11 Public Service Collections and Biological Resource Centers of Microorganisms

David Smith[1] · *Dagmar Fritze*[2] · *Erko Stackebrandt*[3]
[1]CABI, Egham, Surrey, UK
[2]German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany
[3]Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

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Abbreviations *ACM,* The Asian Consortium for the Conservation and Sustainable Use of Microbial Resources (http://www.nbrc.nite.go.jp/e/project01-e.html), a network of Asian culture collections and BRCs (see *Box 11.5*).; *ATCC™,* American Type Culture Collection; Manassas; VA, USA.; *BCC,* Biotech culture collection, Bangkok, Thailand.; *BCCM™/LMG,* Belgian Co-Ordinated Collections of Micro-Organisms, Laboratorium voor Microbiologie, University Gent, Belgium.; *BCCUSP,* Brazilian Cyanobacteria Collection, University Sao Paulo, Brazil.; *BRC,* In the context of this chapter defined as a microbial Biological Resource Center (sensu OECD), a CC running under a defined quality management system which yet needs to be agreed upon by the stakeholders.; *CABI,* CAB International, Egham, UK; *CABRI,* Common Access to Biological Resources and Information (www.cabri.org), a EU-funded network of eight European Collections (1996–1999), (http://www.cabri.org).; *CBD,* Convention on Biological Diversity (http://www.cbd.int/), a global agreement addressing all aspects of biological diversity: genetic resources, species, and ecosystems. Their protection, sustainable use and access to including benefit sharing of the advantages arising from their use.; *CBS,* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.; *CC,* In the context of this chapter defined as a microbial Culture Collection, a general term of a facility accessioning and maintaining microbial resources (prokaryotes, fungi, yeast), DNA, plasmids, phages, and material derived therefrom. Public Culture Collections provide this material to users. For a comprehensive list of abbreviations see WDCM and http://www.bacterio.cict.fr/collections.html.; *CCAP,* Culture Collection of Algae and Protozoa, Scottish Marine Institute, Oban, Argyll, UK.; *CCMM,* Moroccan Coordinated Collections of Micro-organisms, Morocco.; *CCMP,* Culture Collection of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine USA.; *CCTCC,* Chinese Center for Type Cultures Collections, Wuhan University, Wuhan, Hubei, China.; *CCUG,* Culture Collection of the University of Göteborg, Institute of Clinical Bacteriology, Immunology, and Virology, Göteborg, Sweden.; *CECT,* Colección Española de Cultivos Tipo, Valencia, Spain.; *CGMCC,* China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, PR China.; *CIP,* Collection of the Institut Pasteur, Paris, France; *CPCC,* Canadian Phycological Culture Center (formerly known as UTCC), University of Waterloo, Waterloo, ON, Canada.; *DSMZ,* Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.; *EBRCN,* European Biological Resource Centers Network (http://www.ebrcn.net), a EU-funded network of 15 European culture collections of microorganisms and cell cultures (2001–2004).; *ECCO,* European Culture Collection's Organisation (http://www.eccosite.org), a network of European Culture Collections and BRCs (see *Box 11.4*).; *EMbaRC,* European Consortium of Microbial Resource Centers (http://www.embarc.eu/) networking the BCCM™/LMG, Belgium; *CECT,* Spain; *CIP,* France; *DSMZ,* Germany and two French research collections INRA-CIRM-BP in Tours and CIRM-BIA, Rennes), aiming to improve, coordinate and validate microbial resource center (MRC) delivery to European and International researchers from both public and private sectors (*Box 11.4*).; *ENBI,* European Network of Biodiversity Information (www.enbi.org), an EU funded project established to include all European national nodes of the Global Biodiversity Information Facility (GBIF). (2003–2006).; *ESFRI,* European Strategy Forum for Research Infrastructures a strategic instrument to develop the scientific integration of Europe and to strengthen its international outreach (http://ec.europa.eu/research/infrastructures/index_en.cfm?pg=esfri); *FEMS,* Federation of European Microbiological Societies (http://www.fems-microbiology.org).; *GBIF,* Global Biodiversity Information Facility (http://www.gbif.org/), an international government-initiated and funded initiative focused on making biodiversity data free and openly available online.; *GBRCN,* Global Biological Resource Center Network (http://www.gbrcn.org), a project following work in the OECD to improve access to high quality biological resources and information to support research and biotechnology as a platform for a knowledge-based bio-economy.; *INRA CIRM-BIA,* Center International de Ressources Microbiennes - Bacteries d'Interet Alimentaire, Institut National de la Recherche Agronomique, Rennes, France.; *INRA CIRM-BP,* Center International de Ressources Microbiennes – Bacteries Pathogenes, Institut National de la Recherche Agronomique, Nouzilly, France.; *KACC,* Korean Agricultural Culture Collection, National Institute of Agricultural Science and Technology, Suwon, Republic of Korea.; *KTCT,* Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, Taejon, Republic of Korea.; *LMG,* The Belgian Consortium of Collections of Microorganisms (BCCM™), represented by the Universiteit Gent, Belgium.; *MINE,* Microbial Information Network Europe, an EU-funded network of European culture collections, running between 1986–1989 and 1990–1993.; *MIRRI,* Microbial Resource Research Infrastructure (http://www.mirri.org/), a pan- European distributed research infrastructure established on the European Strategy Forum for Research Infrastructures (ESFRI) road map with the goal to improve access to the microbial resources and services that are needed to accelerate research and discovery processes.; *MOSAICC,* Micro-Organisms Sustainable use and Access regulation International Code of Conduct, an EU-funded project (1997–1999), a tool to support the implementation of CBD at the microbial level, in accordance with other relevant rules of international and national laws.; *MUM,* Microtheca do Universidade do Minho, Braga, Portugal.; *NBRC,* Biological Resource Center, National Institute of Technology and Evaluation, Chiba Pref., Japan.; *NCAIM,* National

