

THE *EX SITU* CONSERVATION OF MICROORGANISMS: AIMING AT A CERTIFIED QUALITY MANAGEMENT

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Summary

This chapter is concerned with the issue of conserving microorganisms as biological resources for the benefit of human life and activities. It presents an overview of global efforts in the identification, conservation, data generation and quality management of microorganisms and points out to the need for increased financial support to this challenging task. While there is enormous biodiversity in some world regions for inventory work, the inadequate resources in these not well developed regions poses problems in establishing resources centers with the required financial support. Biological Resource Centres (BRC) are the 21st century culture collections developed to keep pace with user needs and to provide high quality materials to underpin biotechnology. They provide a repository for key organisms and supply samples and associated information for use for the public good. The size of the task is enormous with only a small proportion of the microorganisms in nature described and the majority not yet culturable in the laboratory. It is apparent that no single center can conserve, preserve, characterize, distribute and have a thorough knowledge of all organisms. This requires a coordinated effort, the sharing of tasks and the implementation of common practices and processes so as to deliver reproducible and common products of high quality from the network. A user would expect common approaches and should not be confused by a plethora of different approaches. The Organisation for Economic Co-operation and Development (OECD) coordinated an effort to bring together best practice and offer guidance for culture collections globally to adopt and implement. Quality management extends across all critical elements of a BRCs tasks from the isolation through preservation to distribution of strains. Several organizations representing culture collections such as the World Federation for Culture Collections (WFCC) have developed guidance for collection operations and these have been drawn together to offer a comprehensive and authoritative set of best practices for BRCs. These practices can be implemented formerly through third party independently audited processes and users are finding this a good way to establish confidence in suppliers. If a network is to operate efficiently it should have a base line for expectations from members in delivering their common tasks. Operating quality management systems comes with advantages but not without costs and many BRCs will require capacity building. Mechanisms need to be established to organize this efficiently and in an affordable way so that benefits outweigh costs. Implemented well a certification system for collections and an efficient networking mechanism will help collections deliver the tools for biotechnology in a legal and operational framework that will benefit all.

1. Introduction

The rationale to establish an *ex situ* collection of microorganisms is due to the recognized role microorganisms play in the environment; not only are they global players in the metabolism of nitrogen, phosphate, oxygen and carbon, but many are also of immense scientific and economic benefit. However, some also cause problems to humans, animals and plants. Such organisms must be conserved and made readily available for research and utilization in academia and industry. The recognized importance of microorganisms in industrial countries is the reason for the concentration of public collections in the Northern hemisphere; in contrast, only a few collections are situated in those areas of the world which are rich in biodiversity. Here, their

establishment appears significant in order to develop the bioeconomy and to provide the platform for training of isolation strategies, identification, biosystematics, and collection management. The number of novel species in bacteriology and mycology is too vast to neglect the “diversity hot spots” on this planet with their rich and untapped reservoirs of metabolic and, hence bioeconomic potential. The human resources, facilities, technologies and knowledge necessary to maintain, preserve and exploit microorganisms require development in order to meet the demands to complete the world’s biodiversity inventory and to harness the world’s genetic resources for the benefit of humankind.

For humankind to benefit from biodiversity it must understand and utilize the potential through the ability to identify and maintain biological resources. Though biodiversity offers more than biotechnology, e.g., understanding the evolution of the tree of life and to educate biosystematists, it is biotechnology that is key to meet the needs of the 21st century. The global taxonomic impediment has been recognized and initiatives are underway to help lead to its resolution. The Convention on Biological Diversity (CBD, <http://www.biodiv.org/convention/default.shtml>) has specifically included microorganisms and the importance of *ex situ* collections, as well as benefit sharing. This development stands in contrast to the reduction in numbers of taxonomists and the high average age of most: facts that do not bode well for the future. However, traditional microbiology finds its alliance in molecular biology and modern non-culture tools which are utilized to recognize the vast diversity of microorganisms. For example only a small fraction of microbes can be grown in culture. To culture the huge numbers of microbial species yet to be discovered requires innovative isolation strategies, automated identification and a high quality global network of bioinformation of properties of organisms already in culture. The small number of 600-800 described type strains of novel prokaryotic species is the best argument for a global effort to unravel the World’s microbial diversity, potentially representing in terms of species numbers, about 50% of total biodiversity. In addition to the human resources there are many other and varied needs to develop capacity to deal with the biodiversity needs. Many organizations and programs are carrying out isolation projects with in a non-coordinated effort from which different microbiological disciplines would benefit; training often lacks clear objectives for the long-term and quite often the training is in vain because the recipients move on or change careers. Some Governments are investing in biodiversity where others, although recognizing the value of biodiversity, don’t know where to begin.

