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://wdcm.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://wdcm.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:

1. *Ex situ* conservation strategies are designed to support *in situ* conservation programs and to meet obligations to conventions, treaties and national law
2. Mechanisms to derive benefits from GR exploitation to support biosystematics, collection maintenance, conservation and fundamental research are implemented
3. Plans for the conservation of endangered or critical biodiversity elements must be designed
4. Collection accession policies are be coordinated
5. A coordinated approach to discover and understand microbial communities is needed
6. A coordinated policy for funding *ex situ* conservation programs and *ex situ* collections is required
7. 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
8. A Framework for coordinated research, knowledge development and gap analysis is needed
9. A better understanding on the genetic resource needs of industry, education, research leading to a bioeconomy
10. A priority list of actions to protect and utilize biological resources is required
11. A program is needed to raise the awareness of the importance of GR

3. Size of the Task

The tasks of microbial culture collections are enormous whilst funding for them and the associated biosystematics and fundamental research is on the decline. There are thought to be less than 1% of the estimated fungi held in culture collections, it is estimated that there are over 1.5 million fungi in the world but less than 100 000 are described by Hawksworth, in 2001. At the current rate of discovery it will take 700 years to describe them all. The number of bacterial cells on this planet has been estimated to reach $6x10^{30}$, probably representing at least 10^9 genospecies. With an annual rate of about 600-800 newly described bacterial species a complete inventory will be achieved one day. However, in both groups of organisms there is also strain diversity to be considered making the potential numbers huge. Genomics and post

genomics and other developing areas in bioinformatics are placing enormous demands on researchers and collections making it imperative that information generation, maintenance of *ex situ* microbial diversity as well as isolated DNA are coordinated and tasks are shared. Microbiologists and microbial collection managers and staff must work together to meet these new and increasing demands for conservation and sustainable utilization of microbial diversity.

Biological resources (living organisms, cells, genes, and the related information) are the essential raw materials for the advancement of biotechnology, human health, and research and development in the life sciences. Governments and industry are making large investments in recovering biological resources from nature and in exploring and engineering these resources. These investments must not be lost and their results must remain accessible so as to reap scientific, economic and medical benefits. The growing world-wide demand for biological resources provides good reasons for greatly increasing the number and quality of BRCs. Only a very few large national centers are currently able to perform a comprehensive role. The development, expansion and survival of these BRCs face many challenges. These include: the molecular revolution (genomics and the information revealed by DNA sequencing), accelerating efforts to conserve biodiversity, funding uncertainties that threaten stability, the need for adequate quality assurance and constraints on access to biological resources within countries and across international borders resulting from private industry's protection of investments and industrial secrecy, import/export regulations, intellectual property rights, safety issues and ethical concerns about the uses of genes and other biological resources.

There is a need to conserve the world's biodiversity through coordinated *in situ* and *ex situ* programs. However, it is difficult to assess the effectiveness of *in situ* conservation of microorganisms when we have so little information on the extent of microbial diversity. Microorganisms are subject to relatively rapid evolution and adapt and change to environmental factors that in some cases challenge their very existence. The application of modern genomic techniques to assess microorganisms has revolutionized bacterial identification but present problems in mycology. It is hampered by limited information and the fact that DNA is not always easily released from the fungal cell impeding population genetic studies; also, information on DNA sequences is extremely helpful in deciphering certain evolutionary trends and flow of genetic information but sequences alone will not indicated its expression *in situ*. Additionally, WDCM data shows that 50% of fungal holdings are only represented in one collection. The situation is not significantly different with the realm of prokaryotes as only the type strains of scientific and commercial interests are widely distributed. It is not always possible to go back to a location and isolate the same species with exactly the same properties. There are so many threats to biodiversity, these include habitat loss or land use change for example where forests are converted to farmland, the over-exploitation of natural resources such as harvesting lichens from rainforest and pollution.

Another problem originates due to the lack of coordination between granting bodies and grant recipients. How many expensive expeditions have been granted to a single team working on a small fraction of diversity when the participation of other teams working on a broader spectrum of diversity could have added so more information? How many scientists collect and store cultures with little records on taxonomy and characterization? The long-term maintenance of a lifetime's collection is in risk of

destruction the moment the scientists retires or changes interest. Collections do not have the funding and manpower to rescue collections when they are not accompanied with some basic electronic information on origin, name of isolator, date, phylogenetic position of description that would allow the affiliation of strains to a genus. It should be the role of granting bodies to assure that part of the grants is used for providing at least this level of bioinformatics.

BRCs must 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 genomes. Genomics leads to the amplification of biodiversity in the form of clones containing fragments of whole genomes. Sequencing the genome of a single human cell generates tens of thousands of new entities (*e.g.* yeast containing fragments of the human genome) that need to be conserved and distributed by BRCs. Similarly, each bacterial cell sequenced means hundreds of such new entities for BRCs.

Genomics studies are generating extraordinary amounts of information and taxing the capabilities of informatics for analyzing and using data. Biologists and biotechnologists will spend the next few decades understanding and exploiting the information provided by these genome-sequencing efforts. These sequence data and their by-products – *e.g.* genome libraries – have to be preserved and made easily accessible. The quest to obtain information on each of the thousands of genes, gene products and other characteristics of each organism highlights the daunting task of storing, maintaining and disseminating this information faced by BRC data banks. Similarly, many products of genetic modification – ranging from genetically engineered bacteria to transgenic plants and animals – must be preserved for scientific investigations and for commercial applications of biotechnology, as well as for regulatory and safety purposes.

4. Quality Management

Although the OECD BRC Task Force aspired to attain high quality proven through accreditation, it wished to base it on existing systems and internationally accepted scientifically based quality criteria. To this end the expertise of organizations such as the WFCC, EU projects such as the EU project (QLRT-2000-00221) European Biological Resource Centres Network (EBRCN), the Common Access to Biological Resources and Information (CABRI) consortium, and several key culture collections were brought into the discussions. The standards being drawn up by the BRC Task Force are thus based on criteria accepted to be best practice. However, what mechanisms might be adopted to ensure user confidence that these practices are being followed.

One system that might be adopted by the national Governments where the BRCs reside might be that of the International Standards Organisation (ISO) see their web site for further information http://www.iso.ch/iso/en/ISOOnline.frontpage. Currently there are a number of standards or guides that could be used that cover at least part of the activities of BRCs. Several collections have already adopted ISO 9000 series certification, a system that ensures quality through critical management of processes. The system requires that procedures and practices are documented and that auditing procedures are put in place to ensure that what is said is done is actually carried out.

The question should be asked, why is this necessary for BRCs and is there a need to go further? The Global System for Biological Resource Centres needs a common standard that can be worked to by all its members to ensure conformity and therefore at the very least the system chosen must be based on a common general standard. There are a number of general standards already available being adopted by single collections and national or international consortia of collections.