Collection of Agricultural and Industrial Microorganisms, Department of Microbiology and Biotechnology, University of Horticulture and Food Industry, Budapest, Hungary.; *NCCB,* Netherlands Culture Collection of Bacteria, Utrecht, The Netherlands.; *NCIMB,* National Collection of Industrial and Marine Bacteria, National Collections of Industrial, Food and Marine Bacteria, Aberdeen, UK.; *NCTC,* National Collection of Type Cultures, Central Public Health Laboratory, London, UK.; *NRRL,* Northern Regional Research Center, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, US Department of Agriculture, Peoria, Illinois, USA.; *OECD,* Organisation for Economic Cooperation and Development (http://www.oecd.org/).; *SAG,* Culture Collection of Algae Sammlung von Algenkulturen, University Göttingen, Göttingen, Germany.; *UCL,* The Belgian Consortium of Collections of Microorganisms (BCCM™), Université Catholique de Louvain, Belgium.; *WDCM,* World Data Center of Microorganisms, an activity of the WFCC, providing an electronic gateway to databases on microbes and cell lines and resources on biodiversity, molecular biology and genomes (see *Box 11.3*).; *WFCC,* World Federation for Culture Collections (http://www.wfcc.info/), a Federation within the International Union of Microbiological Societies (IUMS, http://www.iums.org) (see *Box 11.2*).

Introduction

Culture collections have been preserving organisms and supplying them for research and development for over a century. The term "culture collection" actually does not reflect a common standard, however, since tasks, holdings, size, funding system, affiliation, mandate, and other parameters differ widely. Though the basic principles of operation are the same, namely, accessioning, maintenance, and provision of microorganisms, collections may significantly differ from each other, and hardly any two collections operate under the same system. To name a few extremes, printed or electronic catalogues may be missing completely, while other collections display their holdings electronically in a most professional way, while collections may maintain a very regional selection of microbial strains of a narrow taxonomic or physiological range; others try to accession the complete range of validly named type strains, and yet others access their strains from geographically diverse regions; some collections are affiliated and funded as part of an academic institute; others receive strong governmental support, while others are in charge of nonpublic strains used by the biotech industry. Though the range of collection types is vast and many are not even visible to the public, information is available for those 592 collections (status May 2011, including those with non-microorganism holdings) which are registered at the MIRCEN-World Data Center for Microorganisms (WDCM) (http://www.wfcc.info/index.php/wdcmdb/) which is overseen by the World Federation for Culture Collections (WFCC). Statistics on these collections and summaries of kind and number of holdings, number of staff, funding system, and services offered are compiled in the WDCM.

