

Microbe Division/Japan Collection of Microorganisms



Head, Yoshimi Bennno

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 yet-unexploited microbes, screening systems for valuable microbes and culture-independent analysis of the microbes.

Activities Overview

- 1) Collection, preservation and distribution of the microbial strain(s)
- 2) Deposit of new microorganism(s) and issuance of the 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
 - 1) Confirmation of purity and the characteristics of deposited strain(s)
 - 2) Genetic monitoring of the deposited strain(s)
 - 3) Collection and distribution of microbial information related to deposited strain(s)
 - 5) Transference of the microbial strain(s) 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 BRC/JCM Catalogue per 3 years.
3. Publication of BRC/JCM Newsletter every year.

2003 ~ 2005

Members

Head

Yoshimi Benno, DVM, Ph.D. (2004.7 ~)

Senior researchers

Yoshimasa Kosako, Ph.D. (2004.7 ~)

Masako Takashima, Ph.D. (2004.7 ~)

Gen Okada, Ph.D. (2004.7 ~)

Motofumi Suzuki, Ph.D. (2004.7 ~)

Takuji Kudo, Ph.D. (2004.7 ~)

Takashi Ito, Ph.D. (2004.7 ~)

Technical staffs

Tsutomu Ohwada (2004.7 ~)

Tomoko Tsuzuki (2004.7 ~)

Kayo Kusaoke (2004.7 ~)

Yumi Oshida (2004.7 ~)

Research staffs

Mitsuo Sakamoto, Ph.D. (2004.7 ~)

Maki Kitahara, Ph.D. (2004.7 ~)

Hidenori Hayashi, Ph.D. (2004.7 ~)

Shinji Sakata, Ph.D. (2004.7 ~)

Fellowship members

Hideo Hasegawa, Ph.D. (2004.7 ~)

Mariko Hayakawa (2005.9 ~)

Emiko Iwata (2005.9 ~)

Jun Shimizu, Ph.D. (2004.7 ~)

Hitomi Ito (2005.9 ~)

Guest researchers

Madalin Iancu Enache (JSPS), Ph.D. (2004.7 ~)

Ismet Ara (JSPS), Ph.D. (2004.7 ~)

Trainees

Toshitaka Odamaki (2004.7 ~)

Seigo Nakaya (2004.7 ~ 2005.3)

Toshiya Maruo (2004.11 ~)

Ryouko Kibe (2004.7 ~ 2005.10)

Mayuko Ohnishi (2004.11 ~)

Miyoka Ide (2004.11 ~)

Kensaku Shibata (2005.4 ~)

Keita Sakuma (2004.7 ~)

Shouko Kuwazuru (2004.7 ~ 2005.10)

Miho Ohkouchi (2004.11 ~ 2005.11)

Itaru Dekio (2004.11 ~)

Naoto Yoshikawa (2004.11 ~)

Tomonori Ikegami (2004.11 ~)

Mariko Kibe (2004.7 ~ 2005.10)



Kuwazuru Sakuma Bakir Enache Yoshikawa Sakata Yamazaki Kitahara Ide Suzu
Maruo Kibe Hayashi Ichikawa Owada Nakai Sakamoto Hasegawa Tuzuki Oshida Okano
Kusaoke Toma Ismet Takashima Fijikawa
Kudo Itoh Benno Kosako Suzuki Okada

Specific aim Business Plan & Results

1) Collection and preservation of the microbial strain(s)

Deposit of new microorganism(s)

To contribute domestic, regional, and global improvements of conservation of microbiological resources in cooperation with other culture collections and institutions, JCM works collection, preservation and distribution of authentic microorganisms to researchers in the fields of life sciences and biotechnology as well as research on microbial taxonomy. The isolation of microorganisms from natural sources, their phenotypic characterization and phylogenetic analyses are carried out to determine the microbial biodiversity and to evaluate the currently recognized taxonomic systems and criteria. From 1st April, 2004 to 31st March, 2005, JCM accessioned 787 strains, and supplied 4119 strains to researchers. As of the end of March 2005 a total of 11919 strains (4799 bacteria, 2650 actinomycetes, 253 archaea, 1808 filamentous fungi, 2388 yeasts, and 21 others) were listed in JCM. JCM also provides an interface for access to the culture collection by users by accepting strains for deposit and distributing cultures (JCM homepage and on-line catalogue).

2) Preservation of the microbial strain(s)

To preserve and maintain microorganisms safely and correctly, BRC/JCM employed two methods for preservation, freezing and freeze-drying. For freezing, a harvested culture is suspended in cryoprotectant, placed in a serum tube, and kept in a deep-freezer (-80°C) and the vapor phase of liquid nitrogen (ca -175°C). Fundamentally, ten tubes are made 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 a risk of emergency, and the remaining one is used for the 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 most reliable and applicable method for preservation of microorganisms with some exception, BRC/JCM employed this method for all microorganisms held.

Freeze-drying is also well known method to preserve microorganisms. BRC/JCM has improved a commercially available freeze-drying machine to the system with manifold (Japan Patent 59-141735). As fifteen ampoules are able to put on one manifold, 15 ampoules that are one batch for making ampoules can be made under the same condition for one strain. Using our manifold system, a total of 105 ampoules (15 each for 7 strains) can be made as our system equips with seven manifolds. Once ampoules are made and checked after acceleration test at 37°C for 2 weeks, we keep these ampoules in a cold room and use them to send the cultures out for distributing in answer to request.