Examples of existing standards for microbial and cell culture collections are:

- The WFCC *Guidelines for the establishment and operation of collections of microorganisms (*http://wdcm.nig.ac.jp/wfcc/index.html).
- The Microbial Information Network for Europe (MINE) project standards for the member collections.
- UKNCC quality management system (http://www.ukncc.co.uk).
- Common Access to Biological Resources and Information (CABRI) guidelines (http://www.cabri.org).

There are also standards that can be applied to microbiology laboratories such as Good Laboratory Practice (GLP), ISO 17025, ISO Guide 25 and, as described above, the ISO 9000 series. Industry is expressing the need for quality control and standards within collections. Although publications on collection management and methodology give information on protocols and procedures the UKNCC quality management system goes further toward setting minimum standards. Additionally, the Common Access to Biological Resources and Information (CABRI) electronic catalogue project (http://www.cabri.org) has made available a set of guidelines to aid collections to put in place best practice. These cover critical elements in the handling, storage, characterization and distribution of microorganisms and cell cultures and the handling of associated information. Setting required minimal and preferred standards for these elements would ensure reproducibility.

The OECD BRC Task Force considered different systems that could be applied but have decided that whatever system is selected that the rules and criteria are based upon the implementation of the *OECD best practice*. This is formulated at two levels, the general criteria that can be applied to all BRCs and organism domain specific criteria that are applied to BRCs based on the biological materials they hold. The domains cover microorganisms, including viruses, and animal, plant and human genetic material, cell lines and tissues as outlined in the OECD definition. The concept of the BRC is that it must comply with relevant national, agreements, policies, frameworks and recommendations and the regulations of the countries of supply or receipt when moving biological materials across national boundaries.

In discussions with accreditation bodies, one particular guide was recommended immediately. ISO Guide 34, *General requirements for the competence of reference material producers* seems to be the most suitable to provide guidance for the accreditation of BRCs. This goes a step further from ISO 9000 as it not only addresses the management and processes but extends to the product too. However, this guide was written for defined reference materials used for the calibration of measuring equipment and for the evaluation or validation of measurement procedures such as pharmacopoeia standards and substances. Considering the enormous holdings in some public collections it is out of question that the biological material will be manageable under ISO Guide 34, even if restricted to type strains only. There are some differences

that may confuse or cause problems when referring to living biological material. We must therefore consider if all holdings of a BRC would be regarded as reference material in the same context. It may well be that the equivalent is type material or ex-type material or authenticated strains only. Assignment of property values and their uncertainties may be problematic but again suitable values could be established for living materials. The Guide also states that the reference material producer shall use documented procedures based on accepted statistical principles for the assignment of property values and lays down the procedures on which this should be based. Many of these principles cannot be applied directly and this must be taken into account in documents giving guidance to the accreditation procedure for BRCs.

The benefits of developing into a BRC are logical; these include being able to join all initiatives of international collaboration, participate in strategic planning for scientific research on biodiversity, and to increase the chance of receiving long-term financial support. Coordinated actions that will enable survival in an internationally competitive environment and that follow from the transition include:

- Decision making
- Improvement of research profile
- Incorporation of the latest technologies
- Harmonization of catalogues and databases
- Development of new systems and technologies
- Access to affordable products and services
- Conservation of biodiversity

5. Information Technology

There are enormous possibilities for generating information on microorganisms from descriptive text on morphology, information on isolation and geographic location, host and substrate etc., to digital images, metabolic and genomic data. Culture collections must utilize available technology to ensure adequate storage, analysis and presentation. Historically, culture collections have maintained and published catalogues that usually contain the name of the organism, information on the host or substrate, geographical location, who isolated and deposited it, some level of characterization and literature. More recently this information has been released electronically and presented over the Internet. There are numerous information provision initiatives that enable collections to present their data such as the World Data Centre for Microorganisms (http://wdcm.nig.ac.jp), the Global Information Facility (http://www.gbif.org), EMBL and GENBANK. To ensure the data provided by culture collections is authentic and of high quality and relevant they must institute quality assurance measures for recording, management and exchange.

5.1. Data Quality Management

A key source for information for culture collection data management is the Common Access for Biological Resources and Information (CABRI) web site (http://www.cabri.org). The collections that are involved in this electronic catalogue partnership must provide mechanisms to authenticate information they store and provide via the CABRI web site. There must be a standard for data format and recording. In the CABRI format, bacteria and filamentous fungi and yeasts data are entered according to the Microbial Information Network Europe (MINE) published

format. A description for each field exists, detailing the content, its format, the type of information for example if it is structured, repetitive or numbered, whether it is a specific output from the field or if the field is linked to others. Details of who is responsible for the modification ensuring they have the qualifications and experience to do this is also noted. The details also include the source of the information, authentication and consistency tests carried out and recorded details of "who, how and when" modifications have been made. An example of how data can be authenticated and checked for consistency is using the validated electronic catalogue of names (ECAT) produced by Species 2000 for GBIF. Often databases can be set up to check entries against formatting requirements, a thesaurus containing the correct terms that are to be entered and accepted names, such as ECAT.

5.2. Typical Information Generated on Microorganisms

There are several ways to characterize strains taking advantage of new technologies this results in several different types of information that need to be managed stored, analyzed and delivered. Images can provide added value, for example to agriculturally important microorganisms where photomicrographs, photographs of disease symptoms, electrophoretic gels, chromatographic plates, and profiles from HPLC, MALDI TOF or similar equipment can be stored. Added value to such information can be gained by use of image analysis equipment. Recording and storage of metabolic profiles can be used in several organism types to aid their identification for example with bacteria and yeasts in particular. Genomic information is valuable particularly if sequence information can be compared.

5.3. Information Provision and Exchange

There are several opportunities for collections to distribute their data. Many choose to place interactive databases on the Internet. Greater value can be gained by networking such databases for example through GBIF and WDCM. There is a need for further information over and above that provided by electronic catalogue databases for culture collections and their user communities. Information portals are established to do this exemplified by the World Federation for Culture Collections (WFCC) (http://www.wfcc.info) linking to the WDCM.

The following sections describe the state of the art preservation and long term maintenance technologies, secondly, the molecular quality control of incoming, outgoing and maintained resources and thirdly the management of bioinformatics. These points will address the technologies already in place in some larger public collections though the procedures may differ in detail. Other important items not discussed below encompass the traditional aspects of characterization and identification (culture based tests, pathogenicity testing, the use of automated identification systems, chemotaxonomy) and the application of state-of-the-art taxonomy.

6. Preservation

6.1. Background

The primary objective of preserving and storing an organism is to maintain it in a viable state without morphological, physiological, or genetic change until it is

required for future use. Ideally, complete viability and stability should be achieved, especially for important research and industrial isolates. Preservation techniques range from continuous growth methods through to methods that reduce rates of metabolism to the ideal situation where metabolism is suspended. Continuous growth techniques involve frequent transfer from depleted to fresh nutrient sources, which initially provide optimum growth conditions. The need for frequent sub-culture can be delayed by storing cultures in a refrigerator, freezer (at -10°C to -20°C), in glycerol, under a layer of paraffin oil or in water (organisms-dependant). These are not considered here as they are mainly used for short-term maintenance as longer storage allows deterioration and loss of properties. Drying of the whole cell, propagule or resting stage (e.g., spores, cysts or sclerotia) of an organism can be achieved by air drying, in or above silica gel, in soil or sand. This not always successful and often only low numbers of cells recover.

