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Microbial Diversity and the Role of Culture Collections

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Microorganisms are not only of value for the production of useful substances; they also play unique roles in element cycles with plants and animals. Microorganisms are also significant gene pools, and these gene pools must not be lost. From this point of view, microorganisms can be regarded as the cultural heritage and the cultural property, and they must be transferred to the next generation in a normal and healthy condition. Therefore, reliable culture collections are needed as the depository and for the further study and application of the cultures.

INTRODUCTION

Recently, the term of "biological diversity" or "biodiversity" has become well known to the public. Biological diversity means variability among living organisms from all sources including terrestrial, marine, and other aquatic ecosystems. This includes diversity within species, between species, and of ecosystems (Convention on biological diversity, Rio de Janeiro, 1992). In addition, biological diversity involves the great number of biological species on the earth. Plants, animals, and microorganisms are consistent elements of ecosystems, and play important roles in element cycles and other functions to sustain an active and clean earth. However, biological diversity has been reduced significantly by certain human activities.

From a global point of view, tropical rain forests occupy only 3% of the surface of the earth, but more than 50% of the biological species inhabit such areas. A survey found more than 300 species of leaf-feeding insects on a single tropical tree and about half of the species were undescribed. In addition, ants belonging to 43 species and 26 genera were recognized on a single leguminous tree, the number of species found being comparable to that in the whole of England. These findings indicate close association among plants, animals, and microorganisms, and the richness of biological diversity of tropical rain forests. Microorganisms are an important part of ecosystems on the earth, and are responsible for nitrogen fixation, bioremediation of pollutants, and soil building, and the life of plants and animals, and even humans depends on the activities of microorganisms. Food chains in nature cannot be completed without microorganisms. The development of modern biotechnology aspires to isolation of new microorganisms and improvement of their attributes. This is due to a great extent to the genetic resources resulting

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from microbial diversity. However, microorganisms have been overlooked compared with plants and animals because of their microscopic life forms.

MICROORGANISMS AND THEIR DISTRIBUTION IN BIOLOGICAL SYSTEMATICS

Needless to say, the discovery of first microorganisms owes much to the work of Antonie van Leeuwenhoek in 1677. However, microbial activities and functions were made evident through the study of lactic acid fermentation by Pasteur, L. in 1857 and the study of anthrax by Koch, R. in 1876. Rhizobia were isolated and identified by Beijerinck, M. W. in 1888, and thus microorganisms were recognized to be important in agriculture. These studies were carried out about 200 years after the discovery of the first microorganisms. The major studies in microbiology originated in fermentations, diseases in humans and animals, and agriculture. Thus microbiology was deeply concerned with human life from the outset, and lacked a period of study of natural history. As a result, studies of distribution of microorganisms have been limited to certain subjects. Incidentally, the word "microbe", now in common use, was first introduced by S_dillot, C.-E. in 1878.

Haeckel, E. (1) separated living organisms into three groups, plants, animals, and protista in 1866, and primitive organisms were included in protista. This was 12 years before the S_dillot's use of "microbe". Therefore, a substantial concept of microorganisms probably did not exist at that time, and microorganisms in a modern sense were scattered into plants and protista. With time, procaryotic and eucaryotic organisms came to be distinguished on the basis of cell anatomy, and in 1962 Stanier, R. Y. and van Niel, C. B. (2) introduced the concept of a bacterium as a procaryotic organism in microbiology. In 1969, Whittaker, R. H. (3) proposed a five-kingdom system consisting of kingdoms of plants, fungi, animals, protista, and monera for all organisms on the basis of their energy-yielding systems and cell anatomy. Microorganisms are distributed in the kingdoms of monera, protista, fungi, and a part of plants. Recently, evolutionary relationships of living organisms have been clarified on the basis of ribosomal RNA sequences and other data. Woese C. R. et al. (4) noted in 1990 that bacteria are distant from plants and animals, and by contrast plants and animals are not so far from each other. Therefore, they established a new superior concept of domains over the kingdom, and proposed three domains, Archaea, Bacteria, and Eucarya. In a modern sense, microorganisms encompass bacteria, actinomycetes, cyanobacteria, etc. in the domain Bacteria; methanogens, extremely thermophilic organisms, extremely halophilic organisms, etc. in the domain Archaea; and molds, yeasts, basidiomycetes, algae, and protozoans, etc. in the domain Eucarya. Microorganisms are regarded as collections of evolutionarily different organisms.