Every data concerning preservation was kept in a database, and the data is compared with actual tubes or ampoules carefully 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 deposition of type strains in culture collections and their availability to the public prior to acceptance of the manuscript or citation for the list. In response to this policy and upon a depositor's request, JCM will issue a certificate to him/her or one of the authors of a manuscript describing a new taxon after confirmation of the viability and purity of the deposited culture and also the identity of the culture preserved in JCM.

2003 ~ 2005

Research Subjects:

- 1) Search of new microbes as bioresource
- 2) Development of new screening systems for valuable microbes related to human health and environment.
- 3) Development of rapid isolation, cultivation, classification and preservation of extremophiles and yet-unexploited microbes.
- 4) Development of preservation of yet-unexploited microbes by clone as cDNA.
- 5) Development of culture-independent analysis 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 biosciences, biotechnologies and environmental preservation: for examples, speculation of models to infer early life forms on the planet, understanding of the adaptive machinery and involvement in biogeochemical cycles in extreme environments, and biotechnological applications such as heat-stable enzymes and methanogenic processing. In order to support such frontiers of researches, 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 isolation and cultivation technologies. We are also holding training courses for those who are wishing to deal with these fastidious microorganisms.

2) Actinomycetes:

Gram-positive bacteria are broadly classified into two clades and one of them is called the class Actinobacteria, which is constituted of about 150 genera and 1500 species of actinomycetes. Actinomycetes have been utilized from long ago as producers of antibiotics, enzymes, amino acid seasonings, etc., and some actinomycetes have been known as decomposers of persistent organic pollutants. We have collected more than 90% of type strains of actinomycetes from domestic and foreign researchers and culture collections in order to satisfy all sorts of demands of users. We also newly isolate actinomycetes from various environments to give originality to the JCM collection.

3) Aerobic bacteria:

A numerous number of *Bacillus* and related genera on the Culture Collection of the Institute of Applied Microbiology (IAM), the Tokyo University were transferred from the IAM to JCM on 1980s. On the basis of the microbe transfer to JCM, JCM could keep and distribute these microorganisms. So, the distributed number of *Bacillus* and related genera is the best one compared with that of another JCM strains. Especially, the JCM staff actively proposed the new genus and species in *Bacillus* and related genera from environmental materials and foods. The JCM staffs hope that *Bacillus* and related genera will be availed to develop the food science and the environmental subjects.

Considering health and environment, collection, preservation and distribution of the Gram-negative strains was focused on the Proteobacteria. Among the proteobacterial strains, there are many strains, such as *Escherichia*, *Salmonella*, *Yersinia* etc, which are categorized on BSL2. *Campylobacter*, *Helicobacter* and *Listeria* etc, which are difficult to obtain from other culture collection in Japan, are also distributed to users. In order to increase the

number of such microbe, we endeavor to collect them. As a part of research and business, we are going to collect all validated species of the *Pseudomonas* strains. Using them, reclassification of the genus *Pseudomonas* is carrying out and the database for simple identification is being constructed.

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

On the basis of the microbe transfer to JCM, JCM could kept and distributed anaerobic bacteria and lactic acid bacteria. Then, the numerous researchers on the world deposited some strains, which are deposited as a new species. Since the official distribution of the JCM strains, the major distributed microbe from JCM is lactobacilli. So, the distributed number of lactobacilli is the best one compared with that of another JCM strains. The collection and distribution of anaerobic bacteria was also maintained year by year. These microorganisms were contributed to the research for anaerobes.

Especially, the JCM staff actively proposed the new genus 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:

- 1) Search of new LAB and anaerobes as a resource,
- 2) Development of new screening systems for valuable LAB and anaerobes related to human health and environment.,
- 3) Development of rapid isolation, cultivation, classification and preservation of LAB and anaerobes, and
- 4) Development of culture-independent analysis for microbial diversity including LAB and anaerobes.

The JCM staffs hope that LAB and anaerobes distributed will be availed to develop the life science and the environmental subjects.

5) Yeasts:

Our yeast collections concerning salt-tolerant *Debaryomyces* strains isolated from Japanese traditional foods, frass of woods and related insects, and ballistoconidium-forming yeasts in the phyllosphere contribute to the construction of molecular taxonomic systems of yeasts and the elucidation of yeast species diversity.

The collection starts to be enriched by the yeast resources associated with human health and environmental conservation.

6) Filamentous fungi:

Japanese have associated with “koji mold” (a group of filamentous fungi) for brewing sake, bean paste, soy sauce, etc. Mushrooms, molds and yeasts are called “fungi” collectively. Although more than 80,000 fungal species were reported, what used for application of food, medical supplies, etc. is extremely restricted. Cooperating with researchers in and outside Japan, the filamentous fungal section of JCM performs collection, preservation and distribution of useful or unique strains for basic/applied scientific research, rare or not-yet cultivated species, etc. Researches on fungal classification, phylogeny, and preservation are also undertaken. We continue to try hard so that JCM strains obtained from various environments can give you a help.

Trainee service

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

「The Culture Collection Management in BRC-JCM」



Bacterial cell harvest



Dispense the cell suspension into ampoules



Freezing-dryer (Pat. S59-141735)



Freezing-drying and sealing



Ampoules in a cabinet at 4°C



Ampoules in bags



Liquid nitrogen tanks



Freezing preservation in a liquid nitrogen tank

Publications (Original Papers)

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