Microbe Division / Japan Collection of Microorganisms

Head
Yoshimi BENNO, D.V.M., Ph.D.

Goal

Microbes are closely related to human health and environment. The Microbe Division / Japan Collection of Microorganisms has contributed to bioscience and biotechnology as one of the leading Culture Collections in the world since 1981. A wide range of microbes relevant to human health and environment are collected, preserved and distributed using new technologies and by collaboration with researchers and the Culture Collections. Efforts have been also made to search the new microbes and to develop technologies for handling extremophiles and uncultured microbes, screening systems for valuable microbes and culture-independent analysis of the microbes. A total of preserved strains are 18,500 strains. Approximately 3,200 strains were annually distributed to the Universities and Research Institutes in Japanese and overseas.

Type strains and related strains of lactic acid bacteria, anaerobic bacteria, aerobic bacteria, actinomyces , archea, extremphiles, yeasts and fungi for human health and environmental research are collected to BRC-JCM. Distribution of the genomic DNA from microbe also started by the combination of the Microbial Division with the Gene Engineering Division. BRC-JCM acquired the ISO9001•2000 certification for the quality control of microbial strains on August 2007. The BRC-JCM activities include training course, the homepage renewals, publications of mail-news, the JCM newsletter and JCM catalogue. By these activities, microbial researches in Japan and overseas will be developed and be aligned our international initiatives with share and relations to the Asian Microbial Culture Collections.

Activities

Overview

1. Collection, preservation and distribution of the microbial strain(s)
2. Deposition of new microorganisms and issuance of JCM certification for deposition and availability of the type strain to new taxon proposed
3. Freezing and freeze-drying preservation of microbial strains for the safety purposes
4. Quality control of the microbial resources
   a. Confirmation of purity and characteristics of deposited strains
   b. Genetic monitoring of deposited strains
   c. Collection and distribution of microbial information related to deposited strains
5. Transference of microbial strains is practiced according to the material transfer agreements (MTAs)
6. The JCM management is practiced according to the Regulations of Microbial Usage in RIKEN

Information services

1. Homepage (http://www.jcm.riken.jp).
2. Publication of the JCM mail-news every month
3. Publication of the JCM Newsletter every year.
4. Publication of the JCM Catalogue per 3 years.

Members

Head

Yoshimi BENNO, D.V.M., Ph.D. (2004.7~)

Senior Research Scientist

Yoshimasa KOSAKO, Ph.D. (2004.7~)
Motofumi SUZUKI, Ph.D. (2004.7~)
Masako TAKASHIMA, Ph.D. (2004.7~)
Takuji KUDO, Ph.D. (2004.7~)
Gen OKADA, Ph.D. (2004.7~)
Takashi ITO, Ph.D. (2004.7~)

Senior Technical Scientist

Tsutomu OHWADA (2004.7~)

Contract Researcher

Mitsuo SAKAMOTO, Ph.D. (2004.7~)
Maki KITAHARA, Ph.D. (2004.7~)

Visiting Scientist

Hideo HASEGAWA, Ph.D. (2004.7~)
Itaru DEKIO (2007.4~)

Agency staff

Kayo KUSAOKE (2004.7~)
Tomoko TSUZUKI (2004.7~)
Yumi OSHIDA (2004.7~)

Student Trainee

Toshiya MARUO (2004.11~)
Tomonori IKEGAMI (2004.11~2008.2)

Oshida, Kusaoke, Baba, Tsuzuki, Matsumoto, Fujikawa
Takashima, Suzuki, Owada, Kitahara, Ito, Sakamoto
Okada, Suzuki, Benno, Kosako, Kudo

Specific Aims

Business Plan & Results

1. Collection and preservation of the microbial strains

Deposit of new microorganisms:

To contribute to the domestic, regional, and global improvements in the conservation of microbial resources in cooperation with other culture collections and institutions, JCM collects, preserves and distribute of authentic microorganisms to researchers in the fields of life sciences and biotechnology as well as of microbial taxonomy. The isolation, phenotypic characterization and phylogenetic analyses of microorganisms from natural sources are carried out to determine microbial biodiversity and to evaluate the currently recognized taxonomic systems and criteria. As of the end of November, 2008 a total of 18,533 strains (8,281 bacteria, 3,475 actinomycetes, 411 archaea, 2,969 filamentous fungi, 3,375 yeasts and 22 others) were held in JCM.