The methods selected by BRCs must suspend metabolism, which normally involves reducing the water content available to cells by dehydration or cryopreservation. Freeze-drying (lyophilization) is the sublimation of ice from frozen material at reduced pressure and requires storage in an inert atmosphere either under vacuum or at atmospheric pressure in an inert gas. Cryopreservation generally implies storage at temperatures that impede chemical reactions of around -70°C and below. This can be achieved in mechanical deep freezers (some are capable of reaching temperatures of -150°C) or in/above liquid nitrogen. To achieve an adequate suspension of metabolism to a point where no physical or chemical reaction can occur requires storage at temperatures of below -139°C.

Desiccation has been used successfully for the preservation of many microorganisms, mostly fungi. The removal of water suspends metabolism of the cell. Bacterial and fungal spores have lower water content than vegetative hyphae and are able to withstand desiccation, reviving when water becomes available. The techniques considered here involve the use of silica gel, freeze-drying and a technique often known as L-drying. After a suitable preservation technique is selected and the strains successfully stored a distribution and seed stock should be kept. The size of the stock depends upon the anticipated distribution. Enough replicates must be maintained to ensure that preserved strains have undergone a minimum number of transfers from the original. Wherever possible, an original should be preserved without subculturing. The seed stock should be stored separately from the distribution stock. It is also advisable to keep a duplicate collection in another secure building or site as a reserve. An inventory control system should be used to ensure that cultures remain in stock for distribution or use. After preservation, the viability, purity, and identity should be rechecked and compared with the original results before the culture is made available outside the collection. The viability, purity and stability of strains must be assessed before and after preservation and during storage.

Avoidance of selection of variants from within the population, strain deterioration and contamination are important when growing strains for use and essential when maintaining cultures for long periods. There are a number of preservation techniques suitable for fungi, however many criteria should be assessed before preserving an isolate. No preservation technique has been successfully applied to all microorganisms, although storage in liquid nitrogen appears to approach the ideal. However, changes in physiology and genetic stability may occur in some isolates of fungi, so optimal preservation protocols may have to be established. Most fungi that

grow well in culture survive cryopreservation in liquid nitrogen including non-sporulating fungi although isolates that grow poorly tend to do less well. Organisms that have yet to be cultured in the laboratory or those that require growth on their host can also be successfully preserved, for example pathogenic organisms can be preserved in infected tissue. Of the fungi only sporulating cultures survive well in silica gel storage, and spores with thin walls and high water content or those with appendages do less well. Centrifugal freeze-drying allows only the more robust spores to survive. Some sclerotia and other resting stages, and even in a few cases sterile mycelia, have been known to survive freeze-drying. In general, yeast cells are considered to be robust, tolerant of unfavorable conditions, nutritionally undemanding, and readily managed in industry. It is incorrectly assumed that they are easy to maintain as a number of preservation and maintenance methods result in poor viability and instability of properties. Factors affecting survival are becoming better understood at the sub-cellular level, but many strains from a wide range of species remain difficult to preserve. The relative poor performance of yeasts post preservation may be partly attributed to the large size of cells compared with bacteria and the absence of the resistant spore types produced by many of the higher fungi. In the light of present knowledge, high survival rates can best be achieved by careful attention to the techniques used for preservation. For example, growth conditions, suspension media, choice of cryoprotectant and cooling rates.

Percentage survival of the total population, following subculture and drying is generally low, although ‘cultures’ may appear viable. Viability following freeze-drying is also frequently poor, but may be improved for some strains by careful selection of the suspension medium. In contrast, survival following storage in liquid nitrogen is high, often reaching levels of 100%. There is no apparent relationship between survival and taxonomic position, and the factors determining survival are strain specific. Therefore, a preservation method that is satisfactory for one strain of a species may be unsuitable for others. If strain stability is of paramount importance the choice of maintenance method becomes critical. Any method that enables cell division to occur during storage should be rejected. It has been shown that the morphological, physiological and industrial characters of filamentous fungi and yeasts generally remain unchanged following freeze-drying, although little work has been carried out at the molecular level and there are occurrences of instability being recorded as these techniques are employed to assess recovery. Liquid nitrogen remains the method of choice for stability of most fungi.

Again freeze-drying is often the method of choice for bacteria providing a method that gives excellent recovery and ampoules that are easy to distribute requiring simple storage conditions. Another method sometimes used to preserve organisms whilst allowing easy distribution is the gelatin disc technique. Occasionally, cultures are distributed as active cultures resuscitated from cryopreservation onto a suitable nutrient media. Cryopreservation is generally only used for isolates that are difficult to preserve. Freezing, especially to ultra-low temperatures in or above liquid nitrogen is generally considered to be the least damaging preservation technique for bacteria. The lower the storage temperature the better the long-term survival and stability. Storage at -20°C is not generally recommended and storage in -70°C or -80°C freezers can be used if liquid nitrogen is not available. Many collections preserve plasmids, bacteriophages and genetically modified organisms in liquid nitrogen. Preserving them in this way reduces the chance of potential contamination which can result from aerosols produced during lyophilization or when opening ampoules.

From a logistic point of view, any dried preserved material is advantageous over material stored in liquid nitrogen. Material of the latter method must be reactivated in medium of optimal growth before shipment, while dried material can be sent directly from the shelf with no additional intermediate handling. As a consequence, costs of shipment of an active culture are significantly higher than those of e.g., a glass ampoule. If handling fees and additional costs are not the limiting factor, then the customer will prefer receiving an actively growing culture.

6.2. Silica Gel Storage

The silica gel method has been applied to fungi and has proved to be very successful. At CABI., Sporulating fungi have been stored for 7-20 years in silica gel and appear to remain morphologically stable after resuscitation. The technique is relatively simple and involves the inoculation of a suspension of fungal propagules onto cold silica gel. The culture will then dehydrate to enable storage without growth or metabolism.

Silica gel storage has a number of advantages as it is cheap, simple and does not require expensive apparatus. Cultures are relatively stable, allowing a wide range of sporulating fungi (including representatives of the *Basidiomycota*) to be successfully preserved. Penetration by mites is unlikely, as they cannot survive the dry conditions encountered. Repeated inocula can be removed from a single bottle. However, it is recommended that a stock bottle is established in case of contamination during retrieval. There are some disadvantages of silica gel storage. It is limited to sporulating fungi and is unsuitable for *Pythium*, *Phytophthora* and other *Oomycota*, mycelial fungi or fungi with delicate or complex spores. This limitation does not apply to yeast cultures. There is a possibility of introducing contaminants by repeated retrievals.