Before the globalization of information by the internet, printed catalogues were the only means by which the users could have an insight into collection holdings. Thus, collections usually served the national market, and only a few highly visible collections operated on an international level. The common goals of collections and their same basic operations for collection functioning triggered the need for better cooperation which started after the mid-1980s. National networks were created, some of which, such as the Belgian Network and more recently the Chinese and Brazilian networks, proved successful, while others, such the British and the US networks of culture collections, passed through some stormy times. Regional networks have operated since the early 1980s, first in Europe but now also in East Asia. The need for better harmonization among those collections which provide users in academia and bio-industry with biological material was recognized by Organization for Economic Cooperation and Development *(OECD)*. From 1998, this organization, together with collection managers and representatives of governments and industry from member and associated states, developed a strategic plan to improve the quality of management and operation of collections for their own benefit and for the benefit of the user, specifically to play a key role in underpinning the knowledge-based bio-economy. The growing market in Biotech R&D in the USA, China, India, Brazil, and Europe required the improvement of the operation of providers of biological material as their authenticity, purity, and the availability of associated database resources were considered indispensible for the success of downstream processes.

As a result, the role of those collections that agreed to follow the route of higher quality has changed dramatically, culminating in the introduction of the term Biological Resource Center (BRC) to reflect the delivery of services and products compliant with a standard agreed by national authorities. BRCs focus on the following quality criteria:

- *Achieving the primary objective* to maintain strains in a viable state without morphological, physiological, or genetic change
- *Implementing best practice in the provision of services by ensuring:*
  - Authentication of biological materials
  - Validity of data
  - Continued availability and reproducibility of materials
  - Safe and legitimate shipping
  - Legitimate acquisition of biological material
  - Compliance with biosafety and biosecurity guidance
  - Protection of intellectual property rights, particularly for patents
- *Applying long-term methods of preservation essential to ensure availability of biological materials for the long-term*
  - Selection of most suitable method
  - Optimization
  - Viability, purity, and stability

Meeting the requirements of BRC status requires investment and change. While it will be feasible for some of the well-funded public service collections to implement international accepted

guidelines, others will need more support, both strategically as well as financially. The mandate to develop a framework for the evolution of collections to BRC status is being undertaken by the Global Biological Resource Center Network, which is supported by regional activities (e.g., MIRRI in Europe). Additional expertise in various areas of collection-related issues, such as the CBD, intellectual property issues, material transfer agreement, biosecurity, and the like, is being harnessed to deliver common policy and strategy for implementation. An overview of the legal and regulatory environment in which microbiologists and in particular microbial service collections dwell and their reactions is given in Fritze (2010).

This chapter will highlight some of the recent developments, focusing on the core activities of any type of collection of microorganisms, and will describe in detail the way forward to achieve the goal of their global networking.

Prokaryotic Holdings in Public Service Collections

On 30 May 2011, a total of 1,751,439 microbial resources were listed in the 592 culture collections in 68 countries registered in the WDCM: about 761,000 of them were bacteria and 506,500 of them were fungi. Other holdings embrace bacteriophages, plant and animal viruses, microscopic algae, protozoa, dedifferentiated plant cells, and immortalized human and animal cell lines. Collections of microorganisms maintain two categories of strains with relevance to taxonomy, type strains, and non-type strains. For prokaryotes, the deposition of type strains, the nomenclatural type to which the binominal species designation is linked, is mandatory (Tindall et al. 2006). This means that no name will be valid without the written confirmation of at least two public collections in two different countries that the respective strain has been deposited without restrictions and checked for authenticity by the original depositor (for descriptions before 2006, the type may be available in a single collection only). This procedure was internationally accepted in order to make the type available as reference for scientific studies. Non-type strains can be either authenticated strains of a described species or be any taxonomically less well-identified strain found worthwhile maintaining by scientists and collection managers for their specific properties. A 2011 survey on holdings of prokaryotes in several of the major and some of the smaller public collections (see legend to *Fig. 11.1*) clearly indicated the higher number of deposits of non-type strains over type strains. Over the past 11 years, about 136,000 strains were accessioned by these collections, about 80 % of which were non-type strains. In the same period, 5,412 type strains were validly named and described, meaning that type strains were, on average, distributed to five public collections. Duplication in collections at a reasonable level is considered good practice as backup. The decision to maintain copies of strains depends upon a wide range of scientific and user-related interests: it guarantees the long-term maintenance of the prime reference strain in bacteriology, while the rapid provision of these strains to users at the regional/national level facilitates