The OECD has recognized in their report, *Biological Resource Centres – Underpinning the Future of Life Sciences and Biotechnology* (http://oecdpublications.gfi-nb.com/cgi-bin/oecdbookshop_storefront) the crucial role of Biological Resource Centres (BRCs) as a key element of the scientific and technological infrastructure for the life sciences and biotechnology. This report not only initiated the definition of a BRC but highlights the need for high quality collections as a prerequisite for meeting the requirements of the 21st century. Managed under a yet to be defined common set of quality standards, BRCs will represent a new generation of culture collections and genetic resource banks. BRCs must meet the standards of quality and expertise demanded by the international community of scientists and industry for the delivery of biological information and materials that will enable research and development in biotechnology. The OECD report

also recommends the creation of a Global Biological Resource Centre Network (GBRCN), a consortium of networks presently under discussion. BRCs will have little relevance unless the organisms they hold are recognized by the national and international user as a high quality product in terms of purity, viability, genetic identity and are accompanied by a maximum of relevant bioinformation. This chapter discusses how microorganisms should be preserved and their identities authenticated under defined “best practice” recommendations.

2. Towards a Global Network

Culture Collection organizations such as the World Federation for Culture collections (WFCC) and the European Culture Collection Organisation (ECCO) act as forums for discussion. They bring together a critical mass of collections and users, and attempt to coordinate activities, exchange information and provide technology transfer in order to facilitate progress in this vital task.

2.1. The World Federation for Culture Collections (WFCC)

The WFCC was founded in 1963 and is a multidisciplinary commission of the International Union of Biological Sciences (IUBS) and since the separation of the International Union of Microbiological Societies (IUMS) from IUBS in 1979; it has operated as an inter-union commission (<http://www.wfcc.info>). It seeks to promote activities that support the interests of culture collections and their users. Member collections of the WFCC register with the World Data Center for Microorganisms (WDCM) and there are currently *over 500* member collections (<http://wdcn.nig.ac.jp>) with over 2000 staff. The WFCC has a total membership of around 600 from 62 countries. A congress is held every three years to discuss advances in technology and common policies with regard to biodiversity and the role of culture collections. The WFCC keeps its members informed on matters relevant to collections in its Newsletter and has work programs on patent depositions, postal, quarantine and safety regulations, safeguard of endangered collections, education, publicity, standards and biodiversity. Since 1986, the WFCC has overseen the activities of the WDCM and it is now the data center for the WFCC and the Microbial Resource Centers (MIRCENs) Network. It was established in 1966 and produced the first hard copy volume of the *World Directory of Collections of Cultures of Microorganisms* in 1972, whilst based at the University of Queensland, Australia. The WDCM relocated in 1986 to RIKEN, Saitama, Japan and then again in 1999 to the National Institute of Genetics, Japan. The *World Directory* illustrates some of the data held on the web site (<http://wdcn.nig.ac.jp>); it has indexes by country, main subjects studied, cultures held, the culture availability, their staff, and services offered. The WDCM collections hold in excess of 1 million strains, 44% are fungi, 43% bacteria, 2% viruses, and 11% others (including plasmids, plant, animal cells and algae).

The WFCC is the largest independent global organization that represents professional individuals and culture collections, which preserve biodiversity and enable their proper use. They target living microorganisms, cell lines, viruses and parts and derivatives of them. Key values are authenticity and genetic integrity of the material and validity of the information provided.