A total of 9,884 cultures were distributed to local and overseas researchers from 1st January 2006 to 30th November 2008.

JCM also provides an interface for access to its culture collection by users, as well as for accepting strains for deposit and distributing cultures (JCM homepage and on-line catalogue).

2. Preservation of microbial strains

To preserve and maintain microorganisms safely and correctly, BRC-JCM employs two preservation methods: freezing and freeze-drying. In freezing, a harvested culture is suspended in cryoprotectant, placed in a serum tube, and kept in a deep-freezer (-80˚C) or in the vapor phase of liquid nitrogen (ca -175˚C). Fundamentally, ten tubes are prepared for freezing, 6 are kept in a deep freezer, 2 are in liquid nitrogen, 1 is kept in a liquid nitrogen tank in another place to avoid risk of emergency, and the remaining 1 is used for routine check after freezing. In the liquid nitrogen tank shown in page 68, 7,000 strains (14,000) tubes are kept. Since freezing is known to be the most reliable and applicable method of microbial preservation with some exception, BRC-JCM employs this method for all microorganisms held.

Freeze-drying is also a well-known method to microbial preservation. BRC-JCM has devised an available freeze-drying machine with manifold (Japan Patent 59-141735). Since 15 ampoules can be placed in one manifold, 15 ampoules per batch can be prepared under the same condition for one strain. Using our manifold system, 105 ampoules (15 each for 7 strains) can be prepared as our system is equipped after acceleration test at 37˚C for 2 weeks, we keep them in a cold room and use them to send out cultures for distribution in answer to requests.

All data concerning preservation was kept in a database and carefully compered with actual tubes or ampoules when newly stocked or used for distribution.

3. Issuance of the JCM certification for deposition and availability of the type strain to new taxon proposed

In accordance with the policy of the International Journal of Systematic and Evolutionary Microbiology (IJSEM), authors who wish to propose a new species, a new subspecies or a new combination in IJSEM or to apply for citation of a new name in the Validation List of IJSEM are required to prove the deposition of type strains in culture collections and their availability to the public prior to the acceptance of their manuscript or the citation in a culture collection list. In response to this policy and upon a depositor's request, BRC-JCM will issue a certificate to him/her or one of the authors of a manuscript describing a new taxon after confirming the viability and purity of the deposited culture and the identity of the culture preserved in BRC-JCM.

Research Subjects:

1. Search of new microbes as bioresources
2. Development of new screening systems for valuable microbes related to human health and environment.
3. Development of rapid isolation, cultivation, classification and preserva- tion of extremophiles and uncultured microbes.
4. Development of preservation of uncultured microbes by cloning using cDNA.
5. Development of culture-independent analytical technique for microbial diversity.

Research Subjects by the BRC-JCM Staff

1. Archaea and extremophilic bacteria:

Archaea and extremophiles would play important roles in promising areas of bioscience, biotechnology and environmental preservation: for examples, speculation of models for inferring early life forms on the planet, understanding of the adaptive machinery and involvement in biogeo- chemical cycles in extreme environments, and biotechnological applica- tions such as those to heat-stable enzymes development and methanogenic processing. To support such frontiers of research, the BRC-JCM has been collecting, preserving and distributing strains of archaea and extremophilic bacteria. Furthermore, we are exploring novel archaea and extremophilic bacteria by developing modern isolation and cultivation technologies. We are also holding training courses for those who are wishing to study these fastidious microorganisms.

