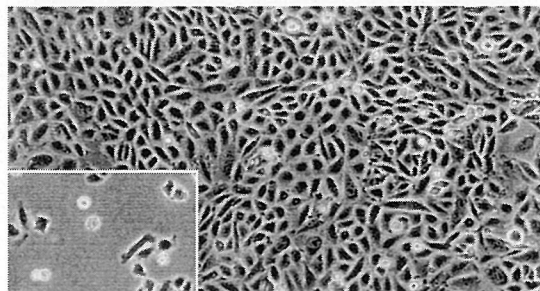
5. IFO 50071 MDCK (NBL-2) Kidney established from normal kidney, susceptible for many viral species



HAMSTER, CHINESE

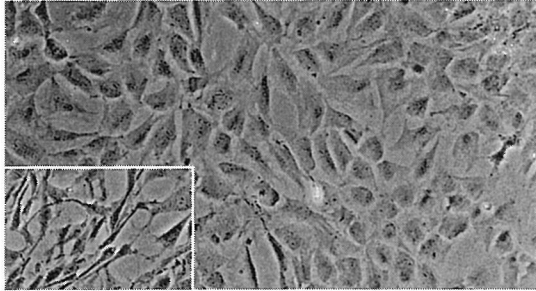


6. IFO 50082 V79 379A Lung lung, fibroblast, widely used in somatic genetics

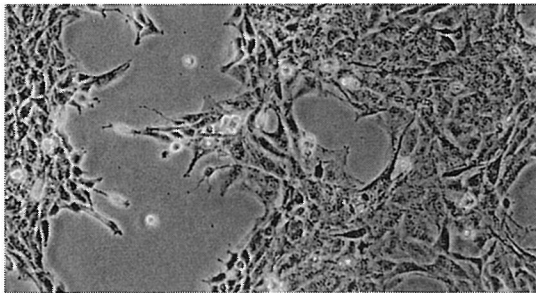


7. IFO 50414 CHO-K1 Ovary widely used for production of recombinant proteins

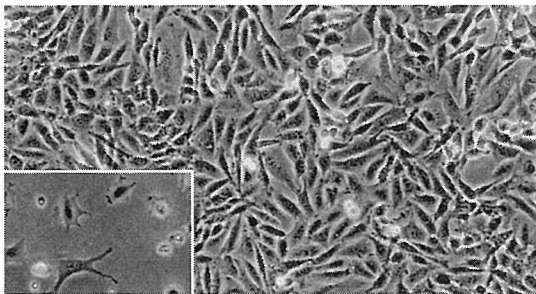
100µm

HAMSTER, SYRIAN

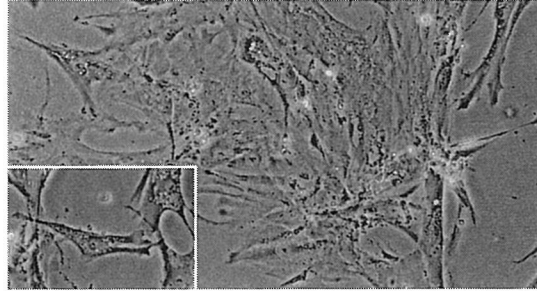
8. IFO 50367 SHOK Embryo fibroblast, contact-inhibited



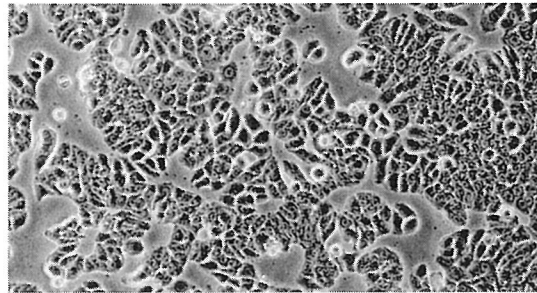
9. IFO 50426 NIL-SF2 Embryo NIL subline adapted to serum-free medium



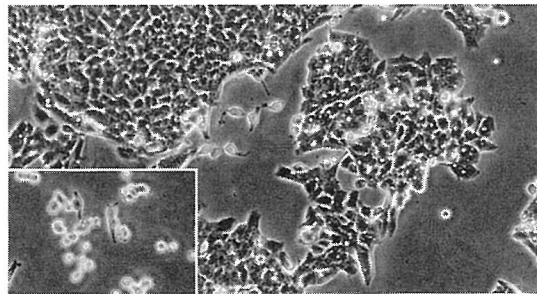
10. IFO 50018 NIL Embryo transformed from hamster fibroblast

HUMAN

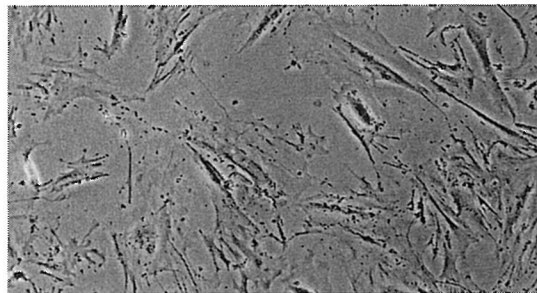
11. IFO 50477 FS-1 Bone marrow stroma bone marrow stromal cell from a myelogenous leukemia patient



12. IFO 50043 WiDr Colon adenocarcinoma



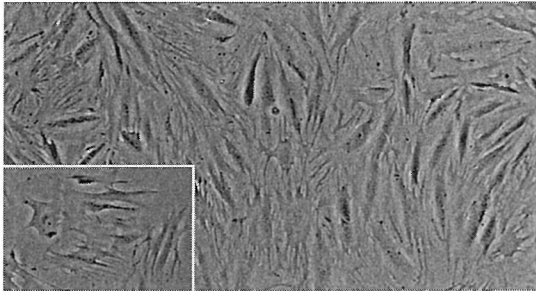
13. IFO 50067 LoVo Colon adenocarcinoma, carcinoembryonic antigen-producing



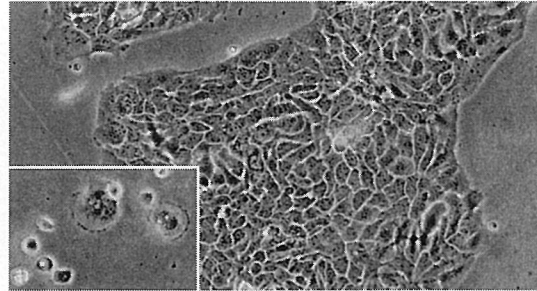
14. IFO 50483 CCD-33Co Colon established from normal colon, fibroblast

100μm

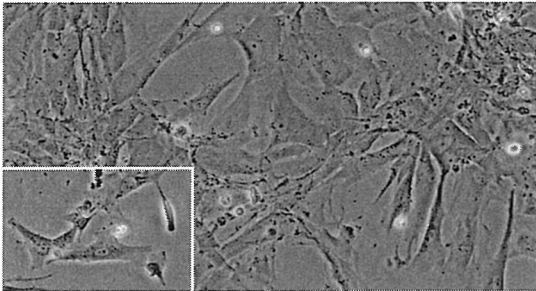
HUMAN



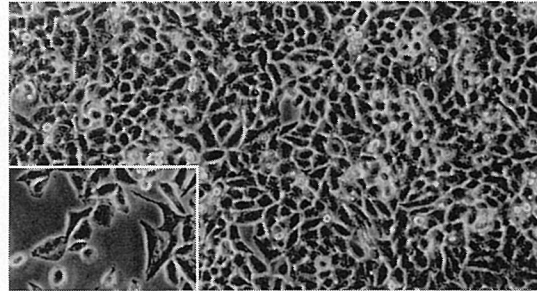
15. IFO 50482 CCD 841 CoN Colon
established from normal colonic tissue, epithelial-like



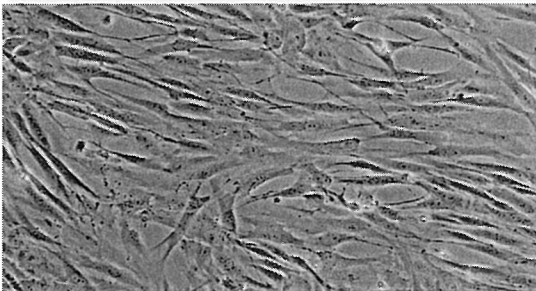
19. IFO 50411 A431 Epidermoid carcinoma
epidermoid carcinoma, high expression of EGF receptor



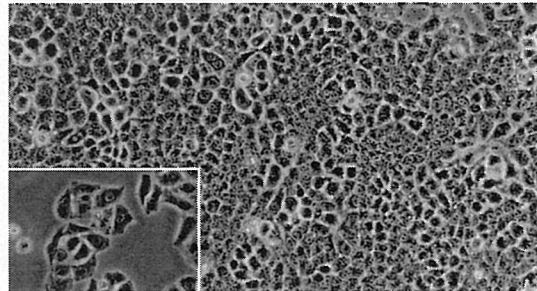
16. IFO 50300 Miz-1 Embryo
limb interstitial tissue, normal diploid fibroblast



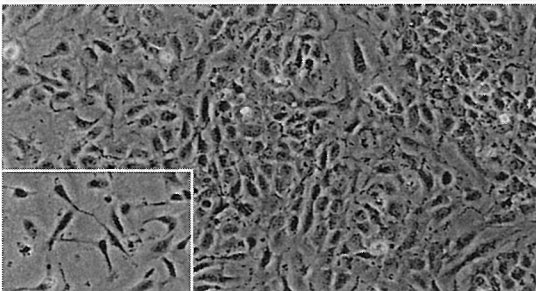
20. IFO 50005 J-111 HeLa marker
originally established from acute monocytic leukemia, HeLa-contaminated



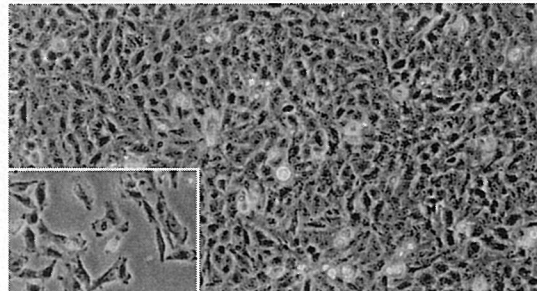
17. IFO 50297 HE-1 Embryo
whole fetus, normal diploid fibroblast



21. IFO 50004 WISH HeLa marker
originally established from amnion, HeLa-contaminated

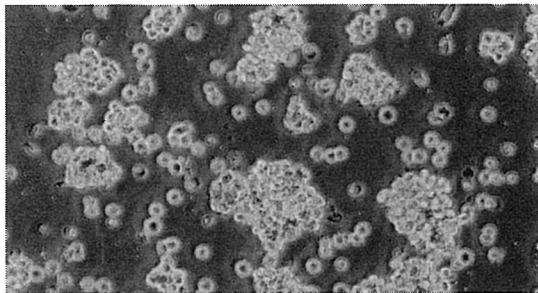


18. IFO 50271 HUV-EC-C Endothelial
normal endothelial cell, umbilical cord

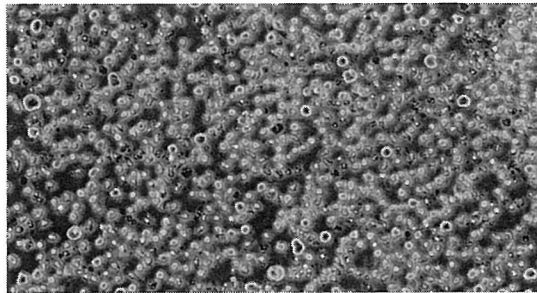


22. IFO 50016 Chang Liver HeLa marker
originally established from liver, HeLa-contaminated

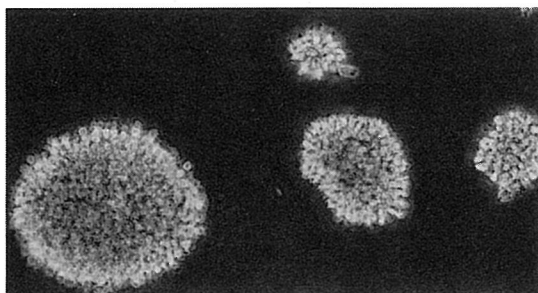
100µm

HUMAN

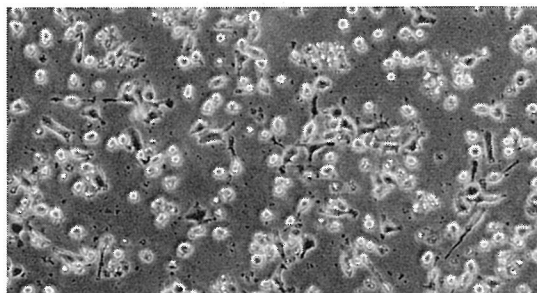
23. IFO 50037 RPMI 1788 Hematopoietic lineage established from normal blood, IgM secreting



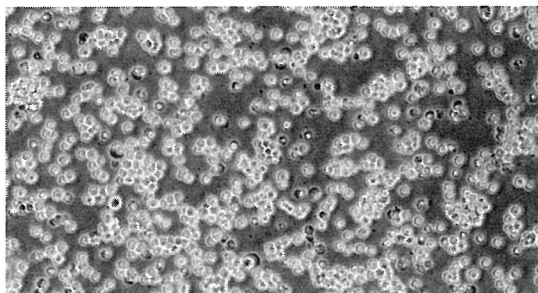
27. IFO 50428 CMK-86 Hematopoietic lineage leukemia, acute megakaryoblastic



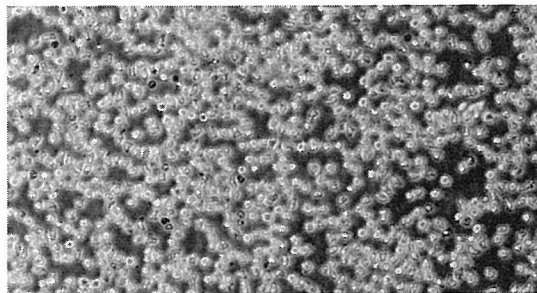
24. IFO 50026 CCRF-SB Hematopoietic lineage leukemia, acute lymphoblastic



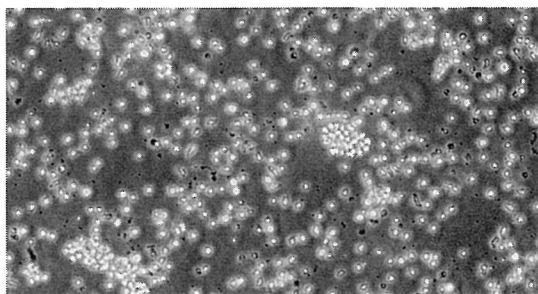
28. IFO 50430 CMK-11-5 Hematopoietic lineage leukemia, acute megakaryoblastic



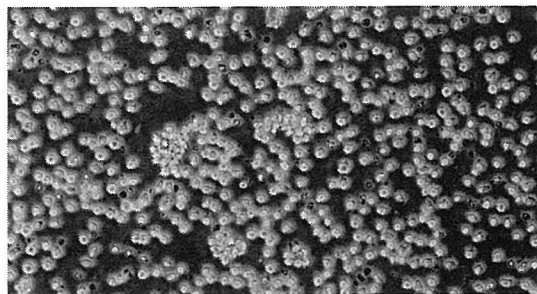
25. IFO 50362 MOLT-4 Hematopoietic lineage leukemia, acute lymphoblastic



29. IFO 50475 NOMO-1s Hematopoietic lineage leukemia, acute monocytic, NOMO-1-derivative adapted to serum-free medium



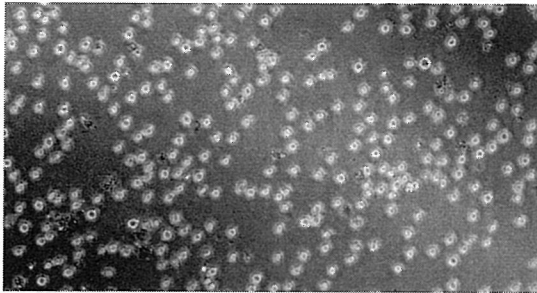
26. IFO 50412 CCRF-CEM Hematopoietic lineage leukemia, acute lymphoblastic



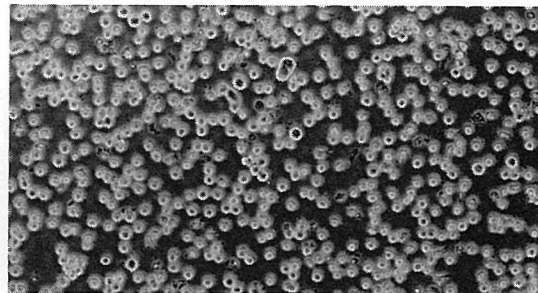
30. IFO 50474 NOMO-1 Hematopoietic lineage leukemia, acute monocytic, promonocytic leukemia cells

100μm

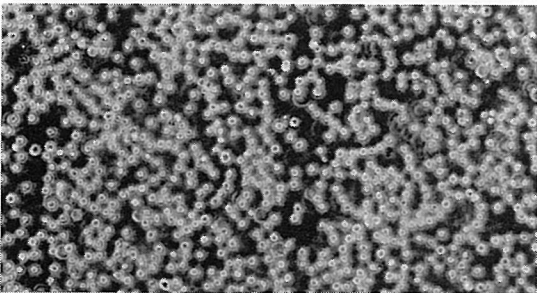
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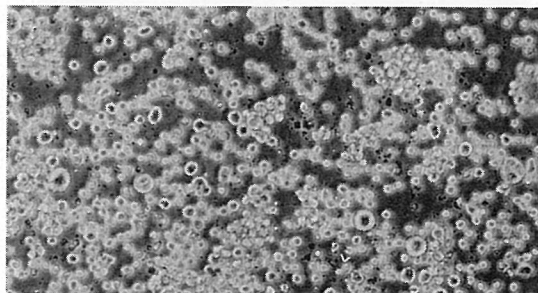
31. IFO 50476 NKM-1 Hematopoietic lineage leukemia, acute myeloid, myeloid leukemia cells, responsive to G-CSF and M-CSF for growth



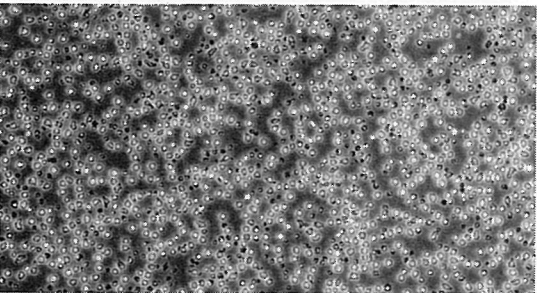
35. IFO 50473 MEG-01s Hematopoietic lineage leukemia, chronic megakaryoblastic, MEG-01s-derivative



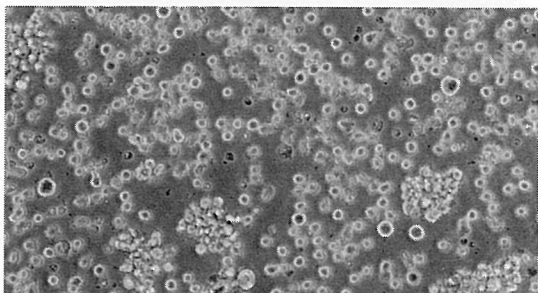
32. IFO 50022 HL-60 Hematopoietic lineage leukemia, acute promyelocytic, This strain is unable to induce differentiation.



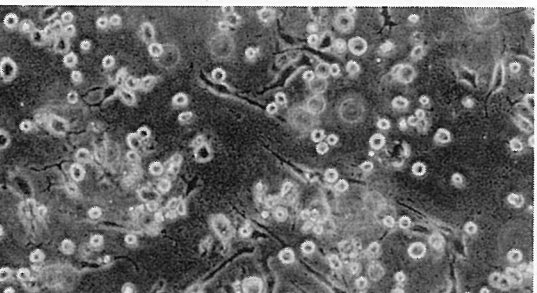
36. IFO 50294 MEG-01SSF Hematopoietic lineage leukemia, chronic megakaryoblastic, MEG-01s-derivative adapted to serum-free medium



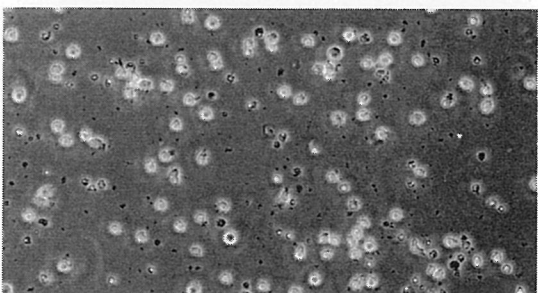
33. IFO 50479 NAGL-1 Hematopoietic lineage leukemia, B cells



37. IFO 50025 IM-9 Hematopoietic lineage leukemia, chronic myelogenous

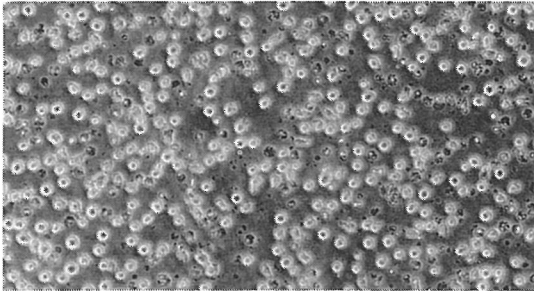


34. IFO 50151 MEG-01 Hematopoietic lineage leukemia, chronic megakaryoblastic

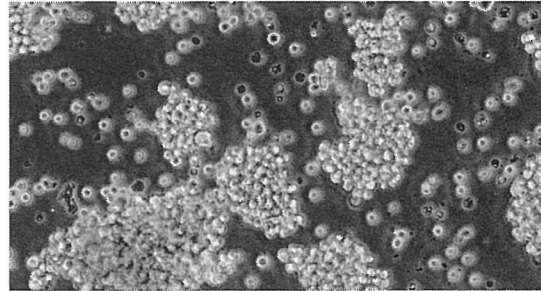


38. IFO 50478 MEG-A2 Hematopoietic lineage leukemia, chronic myelogenous, megakaryoblastic cell line

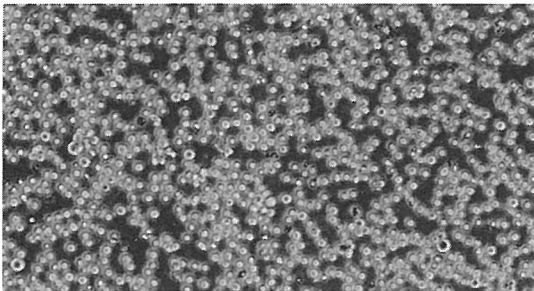
100µm

HUMAN

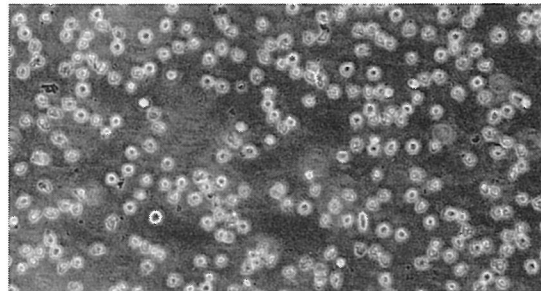
39. IFO 50363 KU812 Hematopoietic lineage
leukemia, chronic myeloid



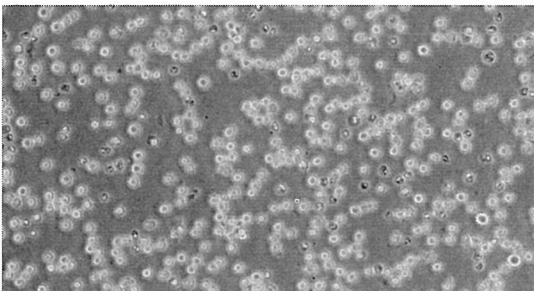
43. IFO 50046 Raji Hematopoietic lineage
lymphoma, Burkitt's



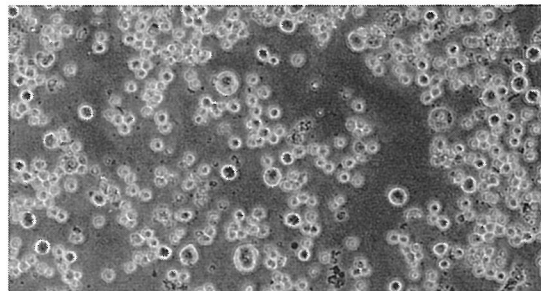
40. IFO 50039 NC-37 Hematopoietic lineage
lymphoblast, Contamination of Raji cells has been suspected.