The WFCC supports the professionals, organizations and individuals with interests in culture collection activities through:

- Networking, providing information and expertise and facilitating communication
- Facilitating access to the collection resources
- Providing training and promoting partnerships
- Encourage the development and implementations of quality and security procedures and the use of common standards and regulations
- Representing member interests in international organizations and forums
- Promoting the establishment of culture collections, their promotion and perpetuation

In the growing international bio-economy, WFCC's members face increasing global demands for worldwide and controlled access to biological resources, public security, industrial quality of their holdings and associated data and long-term genetic stability of the material. Key to the use of microorganisms from culture collections is the retention of their properties as research and development must be based on authentic and well-preserved biological material. It is therefore imperative that there is a quality assurance and regulatory framework for the operations of collections. To date several initiatives have lead to the development of guidelines to ensure best practice. The World Federation for Culture Collection (WFCC) has been helping collections in this respect for over 3 decades and have produced *Guidelines for the Establishment and Operation of Culture Collections* (<http://www.wdcm.nig.ac.jp>). In the 1990's the UK National Culture Collection (UKNCC) initiative drew together a quality management system (<http://www.ukncc.co.uk>). European collections collaborated similarly to produce the Common Access to Biotechnological Resources Information (CABRI) Guidelines (<http://www.cabri.org>). As a result, strains of organisms are supplied from member collections with traceability, conforming to national and international regulatory requirements, and are preserved in such a way as to retain their full potential.

2.2. Organization for Economic Cooperation and Development BRC Initiative

As part of the OECD theme in biotechnology and the environment, a panel of experts was convened to explore the development of biological resource centers (<http://oecdpublications.gfi-nb.com/cgi-bin/oecdbookshop.storefront>). This program encompasses the establishment of Biological Resource Collections (BRC) and the creation of a Global BRC Network. This will require the transformation of traditional culture collections to BRCs operating to internationally agreed criteria and compliant with relevant national law, regulations and policies is a prerequisite for this global network.

The 21st century dawned with the expectation that it would be the century of biotechnology and would particularly see the harnessing of the hidden potential of microorganisms. As the long-term sustainability of collections becomes an evermore-fearsome battle, the issues of quality assurance, biosecurity, biosafety and other regulatory issues pile yet more demands upon the over-stretched culture collection. The task of maintaining representative samples of microbial diversity cannot be achieved by one collection alone and it is imperative that organisms utilized in biotechnology are

maintained in a way that will ensure that they retain their full integrity. Therefore, it is essential that a world-wide network of collections interacts to provide the coverage required by the user. In order that a customer of such a network would get a consistent level of service and quality it is necessary to set standards for all collections to attain. In addition to the drive to improve quality culture collections must now deal with the vast diversity of new genetic entities generated by life scientists as they seek to reveal the genomes of many organisms and to engineer new cells with novel properties.

This increased demand is occurring whilst statistics of the World Data Centre for Microorganisms (WDCM) show a reduction in the number of registered collections (<http://www.wfcc.info>). With fewer biosystematists, it is imperative that organizations such as BioNET International (<http://www.bionet-intl.org/>) and the CBD's Global Taxonomic Initiative (<http://www.biodiv.org/programs/cross-cutting/taxonomy/>) collaborate with the national, regional and world networks of collections to make best use of the world's expertise and develop programs to sustain and improve our taxonomic capacity. Access to information is crucial; there are many initiatives in this area sponsored by the EU and others. The Global Biodiversity Information Facility (GBIF) is developing tools for data access and a collaborative approach to biodiversity information provision, and must explore ways of ensuring, there is no duplication of effort whilst drawing together the limited resources available to provide a resource for the furtherance of science. The WFCC is setting its priorities and most importantly wishes to help coordinate activities to the benefit of collections and their users. For the survival of collections to meet the needs of the coming century partnerships, sharing tasks and responsibilities, and coordination of effort is paramount.