6.3. Freeze-drying (Lyophilization)

Freeze-drying (lyophilization) is a highly successful method for preserving bacteria, yeasts and the spores of filamentous fungi. During the freeze-drying process, water is removed directly from frozen material by sublimation under vacuum. If carried out correctly, freeze-drying will prevent shrinkage, structural change and help retain viability. There is a vast array of freeze-drying equipment available, ranging from laboratory bench models through to pilot scale and huge industrial installations. Freeze-drying should be optimized for different organisms and cell types. If this is done it should be successful for the majority of bacteria, sporulating fungi, and yeasts. It is generally unsatisfactory for eukaryotic microalgae as levels of post preservation viability are unacceptably low. More protocol development is required to achieve successful lyophilization for algae and protozoa, although cyanobacteria are more likely to survive.

Lyo-injury can occur during the cooling and/or drying stages. The phase changes encountered during the drying process can cause the liquid crystalline structure of the cell membranes to degenerate to the gel phase, which disrupts the fluid-mosaic structure of the membrane. This causes leakage of the membrane, which may culminate in cell damage. Optimal survival can be improved with the use of a suitable suspension medium. It should be readily available, easy to prepare and provide protection during the freeze-drying process (i.e. to protect the spores/cells from ice damage during cooling and storage problems such as oxidation). Skimmed milk is a

suitable protectant for fungi and is sometimes used in combination with inositol. Saccharides such as trehalose protect membranes by attaching to the phospholipids, replacing water and lowering the transition temperature. Other suspending media can be used when preserving bacteria and yeasts with many collections using their preferred preservation base.

The recommended final moisture content following drying is between 1 and 2% (w/v). To monitor freeze-drying a means of measuring vacuum both in the chamber and close to the vacuum pump is required. Comparing the measurements will allow the determination of the end point of the drying process. When the values are equal, water has ceased to evaporate from the material being dried and drying is probably complete. This is confirmed by determining the residual water content. This can be done by dry weight determination or by the use of chemical methods such as the Karl Fischer technique. The freezing point of the material should be determined and the temperature monitored during freeze-drying. The sample temperature must not rise above the melting point until most of the water has been removed. To ensure that a high quality product is produced and maintained the equipment used must be reliable and conditions reproducible from batch to batch.

The technique of centrifugal freeze-drying, which relies on evaporative cooling, can be used successfully for the storage of many sporulating fungi, as well as bacteria and yeasts. However, this is not a method that can be adapted and changed easily, as it is dependent upon the scope of the equipment. Optimization of cooling rate to suit the organism being freeze-dried can be applied using a shelf freeze-drier. The sealing of the ampoules or vials is most important and heat sealed glass is preferred to butyl rubber bungs in glass vials as these may leak over long-term storage and allow deterioration of the freeze-dried organism. There are many advantages of freeze-drying over other methods, including the total sealing of the specimen and protection from infection and infestation. Cultures generally have good viability/stability and can be stored for many years. Ampoules take up little space and can be easily stored. In addition, cultures do not have to be revived before postal distribution. However, there are disadvantages, some isolates fail to survive the process and others have reduced viability and genetic change may occur though unless high viability is retained it is difficult to differentiate between this and selection of spontaneous mutants by freeze-drying. Ampoules of freeze-dried organisms must be stored out of direct sunlight and chilled storage will reduce the rate of deterioration and should extend shelf-life. However, the process of lyophilization is relatively complex, can be time-consuming and may be expensive.

Many collections use a a two-stage centrifugal freeze-drying process. At CABI this has been used since 1966 and a shelf freeze-drying protocol was introduced in 1982 that allows optimization of protocols for different strains. Freeze drying of sporulating fungi such as the Ascomycota and mitosporic fungi is routinely undertaken, but is not so suitable for the Oomycota and other non-sporulating cultures. Although it is only spores and conidia that are routinely freeze dried, research has been carried out to establish whether lyophilized hyphae can be revitalized successfully after preservation. Investigations by Tan and coworkers in 1991 gave mixed results. Some cultures did not survive at all and others showed only limited viability. Success with freeze-drying varies between isolates of the same species. In general those fungi that grow and sporulate well in culture survive the process, while weak or deteriorated isolates tend to fail. It may therefore be misleading to state categorically that one

particular species will not survive freeze-drying. In general the young vegetative hyphae of fungi do not survive freeze-drying. At CABI it has been found that sterile ascomata, chlamydospores, sclerotia and in some few cases stroma and resting mycelium have survived. However, in general it is only the spores (e.g., conidia, ascospores, and basidiospores) that survive.

Freeze-drying is a generally accepted method for yeast storage, although viability is generally low, typically between 1 and 30%, as compared to >30% for those of yeast preserved frozen in liquid nitrogen. Some strains have been stored for 30 years at the National Collection of Yeast Cultures (NCYC) in the UK and for longer periods by other laboratories. There are several yeast genera, including *Lipomyces*, *Leucosporidium*, *Brettanomyces*, *Dekkera*, *Bullera*, *Sporobolomyces* and *Rhodosporidium* that have particularly low survival levels and frequently cannot be successfully freeze-dried by the standard method. However, some improvements have been made recently using trehalose as a protectant. Survival of yeasts following freeze-drying is remarkably strain specific and generalizations regarding survival levels should be viewed with caution. Nevertheless, all cultures maintained at NCYC, and covering nearly all yeast genera, have been recovered successfully, although the percentage survival of the population is generally low.

Freeze-drying is the universal method employed for the preservation of bacteria although there are a number of modifications to the basic freeze-drying procedure that can be used. The UKNCC bacteria collections use modified techniques. The majority of bacteria survive freeze-drying well, but a few species can sometimes give disappointing results. This may be due in some cases to difficulties in obtaining adequate pre-drying growth. Cultures which often prove more difficult than others include *Aquaspirillum serpens*, *Clostridium botulinum*, *C. chauvoei*, *C. novyi*, *C. putrificum*, *C. scatologenes*, *Helibacter pylori* and *Peptococcus heliotrinreducans*. Additionally, some lesser problems may be encountered with *Bacteroides melaninogenicus*, *Haemophilus canis*, *H. suis*, *Leptotrichia buccalis*, *Mycobacterium microti* and *Neisseria gonorrhoeae*.

6.4. L-drying

Liquid drying (L-drying) is a useful alternative method of vacuum drying for the preservation of bacteria that are particularly sensitive to the initial freezing stage of the normal lyophilization process. The intrinsic feature of this process is that cultures are prevented from freezing; drying occurs direct from the liquid phase. At the National Collection of Industrial and Marine Bacteria (NCIMB) bacteria such as *Spirilla* and *Azomonas insignis* have been preserved by L-drying. These organisms are particularly sensitive to freeze-drying, but L-dried cultures have survived with good recovery levels for up to fifteen years. L-drying can, therefore, be considered as a suitable alternative to freeze-drying for bacteria that are susceptible to damage by freeze-drying.