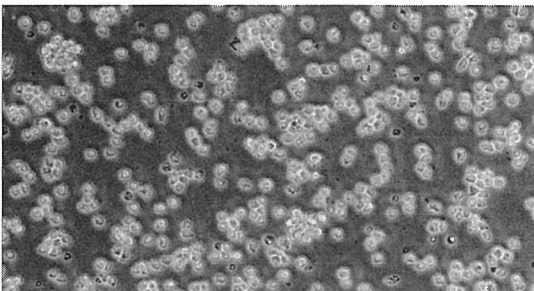
44. IFO 50038 U-937 Hematopoietic lineage
lymphoma, histiocytic



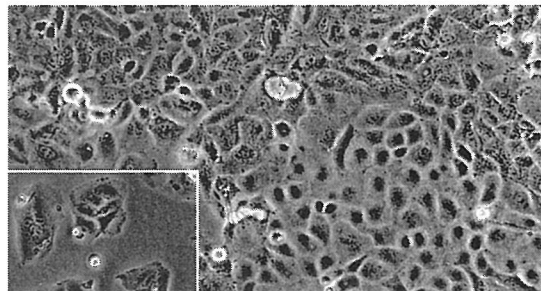
41. IFO 50028 EB-3 Hematopoietic lineage
lymphoma, Burkitt's



45. IFO 50013 RPMI 8226 Hematopoietic lineage
myeloma, multiple



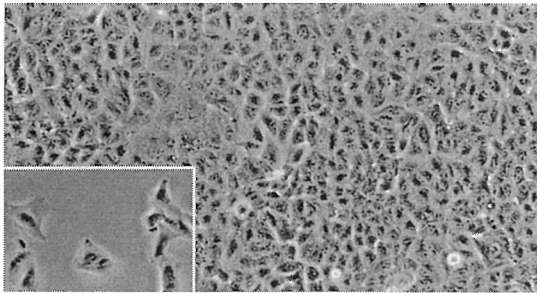
42. IFO 50040 Namalwa Hematopoietic lineage
lymphoma, Burkitt's



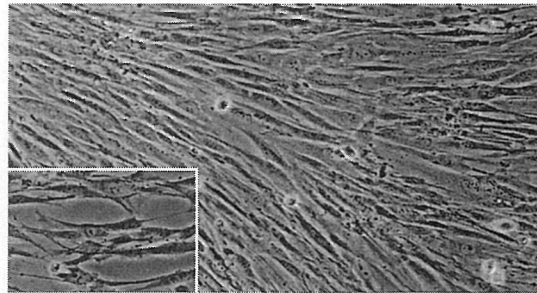
46. IFO 50069 Alexander cells Liver
hepatoma, HBs antigen-positive

100μm

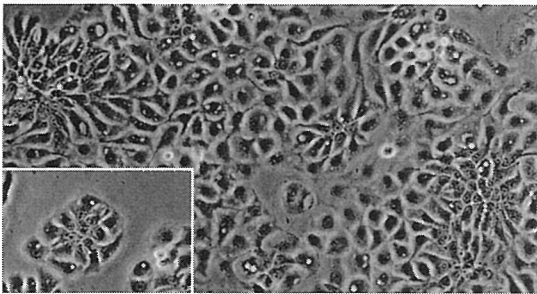
HUMAN



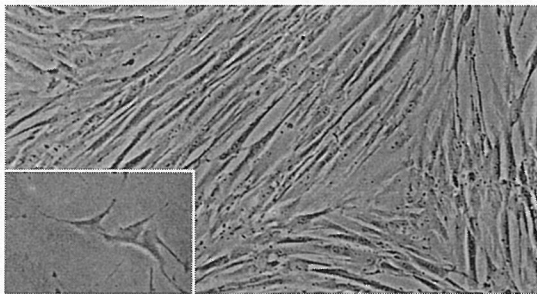
47. IFO 50153 A549 Lung
adenocarcinoma



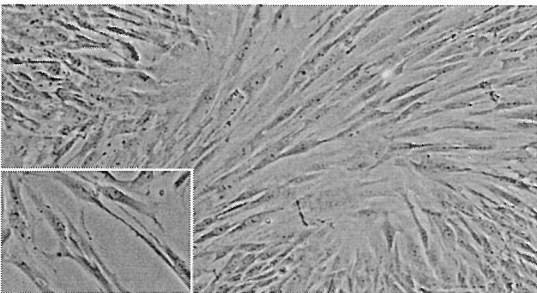
51. IFO 50074 HFL 1 Lung
fetus lung normal diploid fibroblast



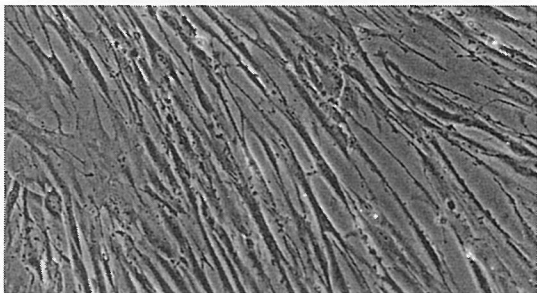
48. IFO 50358 KNS-62 Lung
bronchial squamous carcinoma, metastasis to brain



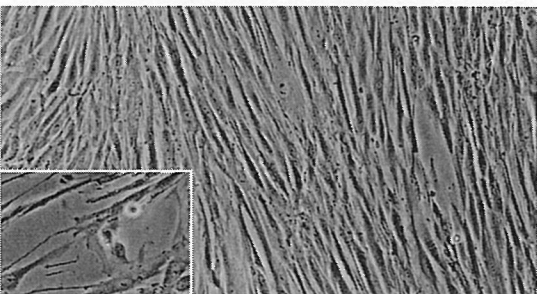
52. IFO 50075 WI-38 Lung
fetus lung normal diploid fibroblast



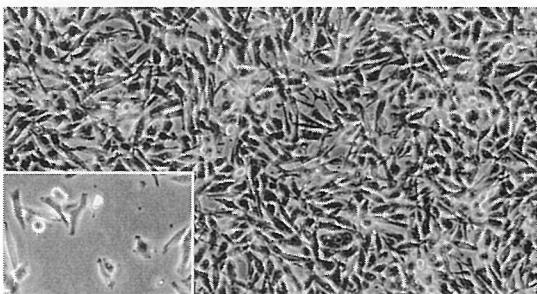
49. IFO 50072 MRC-9 Lung
fetus lung normal diploid fibroblast



53. IFO 50089 Flow 2000 Lung
fetus lung normal diploid fibroblast

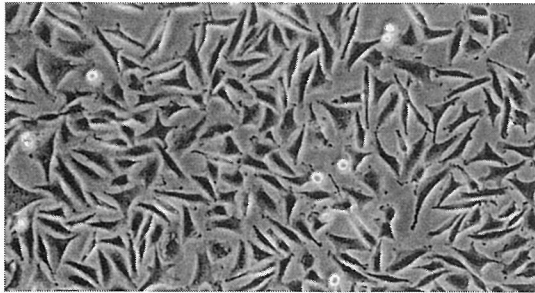


50. IFO 50073 MRC-5 Lung
fetus lung normal diploid fibroblast

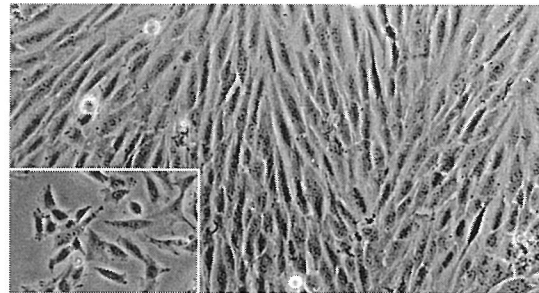


54. IFO 50009 G-361 Melanoma
malignant melanoma

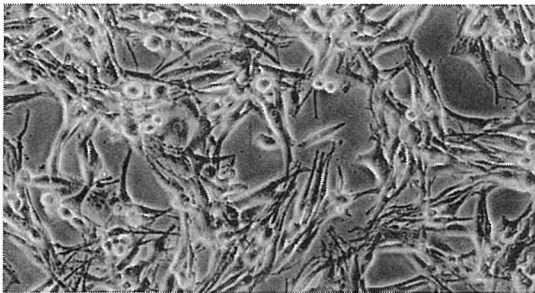
100µm

HUMAN

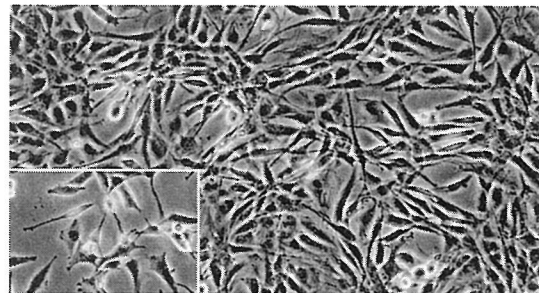
55. IFO 50276 A2058 Melanoma
malignant melanoma, metastasis to lymph node



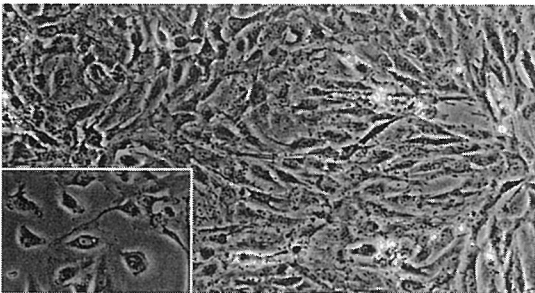
59. IFO 50286 SF126 Nervous system
astrocytoma, GFAP-negative



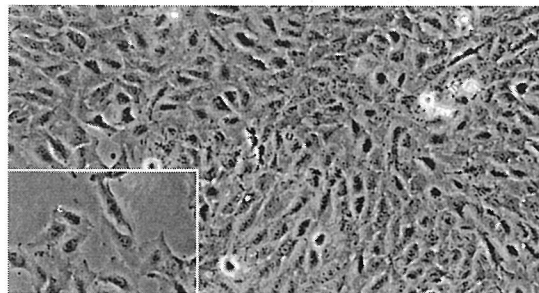
56. IFO 50321 GAK Melanoma
malignant melanoma, metastasis to lymph node



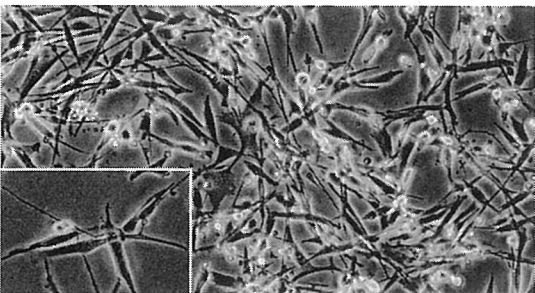
60. IFO 50289 Becker Nervous system
astrocytoma, GFAP-negative



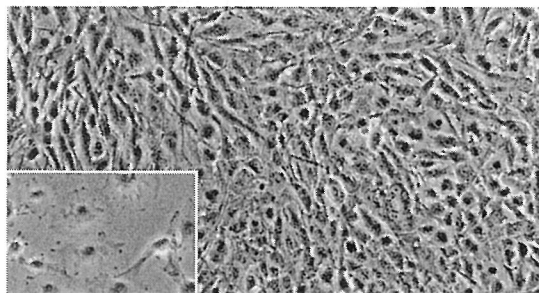
57. IFO 50344 SK-MG-1 Nervous system
astrocytoma, adapted to serum-free medium, derived from
Marcus cells



61. IFO 50290 Marcus Nervous system
astrocytoma, GFAP-negative



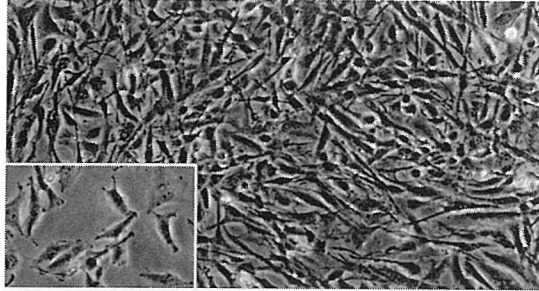
58. IFO 50435 KINGS-1 Nervous system
astrocytoma, anaplastic, GFAP, S-100, vimentin, CD13 and
HNK-1-positive



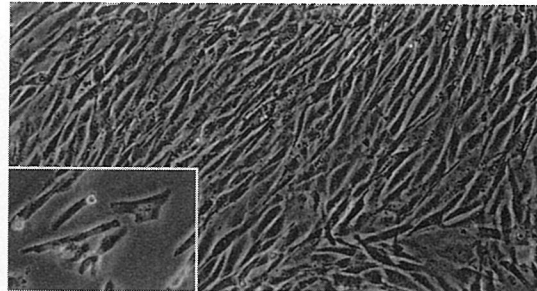
62. IFO 50285 U-251 MG (KO) Nervous system
astrocytoma, GFAP-positive

100μm

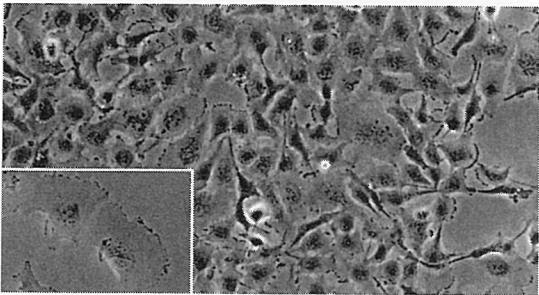
HUMAN



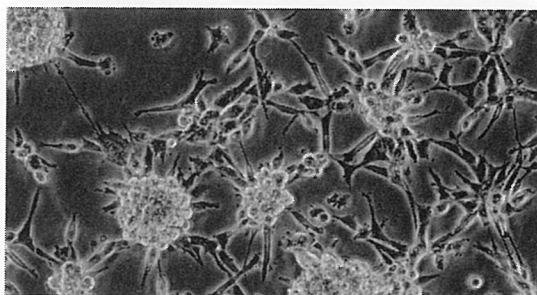
63. IFO 50288 U-251 MG Nervous system astrocytoma, GFAP-positive



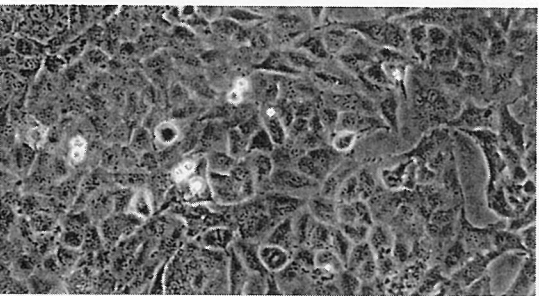
67. IFO 50493 YH-13 Nervous system glioblastoma, GFAP and S-100-positive, ACNU(a cancer chemotherapy drug)-resistant



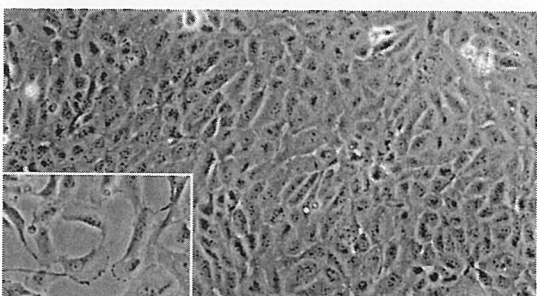
64. IFO 50361 B2-17 Nervous system astrocytoma, thymidine kinase-deficient mutant of U251 MG cells



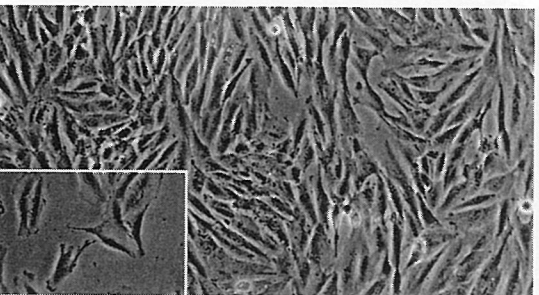
68. IFO 50492 AM-38 Nervous system glioblastoma, GFAP and S-100-positive, ACNU(a cancer chemotherapy drug)-sensitive



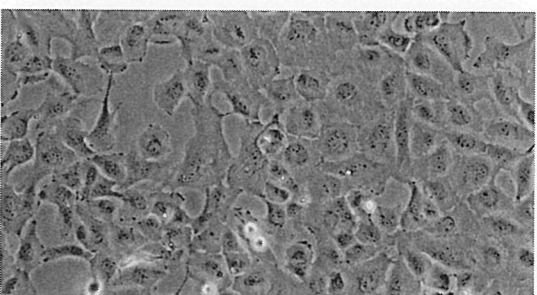
65. IFO 50303 T98G Nervous system glioblastoma



69. IFO 50368 no.10 Nervous system glioma, anaplastic, GFAP-positive

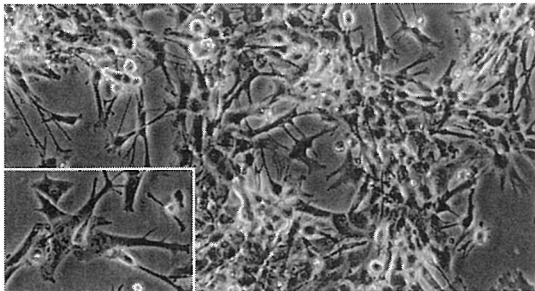


66. FO 50436 KS-1 Nervous system glioblastoma

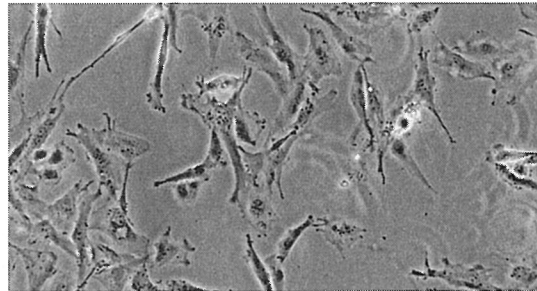


70. IFO 50369 no.11 Nervous system glioma, anaplastic, GFAP-positive

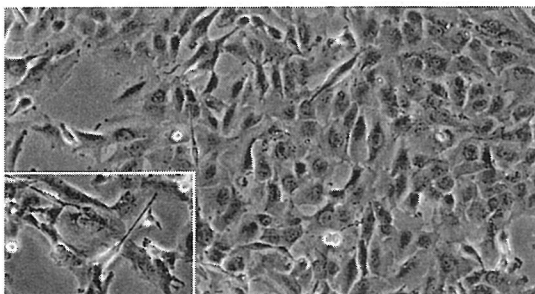
100µm

HUMAN

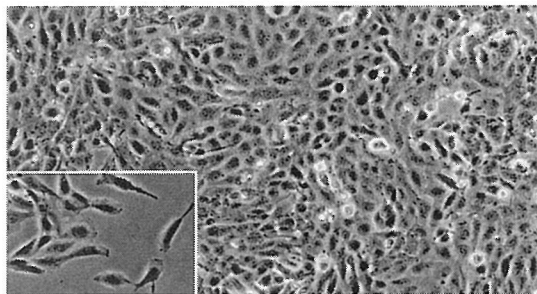
71. IFO 50444 KNS-81-FD Nervous system glioma, derivative of KNS-81 adapted to serum-free medium



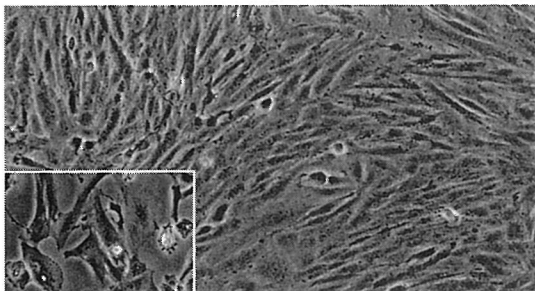
75. IFO 50434 KALS-1 Nervous system glioma, GFAP, vimentin and CD13-positive



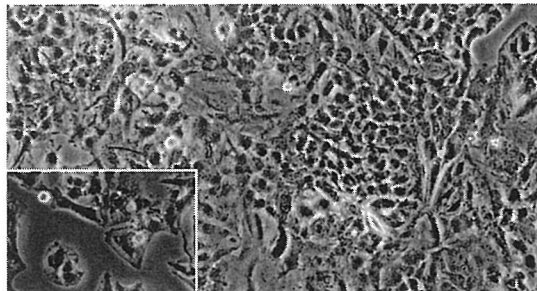
72. IFO 50467 NMC-G1 Nervous system glioma, FGF-9-producing



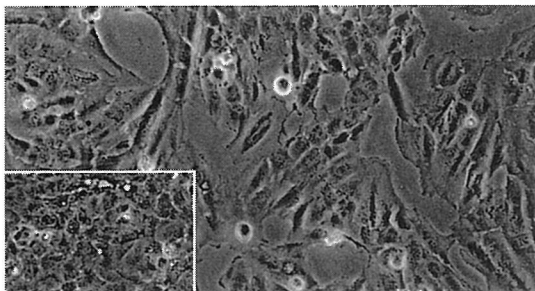
76. IFO 50489 GB-1 Nervous system glioma, GFAP, vimentin and fibronectin-positive



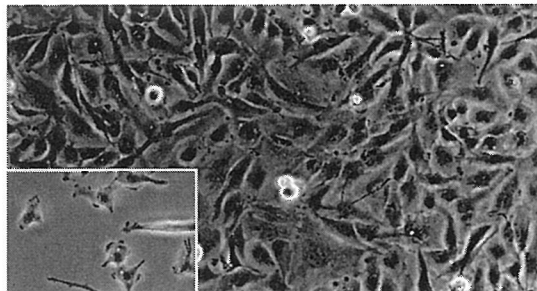
73. IFO 50359 KNS-81 Nervous system glioma, GFAP and S-100-positive, NSE-negative



77. IFO 50356 KNS-42 Nervous system glioma, GFAP-positive, S-100 and NSE-negative



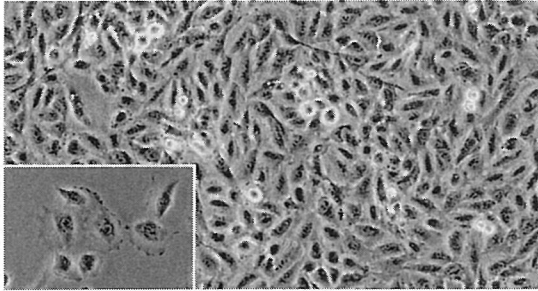
74. IFO 50357 KNS-60 Nervous system glioma, GFAP, S-100 and NSE-negative



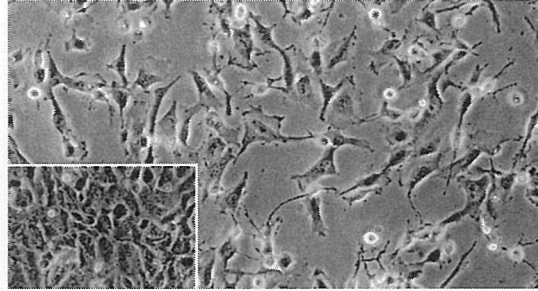
78. IFO 50360 KNS-89 Nervous system gliosarcoma, GFAP and NSE-positive, S-100-negative

100μm

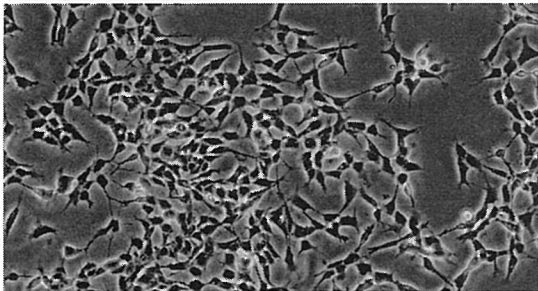
HUMAN



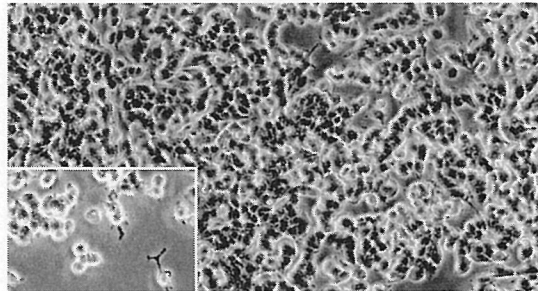
79. IFO 50355 ONS-76 Nervous system medulloblastoma