THE NUMBER OF MICROORGANISMS AND BACTERIAL NOMENCLATURE

Broadly speaking, the number of species of organisms hitherto described is 1,500,000, and the actual number is estimated at 10,000,000. Hawksworth, D. L. (5) calculated the total number of known species of algae, bacteria, fungi, protozoans, and viruses to be 157,000, and estimated conservatively the actual number of such microorganisms to be 1,820,000. An extremely large difference in the numbers of species is found between the number of bacteria and the number of fungi. About 3,600 bacterial species appear on references, and by contrast about 69,000 fungal species are known according to the Hawksworh's data. This is due to the different nomenclature used for bacteria and fungi.

The International Committee on Systematic Bacteriology (ICSB) of International Union of Microbiological Societies (IUMS) revised the international code of nomenclature of bacteria (the bacteriological code) in 1976 (6) and 1990 (7), and decided a new starting date, January 1, 1980, for the nomenclature of bacteria instead of the old starting date, May 1, 1753. In connection with the new starting date, ICSB approved the bacterial species of which type strains were deposited with and available from culture collections, and published the names of bacteria on an "Approved lists of bacterial names" (8) in 1980, which contained about 1,800 species and about 300 genera.

All names not on the approved list lost their standing in nomenclature of bacteria. All new names have since been published only in the International Journal of Systematic Bacteriology (IJSB). The valid dates of the publication of bacterial names were agreed to be the dates that the new names appeared in the IJSB. There are over 800 microbiological publications in which papers dealing with nomenclature may appear, but the listing of all new names and combinations in the IJSB has now become a complete source for nomenclature of bacteria.

In addition, nomenclatural type strains of the new species have to be designated for valid publication. A type culture is made up of living cultures of organisms which are descended from a strain designated as the nomenclatural type. A culture of the type strain should be deposited with at least one of the permanently established and reliable culture collections, from which it would remain readily available. Since 1980, the number of bacterial taxa, by about 150 species and about 80 genera, increased each year.

THE CONCEPT OF BACTERIAL SPECIES

Biological diversity has arisen as part of the evolution of organisms, and the smallest unit of microbial diversity is a species. Since the days of Carl von Linn_, biological species have been defined typologically as morphospecies. In addition, interbreeding and geographical isolation are considered to be elements in the concept of biological species. However, bacteria lack sexuality, fossil records, and other attributes that are used for defining the species of plants and animals. Thus bacterial species have been defined for several decades as a group of similar strains that are distinguished sufficiently from other similar groups of strains by genotypic, phenotypic, and ecological characteristics. On the other hand, the ad hoc committee on the reconciliation of approach to bacterial systematics of the ICSB (9) recommended in 1987 that bacterial species would include strains with approximately 70% or more DNA-DNA relatedness and with 5ÅKor less in thermal stability, Å¢Tm. This concept of bacterial species is widely accepted by bacteriologists. In this sense, a bacterial species is a genomic species based on DNA-DNA relatedness.

MICROBIAL DIVERSITY IN APPLICATION OF MICROORGANISMS

To date, more than 69,000 species in 5,100 genera of fungi, and about 3,600 species in about 700 genera of bacteria have been described in the literature. However, it is surprising to learn how small the number of microbial taxa appear in references to the application of microorganisms.

Alcoholic beverages were made for a long time before the existence and functions of microorganisms were recognized. Many interesting fermented foods are known in every region and every country. Such food production has been developed on the basis of region-originated and nation-originated ideas and specialties in the application and control of microorganisms. The originality and creativity of traditional fermentations have been transferred from generation to generation within the framework and circumstances of particular culture. In addition, particular microorganisms have been used for the production of traditional fermented foods. Saccharomycopsis species are important as a starter called "loogpang" for fermentation in Thailand, and they cause hydrolysis of starch. The roles of such yeasts were more recently ascertained. Mucor and Rhizopus are widely employed for the fermentation in China, Indonesia, and other areas. Aspergillus oryzae and other Koji molds are traditionally used for the production of sake, soy sauce, miso (fermented soybean paste), and other fermented foods in Japan.