No one collection, or country for that matter, will be able to meet these challenges alone. The OECD Report on BRCs stresses that to cope with the massive expansion of biological resources, including living biological materials and data on genomics, BRCs need to:

- Contribute to the coordination of efforts to conserve biodiversity and to provide access to natural and engineered biological resources.
- Assist in the development of a coordinated international system for decision making to guide appropriate acquisition, maintenance and distribution of biological resources so as to avoid unnecessary duplication of effort while preserving critical levels of biodiversity.
- Modernize to incorporate the latest developments in web-based electronic communication, bioinformational science and informatics technologies.
- Coordinate and unify catalogues and databases to meet the requirements of science in the developing post-genomics era.
- Develop new systems and technologies for the long-term maintenance and distribution of large numbers of diverse biological resources.
- Coordinate curation, as well as development and networking of informatics tools for data analysis, comparison and visualization.
- Ensure that the scientific community has access to affordable products and services.

If the user benefits from the accreditation of culture collections through better access to

authentic and reproducible materials in a transparent and traceable way, how does the collection benefit? There is an ever-increasing demand for authentic reference materials as more and more industries are adopting certification or accreditation as a means to demonstrate quality and competence. This may be the driving force for the business elements of a collection's strategy for long-term sustainability but it is also an increasing requirement to satisfy the sponsors of research who seek high quality science and solutions. The ability to demonstrate the competence to carry out and manage high quality research is being recognized by Research Councils and Government Departments in the UK and throughout the world. Third party evaluation through accreditation or certification may be the only way to demonstrate this.

2.3. Microbiological Resource Centres (MIRCEN)

In 1974 UNEP, UNESCO and ICRO established the MIRCEN network. The objectives of this network are to preserve and exploit microbial gene pools, make them accessible to developing countries and to carry out research and development in environmental microbiology and biotechnology. The 34 MIRCENs carry out various activities to meet these ends including training and the provision of information. Further details can be obtained from The MIRCEN Secretariat, Division of Scientific Research and Higher Education, United Nations Educational Scientific and Cultural Organisation (UNESCO), 7 Place de Fontenoy, 75700 Paris, France, Tel: 010 331 4568 3883 Fax: + 331 430. Information on the MIRCEN fellowships is available on <http://www.unesco.org/science/life/life1/rcenform.htm>. The production of MIRCEN News in 1980 helped publicize the activities of the network; this has now broadened in content and is published as the World Journal of Microbiology and Biotechnology.

The task of maintaining biodiversity must be shared. There are vast numbers still to be discovered the majority of which are not yet culturable. If it were merely the conservation of these organisms of concern then *in situ* conservation may go some way to achieve this. However, it is clear that a better understanding of the microbial diversity is required, in particular to enable us to harness their properties for the benefit of humankind at a time when other natural resources are depleted. Additionally, disease is killing thousands of people every day and similar numbers are starving. No one collection or country can tackle this alone.

It is essential that:

- *Ex situ* conservation strategies are designed to support *in situ* conservation programs and to meet obligations to conventions, treaties and national law
- Mechanisms to derive benefits from GR exploitation to support biosystematics, collection maintenance, conservation and fundamental research are implemented
- Plans for the conservation of endangered or critical biodiversity elements must be designed
- Collection accession policies are be coordinated
- A coordinated approach to discover and understand microbial communities is needed
- A coordinated policy for funding *ex situ* conservation programs and *ex situ* collections is required

- There is a coordinated effort to identify uses of biodiversity and to harness income streams to fund fundamental research, collection maintenance, biosystematics, and to support a bioeconomy
- A Framework for coordinated research, knowledge development and gap analysis is needed
- A better understanding on the genetic resource needs of industry, education, research leading to a bioeconomy
- A priority list of actions to protect and utilize biological resources is required
- A program is needed to raise the awareness of the importance of GR

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Web site links

Biological Resource Centres – Underpinning the Future of Life Sciences and Biotechnology (<http://oecdpublications.gfi-nb.com/cgi-bin/oecdbookshop.storefront>).

OECD best Practice Guidelines for Biological resource Centres. www.wfcc.nig.ac.jp/Documents/OECD.pdf

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Bibliographical Sketches

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Erko Stackebrandt is a microbiologist by training. He has a chair in Bacterial Systematics at the Technical University of Braunschweig, Germany and he is the Head of the German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig. His research focus is on bacterial systematics, phylogeny and ecology