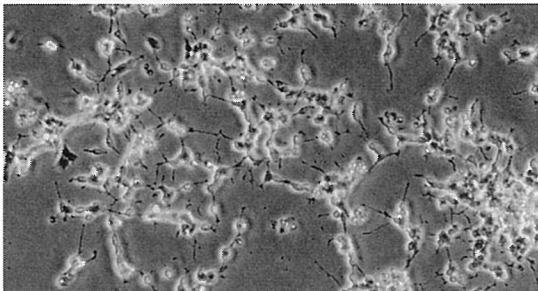
83. IFO 50433 KP-N-S19s Nervous system neuroblastoma, from lymph node, smooth muscle marker-expressing



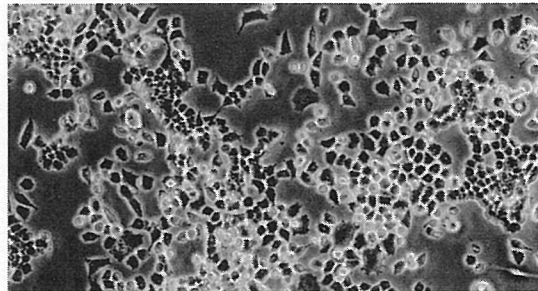
80. IFO 50283 IMR-32 Nervous system neuroblastoma



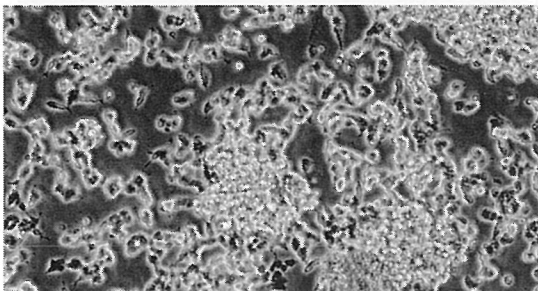
84. IFO 50295 NB-1 Nervous system neuroblastoma, neuronal differentiation



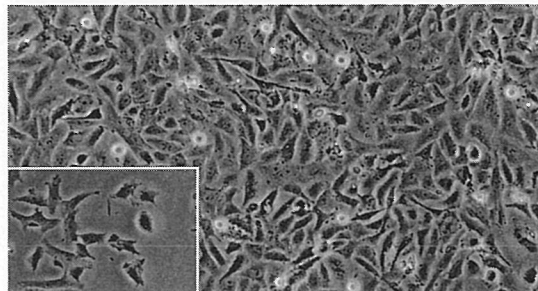
81. IFO 50432 KP-N-RT-BM-1 Nervous system neuroblastoma, from bone marrow, neuronal and Schwannian differentiation



85. IFO 50494 KK-2 Nervous system primitive neuroectodermal tumor, NSE and PGP9.5-positive, NF and S-100-negative

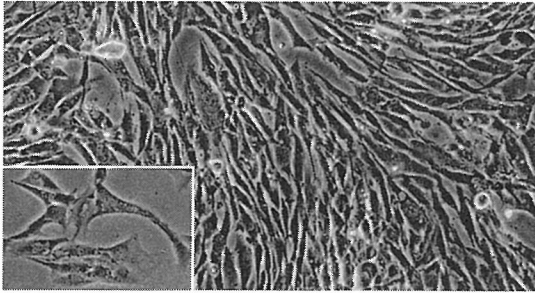


82. IFO 50431 KP-N-YN Nervous system neuroblastoma, from lymph node, smooth muscle marker-expressing

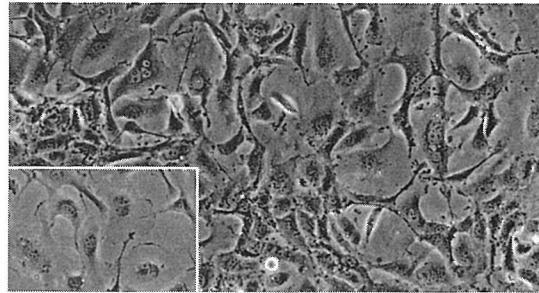


86. IFO 50106 HOS Osteosarcoma

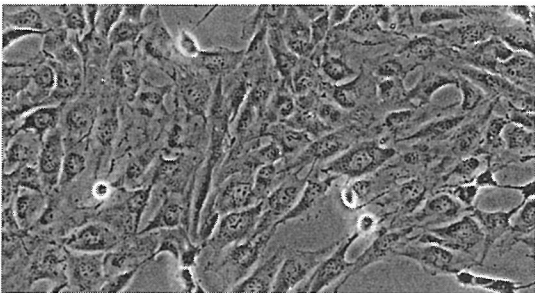
100µm

HUMAN

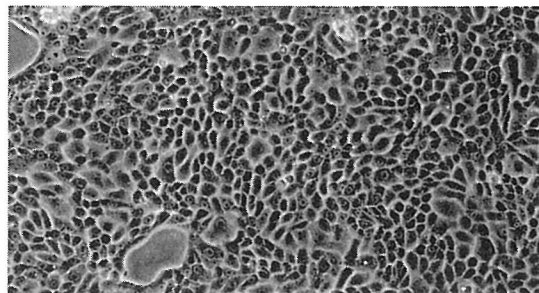
87. IFO 50107 G-292 clone A141B1 Osteosarcoma
osteosarcoma



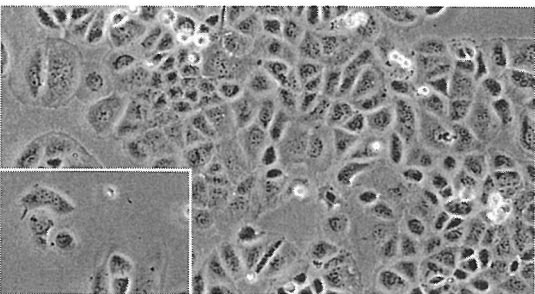
91. IFO 50317 RKN Ovary
leiomyosarcoma



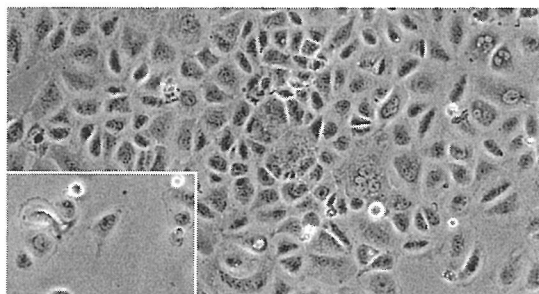
88. IFO 50108 MG-63 Osteosarcoma
osteosarcoma



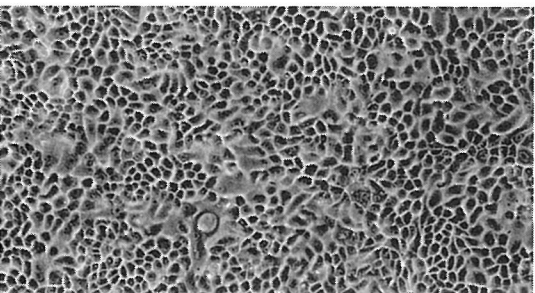
92. IFO 50316 RMG-II Ovary
mesonephroid cancer



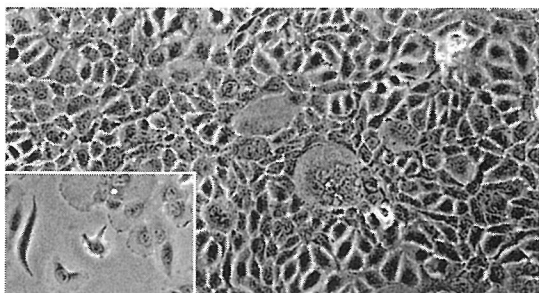
89. IFO 50318 RTSG Ovary
adenocarcinoma, poorly differentiated



93. IFO 50319 RMUG-L Ovary
mucinous cystadenocarcinoma



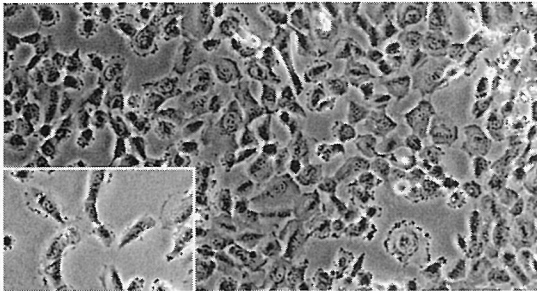
90. IFO 50315 RMG-I Ovary
clear cell carcinoma



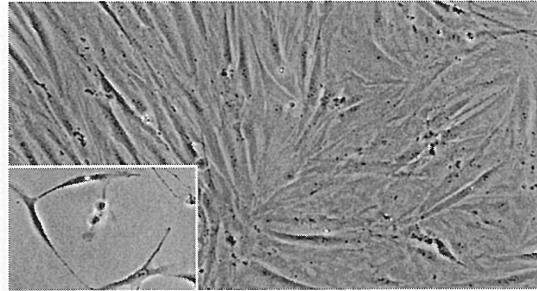
94. IFO 50320 RMUG-S Ovary
mucinous cystadenocarcinoma

100μm

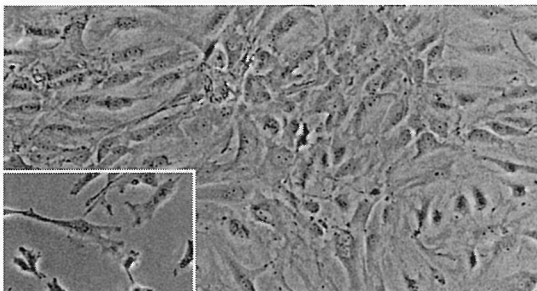
HUMAN



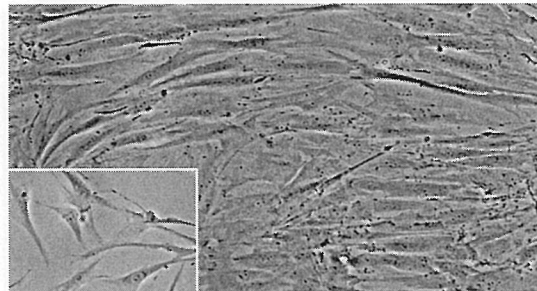
95. IFO 50354 HT-1080 Sarcoma acetabulum, fibrosarcoma



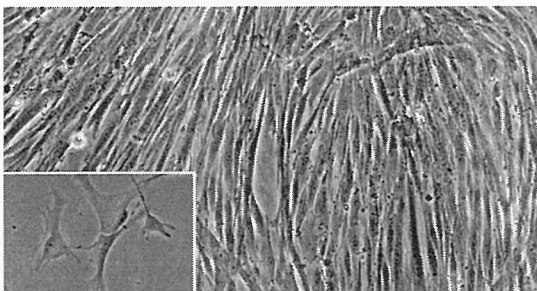
99. IFO 50418 ASF-4-1 Skin skin normal diploid fibroblast



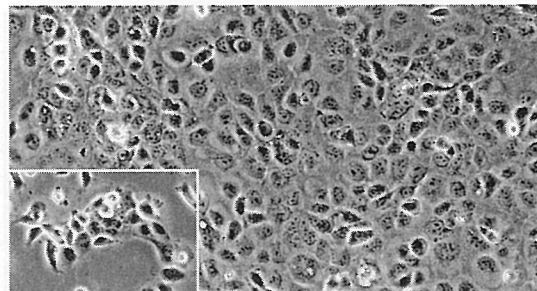
96. IFO 50488 OUMS-27 Sarcoma chondrosarcoma, type 1, 2 and 3 collagens; type 9 and 11 collagen mRNA; proteoglycan-producing



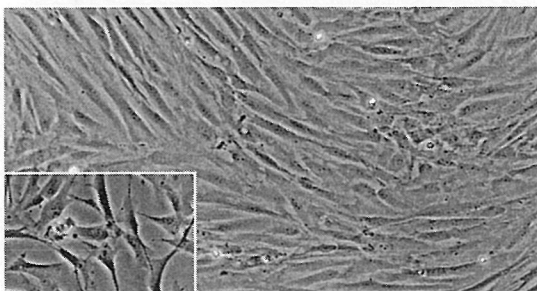
100. IFO 50419 ASF-4-2 Skin skin normal diploid fibroblast



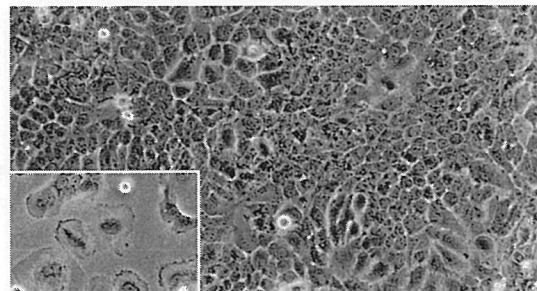
97. IFO 50079 Flow 7000 Skin skin normal diploid fibroblast



101. IFO 50007 Ca Ski Uterus cervical epidermoid cancer

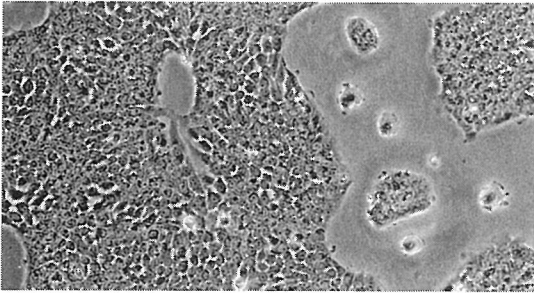


98. IFO 50350 Hs68 Skin skin normal diploid fibroblast

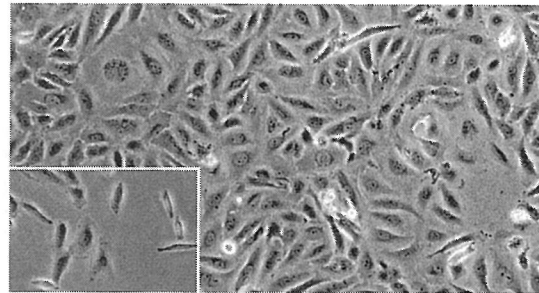


102. IFO 50308 SKG-I Uterus cervical epidermoid cancer

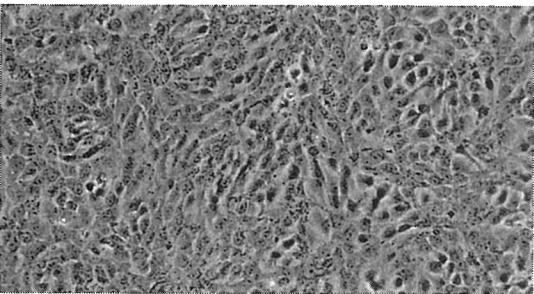
100µm

HUMAN

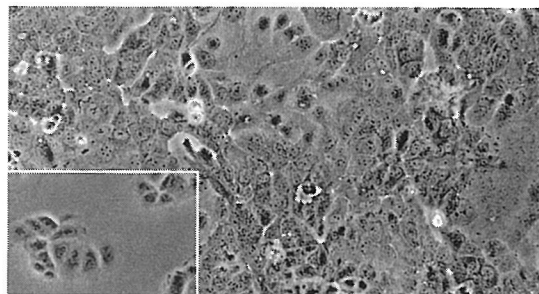
103. IFO 50310 SKG-IIIa Uterus
cervical epidermoid cancer



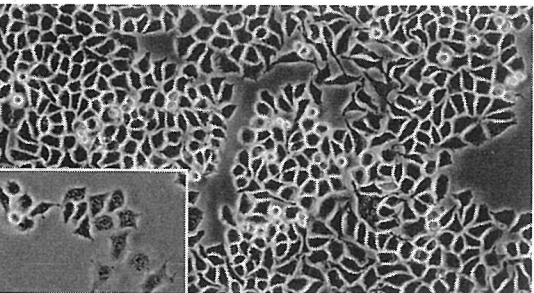
107. IFO 50323 BOKU Uterus
cervical squamous cell carcinoma



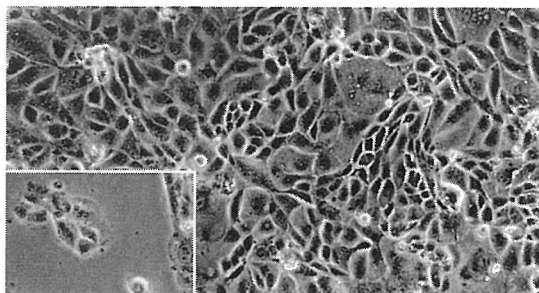
104. IFO 50311 SKG-IIIb Uterus
cervical epidermoid cancer



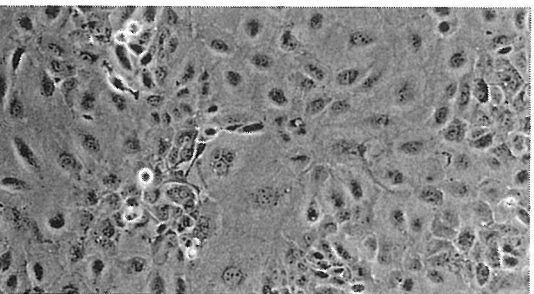
108. IFO 50312 SNG-II Uterus
endometrial adenocarcinoma



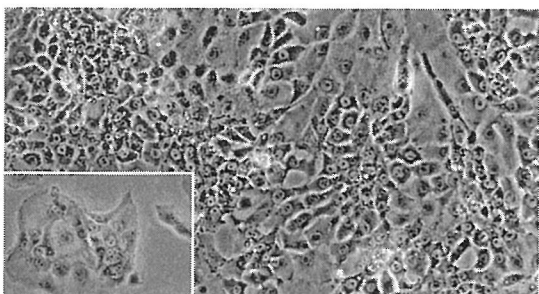
105. IFO 50011 HeLa S3 Uterus
cervical epithelioid carcinoma



109. IFO 50313 SNG-M Uterus
endometrial adenocarcinoma



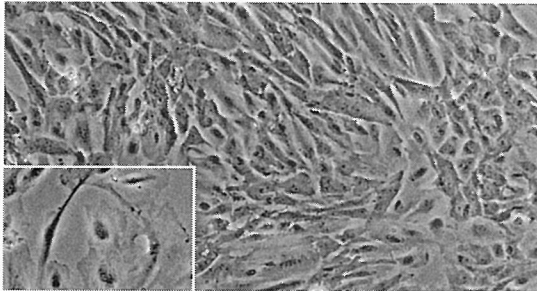
106. IFO 50309 SKG-II Uterus
cervical squamous carcinoma



110. IFO 50322 NJG Uterus
gestational uterine choriocarcinoma

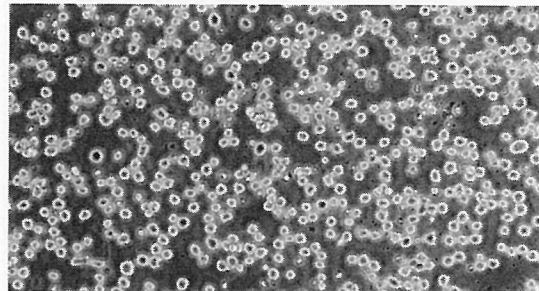
100μm

HUMAN

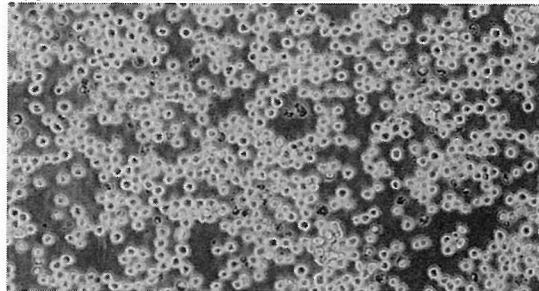


111. IFO 50314 SKN Uterus leiomyosarcoma

HYBRID

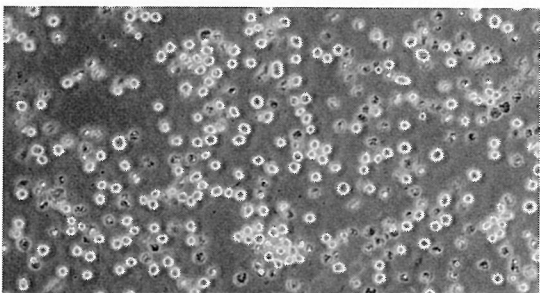


114. IFO 50279 NKY113 Antibody-producing hybridoma MSW113 antibody, recognizing a cancer-associated oligosaccharide

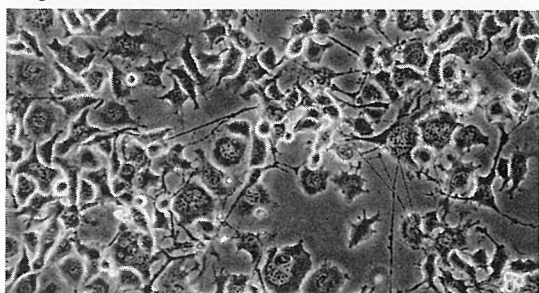


115. IFO 50466 RC1 Antibody-producing hybridoma RC1 antibody, recognizing undifferentiated neural cell-specific antigen

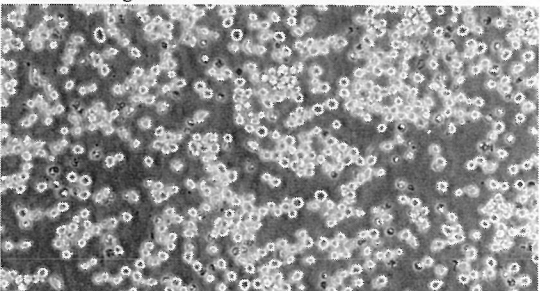
HYBRID



112. IFO 50090 4G12 hybridoma Antibody-producing hybridoma (human x mouse) 4G12 antibody, preferentially reacting with lung carcinoma-associated antigen

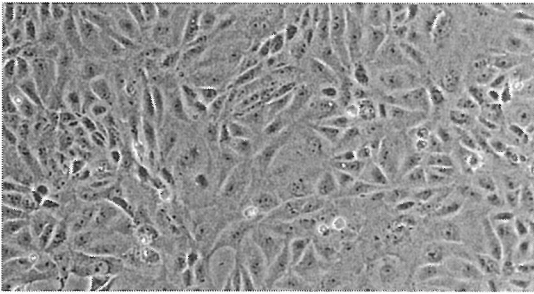


116. IFO 50221 N18-RE-105 Nervous system N18TG2 (mouse neuroblastoma) x Fischer rat 18 d embryonic neural retina

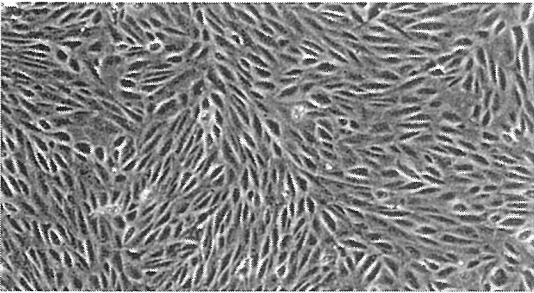


113. IFO 50284 A2B5 clone 105 Antibody-producing hybridoma A2B5 antibody, recognizing a glycolipid expressed in neural tissue

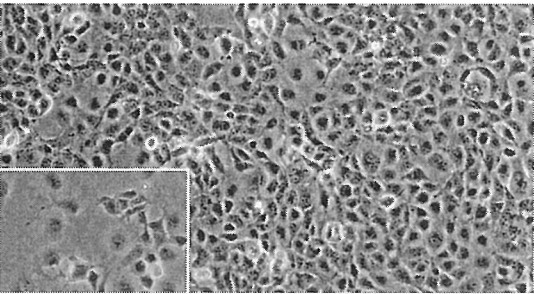
100µm

MONKEY, AFRICAN GREEN

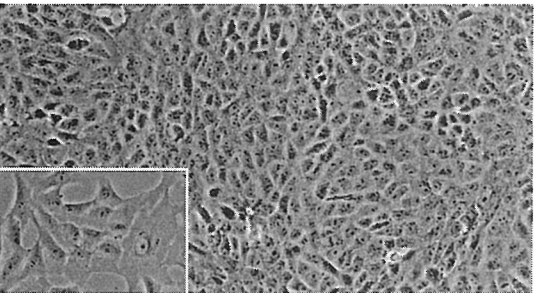
117. IFO 50410 VERO 76 Kidney
subclone of Vero cells