6.5. Maintenance of Bacteria in Gelatin Discs

Bacteria are suspended in melted nutrient gelatin, drops of which are allowed to solidify in Petri dishes. The drops are freeze-dried, or dried over a desiccant, and the resultant flat discs are stored over silica gel. When required, a single disc is placed in warmed broth and the resulting suspension plated onto a suitable growth medium. The method is not particularly suitable for storage of numerous strains over long-periods.

However, it is invaluable for storage of a limited number of frequently used strains, such as those used for quality control of media or reagents. The method therefore has advantages over both active subculture on slopes and freeze-drying in ampoules. A number of organizations provide standard strains of microorganisms in this form at a cheaper rate than freeze-dried cultures. In addition, the Czechoslovak Culture Collection (CCM) makes available 20 different strains for control purposes or for use in diagnostic laboratories. It also prepares discs as a service to customers. Advantages of storage using gelatin discs include their ease of use and storage (30 or 40 discs can be kept in a 14mm screw-capped vial). As the discs are kept dry there is no opportunity for growth of any contaminants introduced during sampling so they remain free from contamination. Characters remain relatively stable, because the bacteria are not growing there is no opportunity for mutation and selection. Various species of Enterobacteriaceae and Staphylococci, and strains of *Pseudomonas aeruginosa* and *Corynebacterium diphtheriae* have been successfully preserved for at least 4 years although this method has not been successful with more delicate.

6.6. Cryopreservation

The ability of living organisms to survive freezing and thawing was first realized in 1663 when Henry Power successfully froze and revived nematodes. Polge and his coworkers became in 1949 the first "modern day" scientists to report the freezing of living organisms when they successfully froze and thawed avian spermatozoa. Liquid nitrogen is the preferred cooling agent for cryopreservation, although liquid air or carbon dioxide can be used. Lowering the temperature of biological material reduces the rate of metabolism until, when all internal water is frozen, no further biochemical reactions occur and metabolism is suspended. Although little metabolic activity takes place below -70°C, recrystallization of ice can occur at temperatures above -139°C and this can cause structural damage during storage. Consequently, the storage of microorganisms at the ultra-low temperature of (-190°C to 196°C) in or above liquid nitrogen is the preferred preservation method of many scientists. Provided adequate care is taken during freezing and thawing, the culture will not undergo change, either phenotypically or genotypically.

Choice of cryoprotectant is a matter of experience and varies according to the organism. Cryoprotection is achieved by:

1. Non-critical volume loss by the reduction of ice formation.
2. An increase in viscosity, which slows down ice crystal growth and formation and solute effects.
3. Reduction of the rate of diffusion of water caused by the increase of solutes.

Glycerol 10%(v/v) gives very satisfactory results but requires time to penetrate the organism; some fungi are damaged by this delay. Dimethyl sulfoxide (DMSO) penetrates rapidly and is often more satisfactory. Sugars and large molecular substances, such as polyvinyl pyrrolidine (PVP) have been used but in general have been less successful. Trehalose may be better remains quite expensive. Establishing the optimum cooling rate has been the subject of much research. Slow cooling at 1° C min^{-1} over the critical phase has proved most successful, but some less sensitive isolates respond well to rapid cooling, preferably without protectant. Slow warming may cause damage owing to the recrystallization of ice, therefore rapid thawing is

recommended. Slow freezing and rapid thawing generally give high recoveries for fungi.

As with other methods of preservation liquid nitrogen cryopreservation has advantages and disadvantages. Advantages include the length of storage, which is considered to be effectively limitless if storage temperature is kept below -139°C. The majority of organisms survive well, giving the method a greater range of successful application. Organisms remain free of contamination when stored in sealed ampoules. Disadvantages of liquid nitrogen storage include the high cost of apparatus such as refrigerators and a continual supply of liquid nitrogen. A regular supply cannot be obtained in some parts of the world and therefore the technique cannot be used. If the supply of nitrogen fails (or the double-jacketed, vacuum-sealed storage vessels corrode and rupture) then the whole collection can be lost. There are also safety considerations to be made, the storage vessels must be kept in a well-ventilated room, as the constant evaporation of the nitrogen gas could displace the air and suffocate workers.

Cryopreservation has been used for the preservation of fungi since the 1960's, although early work involved a very simple procedure. Storage at –196° C in liquid nitrogen or at slightly higher temperatures in the vapor phase is now commonly employed by most collections. Generally a cooling rate of -1° C min^{-1} with 10% (v/v) glycerol as a cryoprotectant is applied and to date, over 4 000 species belonging to over 700 genera have been successfully frozen. No morphological or physiological change has been observed in the 9 000 fungal isolates stored. However, some members of the Oomycota and Basidiomycota survive cryopreservation less well than sporulating fungi and it is anticipated that by employing species-specific cooling rates may provide improved viability.

7. Quality Control based on Molecular Characterization

Culture collections must harness available techniques to characterize, authenticate and screen microorganisms. It is important to assess their properties to enable their use in agriculture and for the properties to be harnessed to provide microbial solutions. Techniques have been derived from a wide range of different biological disciplines including biochemistry, bacteriology, mycology and ecology to provide anatomical, physiological and biochemical data. The information provided by such methods not only deliver diagnostic characters, but such information may be extended to provide general applications such as purification, identification and screening of properties of industrial importance. Many of the methods are simple and can be applied with minimal facilities others require substantial investment.

There are many methods available for the analysis and characterization of nucleic acids. The techniques described have diverse applications including gene cloning, screening of gene expression, species definition, study of interactions, phylogeny, study of degradation processes, detection of metabolite producers, studies of biodiversity and identification of species in natural samples. General molecular procedures such as restriction endonuclease digestion, cloning, hybridization and sequencing are applicable to microbial DNA. Fingerprinting techniques have allowed scientists to separate taxa that are difficult to distinguish using anatomical or morphological criteria. Common techniques that utilize the Polymerase Chain

Reaction (PCR) include RAPDs (Random Amplified Polymorphic DNA), AFLP (Arbitrary Fragment Length Polymorphism), ARDRA (Amplified Ribosomal DNA Restriction Analysis), or SSCP (Single Strand Conformation Polymorphism). Non-PCR techniques such as RFLPs (Restriction Fragment Length Polymorphism) or electrophoretic karyotyping of chromosome size and number may also be applied. More recent technological developments are based on the knowledge about the presence of variable number tandem repeats of the microsatellite DNA. This method, like the multi-locus-sequence-typing is more often used in studies on epidemiological spread and population structure of pathogens but less in routine collection work on purity and authenticity check.

Many techniques require purified DNA, which must be extracted from cells with minimal mechanical disruption to the DNA. Most common methods use a cell lysis solution followed by purification and storage in an appropriate buffer. Simple-to-use kits are now widely available to extract human, animal, bacterial and fungal DNA. However, nucleic acids are sometimes more difficult to obtain from some fungi, primarily because of the nature of their cell walls. Methods using cetyltrimethyl ammonium bromide (CTAB) are often used for extracting total genomic DNA from plants and filamentous fungi and have proved successful for many fungal genera especially those that produce excess extracellular polysaccharides.