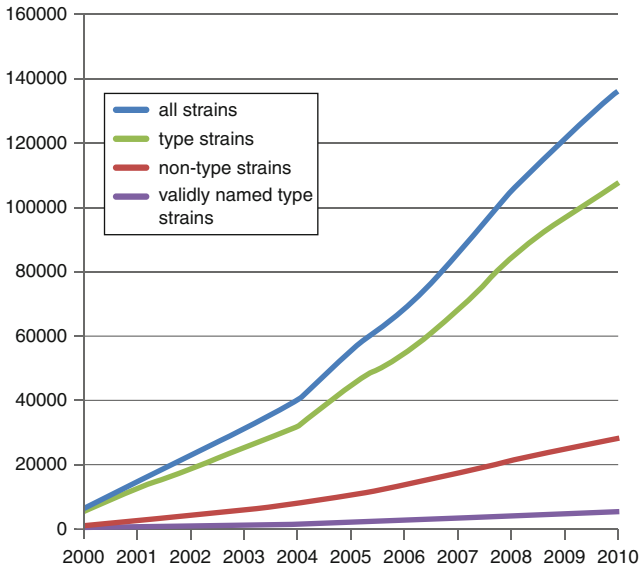

Fig. 11.1
Cumulative display of accessions of type and non-type strains by selected public collections over a period of 11 years. Collections included are ATCC™, USA; BCC, Thailand; BCCM™/LMG, Belgium; CCM, Czech Republic; CCMM, Morocco; CCUG, Sweden; CCTCC and CGMCC, China; CECT, Spain; CIP, France; DSMZ, Germany; KACC and KCTC, Korea; NBRC, Japan (starting 2002); NCAIM, Hungary; NCCB, the Netherlands; and NCIMB, the UK

access and overcomes costly shipping and lengthy documentation procedures. The graph in *Fig. 11.1*, showing the cumulative accessions, clearly displays the significant uptake of non-type strains from 2005 on which may be a reflection of increased interest in biodiversity studies and the emergence of the bioeconomy. The same trend is visible in the duplication of numbers of new type strains around 2005, a direct reflection of the isolation of novel pheno- and genotypes. It must be noted that not all public collections follow the same accessioning strategy: while some (e.g., BCC, CCUG, CGMCC, CCMM, CCTCC, or NCCB; see Abbreviations) concentrate on the deposition of non-type strains (≥75 %), others (e.g., ATCC™, BCCM™/LMG, CCM, CECT, DSMZ, CIP, KACC, NCAIM, or NBRC) accession about as many type strains as non-type strains (35–60 %), while among the collections surveyed, only NCIMB and KCTC concentrate on the deposition of type strains (70–75 %).

While deposition of type strains is free of charge, the increasing number of newly described species (*Fig. 11.1*) challenges collections by increasing the necessary manpower and maintenance costs. These costs include not only the administrative responsibilities according to the CBD and other rules and regulations (Smith et al. 2008) but also identification, authentication, maintenance, and long-term storage, as well as the generation of accompanying bio-information and regular technological updates and training. Some revenues are generated by providing strains to users, but this income does not cover the costs involved in state-of-the-art maintenance of resources. This indicates that even with the descriptions reaching a plateau in the next years (due to the lack of systematists, not of novel

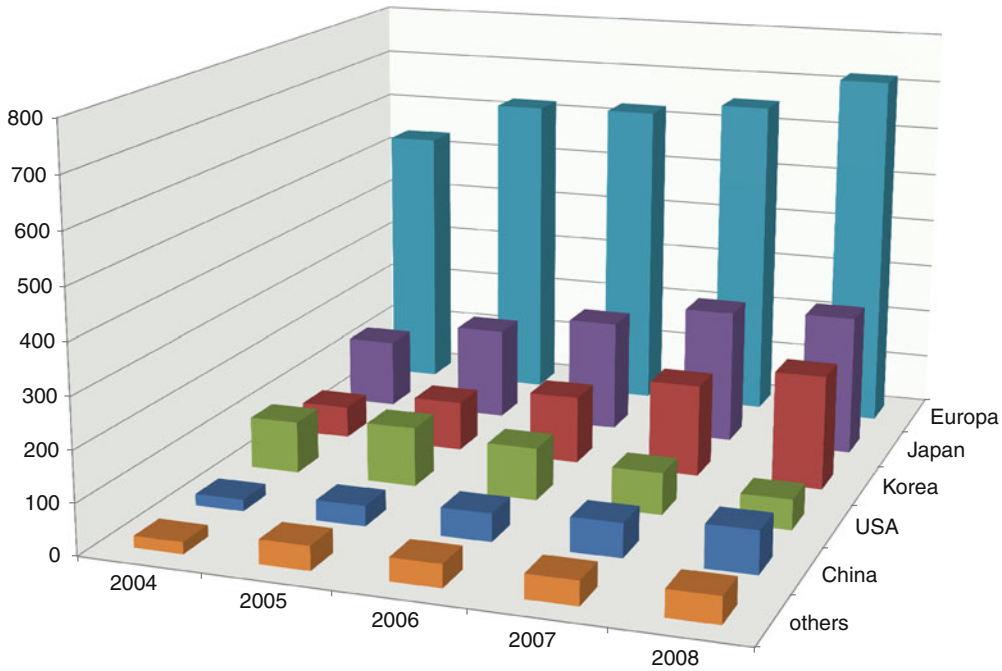

Fig. 11.2
Deposition of new strains in culture collections per country/region between 2004 and 2008 (Courtesy of Ken Suzuki, NBRC, Japan)