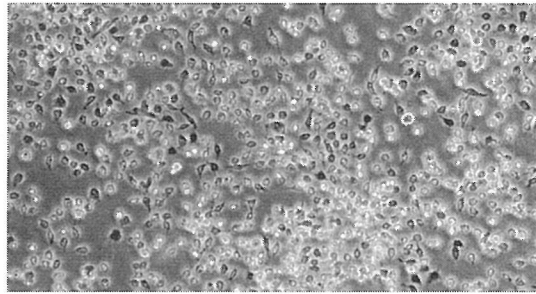
118. IFO 50413 BS-C-1 Kidney
suitable for transfection, especially for SV40



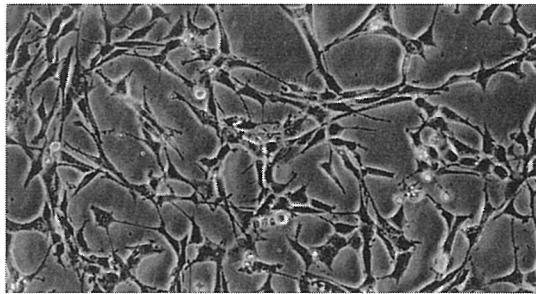
119. IFO 50068 COS-7 Kidney
transformant of CV-1 cells by origin-defective SV-40, SV-40
large T antigen-expressing



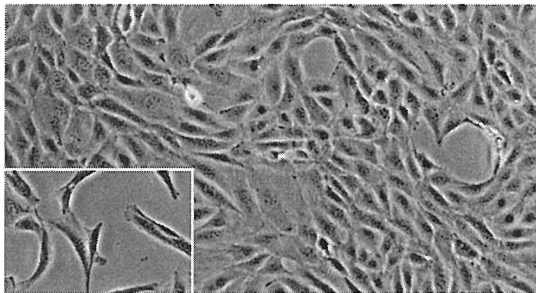
120. IFO 50471 Vero Kidney
useful to assay mycoplasmas

MOSQUITO

121. IFO 50010 C6/36 Hatched larvae
useful for replication of flavivirus

MOUSE

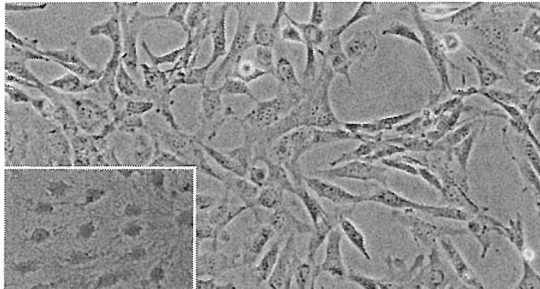
122. IFO 50041 MBT2 Bladder
bladder carcinoma



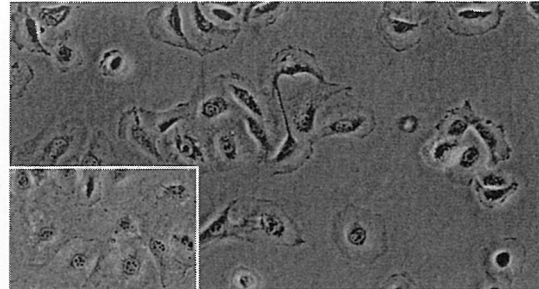
123. IFO 50042 C3H/MCA clone 15 Embryo
3-methylcholanthrene-transformed C3H/10T1/2

100µm

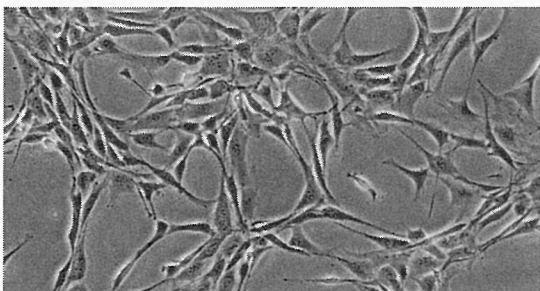
MOUSE



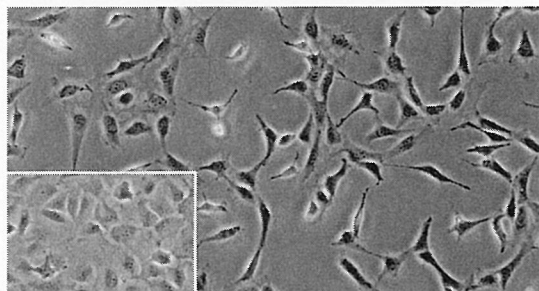
124. IFO 50416 3T3-L1 Embryo
fibroblast, 3T3-Swiss derivative, adipocyte differentiation



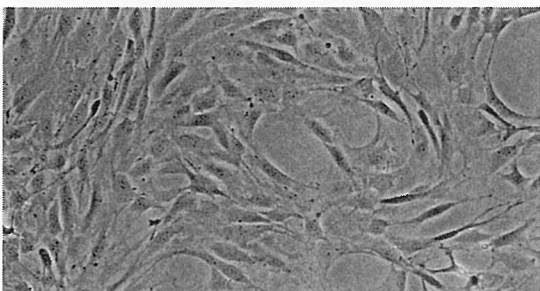
128. IFO 50021 A31-714 C4 Embryo
fibroblast, subclone of Balb/3T3 A31



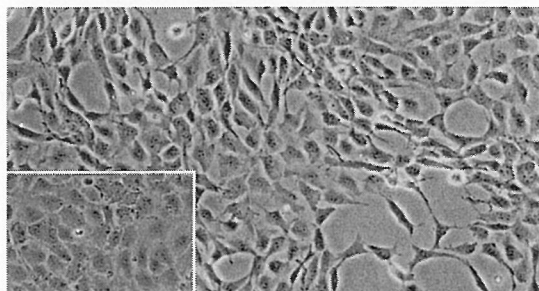
125. IFO 50019 NIH 3T3 p-7 cl-3 Embryo
fibroblast, contact-inhibited



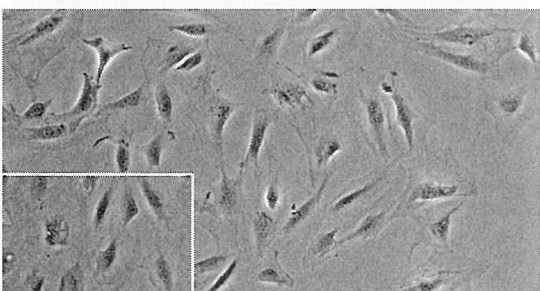
129. IFO 50298 Balb/c 3T3 A31-1-1 Embryo
fibroblast, subclone of Balb/3T3 A31



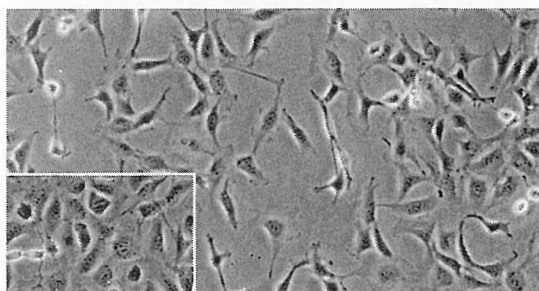
126. IFO 50415 C3H/10T1/2, clone8 Embryo
fibroblast, contact-inhibited



130. IFO 50299 Balb/c 3T3 A31-1-13 Embryo
fibroblast, subclone of Balb/3T3 A31

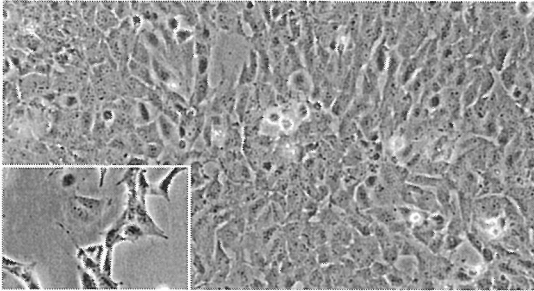


127. IFO 50417 3T3-Swiss albino Embryo
fibroblast, contact-inhibited

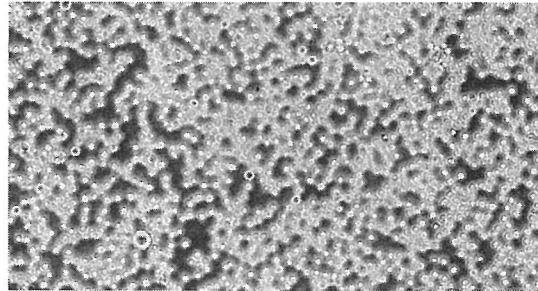


131. IFO 50070 Balb/3T3-A31-1-1 Embryo
fibroblast, subclone of Balb/3T3-A31

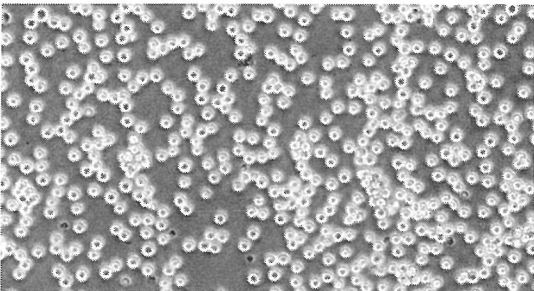
100µm

MOUSE

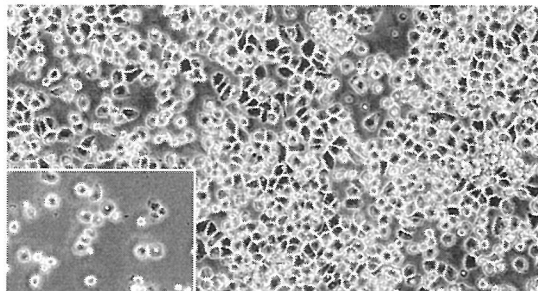
132. IFO 50374 TKD2 Endothelial endothelial cell, tsSV40 large T-immortalized



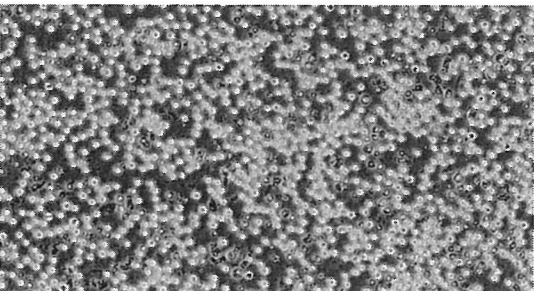
136. IFO 50006 M-1 Hematopoietic lineage spontaneous myeloid leukemia, differentiation into macrophage/granulocyte



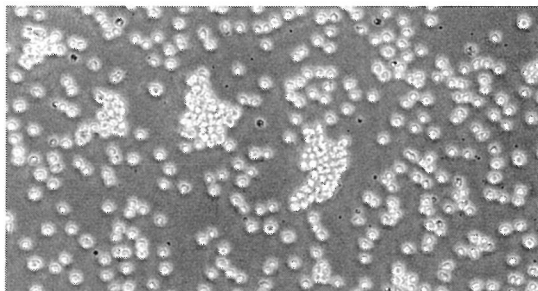
133. IFO 50036 P388D1 Hematopoietic lineage lymphoid neoplasm, derived from P388



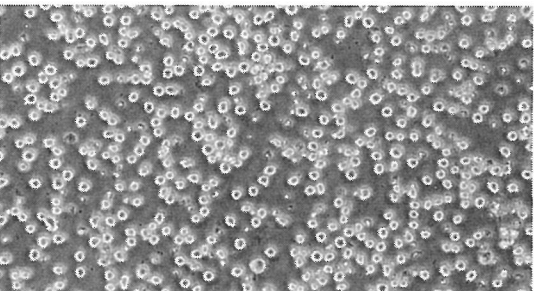
137. IFO 50008 Mm1 Hematopoietic lineage spontaneous myeloid leukemia, M-1-derivative, macrophage-like phenotype



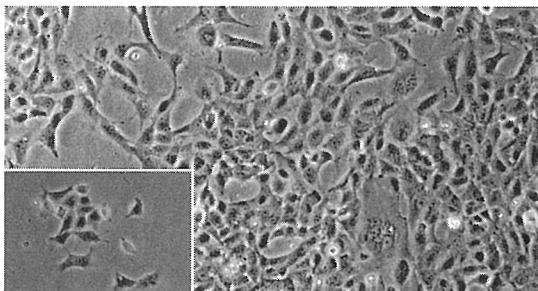
134. IFO 50267 PB-3c Hematopoietic lineage mast cell line



138. IFO 50111 GRSL Hematopoietic lineage thymic leukemia



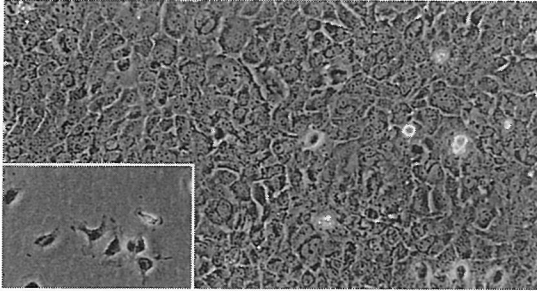
135. IFO 50296 WEHI-3b Hematopoietic lineage myelomonocyte



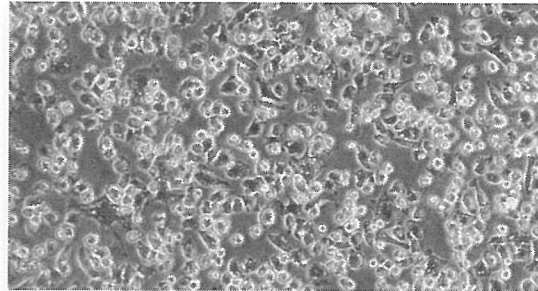
139. IFO 50373 TKC2 Kidney tubule cell, tsSV40 large T-immortalized

100µm

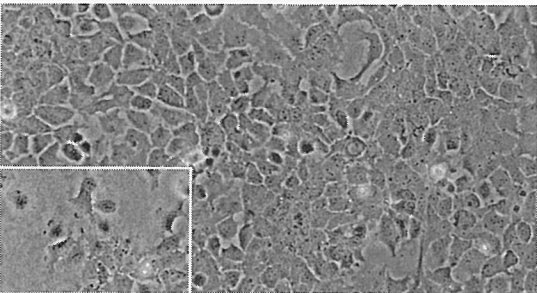
MOUSE



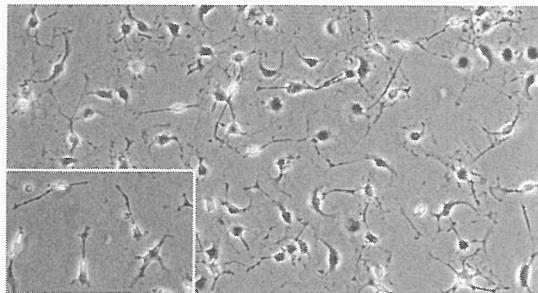
140. IFO 50379 TLR2 Liver hepatocyte, tsSV40 large T-immortalized



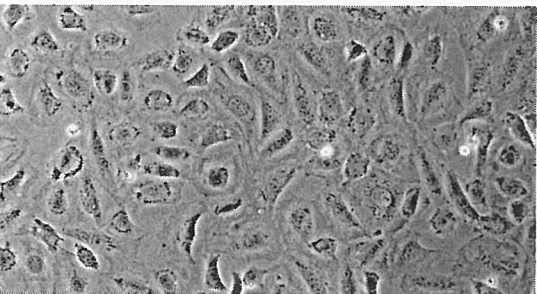
144. IFO 50080 NCTC clone 1469 Liver normal liver-derived



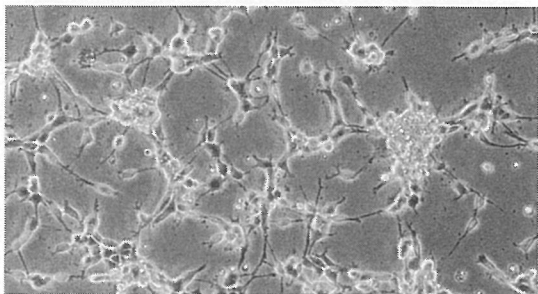
141. IFO 50380 TLR3 Liver hepatocyte, tsSV40 large T-immortalized



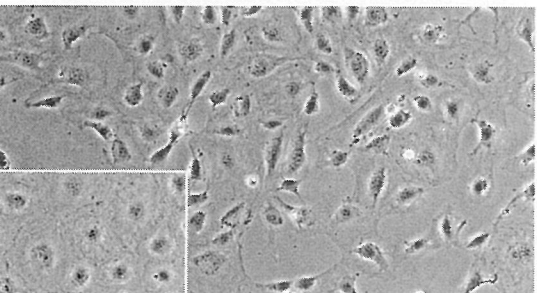
145. IFO 50161 KT-5 Nervous system astrocyte cell line



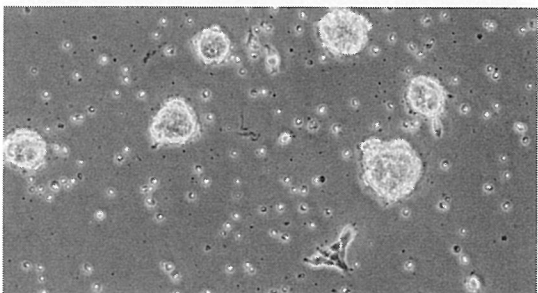
142. IFO 50377 FLS3 Liver liver stromal cell line



146. IFO 50424 AP-16 Nervous system astrocyte-progenitor cell line

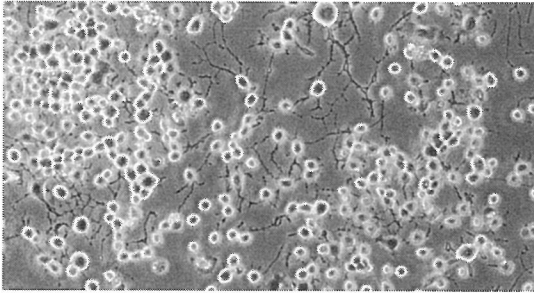


143. IFO 50378 FLS5 Liver liver stromal cell line

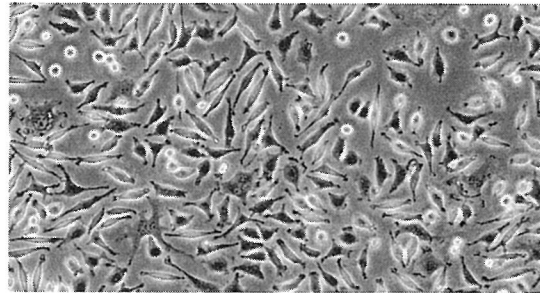


147. IFO 50472 MEB5 Nervous system CNS stem cell line, HPV type 16 E7-immortalized

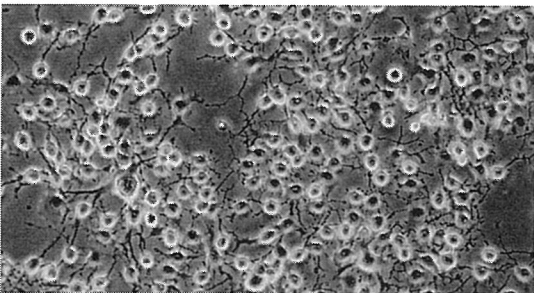
100µm

MOUSE

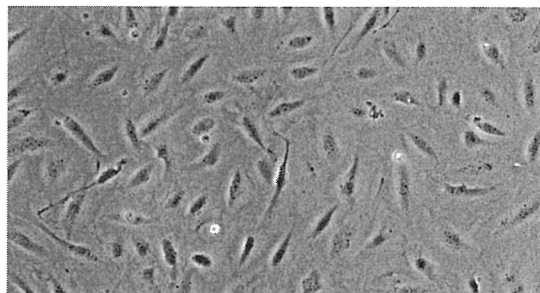
148. IFO 50081 Neuro-2a Nervous system
neuroblastoma



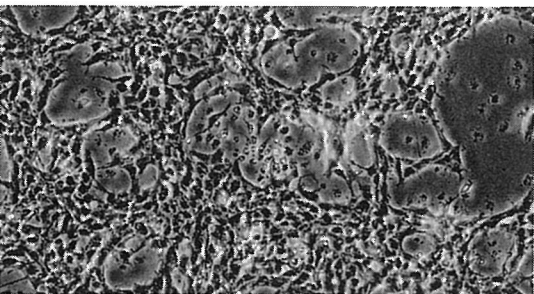
152. IFO 50409 NCTC clone 929 Skin
fibroblast, subclone of strain L



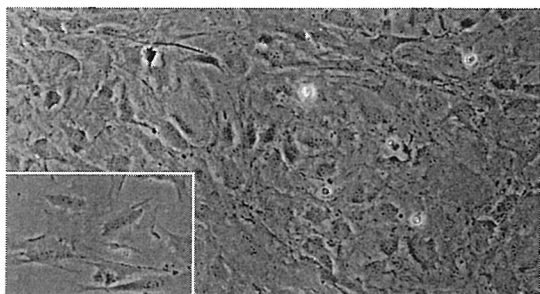
149. IFO 50495 Neuro-2a TG Nervous system
neuroblastoma, 6-thioguanine-resistant, HAT-sensitive



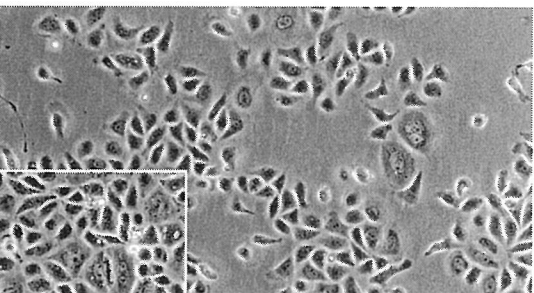
153. IFO 50375 MSS31 Spleen
spleen stromal cell line



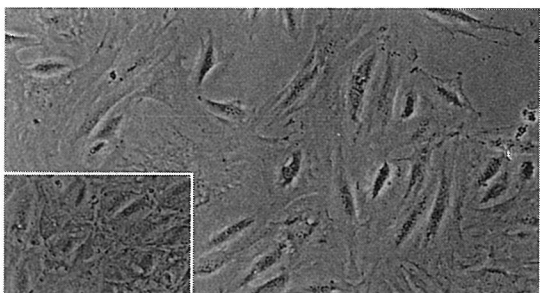
150. IFO 50293 MA-89 Nervous system
normal mixed glial culture



154. IFO 50376 MSS62 Spleen
spleen stromal cell line



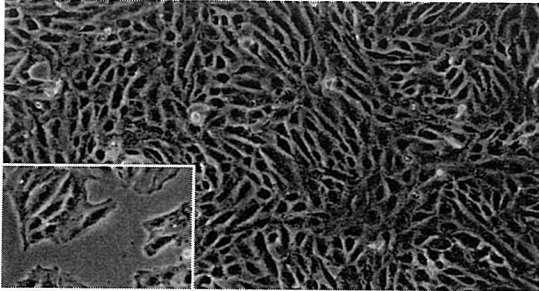
151. IFO 50484 DBC1.2 Olfactory epithelium
dark (horizontal) basal cell-like, keratin-positive



155. IFO 50248 IT-79MTNC3 Thymus
thymic nurse cell

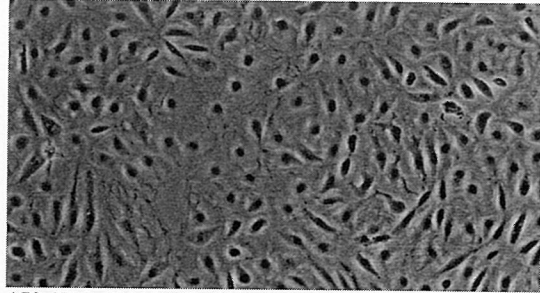
100µm

PIG

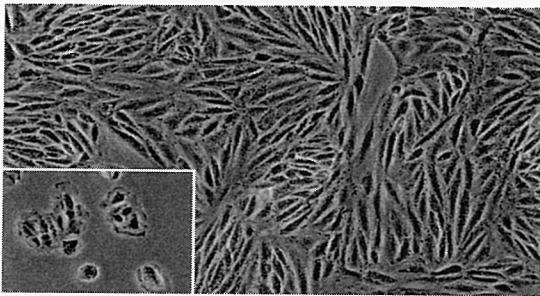


156. IFO 50421 PKS Kidney
susceptible to Vaccinia virus

POTOROO(RAT KANGAROO)