Sequencing is a powerful technique that allows scientists to read the nucleic acid make-up of individual genes. At least some sequence information is essential for gene cloning and genetic manipulations. Many molecular laboratories within culture collection have facilities for sequencing, but there are many commercial companies who operate external sequencing services for individuals who have not got their own facilities. Sequencing has been used in systematics to define groupings at the most basic level through the sequences of specific gene regions such as the subunits of the ribosomal gene clusters, allowing proper phylogenetic analyses of taxa to be undertaken.

It is the advantage of molecular methods to be fast, reproducible, and affordable. Similarly important is the option to establish cumulative sequence databases without which the revolution in microbial systematics would have not been happened. Once sequence data have been obtained, they can be analyzed; either rapidly by BLAST analysis, giving an approximation of similarity of the sequence and its closest relatives, or more precise aligning them to a set of sequences downloaded from public databases (e.g. EMBL or NCIB databases). The most widely used molecule is the ribosomal RNA gene more than 250.000 partial and complete sequences are available for comparison. It’s chimerical structure of highly variable and highly conserved regions allow recognition of closely related species and new members of higher taxa, respectively. While in bacteriology almost complete 16S rRNA gene sequences are necessary for the description of novel species, partial 16S or 18S rRNA gene sequences are sufficient to verify the authenticity of species. This strategy is used in the DSMZ for checking whether the sequence of a new accession into the collection is identical with the sequence of the same type strain which had been deposited in a database by the depositor. Authenticity is approved when the similarity ranges between 99.8 and 100% and the deposition routine will be continued. In the case of slightly lower similarity values the complete sequences will be manually aligned according to idiosyncrasies of the secondary structure and analyzed in order to correct mistakes in the deposited sequence. With more than 1% sequence divergence between

the publicly deposited and the newly generated sequences the depositor will be informed and a new strain requested. Following long term deposition one ampoule of the strain will be returned to the depositor to allow verification of strain identity. The slight delay in the deposition procedure safeguards the identity for the good of science. Until now the gold standard molecule for this kind of tests in bacteriology is the ribosomal RNA because two sets of conservative PCR primers amplify the genes of members of both kingdoms, Bacteria and Archaea. For more closely related taxa the intra-cistronic ITS) regions have been found discriminatory.

Though ideal for rapid assessment of phylogenetic identity, the sequence of ribosomal RNA genes is too conservative to discriminate between strains of a species. As long as the species is defined only by a single strain, the type strain, there will be sufficient nucleotide differences compared to the homologous sequence of its closest relative. In those cases, however, were specific genomic and metabolic differences are described for strains of a species more discriminative methods must be applied to unambiguously verify the authenticity. Such examples are large strain sets of species with relevance to hospitals, food or bioremediation (e.g. *Staphylococcus aureus, Escherichia coli, Lactobacillus delbrueckii, Pseudomonas aeruginosa, Burkholderia cepacia*). Historically, chemotaxonomic markers were considered to be too conservative while phenotypic properties are not reliable. A solution to the problem was found in the generation of DNA typing patterns (listed above). These patterns, however, show certain drawbacks when used for routine purposes. Firstly, individual sets of PCR primers and/or restriction enzymes are necessary to cover members of genera and the reproducibility and intra-laboratory quality of the electrophoretic banding patterns are not always of high standard. Consequently, cumulative database are missing that would allow identification of novel organisms. An exception is the automated RiboPrint technology (Qualicon, Dupont, Wilmington), a robot that generates restriction pattern of prokaryotic rRNA operons under highly controlled conditions. In principle cell mass in the order of a bacterial colony is lysed, and the robot performs the following four steps: restriction of DNA with an enzyme of choice, one-dimensional separation of resulting fragments on a small membrane, hybridization of the bands with labeled rRNA probes and detection of labeled bands, representing the rDNA fragments via a CCD camera. The hybridization pattern is electronically transferred to the RiboPrint database and the similarity determined with other patterns constantly accumulating in the database. It is the availability of restriction sites outside the rRNA genes, i.e., the spacer and flanking regions of the operon that results in the higher discrimination of strains of a species than that seen by sequence analysis of rRNA genes. This method, which also could be executed manually, is used in the DSMZ quality control in the framework of the ISO 9001:2000 Management System for checking the authenticity of batches of lyophilized cultures and strains maintained under N_2. Only when strains have passed this rigorous regime are they released for sale.

Though ease of use, availability of a cumulative database and high reproducibility are positive factors of the automated RiboPrint method, high costs for consumables and maintenance are prohibitive factors for a wide application of the system. With the introduction of mass spectroscopic methods into the era of biosafety and biosecurity, e.g., the rapid search for select agents, a novel method was introduced into systematics in the early 2000. Up to then mainly used in proteomics, matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI TOF) combines the advantages of a low cost technology with high resolution of masses of ionized

proteins in the range of 2.000 – 20.000 Da. This method is already widely used for the classification and identification of microorganisms, with applications in clinical diagnostics, taxonomical research, or food-processing quality control. In addition method robustness has been demonstrated for a broad range of growth media and conditions, and stages of the life cycle. Comparison between dendrograms of protein mass similarity with 16S rRNA gene sequence similarities revealed a high correlation among clusters of highly related strains. The reproducibility and the underlying phylogenetic relevance is based on the measurement of constantly expressed high-abundant proteins, such as ribosomal proteins. The DSMZ uses this technology within their research program for the rapid assessment of taxonomic novelty, affiliation of strain to known species for dereplication purposes and, like the ATCC, in their quality control to assess identity of charges.

8. Capacity Building

"Capacity building" means different things to different people. Capacity building is the development of core skills and capabilities, such as leadership, management, finance and fundraising, programs and evaluation in order to build effectiveness and sustainability. It is the process of assisting an individual or group to identify and address issues and gain the insights, knowledge and experience needed to solve problems and implement change. Capacity building is facilitated through the provision of technical support activities, including coaching, training, specific technical assistance and resource networking.