2. Actinomyces:

Gram-positive bacteria are broadly classified into two clades, one of which is called the class *Actinobacteria*, which constitutes of about 200 genera and 1800 species of actinomycetes. Actinomycetes have long been utilized as producers of antibiotics, enzymes, amino acid seasonings, and other valuable products; some actinomycetes have been known as decomposers of persistent organic pollutants and considered to play important roles in the geochemical cycles. We have collected more than 90% of the type strains of actinomycetes from local and foreign researchers and from culture collections to meet demands of all types of users. We also recently isolated actinomycetes from various environments and are conducting taxonomic studies on these isolates to confer originality to the BRC-JCM collection.

3. Aerobic bacteria:

472 strains obtained from the National Institute of Health, Japan have been registered and preserved. Among them, 52 have been distributed after the confirmation of their biochemical characteristics and 16S rDNA sequences. These strains belong to the family Enterobacteriaceae. Most of strains are categorized as BSL2. They were collected 30 years ago for taxonomical purposes. They are very useful for users because they maintain their characters with time.

4. Anaerobic bacteria and lactic acid bacteria(LAB):

On the basis of the agreement of microbe transfer to BRC-JCM, we could keep and distribute anaerobic and lactic acid bacteria. Numerous researchers in the world deposited some strains as new species. Since the start of the official distribution of the JCM strains, the major group of microbes distributed from JCM is lactobacilli. Thus, the number of lactobacilli distributed is the largest among those of other BRC-JCM strains. The collection and distribution of anaerobic bacteria is maintained yearly. These microorganisms have contributed to research for anaerobic bacteria.

Of note, the JCM staff actively proposed the new genera and species of some isolates from intestine and oral cavity in humans and animals.

On the research activity in BRC-JCM, we can offer the following subjects:

a. Search of new LAB and anaerobic bacteria as resource
b. Development of new screening systems for valuable LAB and anaerobic bacteria related to human health and environment
c. Development of rapid isolation, cultivation, classification and preserva- tion of LAB and anaerobic bacteria
d. Development of culture-independent analytical techniques for microbial diversity including LAB and anaerobic bacteria.

The JCM staffs hope that LAB and anaerobic bacteria distributed will be availed for the development of life science and the environmental subjects.

5. Yeasts:

a. Development of yeast resources involved in the degradation of azo dye.

Three novel yeast species with azo dye-decolorizing activity were isolated from soil and related samples. One is a new species of the genus *Cryptococcus*, which is closely related to *Cryptococcus saitoi*. The other two are novel species of the genus *Geotrichum*, which are closely related to *Geotrichum klebahnii* and *Geotrochum vulgare*, respectively. These three species showed more than 90% de-colorization rates in an azo-dye, Orange I.

b. Development of yeast resources involved in wood conservation.

Five new yeast species, *Ambrosiozyma kamigamensis*,

Ambrosiozyma neoplatypodis, *Candida kashinagacola*, *C. pseudovanderkliftii*, and *C. vanderkliftii* were isolated from galleries of the ambrosia beetle, *Platypus quercivorus*, in a specimen of *Quercus laurifolia*. *Candida kashinagacola* was frequently isolated from the beetle galleries of *Platypus quercivorus* in three different host trees (*Quercus serrata*, *Q. laurifolia* and *Castanopsis cuspidata*), thus indicating that this species is a primary ambrosia fungus of *P. quercivorus*.

6. Filamentous fungi:

Japanese have associated with "koji mold" (a group of filamentous fungi) for brewing sake, bean paste, soy sauce, and other fermented products Mushrooms, molds and yeasts are called "fungi" collectively. Although more than 80,000 fungal species were reported, their application in food, medical supplies, and similar products is extremely restricted. Cooperating with researchers in and outside Japan, the filamentous fungal section of BRC-JCM collects, preserves and distributes useful or unique strains for basic and applied scientific research, including rare or uncultured species. It also conducts research studies on fungal classification, phylogeny, and preservation. Here at BRC-JCM, we are always working hard to ensure that the BRC-JCM strains obtained from various environments can provide researchers with the much-needed help with their research.