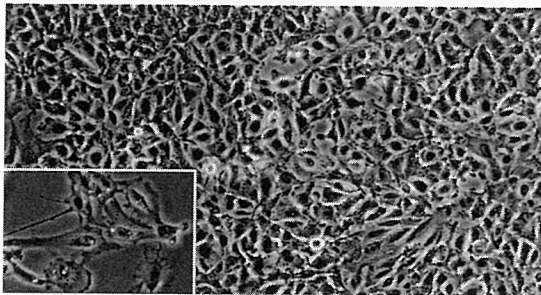


159. IFO 50012 Pt K2 Kidney
kidney, epithelial-like

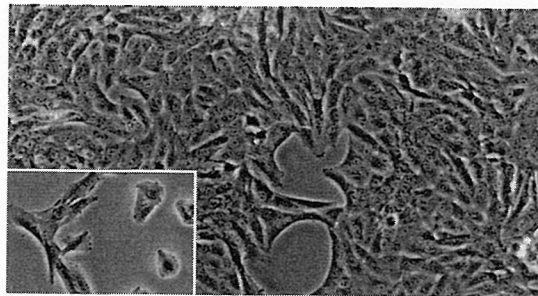


157. IFO 50422 PKF Kidney
susceptible to Vaccinia virus

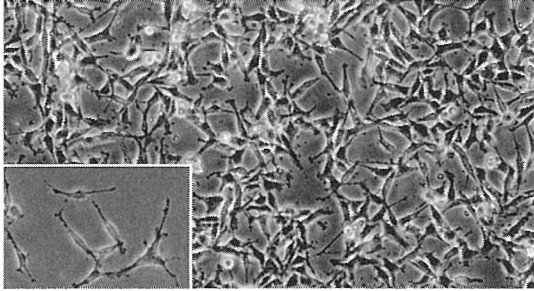
RABBIT



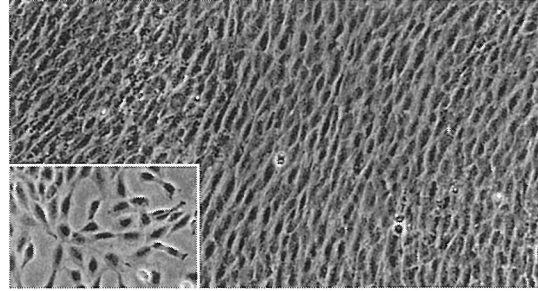
158. IFO 50423 PKR Kidney
susceptible to Vaccinia virus



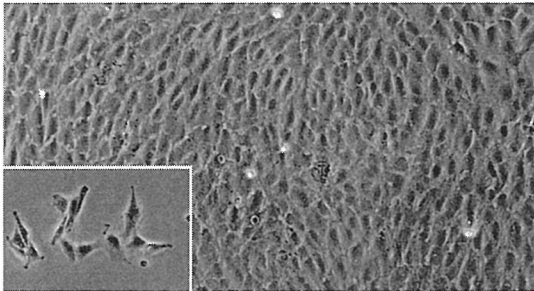
160. IFO 50020 SIRC Cornea
susceptible to rubella virus

RAT

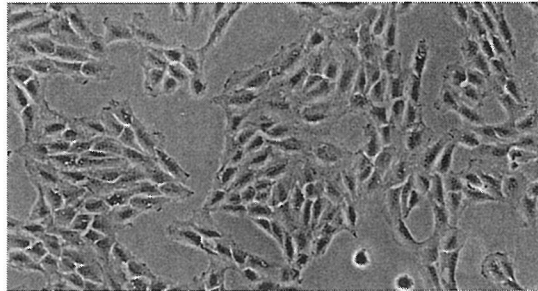
161. IFO 50277 5RP7 Embryo
fibroblast transformed by c-Ha-ras oncogene



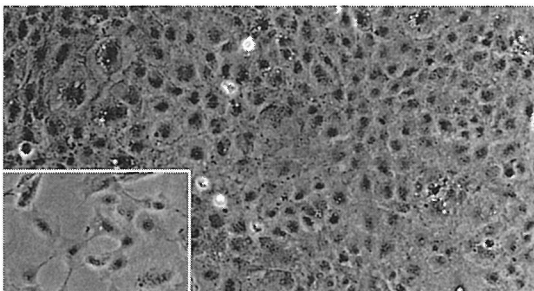
165. IFO 50098 F2408-No.3 Embryo
HGPRT-deficient F2408 cell line



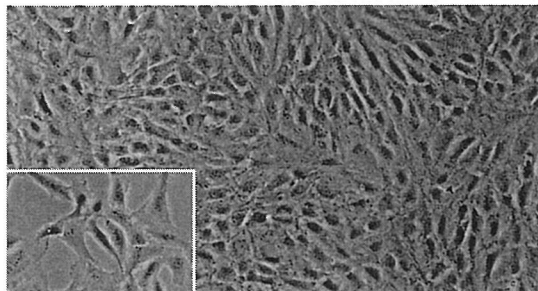
162. IFO 50015 F2408 Embryo
fibroblast-like



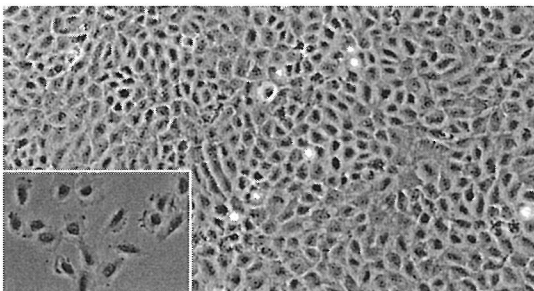
166. IFO 50102 F2408-No.7 Embryo
HGPRT-deficient F2408 cell line



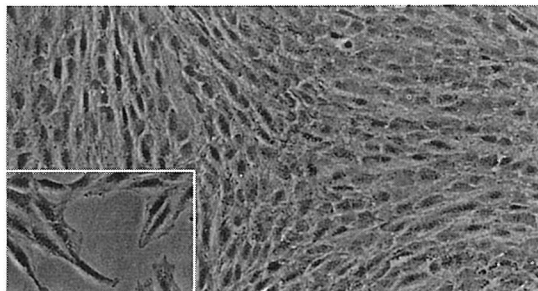
163. IFO 50099 F2408-B812 Embryo
HGPRT(-), TS for transformation by Moloney MSV



167. IFO 50282 RAT-2 Embryo
thymidine kinase deficient RAT-1 (F2408) cell line



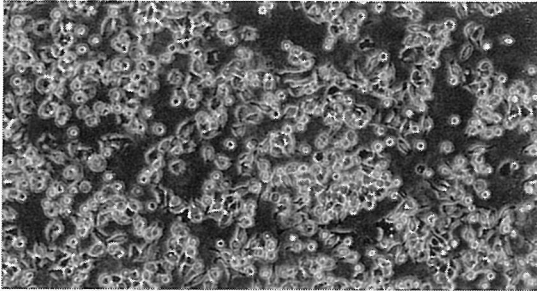
164. IFO 50100 F2408-B993 Embryo
HGPRT(-), TS for transformation by Moloney MSV



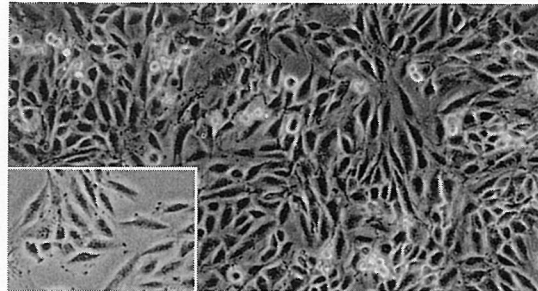
168. IFO 50101 F2408-No.20 Embryo
thymidine kinase-deficient F2408 cell line

100μm

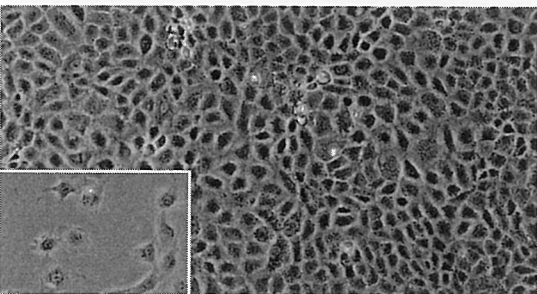
RAT



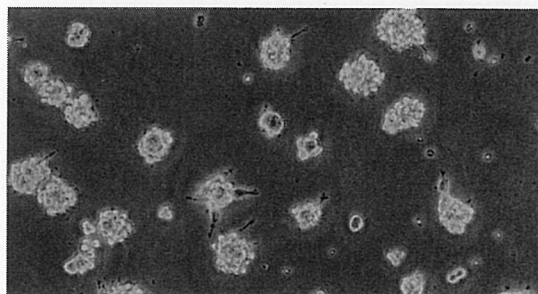
169. IFO 50034 RBL-1 Hematopoietic lineage
basophilic leukemia



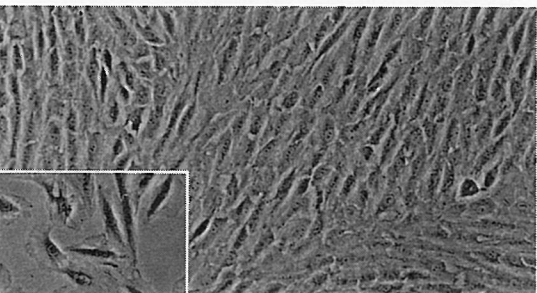
173. IFO 50364 L6 Muscular system
skeletal muscle myoblast



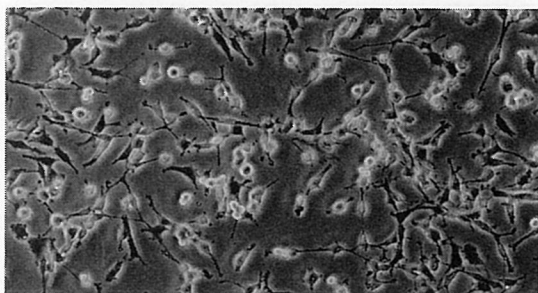
170. IFO 50480 NRK-52E Kidney
epithelioid clone of NRK cells



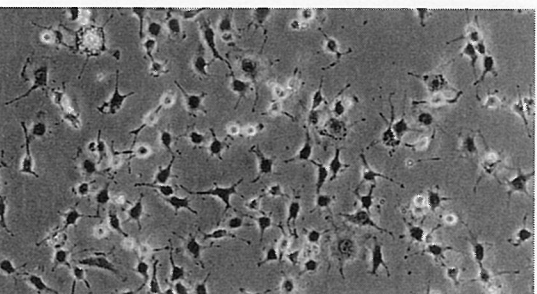
174. IFO 50425 C6-SF2 Nervous system
C6 subline adapted to serum-free medium



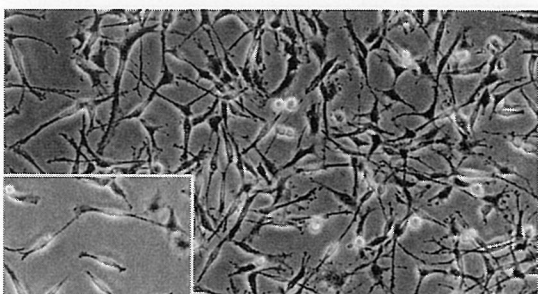
171. IFO 50481 NRK-49F Kidney
fibroblastic clone of NRK cells, useful for transformation assays



175. IFO 50110 C6 Nervous system
glial tumor, GFAP-positive

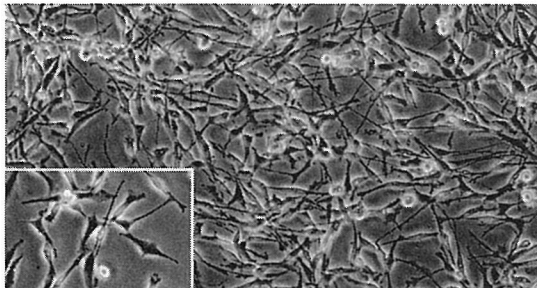


172. IFO 50280 77N1 Kidney
transformant of NRK cells by avian sarcoma virus B77

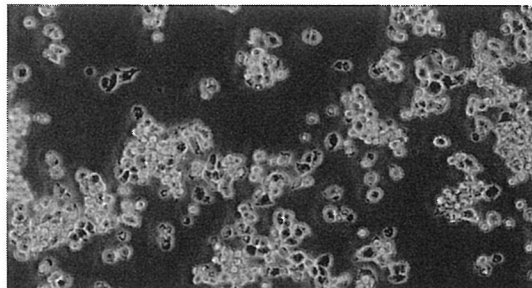


176. IFO 50491 RNB Nervous system
neonatal rat astrocytes, GFAP-positive

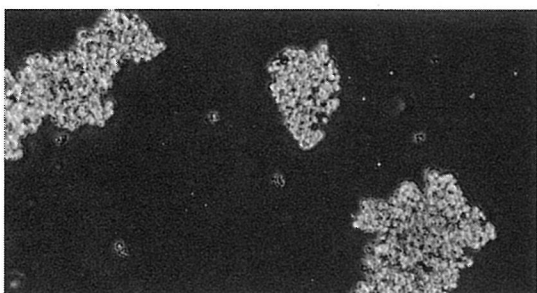
100µm

RAT

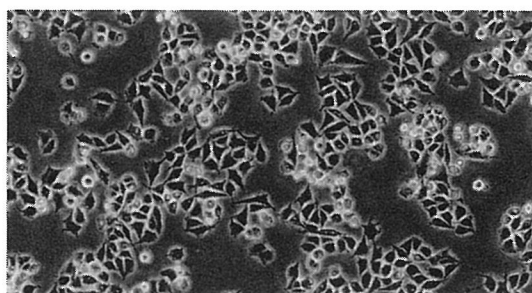
177. IFO 50490 MDM2-RNB Nervous system neonatal rat astrocytes, GFAP-positive; human MDM2 protein-expressing; G418resistant



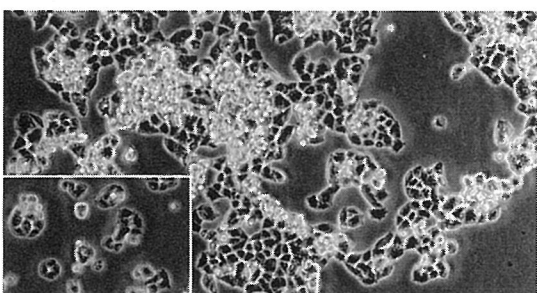
181. IFO 50269 GH1 Pituitary pituitary tumor, somatotrophin-secreting



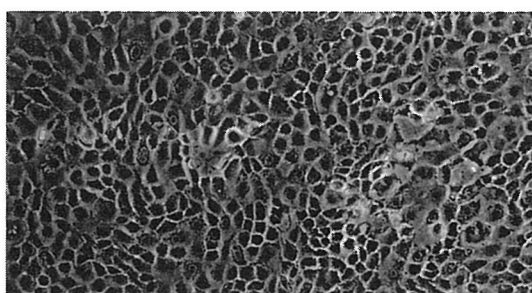
178. IFO 50278 PC-12 Nervous system pheochromocytoma, neuronal differentiation by NGF



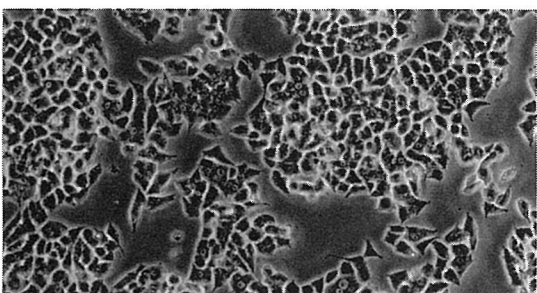
182. IFO 50017 XC Sarcoma useful for assay of murine leukemia virus



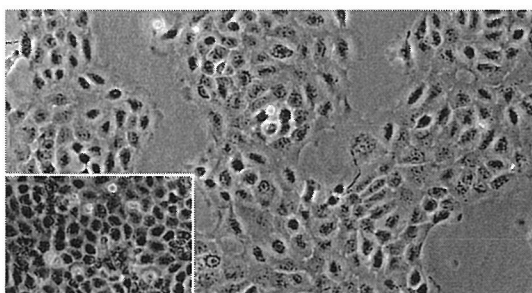
179. IFO 50496 PC-12 TG Nervous system pheochromocytoma, neuronal differentiation by NGF, 6-thioguanine-resistant, HAT-sensitive



183. IFO 50076 IT-45R1 Thymus T cell differentiation factor producing



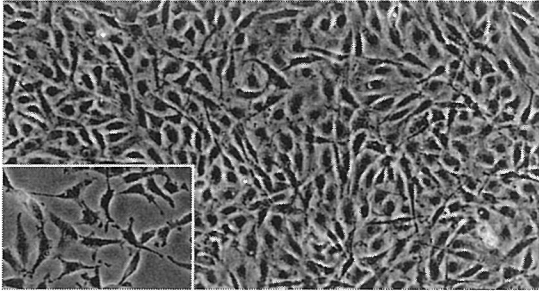
180. IFO 50105 GH3 Pituitary pituitary tumor, somatotrophin-secreting



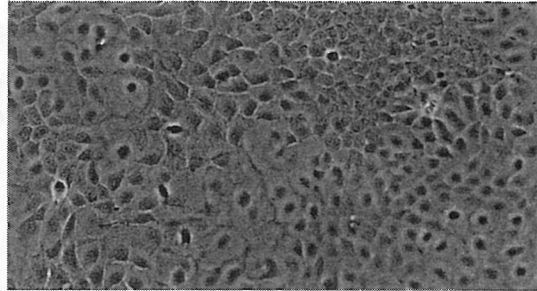
184. IFO 50078 IT-26R21 Thymus T cell differentiation factor producing

100μm

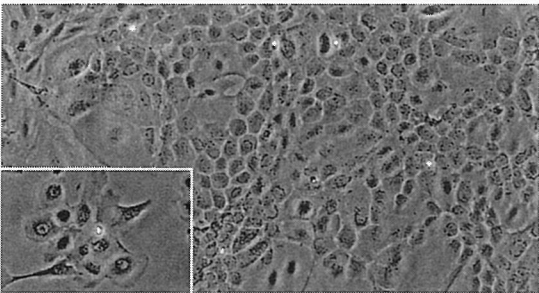
RAT



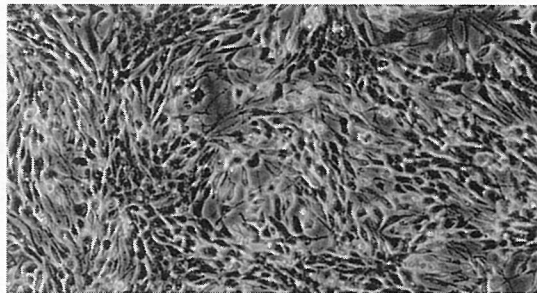
185. IFO 50077 IT-45R91 Thymus
thymic fibroblast



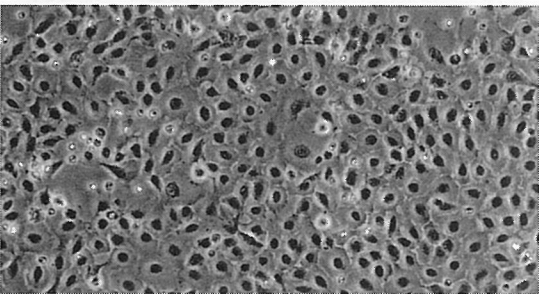
189. IFO 50120 TaD-1-3 Thymus
thymoma, keratin-positive



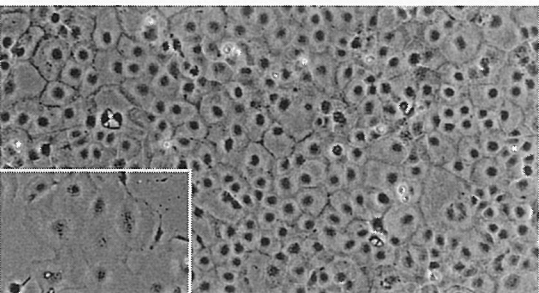
186. IFO 50103 TuD-1 Thymus
thymic stroma, keratin-positive



190. IFO 50270 6-23 clone 6 Thyroid
medullary thyroid carcinoma, neurotensin and calcitonin-producing



187. IFO 50152 TuD-1-3 Thymus
thymic stroma, keratin-positive



188. IFO 50112 TaD-1 Thymus
thymoma, keratin-positive

100µm

CATALOGUE OF NEWLY ACCEPTED STRAINS

FEBRUARY 1996 - DECEMBER 1998

The cultures involved in the following catalogue can be distributed under the same condition as strains listed on the IFO LIST OF CULTURES 10th Edition

IFO No	Name	Temp	Med
(T=Type strain)			
1139	<i>Saccharomyces castellii</i> FMJ -- NRRL Y-664.	28	108
9230	<i>Penicillium chermesinum</i> RTCI (H. Fukuda).	24	1
10894	<i>Nadsonia fulvescens</i> var. <i>elongata</i> JCM 9991 -- (T. Nakase) -- NRRL Y-991.	17	108
10895	<i>Nadsonia fulvescens</i> var. <i>fulvescens</i> JCM 10023 -- (T. Nakase; CK 242, moss).	17	108
10896	<i>Yamadazyma farinosa</i> Dept. Chem., Keio Univ. (H. Ohta; Ku 3040) -- IAM 4682 -- RIFY 7092 -- K. Kodama.	24	108
10897	<i>Kluyveromyces africanus</i> IFO (K. Ueda; U96-103-2; soil; Iriomote Island).	24	108
10898	<i>Kluyveromyces africanus</i> IFO (K. Ueda; U96-105-2; soil; Iriomote Island).	24	108
10913	<i>Stephanoascus ciferrii</i> JCM 2984 -- M. Suzuki, T-5-1 -- W. Daengsubha, Ma-kam-pom dong (pickled emblic myrabolan), Thailand.	24	108
10914	T <i>Kawasakiarxii</i> CBS 7333 -- J.P. van der Walt, soil of forest, South Africa.	24	108
10915	T <i>Saccharomyces kunashirensis</i> CBS 7662 -- I.P. Bab'eva, soil, USSR.	24	108
10916	<i>Saccharomyces</i> sp. CBS 7996 -- A.N. Hagler, water in tank of <i>Quesnelia quesneliana</i> .	24	108
10917	<i>Saccharomyces</i> sp. CBS 7997 -- A.N. Hagler, water in tank of <i>Quesnelia quesneliana</i> .	24	108
10918	<i>Stephanoascus ciferrii</i> CBS 7409 -- CCY 29-170-1, liver of <i>Bufo granulatus</i> , Brazil.	24	108