Recently, a survey was made on the application of microorganisms in the production of foods, food additives, enzymes, and related materials, with the exception of antibiotics, in applied microbiology (10). Of about 700 bacterial genera, 38 genera including 3 genera of actinomycetes have been used for the production of foods, food additives, enzymes, and other products. Lactic acid bacteria are widely used in the food industry. Of about 60 yeast genera, 23 genera have been employed, particularly Saccharomyces cerevisiae strains, which are most frequently used for the production of alcoholic beverages throughout the world. Of about 5,100 fungal genera, only 26 genera are used for the production of foods, enzymes, and other products.

Nowadays the production of several kinds of indigenous foods has been developed into the modern industry. In addition, microbial production of organic acids, antibiotics, amino acids, nucleosides, nucleotides, and enzymes has deep roots in the field of applied microbiology. These successes have in great part arisen from the study of the traditional fermentation processes.

DEVELOPMENT OF THE JAPANESE MICROBIAL INDUSTRY

Japanese traditional fermentations were recorded about 1,000 years ago, but studies of applied microbiology started about 120 years ago. Japanese microbial industry can be divided into four major categories with respect to its origin (11, 12, 13).

1) Traditional fermentation

Sake, soy sauce, and fermented soybean paste are made by employing Koji molds, yeasts, and lactic acid bacteria. Sake is an alcoholic beverage made from rice, and shouchu is a distilled spirit made from rice, sweet potato, and other starchy materials. Both soy sauce and fermented soybean paste are fermented foods of soybean, and still important seasonings for the Japanese. Sake and soy sauce are now made on modern industrial lines and some processes are automatically controlled.

2) Fermentation industry introduced from overseas

Brewing of beer and making of wine and other beverages were introduced from overseas. Production of citric acid by Aspergillus strains opened a new field of microbial application. In addition, the introduction of acetone-butanol fermentation strongly influenced the development of the Japanese microbial industry, and the construction of large-scale fermentors and studies of bacteriophages were initiated in applied microbiology in Japan. Such industrialization seems to be a significant step forward from food industry to nonfood industry.

3) Modern microbial industry

After World War II, penicillin production was introduced from the United States. Penicillin production was successfully industrialized in a short time, and research has been energetically pursued new antibiotics. As a result, large numbers of new antibiotics have come onto the market.

Microbial production of amino acids, nucleosides and nucleotides is the impressive achievement in modern biotechnology in Japan. The Japanese have widely used dried sea tangle, Laminaria, "kombu" in Japanese, as a flavoring material for a long time. A substance with the flavor of "kombu" was crystallized as monosodium glutamate about 90 years ago. After this study, sodium glutamate was commercially produced by the hydrolysis of wheat protein, followed by the hydrolysis of soybean protein. Microbial production of glutamic acid was then attempted directly from glucose, and this was succeeded in producing it by employing Corynebacterium glutamicum strains in 1957. Microbial production of lysine and other amino acids was achieved by employing mutants in which parts of the metabolic pathways were blocked.

In addition, the Japanese have used dried skipjack tuna as a flavoring material. Skipjack tuna is a kind of sea fish, Euthynnus pelamis, and dried skipjack tuna is called "katsuobushi" in Japanese. A compound with the flavor of "katsuobushi" was found to be inosinic acid about 90 years ago. Inosinic acid was first produced by hydrolysis of yeast RNA by a fungal enzyme in 1951. The microbial production of nucleotides was later attempted. One research group succeeded in the production of inosine and guanosine by mutants of Bacillus species, and 5'-nucleotides were produced by chemical phosphorylation. Another group attempted to produce 5'-nucleotides directly from glucose, and succeeded in doing so by using strains of Corynebacterium species and improved media. At present, 5'-nucleotides are produced by these methods and are on the market.

4) Gene manipulation and new biotechnology

The development of techniques of gene manipulation has made it possible to produce novel organisms and useful substances. Besides microorganisms, animal cells are also the target of such studies. The Ministry of International Trade and Industry and other ministries have authorized more than 550 industrial application proposals of gene engineering organisms in the past 11 years. An erythropoteitic hormone, erythropoietin (EPO), is commercially produced by using gene-engineered organisms and employed for hemodialysis.

These microbial industries originated in Japan, and their successes are in great part due to the studies of traditional foods and fermentations. In other words, these studies stimulate basic research, while new findings in basic research bring ideas for new fermentations and new biotechnologies. From such a background, the modern microbial industry has developed in Japan.