Trainee service

1. Culture and preservation methods for strict anaerobic bacteria
2. Molecular analytical techniques of human fecal microbiota by terminal-RFLP method

Publications

【Original Papers】(*Peer reviewed journals)

1. Jongrungruangchok S., Tanasupawat S., Kitakoop P., Bavovada R., Kobayashi H., Kudo T.: "Identification of *Streptomyces* and *Kitasatospora* strains from Thai soils with geldanamycin production strain." Actinomycetologica 20: 10-14 (2006).*

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3. Salminen S., Benno Y., de Vos W. M.: "International colonisation, microbiota, and future probiotics?" Asia. Pac. J. Clin. Nutr. 15: 558-562 (2006).*

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5. Hashimoto K., Kawasaki H., Akazawa K., Nakamura J., Asakura Y., Kudo T., Sakuradani E., Shimizu S., Nakamatsu T.: "Changes in composition and content of mycolic acids in glutamate-overproducing *Corynebacterium glutamicum*." Biosci. Biotechnol. Biochem. 70: 22-30 (2006).*

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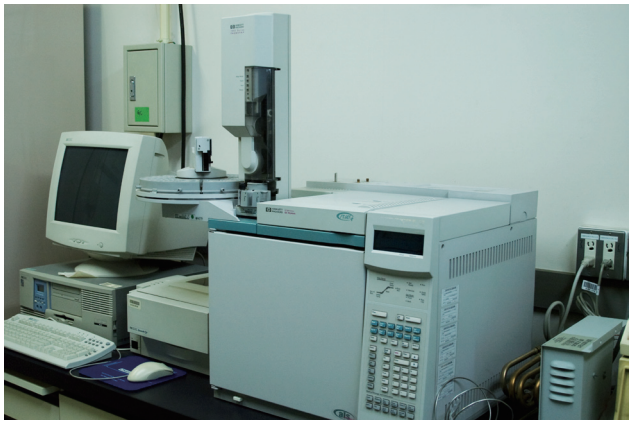
Safety-cabinet



Gas liquid chromatography

Electron microscopy

Extraction of microbial DNA

Liquid nitrogen tank

Preservation check in liquid nitrogen tank

Anaerobic chamber (RIKEN model)

ISO documents

9. Tomita S., Shirasaki N., Matsuyama J., Benno Y., Kiyosawa I.: "Growth behavior of *Clostridium perfringens* cells aggregated by bovine lactoferrin in the presence of lipoteichoic acid." Food Preserv. Sci. 32: 85-89 (2006).*

10. Maruo T., Sakamoto M., Toda T., Benno Y.: "Monitoring the cell number of *Lactococcus lactis* subsp. *cremoris* FC in human feces by real-time PCR with strain-specific primers designed using the RAPD technique." Int. J. Food Microbiol. 110: 69-76 (2006).*

11. Bakir M. A., Kitahara M., Sakamoto M., Matsumoto M., Benno Y.: "*Bacteroides intestinalis* sp. nov., isolated from human faeces." Int. J. Syst. Evol. Microbiol. 56: 151-154 (2006).*

12. Ara I., Kudo T.: "Three novel species of the genus *Catellatospora*, *Catellatospora chokoriensis* sp. nov., *Catellatospora coxensis* sp. nov. and *Catellatospora bangladeshensis* sp. nov., and transfer of *Catellatospora citrea* subsp. *methionotrophica* Asano and Kawamoto 1988 to *Catellatospora methionotrophica* sp. nov., comb. nov." Int. J. Syst. Evol. Microbiol. 56: 393-400 (2006).*