IFO No		Name	Temp	Med
(T=Type strain)				
10919	T	<i>Zygozoma smithiae</i> CBS 7407 -- J.P. van der Walt, frass of ambrosia beetle, <i>Crossotarsus externedentatus</i> , South Africa.	24	108
10920	T	<i>Zygozoma suomiensis</i> CBS 7251 -- E. Tunnela, skin lesion of cow, Finland.	24	108
10921		<i>Torulaspota delbrueckii</i> Dept. Chem., Keio Univ. (T. Sugai; KU 3013; soil).	28	108
10924		<i>Saccharomyces</i> sp. IFO (K. Ueda; U97-433-1; leaf of <i>Kandelia candel</i>).	28	108
10925		<i>Kluyveromyces</i> sp. IFO (K. Ueda; U97-412-1; flower of <i>Elaeocarpus decipiens</i>).	28	108
10926		<i>Kluyveromyces</i> sp. IFO (K. Ueda; U97-412-3; flower of <i>Elaeocarpus decipiens</i>).	28	108
10927		<i>Kluyveromyces</i> sp. IFO (K. Ueda; U97-436-2; leaf of <i>Bruguiera conjugata</i>).	28	108
10928		<i>Kluyveromyces</i> sp. IFO (K. Ueda; U97-437-2; leaf of <i>Rhizophora mucronata</i>).	28	108
10929		<i>Kluyveromyces</i> sp. IFO (K. Ueda; U97-101-2; soil).	28	108
10930		<i>Kluyveromyces africanus</i> IFO (K. Ueda; U97-203-3; sand ball made by crab).	28	108
13183		<i>Streptomyces aureofaciens</i> IFO (K. Nakazawa) -- Lederle Labs, UV-8.	28	228
15159		<i>Sulfolobus</i> sp. Coll. of Liberal Arts & Sci., Kitasato Univ. (T. Itoh; N-8; hot spring mud in Japan).	75	281
15160		<i>Sulfolobus</i> sp. Coll. of Liberal Arts & Sci., Kitasato Univ. (T. Itoh; TA-2; hot spring mud in Japan).	75	281
15161	T	<i>Sulfurisphaera ohwakuensis</i> Coll. of Liberal Arts & Sci., Kitasato Univ. (T. Itoh; TA 1; hot spring mud in Japan).	75	281
15708	T	<i>Microbacterium hominis</i> Lab. de Bacteriol., Univ. Louis Pasteur (D.A. de Briel) -- LCDC 84-209, lung aspirate.	30	203
15709		<i>Microbacterium laevaniformans</i> Lab. de Bacteriol., Univ. Louis Pasteur (D.A. de Briel) -- LCDC 91-039, blood.	30	203

IFO No		Name	Temp	Med
(T=Type strain)				
15828	T	<i>Spirilliplanes yamanashiensis</i> IFO (T. Tamura) -- M. Hayakawa, YU127-1, soil.	28	268
15853	T	<i>Thermobifida alba</i> DSM 43310 -- S.T. Williams, strain E60 (<i>Actinobifida alba</i>) -- CUB 432 -- R. Locci, IPV 1900, soil.	50	227
16051	T	<i>Pilimelia anulata</i> JCM 3090 -- KCC A-0090 -- W.K. Hanton, soil.	28	343
16052	T	<i>Pilimelia columellifera</i> subsp. <i>columellifera</i> JCM 3367 -- G. Vobis, MB-SK6 -- D. Schafer, soil, Peru.	28	343
16055	T	<i>Cellulomonas hominis</i> DSM 9581 -- DMMZ CE40, cerebrospinal fluid.	37	230
16056	T	<i>Gordonia hirsta</i> DSMZ 44140 -- S. Klatte, K718a, packing material of a biofilter.	30	230
16057	T	<i>Gordonia hydrophobica</i> DSM 44015 -- B. Bendinger; 1610/1b; packing material (tree bark compost).	30	230
16058	T	<i>Sphingomonas subarctica</i> DSM 10700 -- R. Herwig, KF1, subarctic area.	30	802
16060	T	<i>Microbacterium thalassium</i> IFO (M. Takeuchi; No.10; soil in mangrove rhizosphere).	30	802
16061		<i>Microbacterium thalassium</i> IFO (M. Takeuchi; No.71; root in mangrove rhizosphere).	30	802
16062	T	<i>Microbacterium halophilum</i> IFO (M. Takeuchi; No.76; root in mangrove rhizosphere).	30	802
16068	T	<i>Gordonia rhizosphaera</i> IFO (M. Takeuchi; No.141; root in mangrove rhizosphere).	30	230
16069	T	<i>Rhodococcus rhodochrous</i> JCM 3202 -- KCC A-0202 -- M. Goodfellow, N54 --IMRU 732 -- R.S. Breed, KMRh -- Kral Collection -- M. Migula.	30	271
16070	T	<i>Micromonospora echinospora</i> subsp. <i>pallida</i> JCM 3133 -- KCC A-0133 -- Y. Okami -- ATCC 15838 -- Schering Corp. -- A. Woyciesjes .	28	227
16079		<i>Streptomyces turgidiscabies</i> Hokkaido Tokachi Agr. Exp. Stn. (K. Miyajima; Sy 9103; potato).	28	227
16080	T	<i>Streptomyces turgidiscabies</i> Hokkaido Tokachi Agr. Exp. Stn. (K. Miyajima; Sy 9113; potato).	28	227
16081		<i>Streptomyces turgidiscabies</i> Hokkaido Tokachi Agr. Exp. Stn. (K. Miyajima; MT 9301; potato).	28	227

IFO No		Name	Temp	Med
(T=Type strain)				
16082	T	<i>Bacillus globisporus</i> JCM 10046 -- CCM 2119 -- J.L. Stokes, W25	20	201
16084	T	<i>Sphingomonas aromaticivorans</i> Dept. Biolog. Sci., Florida State Univ. (D.L. Balkwill; SMCC F199; Saturated subsurface Atlantic coastal plain sediments).	30	802
16085	T	<i>Sphingomonas stygia</i> Dept. Biolog. Sci., Florida State Univ. (D.L. Balkwill; SMCC B0712; Saturated subsurface Atlantic coastal plain sediments).	30	802
16086	T	<i>Sphingomonas subterranea</i> Dept. Biolog. Sci., Florida State Univ. (D.L. Balkwill; SMCC B0478; Saturated subsurface Atlantic coastal plain sediments).	30	802
16095	T	<i>Thermobifida alba</i> JCM 3077 -- KCC A-0077 -- IPV 1900, garden soil.	37	268
16096	T	<i>Thermomonospora chromogena</i> JCM 6244 -- DSM 43794 -- CUB 580 -- IMRU N2900 -- N.S. Agre, 577, soil.	50	266
16102	T	<i>Asiosporangium albidum</i> Sichuan Industrial Inst. Antibiot., Sichuan (H. Runmao; NA 944235; Provice Jiangxi; soil).	28	266
16103	T	<i>Micromonospora halophytica</i> subsp. <i>nigra</i> JCM 8973 -- NCIMB 2225 -- ATCC 33088 -- NRRL 3097 -- A. Woyciesjes, mud, bottom of salt pool.	28	268
16115	T	<i>Cathayosporangium alboflavum</i> SIIA, H. Runmao, NA 945110 soil.	28	227
16118	T	<i>Brachybacterium alimentarium</i> INRA (Tailliez; CNRZ 925) -- K. Schubert , M 180, cheese.	30	203
16119	T	<i>Brachybacterium tyrofermentans</i> INRA (Tailliez; CNRZ 926) -- K. Schubert , M 131, cheese.	30	203
16120	T	<i>Tsukamurella paurometabola</i> ATCC 8368 -- E.A. Steinhaus.	30	203
16126	T	<i>Agrococcus jenensis</i> DSMZ 9580 -- I. Groth, 2002-39/1, soil.	30	264
16127	T	<i>Friedmanniella antarctica</i> DSM 11053 -- P. Hirsch, AA-1042, sandstone.	22	264
16128	T	<i>Janibacter limosus</i> DSM 11140 -- K. Martin, HKI 83, sludge.	30	264
16129	T	<i>Rubrobacter xylanophilus</i> DSM 9941 -- L. Carreto, PRD-1, thermally polluted runoff.	60	264

IFO No	Name	Temp	Med
(T=Type strain)			
16130	<i>Microbacterium</i> sp. Marine Biotechnol. Inst., Co., Ltd. Kamaishi Laboratory (T. Hamada; MBIC 3297; water).	30	802
16131	T <i>Streptomyces stramineus</i> NRRL 12292 -- D.P. Labeda, soil.	28	231
16132	<i>Cytophaga arvensicola</i> IAM 12646 -- H. Oyaizu, IAM, A4L -- Kobe Women's Coll., T. Harada, soil.	30	802
16133	<i>Cytophaga arvensicola</i> IAM 12647 -- H. Oyaizu, IAM, A4S -- Kobe Women's Coll., T. Harada, Segregant from strain A4L.	30	802
16134	<i>Cytophaga arvensicola</i> IAM 12648 -- H. Oyaizu, IAM, A21L -- Kobe Women's Coll., T. Harada, soil.	30	802
16135	<i>Cytophaga arvensicola</i> IAM 12649 -- H. Oyaizu, IAM, A21S -- Kobe Women's Coll., T. Harada, Segregant from strain A21L.	30	802
16137	<i>Cytophaga arvensicola</i> IAM 12651 -- H. Oyaizu, IAM, G8 -- Kobe Women's Coll., T. Harada, soil.	30	802
16138	<i>Cytophaga arvensicola</i> IAM 12653 -- H. Oyaizu, IAM, 469 -- NIAS (K. Kato; isolated from rhizosphere of cucumber root).	30	802
16139	<i>Cytophaga arvensicola</i> IAM 12654 -- H. Oyaizu, IAM, 474 -- NIAS (K. Kato; isolated from rhizosphere of cucumber root).	30	802
16140	T <i>Pseudomonas abikonensis</i> KCTC 2864 -- IAM 12404 -- Cent. Res. Inst. of Electric Power Ind., (S. Yamada; DDA 109; oil soaked soil).	30	802
16141	<i>Sphingomonas</i> sp. KCTC 2881 -- JSS7, mineral water.	30	802
16142	<i>Sphingomonas</i> sp. KCTC 2882 -- JSS26, mineral water.	30	802
16143	<i>Sphingomonas</i> sp. KCTC 2883 -- JSS28, mineral water.	30	802
16144	<i>Sphingomonas</i> sp. KCTC 2884 -- JSS54, mineral water.	30	802
16145	<i>Variovorax paradoxus</i> MBIC 3281, water.	30	802

IFO No		Name	Temp	Med
(T=Type strain)				
16147	T	<i>Actinomadura aurantiaca</i> JCM 8201 -- DSM 43924 -- IMET 9577 -- INA 1933.	28	296
16148		<i>Cellulomonas cellulans</i> JCM 3164 -- KCC A-0164 -- H.A. Lechevalier LL G62 (IMRU 3959, aluminum hydroxide gelantacid).	30	230
16149	T	<i>Rarobacter incanus</i> JCM 6350 -- S. Sato, YLM-32.	30	261
16150		<i>Microbacterium</i> sp. MBIC 3354 -- (T. Hamada; HMD III-3; hot spring).	30	802
16155	T	<i>Micromonospora aurantiaca</i> VKM Ac-1936 -- INA 9442, soil.	28	266
16157	T	<i>Sphingomonas trueperi</i> NCIMB 9391 -- G.R. Anderson, soil.	30	802
16158	T	<i>Sanguibacter kiddieii</i> NCIMB 703025 -- J. Garayzabal, ST-74, dairy cows blood samples.	30	802
16159		<i>Sanguibacter suarezii</i> NCIMB 703023 -- J. Garayzabal, ST-26, dairy cows blood samples.	30	802
16160	T	<i>Corynebacterium mastitidis</i> CECT 4843 -- J.F. Fernandez-Garayzabal, S-8, mastitis in a sheep.	30	346
16161		<i>Corynebacterium durum</i> CCUG 37331 -- P. Riegel, IBS G15036, human sputum.	37	347
16162	T	<i>Corynebacterium singulare</i> CCUG 37330 -- P. Riegel, IBS B52218, human semen.	37	347
16163	T	<i>Corynebacterium imitans</i> DSM 44264 -- G. Funke, DMMZ 2023 -- A. Efstratiou -- D. Kuklinska, human throat.	37	230
16164	T	<i>Demetria terragena</i> DSM 11295 -- I. Groth, HKI 0089, frozen compost soil.	30	230
16165	T	<i>Terracoccus luteus</i> DSM 44267 -- H. Prauser, IMET 7848, soil .	30	230
16166	T	<i>Streptomyces bobili</i> ATCC 3310 -- S.A. Waksman, soil.	28	231
16172	T	<i>Sphingomonas chlorophenolica</i> ATCC 33790 -- R.V. Edgehill, Pentachloro phenol-contaminated soil.	30	802
16183		<i>Bacillus subtilis</i> ATCC 9372 -- N.R. Smith, 1221A -- Bacon Labs. (<i>Bacillus globigii</i> , red strain) -- C. R. Phillips (Camp Detrick) -- E. McCoy.	30	802

IFO No		Name	Temp	Med
(T=Type strain)				
16188	T	<i>Streptomyces tenebrarius</i> ATCC 17920 -- Eli Lilly & Co., A.12253.1, soil.	37	265
16190	T	<i>Saccharopolyspora spinosporotrichia</i> AS 4.198 -- CGMCC (L. Zhiheng; A-50; soil).	28	266
16195	T	<i>Sporichthya brevicatena</i> IFO (T. Tamura) -- Yamanashi Univ. (M. Hayakawa; YU 720-21; soil).	28	266
16196	T	<i>Streptomyces rameus</i> ATCC 21273 -- Kyowa Hakko Co., 154, soil .	28	228
16204		<i>Streptomyces roseovercillatus</i> JCM 4272 -- KCC S-0272 -- IPV 174, soil.	28	227
16205	T	<i>Pseudonocardia sulfidoxydans</i> DSM 44248 -- Reichert, strain 592, biofilter, animal-rendering plant.	28	266
16210		<i>Micrococcus luteus</i> Dept. Microbiol., Faculty of Dentistry, Kagoshima Univ. (T. Monodane; MT).	30	802
16217	T	<i>Rhodococcus opacus</i> JCM 9703 -- DSM 43205 -- IMG -- D. Siebert, 1B.	30	271
16218	T	<i>Rhodococcus percolatus</i> JCM 10087 -- CIP 104964 -- DSM 44240 -- K. Siikanen, HAMBI 1752.	30	227
16224	T	<i>Pseudonocardia asaccharolytica</i> DSM 44247 -- K. Reichert, strain 580, biofilter.	28	266
16225	T	<i>Promicromonospora yunnanensis</i> AS 4.1333, strain 126 (CCCM).	28	266
16231	T	<i>Kineosporia rhamnosa</i> JCM 9954 -- T. Itoh I-132, leave of cat-tail.	28	266
16232	T	<i>Kineosporia succinea</i> JCM 9957 -- T. Itoh I-273, fallen leaves.	28	266
16233	T	<i>Kineosporia rhizophila</i> JCM 9960 -- T. Itoh I-499, root of galingale.	28	266
16234	T	<i>Kineosporia mikuniensis</i> JCM 9961 -- T. Itoh I-463, sphagnum.	28	266
16247		<i>Gordonia rhizosphaera</i> IFO (M. Takeuchi; No.60; soil; rhizosphere of mangrove).	30	802
16250		<i>Micrococcus luteus</i> IFO (M. Takeuchi; No.73; root; rhizosphere of mangrove).	30	802
16255	T	<i>Streptomyces seoulensis</i> JCM 10116 -- S.-O. Kang, IMSNU-1 (=IMSNU 21266; soil).	28	266

IFO No	Name	Temp	Med
(T=Type strain)			
32624	<i>Discosia aquatica</i> IMI 251648 (B.C. Sutton; C142 ; <i>Quercus fusiformis</i>).	24	1
32638	<i>Cercospora smilacis</i> Fac. Med., Univ. Rovira i Virgili (J. Guarro; FMR 4973; leaf of <i>Smilax</i> sp.).	24	8
32646	<i>Diaporthe vaccinii</i> CBS 160.32 -- C.L. Shear, <i>Oxycoccus macrocarpos</i> .	24	8
32648	<i>Epichloe typhina</i> CBS 235.84 -- G.J. Samuels, leaf tissue of <i>Festuca rubra</i> .	24	1
32683	<i>Seiridium cardinale</i> CBS 522.82 -- H.J. Boesewinkel, <i>Cupressus sempervirens</i> .	24	1
32684	<i>Seiridium unicorne</i> CBS 538.82 -- H.J. Boesewinkel, <i>Cryptomeria japonica</i> .	24	8
32689	<i>Monochaetia lutea</i> IMI 168736 -- H.J. Swart -- J. Withers, <i>Acacia pycnantha</i> .	24	1
32900	<i>Eleutherascus lectardii</i> FMR 5751 (J. Gene; soil).	24	2
32901	<i>Saccobolus globuliferellus</i> FMR 5592 (J. Gene; soil).	24	8
32902	<i>Zopfiella karachiensis</i> FMR 5768 (J. Gene; garden soil).	24	8
32903	<i>Zopfiella karachiensis</i> FMR 5769 (J. Gene; garden soil).	24	8
32904	<i>Zopfiella tetraspora</i> FMR 5770 (J. Gene; garden soil).	24	8
32905	<i>Guignardia aesculi</i> CBS 756.70 (H.A. van der Aa; leaf spot in <i>Aesculus hippocastanum</i>).	24	8
32906	<i>Guignardia bidwellii</i> CBS 237.48 (C.J. Alexopoulos; <i>Parthenocissus tricuspidata</i>).	24	8
32907	<i>Guignardia gaultheriae</i> CBS 447.70 (H.A. van der Aa; <i>Gaultheria humifusa</i>).	24	8
32908	<i>Guignardia philoprina</i> CBS 361.68 (leaf litter of <i>Rhododendron</i> sp.).	24	8
32909	<i>Guignardia philoprina</i> CBS 174.77, (<i>Cryptomeria japonica</i>).	24	1
32910	<i>Guignardia reticulata</i> CBS 858.71 (leaf spot in <i>Polygonatum odoratum</i>).	24	17

IFO No (T=Type strain)	Name	Temp	Med
32911	<i>Guignardia vaccinii</i> CBS 126.22 -- C.L. Shear, <i>Oxycoccus macrocarpos</i> .	24	8
32912	<i>Guignardia vaccinii</i> CBS 165.86 (J.A. Stevenson; <i>Oxycoccus macrocarpos</i>).	24	8
32913	<i>Phyllosticta artocarpina</i> CBS 873.72 (S.M. Singh; leaf of <i>Artocarpus heterophylla</i>).	24	1
32914	<i>Phyllosticta capitalensis</i> CBS 226.77 -- (R. Schneider; leaf spot in <i>Paphiopedilum callosum</i>).	24	1
32915	<i>Phyllosticta colocasiicola</i> CBS 205.65 (H.A. van Kesteren; leaf spot in <i>Dieffenbachia</i> sp.).	24	8
32916	<i>Phyllosticta hypoglossi</i> CBS 101.72 (H.A. van der Aa; living <i>Ruscus aculeatus</i>).	24	1
32917	<i>Phyllosticta minima</i> CBS 585.84 (D.H. Defoe; leaf spot in <i>Acer rubrum</i>).	24	1
32918	<i>Lanceispora amphibia</i> IFO (A. Nakagiri; AN-1566; submerged fallen leaf of <i>Bruguiera gymnorrhiza</i>).	24	16
32919	<i>Lanceispora amphibia</i> IFO (A. Nakagiri; AN-1570; submerged fallen leaf of <i>Bruguiera gymnorrhiza</i>).	24	16
32920	<i>Lanceispora amphibia</i> IFO (A. Nakagiri; AN-1116; submerged fallen leaf of <i>Bruguiera gymnorrhiza</i>).	24	16
32922	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 591; mole latrine).	24	7
32923	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 627; mole latrine).	24	7
32924	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 631; mole latrine).	24	7
32925	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 636; mole latrine).	24	7
32926	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 637; mole latrine).	24	7

IFO No	Name	Temp	Med
(T=Type strain)			
32927	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 642; mole latrine).	24	7
32928	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 649; mole latrine).	24	7
32929	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 650; mole latrine).	24	7
32930	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 651; mole latrine?) .	24	7
32931	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 652; mole latrine?) .	24	7
32932	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 655; mole latrine?) .	24	7
32933	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 656; mole latrine).	24	7
32934	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 657; mole latrine).	24	7
32935	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 658; mole latrine).	24	7
32936	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 669; mole latrine).	24	7
32937	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 670; mole latrine).	24	7
32938	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 671; mole latrine?) .	24	7
32939	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 676; shrew-mole latrine).	24	7

IFO No	Name	Temp	Med
(T=Type strain)			
32940	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 677; shrew-mole latrine).	24	7
32941	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 681; mole latrine).	24	7
32942	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 682; mole latrine?) .	24	7
32943	<i>Hebeloma radicosum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 683; mole latrine?) .	24	7
32944	<i>Hebeloma spoliatum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 663; fertilized soil in bush).	24	7
32945	<i>Hebeloma vinosophyllum</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 647; carcass of a dog or cat).	24	7
32946	<i>Coprinus neolagopus</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 653; forest ground dressed with oxamide).	24	7
32947	<i>Coprinus phlyctidosporus</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 665; urea-treated soil in forest).	24	7
32948	<i>Lentinus lepideus</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 646; decaying stump of pine).	24	7
32949	<i>Hypsizygus marmoreus</i> Grad. Sch. Human & Environm. Stud., Kyoto Univ. (N. Sagara; NAO 684).	24	1
32950	<i>Cerinosterus cyanescens</i> IFO (K. Mikata; IFO 10900) -- JCM 2914 -- CBS 357.73, mycosis of human skin.	24	1
32951	<i>Cerinosterus luteoalbus</i> IFO (K. Mikata; IFO 10901) -- JCM 2923 -- CBS 209.48 -- R. Kuhner.	24	108
32952	<i>Sporothrix catenata</i> IFO (K. Mikata; IFO 10902) -- JCM 2921 -- CBS 215.79 -- O. Constantinescu, calf suspected to have trichophytosis.	24	108
32953	<i>Sporothrix curviconia</i> IFO (K. Mikata; IFO 10903) -- JCM 2922 -- CBS 959.73 -- J. Devois, Terminalia ivorensis.	24	108

IFO No	Name	Temp	Med
(T=Type strain)			
32954	<i>Sporothrix foliorum</i> IFO (K. Mikata; IFO 10904) -- JCM 2916 -- CBS 326.37 -- Unilever N.V.	24	108
32955	<i>Sporothrix fungorum</i> IFO (K. Mikata; IFO 10905) -- JCM 2924 -- CBS 259.70, old carpophore of Fomes fomentarius.	24	108
32956	<i>Sporothrix ghanensis</i> IFO (K. Mikata; IFO 10906) -- JCM 2925 -- CBS 755.73, spider with eggs on leaf of Theobroma cacao.	24	108
32957	<i>Sporothrix guttuliformis</i> IFO (K. Mikata; IFO 10907) -- JCM 2919 -- CBS 437.76 -- T. Furukawa, soil.	24	108
32958	<i>Sporothrix inflata</i> IFO (K. Mikata; IFO 10908) -- JCM 2920 -- CBS 239.68, (W. Gams; wheat-field soil).	24	108
32959	<i>Sporothrix insectorum</i> IFO (K. Mikata; IFO 10909) -- JCM 2918 -- CBS 756.73 -- H.C. Evans.	24	108
32960	<i>Sporothrix ranii</i> IFO (K. Mikata; IFO 10910) -- JCM 2917 -- CBS 119.81 -- A.F. Moustafa, tidal salt marsh.	24	108
32961	<i>Sporothrix schenckii</i> var. <i>schenckii</i> IFO (K. Mikata; IFO 10911) -- JCM 2915 -- CBS 359.36 -- D.J. Davis.	24	108
32962	<i>Sporothrix sclerotialis</i> IFO (K. Mikata; IFO 10912) -- JCM 2913 -- CBS 312.77 -- J.W. Veenbaas-Rijks, roots of Lolium perenne.	24	108
32966	<i>Halophytophthora mycoparasitica</i> IFO (A. Nakagiri; AN-1573; submerged fallen leaf of Bruguiera gymnorrhiza).	25	15
32967	<i>Halophytophthora mycoparasitica</i> IFO (A. Nakagiri; AN-1574; submerged fallen leaf of Bruguiera gymnorrhiza).	25	15
32968	<i>Ceratocystis fimbriata</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 94H-1; Ficus carica "Masui Dauphine").	20	1
32969	<i>Ceratocystis fimbriata</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 95S-1; Ficus carica "Masui Dauphine").	20	1
32970	<i>Claviceps purpurea</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 94-1; Hordeum vulgare).	20	1