Capacity building needs to develop culture collections

To facilitate the development of culture collections with respect to their role in conservation and utilization of biodiversity, quality and output there is a requirement for capacity building in several key areas:

- Taxonomy, classifying, naming and identifying elements of biodiversity; a need to focus on neglected groups, e.g. microbial, especially mycological, and entomological)
- Information storage, analysis and distribution
- Coordinated acquisition programs including targeted isolation programs
- Improved technologies e.g. molecular techniques to elaborate classification systems including sequencing, microarray technologies, gene chips etc.
- Characterization and screening for useful properties
- Policies and strategies to comply with international agreements and conventions

There are several initiatives being undertaken to establish, enhance and develop culture collections and create biological resource centers around the world. Some initiatives are directed by Governments and have received significant financial investment. The main drive is to exploit genetic resources to provide solutions to current needs in health, food provision, environment and poverty alleviation: a search for new discoveries to drive a bioeconomy. BRCs provide a basis for coordination and collective effort for benefit on a regional scale, which is essential for realizing and sustainably managing the benefits of microbial diversity. The challenges and capacity needs are far too great to be tackled on a country-by-country basis. There are a number of functions of BRCs that would support a multinational coordinated

approach. They are centers for storage of microbial assets for future use, they characterize these strains both in terms of properties and identity and store, analyse, utilize and disseminate associated information enabling a better understanding of our microbial diversity. The provision of authentic well-characterized strains for research and development is crucial for high quality products. BRCs serve as control points for access to a nation's living natural resources under the requirements of the CBD, support the understanding of a countries indigenous crop pathogens for sanitary and phytosanitary needs in relation to world trade and as centers to control access to dangerous pathogens that could be misused, are therefore important to biosecurity. They also contribute to basic inventories to monitor changes in biodiversity due to threats to ecosystems and the establishment of alien invasive species.

In the context of Biological Resource Centres capacity building is required in the areas of establishment, quality management and networking, sharing tasks and coordinating effort. Furthermore, there are specific needs in taxonomy where the capacity to recognize and characterize organisms is diminished at a time when the need to access and understand biodiversity has increased. The critical reduction in natural resources requires a better understanding and ability to utilize our living natural resources and natural products and biotechnologies can improve national economies and our ability to feed populations and improve health. The characterization and better utilization of our living resources will help develop the bioeconomy. It is essential the capacity to enable this be provided.

9. Conclusion

The search for new active molecules through bioprospecting and its regulation require access and control. BRC's, their capacity and resources, have a major role to play both in providing identification capacity and supporting developing countries to regulate bioprospecting.

There are many other reasons why nations should invest in understanding and managing their biodiversity, and these are reflected in the various international agreements concerning genetic resources, including:

- The Convention on Biological Diversity
- Bonn Guidelines on Access to genetic resources and fair and equitable sharing of the benefits arising out of their utilization
- Budapest treaty on the International Recognition of the deposit of microorganisms for the purposes of patent procedure
- Cartagena Protocol to the Convention on Biological Diversity
- Convention on International Trade in Endangered Species (CITES)
- European Commission council Regulation 3381/94/EEC on the Control of Exports of Dual-Use Goods from the Community
- European Council Directive on Legal Protection of biotechnological inventions
- European Council Directive 90/219/EEC on the Contained use of Genetically Modified Microorganisms
- European Council Directive 90/220/EEC on the Deliberate Release of Genetically Modified Microorganisms

- FAO International Treaty on Plant Genetic Resources for Food and Agriculture
- Global Plan of Action for the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture
- International Plant Protection Convention (IPPC)
- OECD Initiative on biotechnology for sustainable growth and development
- Trade Related Aspects of Intellectual Property Rights Agreement and other Agreements within World Trade Organisation (WTO)
- Trade-related Intellectual Properties (TRIPs) agreement under WTO)
- Sanitary and phytosanitary (SPS) agreement of the WTO.

It is in the national interest to develop a strategic approach to Genetic Resource (GR) conservation and its legitimate and sustainable use. GR are central to biotechnology, sustainable development and for policy on sustainable agriculture. They are an important tool for meeting a wide range of policy objectives, including protecting the rural environment, promoting sustainable rural economies, promoting sustainable and adaptable farming, promoting sustainable management of natural resources and ensuring high standards of animal health and welfare. Biotechnology will challenge many aspects of our life as profoundly as information technologies. The OECD recognize that capturing the economic, environmental, health and social benefits of biotechnology will challenge government policy, public information, law, education and the scientific and technological infrastructure. A framework is needed in order to better coordinate the many activities already carried out by Governments, researchers, NGOs and others.

BRCs are able to address the tricky issue of ownership of biological materials and the intellectual property associated with them in a coordinated way and put in place acceptable procedures to protect IP for individuals, companies, organizations and nations. It is impossible to envisage sharing of efforts unless such rules and procedures are operating through material transfer agreements.

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 we need a better understanding of the microbial diversity in particular to enable us to harness their properties to the benefit of humankind at a time when other natural resources are depleted. Additionally, disease is killing thousands of people per day and similar numbers are starving. No one collection or country can tackle this alone.

It is essential that:

1. *Ex situ* conservation strategies are designed to support *in situ* conservation programs and to meet obligations to conventions, treaties and national law
2. Mechanisms to derive benefits from GR exploitation to support biosystematics, collection maintenance, conservation and fundamental research are implemented
3. Plans for the conservation of endangered or critical biodiversity elements must be designed
4. Collection accession policies must be coordinated

5. A coordinated approach to discover and understand microbial communities are needed
6. A coordinated policy for funding *ex situ* conservation programs and *ex situ* collections is needed
7. Coordinated effort to identify uses of biodiversity and harness income streams to fund fundamental research, collection maintenance, biosystematics and to support a bioeconomy
8. Frameworks for coordinated research, knowledge development and gap analysis need strengthening
9. A better understanding on the genetic resource needs of industry, education, research leading to a bioeconomy
10. A priority list of actions to protect and utilize biological resources is required
11. A program is needed to raise the awareness of the importance of GR

It is evident from the OECD Biological Resource Centre Initiative, activities of the World Federation for Culture Collections, and efforts by CABI International for several of its 44 member countries that capacity building is needed. The limited resources and the need to prioritize activities to focus on providing microbial solutions to our social and economic problems, capacity-building programs must be coordinated to be effective.

Related Chapters

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Glossary

Accreditation	**:** Process that certifies competence, authority, or credibility, ensuring that certified practices are done with competence, behave ethically, and employ suitable quality assurance. One example of accreditation is the accreditation of testing laboratories and certification specialists that are permitted to issue official certificates of compliance with established standards, such as physical, chemical, forensic, quality, and security standards. Organizations that issue accreditation certificates or certify third parties against official standards are themselves formally accredited by national/international standards bodies.
Benefit sharing	**:** Article 15 "Access to Genetic Resources" of the Convention on Biological Diversity (http://www.cbd.int/convention/) indicates the importance sharing benefits:"Each Contracting Party shall take legislative, administrative or policy measures, as appropriate, and in accordance with Articles 16 and 19 and, where necessary, through the financial mechanism established by Articles 20 and 21 with the aim of sharing in a fair and equitable way the results of research and development and the benefits arising from the commercial and other utilization of genetic resources with the Contracting Party providing such resources. Such sharing shall be upon mutually agreed terms".
Biological	**:** Biological Resource Centres (BRCs) are the world's "living