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16. Thawai C., Tanasupawat S., Itoh T., Kudo T.: "*Actinocatenispora thailandica* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*." Int. J. Syst. Evol. Microbiol. 56: 1789-1794 (2006).*

17. Kageyama A., Iida S., Yazawa K., Kudo T., Suzuki S., Koga T., Saito H., Inagawa H., Wada A., Kroppenstedt R. M., Mikami Y.: "*Gordonia araii* sp. nov. and *Gordonia effusa* sp. nov., isolated from patients in Japan." Int. J. Syst. Evol. Microbiol. 56: 1817-1821 (2006).*

18. Tanasupawat S., Pakdeeto A., Namwong S., Thawai C., Kudo T., Itoh T.: "*Lentibacillus halophilus* sp. nov., from fish sauce in Thailand." Int. J. Syst. Evol. Microbiol. 56: 1859-1863 (2006).*

19. Pikuta E. V., Itoh T., Krader P., Tang J., Whitman W. B., Hoover R. B.: "*Anaerovirgula multivorans* gen. nov., sp. nov., a novel spore-forming, alkaliphilic anaerobe isolated from Owens Lake, California, USA." Int. J. Syst. Evol. Microbiol. 56: 2623-2629 (2006).*

20. Lan P. T. N., Sakamoto M., Sakata S., Benno Y.: "*Bacteroides barnesiae* sp. nov., *Bacteroides salanitronis* sp. nov. and *Bacteroides gallinarum* sp. nov., isolated from chicken caecum." Int. J. Syst. Evol. Microbiol. 56: 2853-2859 (2006).*

21. Degawa Y., Shibanuma K., Benno Y.: "The proliferation factors affecting the growth of intestinal bifidobacterium in the presence of corn-fiber components of containing hydrolyzed oligosaccharides." Int. J. Probiot. Prebiot. 1: 203-212 (2006).*

22. Asami Y., Kakeya H., Okada G., Toi M., Osada H. "RK-95113, a new angiogenesis inhibitor produced by *Aspergillus fumigatus*." J. Antibiot. 59: 724-728 (2006).*

23. Fungsin B., Takashima M., Sugita T., Artjariyasripong S., Potacharoen W., Tanticharoen M., Nakase T.: "*Bullera koratensis* sp. nov. and *Bullera lagerstroemiae* sp. nov., two new ballistoconidium-forming yeast species in the Trichosporonales clade isolated from plant leaves in Thailand." J. Gen. Appl. Microbiol. 52: 73-81 (2006).*

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28. Sugita T., Takashima M., Poonwan N., Mekha N.: "*Candida pseudohaemulonii* sp. nov., an amphotericin B- and azole-resistant yeast species, isolated from the blood of a patient from Thailand." Microbiol. Immunol. 50: 469-473 (2006).*

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37. Sakamoto M., Kitahara M., Benno Y.: "*Parabacteroides johnsonii* sp. nov., isolated from human faeces." Int. J. Syst. Evol. Microbiol. 57: 293-296 (2007).*

38. Sakamoto M., Lan P. T. N., Benno Y.: "*Barnesiella viscericola* gen. nov., sp. nov., a novel member of the family *Porphyromonadaceae* isolated from chicken caecum." Int. J. Syst. Evol. Microbiol. 57: 342-346 (2007).*

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48. Pikuta E. V., Marsic D., Itoh T., Bej A. K., Tang J., Whitman W. B., Ng J. D., Garriott O. K., Hoover R. B.: "*Thermococcus thioreducens* sp. nov., a novel hyperthermophilic, obligately sulfur-reducing archaeon from a deep-sea hydrothermal vent." Int. J. Syst. Evol. Microbiol. 57: 1612-1618 (2007).*

49. Sakamoto M., Ohkusu K., Masaki T., Kako H., Ezaki T., Benno Y.: "*Prevotella pleuritidis* sp. nov., isolated from pleural fluid." Int. J. Syst. Evol. Microbiol. 57: 1725-1728 (2007).*

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