THE ROLE OF CULTURE COLLECTIONS

Since the early days of microbiology, astronomical numbers of microorganisms were isolated from a wide variety of natural sources, and used for the scientific research and for the industrial fermentation. However, large numbers of microorganisms had lost in the past, and they are no longer available. Microbiologists often lose microbial cultures that they studied because of the change of their interests and difficulties in keeping the cultures. This is due to the absence of reliable culture collections in which microorganisms are maintained properly and supply them promptly on demand.

Through the study of microbial cultures maintained in the culture collection, potential properties of microorganisms have been developed, and the future perspective of microbiology will be presumed. Effective research needs adequate and reliable sources of properly preserved cultures. In the near future, uncountable numbers of microbial strains will be isolated through the study of microbial diversity, and the attributes of a large number of strains will be improved. Therefore, reliable and well-organized culture collections are needed as the depository and for the promotion of research and application of the strains. In fact, culture collections play a role of the depository of the type strains in bacteriology, and the study of bacterial systematics will not be completed without culture collections consequently.

Major tasks of culture collections are the collection and maintenance of important and useful microbial cultures, supply of the cultures on demand, and preparation of their informative documents. Social needs for culture collections are increasing year by year, and effective and smooth management is required for better services of culture collections. In this point of view, exchange of information and cooperation among culture collections are the most important matter to achieve the purpose of the culture collection.

According to the data of World Data Center for Microorganisms (WDCM) (14), culture collections are distributed in 58 countries in the world, and the number of all the holdings is recorded to be 815,568. However, major public culture collections, which hold a wide variety of microorganisms and distribute them to the public, are estimated to be about 30 collections. In addition, bacterial cultures account for 343,253 (42%), filamentous fungi for 372,304 (46%), viruses for 14,376 (1.8%), cell lines for 5,156 (0.6%), and others for 80,485 (9.9%) by the data of WDCM.

South East Asian countries are rich in microbial diversity, but the exploitation of microorganisms in such areas has not been completed. Large numbers of microbial strains will be isolated from a wide variety of sources in South East Asia, and hundreds of thousands of improved strains in biotechnology will be obtained in the near future. These strains will be deposited with culture collections. This enables culture collections to enrich, and the culture collections will contribute to science and biotechnology in such areas.

Thus improvement of culture collections is critical and crucial for the further development of microbiology, microbial industry, and biotechnology. In addition, good operation and management of culture collections are in great part due to the activity of personnel in the culture collections.

CONCLUSION

Microorganisms are widely used for biological studies, and new advances in biochemistry, genetics, and molecular biology are essentially due to the studies of microorganisms as a model of life. A new era will be opened in biotechnology in parallel with the development of science and technology relevant to microorganisms.

The term of "biological diversity" has become familiar worldwide. Incalculable numbers of microorganisms play important roles in element cycles and the control of pollution on the earth. Microorganisms are not only of value for the production of useful substances; they also play unique roles in element cycles with plants and animals. To a great degree, humans depend on individual microorganisms in biotechnology and diverse ecosystems on the earth. Microorganisms are also significant gene pools, and these gene pools must not be lost. From this point of view, microorganisms can be regarded as a cultural heritage and a cultural property, and they must be transferred to the next generation in a normal and healthy condition.

According to the action statement of "Microbial diversity 21" by International Union of Microbiological Societies (IUMS) and International Union of Biological Societies (IUBS), less than 5% of microorganisms on the earth are recognized, while the remaining 95% are awaiting our exploitation.

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Microbial diversity and the role of culture collections. K Komagata. Exploiting microbial diversity Biodiversity of microbial life: foundations of earthâ€™s biosphere.Â The operations of microbial collections have changed over the last twenty years as a result of the advancement of bioinformatics and the facility to present electronic data over the internet This makes even the smaller collection resources more accessible. View. Show abstract. Biological diversity or biodiversity is actually evolved as part of the evolution of organisms, and the smallest unit of microbial diversity is a species. Bacteria, due to lack of sexuality, fossil records etc., are defined as a group of similar strains distinguished sufficiently from other similar groups of strains by genotypic, phenotypic, and ecological characteristics.Â Culture collections can play a vital role in preserving the genetic diversity of microorganisms. Microbial informationâ€™s including molecular, phenotypic, chemical, taxonomic, metabolic, and ecological information can be deposited on databases. A large number of yet unexplored microorganisms may lead to beneficial informationâ€™s.