IFO No (T=Type strain)	Name	Temp	Med
32971	<i>Claviceps purpurea</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 94-11; <i>Poa annua</i>).	20	1
32972	<i>Claviceps purpurea</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 94-51; <i>Alopecurus aequalis</i> var. <i>amurensis</i>).	20	1
32973	<i>Peziza ostracoderma</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 94-6; forcing soil for tulips).	20	1
32974	<i>Peziza ostracoderma</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 95-2; rice seedling of a pot sheet culture).	20	1
32975	<i>Peziza ostracoderma</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 95-4; rice seedlings of a pot sheet culture).	20	1
32976	<i>Apinisia graminicola</i> FMR 6182 -- Stchigel, soil.	20	1
32977	<i>Ascodesmis porcina</i> FMR 6186 -- Stchigel, soil.	20	1
32978	<i>Chaetomium cymbiforme</i> FMR 5846 -- Stchigel, soil.	20	1
32979	<i>Chaetomium irregulare</i> FMR 5826 -- Stchigel, soil.	20	1
32980	<i>Chaetomium spiralotrichum</i> FMR 5828 -- Stchigel, soil.	20	1
32981	<i>Emericellopsis glabra</i> FMR 5915 -- Stchigel, soil.	20	1
32982	<i>Gelasinospora pseudocalospora</i> FMR 5917 -- Stchigel, soil.	20	1
32983	<i>Gelasinospora novoguineensis</i> FMR 5822 -- Stchigel, soil.	20	1
32984	<i>Thielavia tortuosa</i> FMR 5780 -- Stchigel, soil.	20	1
32985	<i>Aspergillus dimorphicus</i> FMR 5887 -- Stchigel, soil.	20	1
32986	<i>Acremonium furcatum</i> IFO (T. Ito; T. Ito H8-3-12; garden soil).	24	8
32987	<i>Grifola frondosa</i> Hokkaido For. Pro. Res. Inst. (I. Togash i; Gf 85-1 (Etan-no-mai); root of <i>Quercus mongolica</i> var. <i>grosseserrata</i>).	24	1

IFO No (T=Type strain)	Name	Temp	Med
32988	<i>Taphrina coerulescens</i> Fac. Horticul., Chiba Univ. (H. Nagao; K 9701; Quercus phillyraeoides).	25	1
32989	<i>Taphrina coerulescens</i> Fac. Horticul., Chiba Univ. (H. Nagao; K 9702; Quercus phillyraeoides).	25	1
32990	<i>Taphrina coerulescens</i> Fac. Horticul., Chiba Univ. (H. Nagao; K 9703; Quercus phillyraeoides).	25	1
32991	<i>Taphrina coerulescens</i> Fac. Horticul., Chiba Univ. (H. Nagao; K 9704; Quercus phillyraeoides).	25	1
32992	<i>Taphrina wiesneri</i> Fac. Horticul., Chiba Univ. (H. Nagao; C 9201; Prunus x yedoensis).	25	1
32993	<i>Taphrina wiesneri</i> Fac. Horticul., Chiba Univ. (H. Nagao; C 10201; Prunus x yedoensis).	25	1
32994	<i>Taphrina deformans</i> Fac. Horticul., Chiba Univ. (H. Nagao; C 93051; Prunus persica var. vulgaris).	25	1
32995	<i>Rhizopus microsporus</i> var. <i>microsporus</i> CGMCC (AS 3.2674) -- IMAS 8664 (F.-m. Hu; chaff).	28	1
32996	<i>Rhizopus microsporus</i> var. <i>microsporus</i> CGMCC (AS 3.2677) -- IMAS 5431 (F.-m. Hu; swine dung).	28	1
32997	<i>Rhizopus microsporus</i> var. <i>rhizopodiformis</i> CGMCC (AS 3.2675) -- IMAS 3047a (F.-m. Hu; soil).	28	1
32998	<i>Rhizopus stolonifer</i> var. <i>lyococcus</i> CGMCC (AS 3.3459) -- IMAS 7836b (F.-m. Hu; seeds of plant).	24	1
32999	<i>Syncephalastrum monosporum</i> var. <i>cristatum</i> CGMCC (AS 3.3354) -- IMAS (F.-m. Hu; S10; spring soil).	28	1
33000	<i>Syncephalastrum monosporum</i> var. <i>cristatum</i> CGMCC (AS 3.3355) -- IMAS (F.-m. Hu; S11; field soil).	28	1
33001	<i>Syncephalastrum monosporum</i> var. <i>pluriproliferum</i> CGMCC (AS 3.3356) -- IMAS (F.-m. Hu; S12; pit mud).	28	1
33002	<i>Syncephalastrum monosporum</i> var. <i>monosporum</i> CGMCC (AS 3.3357) -- IMAS (F.-m. Hu; S7; millet seeds).	28	1
33003	<i>Syncephalastrum monosporum</i> var. <i>monosporum</i> CGMCC (AS 3.3358) -- IMAS (F.-m. Hu; S8; bone coal).	28	1
33004	<i>Syncephalastrum monosporum</i> var. <i>monosporum</i> CGMCC (AS 3.3359) -- IMAS (F.-m. Hu; S9; soil).	28	1

IFO No	Name	Temp	Med
(T=Type strain)			
33005	<i>Hyalopeziza millepunctata</i> Plant Pathol. Herbarium, Cornell Univ. (R. P. Korf; old stems of <i>Epifagus virginianus</i>).	24	1
33006	<i>Ascochyta aquilegiae</i> Shikoku Nat. Agric. Exp. Stn. (T. Sato; AS1; <i>Delphinium ajacis</i>).	24	1
33007	<i>Stemphylium lycopersici</i> Shikoku Nat. Agric. Exp. Stn. (T. Sato; KS3; <i>Kalanchoe blossfeldiana</i>).	24	1
33008	<i>Botryotinia fuckeliana</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 93, asakano 1; Rape).	24	1
33009	<i>Botryotinia fuckeliana</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 96, ohmugi 1; Barley).	24	1
33010	<i>Microporus vernicipes</i> Lab. Forest Ecol., Grad. School Agric., Kyoto Univ. (T. Osono; MV-SFW1; unidentified twig litter).	24	1
33011	<i>Mycena polygramma</i> Lab. Forest Ecol., Grad. School Agric., Kyoto Univ. (T. Osono; MP-SFM2; decomposed litter).	24	1
33012	<i>Mammaria echinobotryoides</i> Lab. Forest Ecol., Grad. School Agric., Kyoto Univ. (T. Osono; 2BS-8; leaf litter of <i>Fagus crenata</i>).	24	1
33013	<i>Xylaria carpophila</i> Lab. Forest Ecol., Grad. School Agric., Kyoto Univ. (T. Osono; XC-SFM5; cupule litter of <i>Fagus crenata</i>).	24	8
33014	<i>Penicillium crustosum</i> Hokaido Forest Prod. Res. Inst. (I. Togashi; 96201 Pe as <i>Penicillium</i> sp.; mushroom bed of <i>Pholiota nameko</i>).	24	1
33015	<i>Penicillium crustosum</i> Hokaido Forest Prod. Res. Inst. (I. Togashi; 97203 Pe as <i>Penicillium</i> sp.; mushroom bed of <i>Flammulina velutipes</i>).	24	1
33016	<i>Trichoderma harzianum</i> Hokaido Forest Prod. Res. Inst. (I. Togashi; 94110 Tr; Cultivation room of <i>Lentinula edodes</i>).	24	1
33017	<i>Emericella nidulans</i> CBS 288.95.	24	1
33018	<i>Eurotium amstelodami</i> CBS 518.65.	28	9
33019	<i>Aspergillus candidus</i> CBS 283.95.	24	1

IFO No (T=Type strain)	Name	Temp	Med
33020	<i>Aspergillus clavatus</i> CBS 514.65.	24	1
33021	<i>Aspergillus flavus</i> CBS 282.95.	24	1
33022	<i>Aspergillus fumigatus</i> CBS 286.95.	24	1
33023	<i>Aspergillus niger</i> CBS 554.65.	24	1
33024	<i>Aspergillus penicillioides</i> CBS 285.95.	24	3
33025	<i>Aspergillus ochraceus</i> CBS 280.95.	24	1
33026	<i>Aspergillus terreus</i> CBS 601.65.	24	1
33027	<i>Aspergillus versicolor</i> CBS 583.65.	24	1
33028	<i>Emericella pluriseminata</i> FMR 5588 (J. Cano; soil).	24	2
33029	<i>Microthecium levitum</i> FMR 6218 (J. Cano; soil).	24	1
33030	<i>Chaetomidium arxii</i> FMR 6373 (J. Cano; desert soil).	24	2
33031	<i>Sarcopodium circinosetiferum</i> FMR 6354 (J. Cano; garden soil).	24	2
33032	<i>Sarcopodium circinosetiferum</i> FMR 6355 (J. Cano; soil).	24	2
33033	<i>Thanatephorus cucumeris</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 95Kamiichi 1; seedling of <i>Oryza sativa</i>).	24	1
33034	<i>Thanatephorus cucumeris</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 95Kamiichi 3; seedling of <i>Oryza sativa</i>).	24	1
33035	<i>Cercospora victorialis</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 97GiL 1; leaf of <i>Allium victorialis</i>).	24	1
33036	<i>Cercospora victorialis</i> Toyama Agric. Res. Cent. (H. Mukobata; TAC 97GiL 4; leaf of <i>Allium victorialis</i>).	24	1

IFO No	Name	Temp	Med
(T=Type strain)			
33037	<i>Kernia pachypleura</i> UAMH 9026 (S.P. Abbott; SA-M182; leaf of <i>Populus tremuloides</i>).	24	2
33038	<i>Lophotrichus bartlettii</i> UAMH 9036 (S.P. Abbott; SA-M186; dung of skunk).	24	8
33039	<i>Microascus albonigrescens</i> UAMH 8753 (S.P. Abbott; SA-M158; debris of red squirrel).	24	8
33040	<i>Microascus brevicaulis</i> UAMH 7770 (S.P. Abbott; OHS 428; indoor air of honeybee facility).	24	8
33041	<i>Microascus longirostris</i> UAMH 8354 (S.P. Abbott; SA-M52; indoor air of honeybee facility).	24	2
33042	<i>Microascus manginii</i> UAMH 7921 (S.P. Abbott; SA-M73; outside air of honeybee facility).	24	2
33043	<i>Microascus manginii</i> UAMH 9004 (S.P. Abbott; SA-M175; indoor air of office building).	24	2
33044	<i>Doratomyces nanus</i> UAMH 8741 (S.P. Abbott; SA-M167; soil).	24	8
33045	<i>Doratomyces purpureofuscus</i> UAMH 9002 (S.P. Abbott; SA-M3; plate contaminant).	24	8
33046	<i>Doratomyces putredinis</i> UAMH 9028 (S.P. Abbott; SA-M185; dung of skunk).	24	8
33047	<i>Scopulariopsis asperula</i> UAMH 7879 (S.P. Abbott; OHS 207; indoor air of honeybee facility).	24	2
33048	<i>Scopulariopsis asperula</i> UAMH 9029 (S.P. Abbott; SA-M183; dung of skunk).	24	2
33049	<i>Scopulariopsis brevicaulis</i> UAMH 8748 (S.P. Abbott; SA-M155; dung of red squirrel).	24	2
33050	<i>Scopulariopsis brumptii</i> UAMH 8743 (S.P. Abbott; SA-M165; leaf litter of <i>Betula papyrifera</i>).	24	1
33051	<i>Scopulariopsis koningii</i> UAMH 7774 (S.P. Abbott; OHS 41; indoor air of honeybee facility).	24	2
33052	<i>Scopulariopsis koningii</i> UAMH 7918 (S.P. Abbott; SA-M70; mixed air of honeybee facility).	24	2
33053	<i>Scopulariopsis sphaerospora</i> UAMH 7883 (S.P. Abbott; OHS 189; indoor air of honeybee facility).	24	2
33054	<i>Wardomyces anomalus</i> UAMH 8275 (S.P. Abbott; RP-4-1M; indoor air of honeybee facility).	24	8

IFO No (T=Type strain)	Name	Temp	Med
33055	<i>Wardomyces columbinus</i> UAMH 7847 (S.P. Abbott; OHS 209; indoor air of honeybee facility).	24	8
33056	<i>Wardomyces humicola</i> UAMH 8752 (S.P. Abbott; SA-M151; debris of red squirrel).	24	8
33060	<i>Cordyceps heteropoda</i> IFO (A. Nakagiri; AN-1634; ascospores ejected from ascoma).	24	1
33061	<i>Paecilomyces cicadae</i> IFO (A. Nakagiri; AN-1639; conidia from synnema on larva of cicada, <i>Meimuna opalifera</i>).	24	1
33062	<i>Phyllosticta capitalensis</i> CBS 398.80 (G.F. Loundon; orchid).	24	1
33063	<i>Phyllosticta capitalensis</i> IMI 145945 -- Dept. Agric., New Zealand (N.H. Hyde; 3710a; orchid, intercepted from Australia).	24	1
33064	<i>Boothiella tetraspora</i> FMR 6557, sand.	24	8
33065	<i>Emericellopsis synnematicola</i> FMR 6189, soil.	24	2
33066	<i>Lepidosphaeria nicotiae</i> FMR 6559, soil.	24	8
33067	<i>Thielavia coactilis</i> FMR 6405, soil.	24	2
33068	<i>Monochaetia monochaeta</i> IFO (I. Okane; IOC-797; leaf of <i>Rhododendron pulchrum</i> var. <i>speciosum</i>).	24	1
33069	<i>Citrinalia pygmaea</i> IFO (I. Okane; IOC-1237; fallen leaf of <i>Buruguiera gymnorhiza</i>).	24	8
33070	<i>Eudarlucella biconica</i> Life Web, Grad. Sch. Human Environ. Stud., Kyoto Univ. (M. Okada; 96210901; living leaf of <i>Phragmites australis</i>).	24	8
33071	<i>Eudarlucella biconica</i> Life Web, Grad. Sch. Human Environ. Stud., Kyoto Univ. (M. Okada; 96280503; living leaf of <i>Phragmites karka</i>).	24	8
33072	<i>Paraphaeosphaeria michotii</i> Life Web, Grad. Sch. Human Environ. Stud., Kyoto Univ. (M. Okada; 96250903; living leaf of <i>Phragmites australis</i>).	24	8
33073	<i>Paraphaeosphaeria michotii</i> Life Web, Grad. Sch. Human Environ. Stud., Kyoto Univ. (M. Okada; 97160104; living leaf of <i>Phragmites karka</i>).	24	8

IFO No	Name	Temp	Med
(T=Type strain)			
33074	<i>Phaeosphaeria donacina</i> Life Web, Grad. Sch. Human Environ. Stud., Kyoto Univ. (M. Okada; 95410901; living leaf of <i>Phragmites australis</i>).	24	1
33075	<i>Phaeosphaeria donacina</i> Life Web, Grad. Sch. Human Environ. Stud., Kyoto Univ. (M. Okada; 96450503; living leaf of <i>Phragmites australis</i>).	24	1
33076	<i>Phaeosphaeria oryzae</i> Life Web, Grad. Sch. Human Environ. Stud., Kyoto Univ. (M. Okada; 95680104; living leaf of <i>Phragmites australis</i>).	24	8
33078	<i>Penicillium griseoroseum</i> Life Web, Grad. Sch. Human Environ. Stud., Kyoto Univ. (M. Okada; 94-8-3; living leaf of <i>Phragmites karka</i>).	24	1
33082	<i>Aspergillus sojae</i> Kikkoman Co. (Y. Hanya) -- NISL 1478A, seed koji of soy sauce.	30	1
33083	<i>Aspergillus sojae</i> Kikkoman Co. (Y. Hanya) -- NISL 1478B, seed koji of soy sauce.	30	1
33084	<i>Aspergillus sojae</i> Kikkoman Co. (Y. Hanya) -- NISL 1905A, seed koji of soy sauce.	30	1
33085	<i>Aspergillus sojae</i> Kikkoman Co. (Y. Hanya) -- NISL 1909, seed koji of soy sauce.	30	1
33086	<i>Aspergillus sojae</i> Kikkoman Co. (Y. Hanya) -- NISL 1920A, seed koji of soy sauce.	30	1
33087	<i>Aspergillus sojae</i> Kikkoman Co. (Y. Hanya) -- NISL 1939A, seed koji of soy sauce.	30	1
33088	<i>Aspergillus sojae</i> Kikkoman Co. (Y. Hanya) -- NISL 1939B, seed koji of soy sauce.	30	1
50488	OUMS-27 Dept. Cell Biol., Int. Mol. Cell Biol., Okayama Univ., Med. Sch. (M. Namba; human chondrosarcoma, grade III; left proximal humerus).	37	
50489	GB-1 Dept. Neurosurg. Nat. Utano Hospital (T. Morimura; human glioma-derived cells).	37	
50490	MDM2-RNB Dept. Neurosurg. Nat. Utano Hospital (T. Morimura; RNB MDM2; MDM2 gene-transfected neonatal rat astrocyte).	37	
50491	RNB Dept. Neurosurg., Nat. Utano Hospital (T. Morimura; Neonatal rat (Wistar); brain; astrocytes).	37	

IFO No (T=Type strain)	Name	Temp	Med
50492	AM-38 Dept. Neurosurg., Kyoto Pref. Univ. of Medicine (K. Mineura; AM; human glioblastoma -derived cells).	37	
50493	YH-13 Dept. Neurosurg., Kyoto Pref. Univ. of Medicine (K. Mineura; YH; human glioblastoma -derived cells).	37	
50494	KK-2 Dept. Clin. Pathol., School of Medicine, Kitazato Univ. (Y. Dobashi; primitive neuroectodermal tumor-derived cells).	37	
50495	Neuro-2aTG IFO (M. Satoh; Neuro-2aTG; HPRT-deficient mouse neuroblastoma cells).	37	
50496	PC-12TG IFO (M. Satoh; PC-12TG; HPRT-deficient rat pheochromocytoma cells).	37	

LIST OF EXCLUDED STRAINS
Since April 1996

IFO No.	NAME OF TAXON
1145	<i>Torulaspota</i> sp.
3723	<i>Streptomyces netropsis</i>
4200	<i>Aspergillus parasiticus</i>
6820	<i>Ustilago antherarum</i>
12160	<i>Tsukamurella paurometabola</i>
12465	<i>Thermomonospora chromogena</i>
13396	<i>Streptomyces tenebrarius</i>
14068	<i>Micromonospora aurantiaca</i>
14104	<i>Dactylosporangium variesporum</i>
14110	<i>Micromonospora echinospora</i> subsp. <i>pallida</i>
14178	<i>Thermobifida mesouviiformis</i>
14556	<i>Pilimelia terevasa</i>
14840	<i>Microbispora griseoalba</i>
15533	<i>Pilimelia anulata</i>
15554	<i>Actinomadura aurantiaca</i>
15682	<i>Bacillus globisporus</i>
15790	<i>Pilimelia columellifera</i> subsp. <i>columellifera</i>
15880	<i>Planococcus okeanokoites</i>
30112	<i>Aspergillus parasiticus</i>
31337	<i>Termitomyces albuminosus</i>
32433	<i>Platyglaea navispora</i>
32434	<i>Platyglaea navispora</i>
32636	<i>Cylindrotrichum oligospermum</i>

LIST OF MEDIA NEWLY REGISTERED

343 SUCROSE BENNETT'S AGAR

Yeast extract	1	g
Beef extract	1	g
NZ Amine, type A*	2	g
Sucrose	10	g
Distilled water	1	L
Agar	20	g
pH 7.3		

*Sheffield Chemical Co., San Ramon, CA
94583, USA or Wako Pure Chemical Ind.
Ltd., Osaka, Japan.

344 5% PTYG

Peptone	0.25	g
Tryptone	0.25	g
Yeast extract	0.5	g
Glucose	0.5	g
MgSO ₄ ·7H ₂ O	0.6	g
CaCl ₂ ·2H ₂ O	0.07	g
Distilled water	1	L
Agar	15	g
pH 7.2		

345 PPES-II Agar

Peptone	2.0	g
Proteose peptone No.3	1.0	g
Soytone	1.0	g
Yeast extract	1.0	g
Fe(III)-EDTA	0.1	g
Agar	15.0	g
Artificial sea water*	1	L
pH 7.8		

*** Component of artificial sea water**

NaCl	30.0	g
Kcl	0.7	g
MgCl ₂ ·6H ₂ O	10.8	g
MgSO ₄ ·7H ₂ O	5.4	g
CaCl ₂ ·2H ₂ O	1.0	g
Distilled water	1.0	L

346 BHI-Tween Agar

Difco-Heart Infusion Broth	25.0	g
Tween 80	10.0	g
Agar	15.0	g
Distilled water	1	L

347 Blood Agar

Nissui Plate Sheep Blood Agar*

* Nissui pharmaceuticals Co., Ltd.

348 1/2 Trypticase soy Agar

Trypticase Soy	15.0	g
Distilled water	1	L
Agar	15.0	g
pH 7.0		

349 Microcycilus- SpirosomMedium

Glucose	1.0	g
Peptone	1.0	g
Yeast extract	1.0	g
Agar (if needed)	15	g
Distilled water	1	L
pH 6.8-7.0		

350 Acetobacter xylinus medium

Polypeptone*	5	g
Yeast extract	5	g
Glucose	5	g
Mannitol	5	g
MgSO ₄ ·7H ₂ O	1	g
Ethanol**	5	ml
Agar (if needed)	15	g
Distilled water	1	L
pH 6.6-7.0		

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

** Sterilize separately by filtration.

351 IFO medium 351

Polypeptone*	10	g
Yeast extract	2	g
N-Acetyl glucosamine**	30	mg
MgSO ₄ ·7H ₂ O	1	g
Agar (if needed)	15	g
Distilled water	1	L

pH 7.0

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

** Sterilize by filtration

352 Methylobacterium medium

Polypeptone*	10	g
Yeast extract	2	g
Methanol**	5	ml
MgSO ₄ ·7H ₂ O	1	g
Agar (if needed)	15	g
Distilled water	1	L

pH 7.0

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

Scientific Papers, 1 997–1 998

Inhibition of sardine flesh lipoxygenase by a new antioxidant from *Aspergillus terreus*

Y. Ishikawa¹⁾, T. Ito, and K. H. Lee²⁾

J. Jpn. Oil Chem. Soc. 45: 1321–1325 (1996)

1) College of Education, Tottori Univ.

2) School of Pharmacy, Univ. North Carolina at Chapel Hill.