Resource Centres	libraries", offering independent and shared access to authenticated biological materials for research applications in the life sciences in government, industry, and academia. They maintain large and varied collections-including cell lines, microorganisms, recombinant DNA material, and biological media and reagents-and information technology tools that allow researchers to access biological materials.
Common Access to Biological Resources and Information (CABRI)	**:** An EU funded network of European public collections (http://www.cabri.org/) dedicated to the following goals: To provide biological products of quality to the scientific community, by searching the electronic catalogues of the participating collections by a common gateway; to provide quality guidelines (http://www.cabri.org/guidelines.html) to ensure our customers the specific technical procedures used; to expand the resources with other centers having acceptable quality standards. The basis for the OECD best practice guidelines for microbial BRCs
Capacity building	**:** Capacity building is a measure to improve access to genetic resources and benefit-sharing. A database on capacity-building projects has been developed to facilitate information-exchange on ongoing capacity-building activities (http://www.cbd.int/abs/projects.shtml).
Certification	**:** In the context of biological resource centers certification defines the application of the same standard that can be applied to any organization whatever it's holding or service, in any sector of activity, and whether it is a business enterprise, a university facility or a government institute. In contrast to accreditation certification concentrates on improvement and harmonization of management systems in order satisfy the customer's quality requirements, comply with regulations, and meet environmental objectives.
Convention on Biological Diversity (CBD)	**:** The CBD was signed in 1992 in Rio de Janeiro, Brazil and entered into force September 1993. The objectives of the Convention are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding. Microorganisms are specifically mentioned in Article 2. "Use of Terms" and in Article 9 „Ex-situ Conservation"
Cryo-preservation	**:** Cryopreservation is a process where vitrified (addition of cryoprotectants) cells or whole tissues are preserved by cooling to low sub-zero temperatures, typically −196 °C (the boiling point of liquid nitrogen). At these low temperatures, any biological activity, including the biochemical reactions that would lead to cell death, is effectively stopped.
European Biological Resource Centre Network	**:** A follow up of the EU funded CABRI project aiming mainly at the implementation of the principles outlined in the OECD booklet *Biological Resource Centres – Underpinning the Future of Life Sciences and Biotechnology (Online* - http://oecdpublications.gfi-

(EBRCN)	nb.com/cgi-bin/oecdbookshop.storefront). Accessed December 01, 2007) and on the development of a European standard for BRCs, based on present management systems
European Culture Collections Organisation (ECCO)	**:** A consortium of European collections with the mission to support the interests of European collections of microorganisms (including microorganisms, viruses, plant, animal and human cells, as well as replicable parts of these such as genomes, plasmids, cDNAs); to support culture collection users (private and public); and to to support the improvement of scientific and technical standards related to culture collection activities, in close collaboration with the *World Federation for Culture Collections* (WFCC)
***Ex situ* conservation**	**:** Ex-situ conservation of biological resources is the practice of protecting these resources outside their native habitat, typically through their collection and storage in collections, Biological Resource Centres.
Freeze drying	**:** Freeze drying (lyophilization) is a dehydration process used for long-term preservation of microorganisms which makes the material more convenient for storage and transport. Freeze drying works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to gas. The addition of lyoprotectants, typically polyhydroxy compounds such as sugars (mono-, di-, and polysaccharides), polyalcohols, and their derivatives supports the viability of organisms.
L-drying	**:** Liquid drying is a useful alternative method of vacuum-drying for the preservation of bacteria that are particularly sensitive to the initial freezing stage of the normal lyophilization process. The intrinsic feature of this process is that cultures are prevented from freezing; drying occurs direct from the liquid phase.
MIRCEN	**:** A global network of Microbial Resources Centres, existing in the developed and developing countries that participate in a global collaborative networking effort for the harnessing of the beneficial applications of the microbial world for human progress through the vehicle of international scientific co-operation.
Quality control	**:** Quality control is involved in developing systems to ensure products or services are designed and produced to meet or exceed customer requirements.
Quality management	**:** Quality management is a method for ensuring that all the activities necessary to design, develop and implement a product or service are effective and efficient with respect to the system and its performance. Quality management consists of quality control, quality assurance and quality improvement. Quality management is focused not only on product quality, but also the means to achieve it.
World Data Center for Microorganisms (WDCM)	**:** The WDCM, a unit within the WFCC, is an electronic gateway to databases on microbes and cell lines and resources on biodiversity, molecular biology and genomes. The records contain data on the organization, management, services and scientific interests of the

	collections. Each of these records is linked to a second record containing the list of species held. The WDCM database forms an important information resource for all microbiological activity and also acts as a focus for data activities among WFCC members.
World Federation for Culture Collections (WFCC)	**:** The WFCC (http://www.wfcc.info) is a Multidisciplinary Commission of the International Union of Biological Sciences (IUBS) and a Federation within the International Union of Microbiological Societies (IUMS). The WFCC is concerned with the collection, authentication, maintenance and distribution of cultures of microorganisms and cultured cells. Its aim is to promote and support the establishment of culture collections and related services, to provide liaison and set up an information network between the collections and their users, to organize workshops and conferences, publications and newsletters and work to ensure the long term perpetuation of important collections.

Abbreviations

AFLP	**:** Arbitrary Fragment Length Polymorphism
ARDRA	**:** Amplified Ribosomal DNA Restriction Analysis
ATCC	**:** American Type Culture Collection
BRC	**:** Biological Resource Centers
CABRI	**:** Common Access to Biological Resources Information
CBD	**:** Convention on Biological Diversity
CTAB	**:** Cetyltrimethyl ammonium bromide
DMSO	**:** Dimethyl sulfoxide
EBRCN	**:** European Biological Resource Center Network
ECAT	**:** Electronic catalogue [for names]
ECCO	**:** European Culture Collection Organisation
GBIF	**:** Global Biodiversity Information Facility
GBRCN	**:** Global Biological Resource Center Network
GLP	**:** Good Laboratory Practice
GR	**:** Genetic Resource
ICRO	**:** International Cell Research Organisation
ISO	**:** International Standards Organisation
IUBS	**:** International Union of Biological Sciences
IUMS	**:** International Union of Microbiological Societies
MINE	**:** Microbial Information Network Europe
MIRCEN	**:** Microbiological Resource Centers
NGO	**:** Non-governmental Organisation
OECD	**:** Organisation for Economic Cooperation and Development
PCR	**:** Polymerase Chain Reaction
PVP	**:** Polyvinyl pyrrolidine
RAPDs	**:** Random Amplified Polymorphic DNAs

SSCP	: Single Strand Conformation Polymorphism
TRIP	: Trade-related Intellectual Properties
UKNCC	: UK National Culture Collection
UNEP	: United Nations Environment Program
WDCM	: World Data Center for Microorganisms
WFCC	: World Federation of Culture Collections
WTO	: World Trade Organisation

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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

Common Access to Biological Resources and Information (CABRI) guidelines (http://www.cabri.org).

International Standards Organisation (ISO) http://www.iso.ch/iso/en/ISOOnline.frontpage.

UKNCC quality management system (http://www.ukncc.co.uk).

World Federation for Culture Collections (WFCC) *Guidelines for the establishment and operation of collections of microorganisms (*http://wdcm.nig.ac.jp/wfcc/index.html

Biographical Sketches

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