Viability of frozen cultures of Basidiomycetes after fifteen-year storage

T. Ito and A. Nakagiri

Microbiol. Cult. Coll. 12: 67–78 (1996)

Taxonomical position of newly isolated polyethylene glycol-utilizing bacteria

F. Kawai¹⁾ and Mariko Takeuchi

J. Ferment. Bioeng. 82: 492–494 (1996)

1) Department of Biology, Kobe University of Commerce

Electrophoretic karyotyping analysis of yeast chromosome DNAs

K. Mikata

Microbiol. Cult. Coll. 12: 99–103 (1996)

[in Japanese]

L-drying method for long-term preservation of microorganisms

T. Sakane, T. Nishii, T. Ito and K. Mikata

Microbiol. Cult. Coll. 12: 91–97 (1996)

[in Japanese]

Application of the variable region in 16S rDNA to create an index for rapid species identification in the genus *Streptomyces*

M. Kataoka¹⁾, K. Ueda, T. Kudo²⁾, T. Seki¹⁾ and T. Yoshida¹⁾

FEMS Microbiology Letters 151: 249–255(1997)

1) The International Center for Biotechnology, Osaka University

2) Japan Collection of Microorganisms (JCM)

Surface structure of ascospores of genus *Nadsonia* Sydow

K. Mikata and T. Nakase¹⁾

Microbiol. Cult. Coll. 13: 97–102 (1997)

1) Japan Collection of Microorganisms (JCM)

Reclassification of *Cytophaga aprica* (Lewin 1969) Reichenbach 1989 in *Flammeovirga* gen. nov. as *Flammeovirga aprica* comb. nov. and of *Cytophaga diffluens* (ex Stanier 1940; emend. Lewin 1969) Reichenbach 1989 in

***Persicobacter* gen. nov. as *Persicobacter diffluens* comb. nov.**Y. Nakagawa, K. Hamana¹⁾, T. Sakane, and K. Yamasato²⁾

Int. J. Syst. Bacteriol. 47: 220-223 (1997)

1) College of Medical Care and Technology, Gunma University

2) Institute of Applied Microbiology, The University of Tokyo

Collection, detection and isolation of fungi: marine fungi

A. Nakagiri

Nippon Kingakukai Kaiho 38: 105-109 (1997)

[in Japanese]

Retrostium amphiroae* gen. et sp. nov. inhabiting a marine red alga, *Amphiroa zonata

A. Nakagiri and T. Ito

Mycologia 89: 484-493 (1997)

***Lanceispora amphibia* gen. et sp. nov., a new amphisphaeriaceous ascomycete inhabiting senescent and fallen leaves of mangrove**A. Nakagiri, I. Okane, T. Ito and K. Katumoto¹⁾

Mycoscience 38: 207-213 (1997)

1) 2055-27, Oouchmihori, Yamaguchi, Yamaguchi 753-02 Japan

The pathogen of fruit stain disease of pear in Okayama PrefectureH. Nasu¹⁾ and A. Nakagiri

Rep. Okayama Pref. Agric. Exp. St. 15: 25-30 (1997)

1) Okayama Prefectural Agriculture Experiment Station

Gymnastatins, novel cytotoxic metabolites produced by a fungal strain from a spongeA. Numata¹⁾, T. Amagata¹⁾, K. Minoura¹⁾ and T. Ito

Tetrahedron Letters 38: 5675-5678 (1997)

1) Osaka University of Pharmaceutical Sciences

Viabilities of dried cultures of various bacteria after preservation for over 20 years and their prediction by the accelerated storage test

T. Sakane and K. Kuroshima

Microbiol. Cult. Coll. 13: 1-7 (1997)

Promotion of neurogenesis in mouse olfactory neuronal progenitor cells by leukemia inhibitory factor in vitro

M. Satoh and T. Yoshida

Neurosci. Lett. 225: 165-168 (1997)

A new genus the order *Actinomycetales*, *Spirilliplanes* gen. nov., with description of *Spirilliplanes yamanashiensis* sp. nov.T. Tamura, M. Hayakawa¹⁾ and K. Hatano

Int. J. Syst. Bacteriol. 47: 97–102 (1997)

1) Department of Applied Chemistry and Biotechnology, Faculty of Engineering,
Yamanashi University

The phylogeny of strains of species of the genus *Pichia* Hansen (Sacchromycetaceae) based on the partial sequences of 18S ribosomal RNA: the proposals of *Phaffomyces* and *Starmera* the new genera

Y. Yamada¹⁾, T. Higashi¹⁾, S. Ando¹⁾ and K. Mikata

Bull. Fac. Agric. Shizuoka Univ. 47: 23–35 (1997)

1) Department of Agricultural Chemistry, Shizuoka University

Cytotoxic substances produced by a fungal strain from a sponge: physico-chemical properties and structures

T. Amagata¹⁾, Y. Usami¹⁾, K. Minoura¹⁾, T. Ito and A. Numata¹⁾

J. Antibiot. 31: 33–40 (1998)

1) Osaka University of Pharmaceutical Sciences

Identification and characteristics of the yeast strain isolated from sake lees

H. Ando¹⁾, T. Ikegami²⁾ and K. Mikata

Reports of Kyushu National Industrial Research Institute 61: 3817–3825 (1998)

1) Kagoshima Prefectural Institute of Industrial Technology

2) Materials Chemistry of Department Kyushu National Industrial Reserch Institute

Polymerase profiles as chemotaxonomic marker within alpha, beta, gamma, delta, and epsilon subclasses of class *Proteobacteria*: distribution of 2-hydroxyputrescine and homospermidine

K. Hamana¹⁾ and Mariko Takeuchi

Microbiol. Cult. Coll. 14: 1–14 (1998)

1) Department of Laboratory Sciences, Gunma University School of Health Sciences

***Thielavia aurantiaca*, a new species from Japanese soil**

T. Ito, I. Okane and A. Nakagiri

Mycoscience 39: 93–96 (1998).

The latest taxonomy of the genus *Saccharomyces*

K. Mikata

J. Brew. Soc. Japan 93: 858–862 (1998)

[in Japanese]

Establishment of an epidermal growth factor-dependent, multipotent neural precursor cell line

Y. Nakagaito, M. Satoh, H. Kuno, T. Iwama¹⁾, Masao Takeuchi, A. Hakura²⁾ and

T. Yoshida

In Vitro Cell. Dev. Biol. 34: 585-592 (1998)

1) Takeda Chemical Industries, Ltd.

2) Institute for Microbial Diseases, Osaka University

Utilization of RAPD for quality control of bacterial cultures preserved in culture collections

Y. Nakagawa, H. Kawasaki¹⁾, T. Nishii, T. Sakane, A. Yokota¹⁾ and K. Hatano
Microbiol. Cult. Coll. 14: 15-21 (1998)

1) Institute of Molecular and Cellular Biosciences, The University of Tokyo

Diversity of *halophytophthoras* in subtropical mangroves and factors affecting their distribution

A. Nakagiri

In: Proceedings of the Asia-Pacific Mycological Conference on Biodiversity and Biotechnology, pp.109-113, BIOTEC, Thailand (1998)

Zoosporangium development, zoospore release and culture properties of *Halophytophthora mycoparasitica*

A. Nakagiri, I. Okane and T. Ito

Mycoscience 39: 223-230 (1998)

Plants and fungi

I. Okane

J. Antibact. Antifung. Agents 26: 403-411(1998)

[in Japanese]

Endophytic fungi in leaves of ericaceous plants

I. Okane, A. Nakagiri and T. Ito

Can. J. Bot. 76: 657-663 (1998)

A simple PCR method for distinguishing *Saccharomyces cerevisiae* from its sibling species by amplification of the *RPL2* region

S.-L. Ryu¹⁾, K. Mikata, Y. Murooka¹⁾ and Y. Kaneko¹⁾

J. Ferment. Bioeng. 86: 249-252 (1998)

1) Department of Biotechnology, Graduate School of Engineering, Osaka University

β -Casomorphin-5 stimulates neurite outgrowth in a mouse neuroblastoma cell line (Neuro-2a)

M. Sakaguchi¹⁾, K. Murayama¹⁾, K. Yabe¹⁾, M. Satoh, Masao Takeuchi and E. Matsumura¹⁾

Neurosci. Lett. 251: 97-100 (1998)

1) Laboratory of Cell Biology, Osaka University of Pharmaceutical Sciences

Platelet-like particle formation in the human megakaryoblastic leukemia cell lines, MEG-01 and MEG-01s

K. Takeuchi¹⁾, M. Satoh, H. Kuno, T. Yoshida, H. Kondo¹⁾ and Masao Takeuchi
Br. J. Haematol. 100: 436-444 (1998)

1) Osaka Prefectural College of Health Science

Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins et al. in a redefined genus *Microbacterium*

Mariko Takeuchi and K. Hatano
Int. J. Syst. Bacteriol., 48: 739-747 (1998)

***Gordonia rhizosphaera* sp. nov. isolated from the mangrove rhizosphere**

Mariko Takeuchi and K. Hatano
Int. J. Syst. Bacteriol., 48: 907-912 (1998)

Proposal of six new species in the genus *Microbacterium* and transfer of *Flavobacterium marinotypicum* ZoBell and Upham to the genus *Microbacterium* as *Microbacterium maritypicum* comb. nov

Mariko Takeuchi and K. Hatano
Int. J. Syst. Bacteriol., 48: 973-982 (1998)

Phylogenetic Analyses on the strains belonging to invalidated genera of the order *Actinomycetales*

T. Tamura and K. Hatano
Actinomycetologica 12:15-28 (1998)

A New Genus of the Order *Actinomycetales*, *Cryptosporangium* gen. nov., with Descriptions of *Cryptosporangium arvum* sp. nov., *Cryptosporangium japonicum* sp. nov.

T. Tamura, M. Hayakawa¹⁾ and K. Hatano
Int. J. Syst. Bacteriol. 48: 995-1005 (1998)
1) Department of Applied Chemistry and Biotechnology, Faculty of Engineering,
Yamanashi University

Presentation of Papers at Scientific Meetings, 1997–1998

Japan Society for Bioscience, Biotechnology and Agrochemistry (April, 1997, Tokyo)

K. Murayama¹⁾, M. Sakaguchi¹⁾, E. Matsumura¹⁾, Y. Jinsmaa²⁾, M. Yoshikawa¹⁾, M. Satoh and Masao Takeuchi

Relationship between neurite outgrowth stimulating activity and opioid activity of β -casomorphins

- 1) Laboratory of Cell Biology, Osaka University of Pharmaceutical Sciences
- 2) Faculty of Agriculture, Kyoto University

M. Suzuki¹⁾, Y. Nakagawa, S. Yamamoto¹⁾ and S. Harayama¹⁾

Phylogenetic analysis of the *Cytophaga-Flavobacterium* complex and the related bacteria based on *gyrB* sequences

- 1) Marine Biotechnology Institute, Kamaishi Laboratories

Mariko Takeuchi and K. Hatano

Taxonomic study of actinobacteria isolated from the mangrove rhizosphere

K. Ueda and K. Mikata

Identification of group I intron within the 18S rDNA from *Stephanoascus ciferrii* and *Arxula terrestris*

Symposium on preservation and application of microbial resources (May, 1997, Hsin-chu, Taiwan)

Masao Takeuchi

Roles of animal cell banks in life science

The 10th International Symposium for Biology of Actinomycetes (May, 1997, Beijing)

T. Tamura, M. Hayakawa¹⁾ and K. Hatano

A New Genus of the Order *Actinomycetales*, *Kineopolyspora* gen. nov.

- 1) Department of Applied Chemistry and Biotechnology, Faculty of Engineering, Yamanashi University

Mycological Society of Japan (May, 1997, Higashi-hiroshima)

T. Ito and A. Nakagiri

Mycoflora of the rhizosphere of *Bruguiera gymnorrhiza*.

A. Nakagiri, I. Okane and T. Ito

A new Amphispheariaceous fungus from senescent and fallen leaves of *Bruguiera gymnorrhiza*

I. Okane, A. Nakagiri and T. Ito

Successional change of endophytic fungi after leaf fall in ericaceous plants

Japan Society for Culture Collection (June, 1997, Wako)

K. Mikata and T. Nakase¹⁾

Surface structure of ascospores of the ascomycetous yeasts

1) Japan Collection of Microorganisms (JCM)

Japanese Society of Microbial Ecology (July, 1997, Sapporo)

I. Okane

Endophytic fungi of ericaceous plants.

M. Suzuki¹⁾, T. Takadera²⁾, S. Yamamoto¹⁾, H. Sano²⁾, Y. Nakagawa and S. Harayama¹⁾

Diversity of the *Cytophaga/Flavobacterium* complex and the related bacteria isolated from sea weeds based on *gyrB* sequence analysis

1) Marine Biotechnology Institute, Kamaishi Laboratories

2) Marine Biotechnology Institute, Shimizu Laboratories

61st Annual Meeting of the Botanical Society of Japan (September, 1997, Narashino)

D. Honda¹⁾, T. Yokochi²⁾, T. Nakahara²⁾, K. Schaumann³⁾, S. Raghu-kumar⁴⁾, A. Nakagiri and T. Higashihara²⁾

Molecular phylogeny of labyrinthulids inferred from 18S rRNA gene sequence

1) Marine Biotechnology Institute, Kamaishi Laboratories

2) Inst. Lifesci. Technol.

3) Alfred-Wegner-Inst. Pol. Meeresforsch. Germany

4) Nat. Inst. Oceanogr. India

23rd Kinki Agri-high-tech Symposium (October, 1997, Kyoto)

A. Nakagiri

Long term preservation of fungal cultures

3rd Annual Academic Meeting of Japan Association of Mangrove (November, 1997, Tokyo)

A. Nakagiri

Ecology of *Halophytophthora* species inhabiting mangrove brackish water

17th Annual Meeting on Microbial Taxonomy (November, 1997, Tsuchiura)

Mariko Takeuchi

Union of the genera *Microbacterium* and *Aureobacterium* and proposal of new seven species in a redefined genus *Microbacterium*

The Japanese Tissue Culture Association, Autumn Symposium (November, 1997,

Osaka)

T. Yoshida

Proliferation and differentiation of CNS progenitor cells

Symposium on the Institute of Physical and Chemical Research (RIKEN) (November, 1997, Wako)

K. Mikata

Taxonomy of the genus *Saccharomyces*

International symposium on bioactive peptides deived from food and non-food proteins (December, 1997 Kyoto)

M. Sakaguchi¹⁾, K. Murayama¹⁾, E. Matsumura¹⁾, Y. Jinsmaa²⁾, M. Yoshikawa¹⁾, M. Satoh and Masao Takeuchi

Stimulatory effect of β -casomorphins on neurite outgrowth in mouse neuroblastoma cells

1) Laboratory of Cell Biology, Osaka University of Pharmaceutical Sciences

2) Faculty of Agriculture, Kyoto University

The 49th Yeast Research Club (March, 1998, Nishinomiya)

K. Mikata

Morphology and taxonomy of ascomycetous yeasts

Japan Society for Bioscience, Biotechnology and Agrochemistry (April, 1998, Nagoya)

K. Ashiya¹⁾, H. Kawasaki¹⁾, Y. Nakagawa, T. Sakane, Y. Uemura²⁾, S. Krivonogov³⁾, H. Takahara⁴⁾ and T. Seki¹⁾

Analysis of microbial diversity in ancient soil

1) The International Center for Biotechnology, Osaka University

2) School of Letters, Bukkyo University

3) UIGGM, SB, RAS

4) Faculty of Agriculture, Kyoto Prefectural University

K. Uemori¹⁾, H. Ryu¹⁾, D. Honda, Mariko Takeuchi, K. Yokota¹⁾ and J. Sugiyama¹⁾
Phylogenetic analysis of gram-positive bacteria which containing 2,4-diaminobutyric acid in the cell wall

1) Institute of Molecular and Cellular Biosciences, The University of Tokyo

Mycological Society of Japan (May, 1998, Kyoto)

T. Ito, A. Nakagiri and I. Okane

Mycoflora of the rhizosphere of *Salicornia europaea*

A. Nakagiri, I. Okane and T. Ito

Geographical and seasonal distribution of arenicolous marine fungi along the Pacific coast of the Bousou Peninsula

I. Okane, A. Nakagiri, and T. Ito

Host range of an endophytic species of *Guignardia* (anamorph: *Phyllosticta*)

Japan Society for Culture Collection (June, 1998, Higashi-hiroshima)

Y. Nakagawa, T. Nishii, H. Kawasaki¹⁾, T. Sakane, A. Yokota¹⁾ and K. Hatano
Utilization of RAPD for quality control of preserved bacterial cultures

1) Institute of Molecular and Cellular Biosciences, The University of Tokyo

K. Ueda and K. Mikata

Kluyveromyces strains isolated in the Nansei Islands

Asia-Pacific Mycological Conference on Biodiversity and Biotechnology (July, 1998, Hua Hin, Thailand)

A. Nakagiri

Diversity of halophytophthoras in subtropical mangroves and factors affecting their distribution

1st International Symposium on Identification of Microorganisms and their Secondary Metabolites (August, 1998, Seoul)

Mariko Takeuchi

Actinobacteria in mangrove rhizospheres

-isolation, identification and classification-

3rd Congress of the Asian-Pacific Organization for Cell Biology (August, 1998, Osaka)

K. Takeuchi¹⁾, M. Satoh, T. Yoshida and Masao Takeuchi

In vitro formation and function of platelet-like particles from human megakaryoblastic leukemia cell lines, MEG-01 and MEG-01s

1) Osaka Prefectural College of Health Science

The Japanese Association for the Study of Taste and Smell (October, 1998, Koriyama)

M. Satoh

Characterization of olfactory basal cells in culutre

Annual Meeting on Microbial Taxonomy (October, 1998, Kawaguchi-ko)

M. Suzuki¹⁾, Y. Nakagawa, S. Yamamoto¹⁾ and S. Harayama¹⁾

Phylogenetic analysis of *Cytophaga/Flavobacterium/Bacteroides* complex based on the amino acid sequences of *gyrB* and taxonomic consideration of the genus *Marinilabilia*

1) Marine Biotechnology Institute, Kamaishi Laboratories

Endophytics Microbes Workshop (November, 1998, Bogor, Indonesia)

I. Okane

Assemblages of endophytic fungi in leaves of ericaceous plants and their succes-

sional change

The Molecular Biology Society of Japan (December, 1998, Yokohama)

M. Kataoka¹⁾, T. Seki²⁾ and K. Ueda

Two distinct mechanisms cause heterogeneity of 16S rRNA

1) Mitsubishi-Kasei Institute of Life Sciences

2) The International Center for Biotechnology, Osaka University

K. Ueda and K. Mikata

Group I introns and species differentiation of yeast-like fungi

Miscellaneous Scientific Papers, 1997-1998

A. Nakagiri. 1997. Fungi living in the sea. In: Kinoko-no-sekai, Vol. 4, p. 119. Asahi Shinbunsha, Tokyo. [in Japanese]

A. Nakagiri. 1998. Chap. 4, 9. Marine fungi. In: An illustrated guide to mitosporic fungi (Deuteromycetes)...Collection, isolation and identification. (ed. by K. Tubaki), pp. 85-97. IPC, Tokyo. [in Japanese]

A. Nakagiri. 1998. Chap. 4. Ecology of fungi in Chiba Prefecture, 3. River, lake and sea. In: Natural history of Chiba Prefecture, Vol. 4. Plants in Chiba Prefecture 1, -Bacteria, fungi, lichen, algae and moss-, pp. 72-78. Chiba Historical Materials Research Foundation, Chiba. [in Japanese]

Masao Takeuchi. 1998. Cell Banks and Gene Banks in Japan, Chap. 2-3, 6-1, 11-1. 8, 11-1 (ed. by Cell Bank Committee, The Japanese Tissue Culture Association). Kyoritsu Shuppan, Tokyo. [in Japanese]

Amendment of the Plant Quarantine Law for Phytopathogenic Microorganisms

Distribution of many imported phytopathogenic microorganisms that are preserved in the IFO Collection is controlled by the plant quarantine law in Japan. With the amendment of this law, the restrictions on distribution of the following 135 pathogenic strains have been relaxed. They are now available directly from the IFO Collection without authorization of the plant protection station.

Derestricted strains of phytopathogenic microorganisms.

Species name	IFO No.
<i>Aspergillus candidus</i>	4036, 4037, 32248
<i>Aspergillus clavatus</i>	4045, 8605, 8606
<i>Aspergillus flavus</i>	4295, 6343, 7540, 7599, 7600, 30107, 30180
<i>Aspergillus melleus</i>	7541, 32035
<i>Aspergillus niger</i>	4066, 4343, 5374, 5376, 6341, 6342, 6428, 6661, 6662, 8541, 9455, 31638
<i>Aspergillus ochraceus</i>	4069, 4070, 4071, 4072
<i>Aspergillus restrictus</i>	31385
<i>Aspergillus sclerotiorum</i>	5863, 7542, 32036
<i>Aspergillus tamarai</i>	4099
<i>Aspergillus versicolor</i>	4105, 30338, 31639
<i>Ceratocystis paradoxa</i>	6325, 6804
<i>Chaetomium brasiliense</i>	32221
<i>Chaetomium cochliodes</i>	6308, 6309, 6550, 9911
<i>Chaetomium funicola</i>	6555
<i>Chaetomium globosum</i>	5886, 31387
<i>Chaetomium indicum</i>	6558
<i>Endomyces geotrichum</i>	9541, 9542
<i>Eurotium amstelodami</i>	4028, 5817, 31389
<i>Geotrichum candidum</i>	4597, 4599, 5959, 6454, 9538, 9539, 9540
<i>Memnoniella echinata</i>	31007, 31644, 31649
<i>Mucor circinelloides</i> f. <i>griseocyanus</i>	4563
<i>Myrothecium verrucaria</i>	6113, 6133, 6351, 9056, 32264
<i>Penicillium aurantiogriseum</i> var. <i>aurantiogriseum</i>	5847, 7733
<i>Penicillium chrysogenum</i>	4626, 4688, 4689, 4897, 5471, 5472, 5473, 6135, 9250, 9251, 9252, 32029, 32030

(continued)

Species name	IFO No.
<i>Penicillium citrinum</i>	6352
<i>Penicillium claviforme</i>	31392
<i>Penicillium corymbiferum</i>	6092
<i>Penicillium digitatum</i>	7006, 7758
<i>Penicillium expansum</i>	5854, 6096
<i>Penicillium glabrum</i>	31393
<i>Penicillium islandicum</i>	6963, 6964, 7601
<i>Penicillium italicum</i>	5848, 32032
<i>Penicillium rubrum</i>	30542, 32043
<i>Penicillium spinulosum</i>	5853
<i>Penicillium thomii</i>	6109, 31394, 31645
<i>Petromyces alliaceus</i>	5320, 7538, 32230, 32231
<i>Rhizopus oryzae</i>	4707, 4772, 4798, 5378, 5379, 5380, 5384, 5780, 6155, 9364, 31005
<i>Rhizopus stolonifer</i>	5781, 6154, 6300, 30795
<i>Streptomyces flavovirens</i>	3197, 3412, 3716, 12771
<i>Trichoderma harzianum</i>	30543, 31976
<i>Trichothecium roseum</i>	6157, 31647
<i>Wallemia sebi</i>	32277
<i>Zygorhynchus moelleri</i>	4832, 5305

***Aspergillus* Reference Culture Set**

The following eleven strains of *Aspergillus* were received from the Centraalbureau voor Schimmelcultures, which is a member of the International Commission on *Penicillium* and *Aspergillus* (ICPA). These strains are guaranteed by the ICPA members as a reference culture set.

You can use these authorized strains and get other information on these cultures from the ICPA homepage (<http://www.cbs.knaw.nl/ICPA/ICPA.HTM>).

Isolate Numbers of Reference Cultures

Species name	IFO	CBS	IMI	ATCC
<i>Emericella nidulans</i>	33017	288.95	370083	96921
<i>Eurotium amstelodami</i>	33018	518.65	229971	16464
<i>Aspergillus candidus</i>	33019	283.95	78734	13686
<i>Aspergillus clavatus</i>	33020	514.65	321306	10058
<i>Aspergillus flavus</i>	33021	282.95	370082	22546
<i>Aspergillus fumigatus</i>	33022	286.95	370079	96918
<i>Aspergillus niger</i>	33023	554.65	50566	16888
<i>Aspergillus penicillioides</i>	33024	285.95	370081	42692
<i>Aspergillus ochraceus</i>	33025	280.95	370080	96919
<i>Aspergillus terreus</i>	33026	601.65	17294	1012
<i>Aspergillus versicolor</i>	33027	583.65	229970	96920

Ordering cultures

Please specify both species name(s) and its IFO accession number(s) on the order form and send it to IFO by mail or facsimile. All orders and other correspondence should be addressed to:

Institute for Fermentation, Osaka (IFO)

17-85, Juso-honmachi 2-chome, Yodogawa-ku, Osaka 532-8686, Japan

FAX: +81-6-6300-6814

The following fees are charged and should be paid upon receipt of an invoice.

For commercial firms	¥ 8,000
For non-profit organizations	¥ 4,000
For domestic high schools	¥ 1,000

A handling charge of ¥ 5,000 and a shipping charge are added to all orders to be shipped outside of Japan.

Announcement of Change of Fungal Name

The listed name of the following 12 strains was changed from *Aspergillus parasiticus* to *Aspergillus sojae*, because they were proved to be genetically unable to produce aflatoxins.

IFO 4239	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>
IFO 4241	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>
IFO 4243	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>
IFO 4244	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>
IFO 4252	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>
IFO 4274	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>
IFO 4279	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>
IFO 4386	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>
IFO 4391	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>
IFO 4403	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>
IFO 5241	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>
IFO 32074	<i>Aspergillus parasiticus</i>	→	<i>Aspergillus sojae</i>

A further 7 strains (IFO 33082–33088) of *Aspergillus sojae*, which were also proved to be genetically unable to produce aflatoxins, have been deposited in our collection (see Newly Accepted Strains).

Corrections

In the issue of IFO Research Communications No.18, the following corrections should be made.

Page	Line	Type	Should read
42	23	<i>Pencillium</i> sp. 1	<i>Penicillium</i> sp. 1
42	24	<i>Pencillium</i> sp. 2	<i>Penicillium</i> sp. 2
42	25	<i>Pencillium</i> sp. 3	<i>Penicillium</i> sp. 3
42	26	<i>Pencillium</i> sp. 4	<i>Penicillium</i> sp. 4
107	25	<i>taxicarius</i>	<i>toxicarius</i>

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