organisms), the workload of maintaining and providing novel biodiversity under high quality standards remains a huge task for accessioning collections.

As shown in *Fig. 11.2*, European collections were used most frequently as repositories for new strains over the last years (data available from 2004 to 2008). The decline of the USA collections as primary source for deposition is worth noting as is the increased deposition in Korea, Japan, and China, countries that today contribute most to the description of novel species. It is to the advantage of new public collections, mainly in East Asia and Brazil, that their planning fell in the times of increasing awareness of microbial diversity and the genomic revolution, resulting in the proper provision of infrastructure for their future tasks.

Gaps and Strengths

Public collections do not only differ in numbers and types of material deposited, they also differ significantly in the range of taxa maintained. A worldwide comparative analysis of holdings of prokaryotes in WFCC/WDCM member collections, as displayed individually in their respective strain catalogues, is not available; a 2009 survey of some West European collections, members of the EMbaRC project (see Abbreviations), most likely mirrors the situation at a more regional level, such as those existing in North America and East Asia. The range of gaps at the generic level is rather small as the majority of phyla are covered at least by some strains. At the genus level, most phyla are covered above 80 % (*Table 11.1*); only the monogeneric phyla Fibrobacteres and Lentisphaera are not covered in any of these collections, and some of the "rare" (rare in the sense of under-sampled or low diversity) phyla are represented by a few type strains only. The policy of mandatory deposition, however, guarantees that the type strain is available from at least one public collection (e.g., the respective type strains of species of *Fibrobacter* and *Lentisphaera* are held in the ATCC™ and ATCC™ and KCTC, respectively). Obvious gaps detected are within the Tenericutes (formerly Mollicutes) and within Cyanobacteria. Mollicutes embrace primarily parasites of various animals and plants, living within the host's cells. Their maintenance often requires host tissues which are out of range for most resource centers. Collections of Cyanobacteria exist in several countries (e.g., ATCC™ and CCMP, USA; PCC, France; UTCC, HAMBI, Finland; Canada; CCAP, UK; SAG, Germany; BCCUSP, Brazil) and often in conjunction with collections of eukaryotic algae.

At the species level gaps are obvious in the so-called rare species, here, the more specialized collections show their strength, especially in holdings of the extremophiles. These species are usually less frequently requested than species of medical and biotechnological interest, and their maintenance is more demanding than those of the majority of aerobic and heterotrophic species in the phyla Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes. Other gaps are also seen among some of the genera of obligate endosymbionts and pathogens, as well as among the obligate chemolithotrophs.

Only 12 % of all genera described are not covered by any of the six EMbaRC collections surveyed. These are either those recently described, embracing obligate endosymbionts or fastidious pathogens. More telling than genus numbers, however, is the availability of type strains and range of diversity covered at the strain level. In this respect, too, collections differ widely from each other (for EMbaRC collections, see *Table 11.2*). Certainly, the history of

Table 11.1
In percentage coverage of bacterial diversity at the genus level by six EMbaRC collections (Survey from 2009)

Taxon	Number of described genera	Number of genera covered	Percent coverage
Archaea	91	86	**95**
Bacteria			
Aquificae	12	12	**100**
Thermotogae	6	5	**83**
Thermodesulfobacteria	4	4	**100**
Deinococcus/Thermus	6	6	**100**
Chrysiogenetes	1	1	**100**
Chlorobi	3	3	**100**
Chloroflexi	13	10	**77**
Thermomicrobia	1	1	**100**
Nitrospirae	3	2	**67**
Deferribacteres	6	5	**83**
Synergistetes	5	3	**60**
Planctomycetes	9	7	**78**
Fusobacteria	9	7	**78**
Chlamydiae	5	1	**20**
Spirochaetes	14	7	**50**
Fibrobacteres	2	0	0
Acidobacteria	6	5	**83**
Verrucomicrobia	13	8	**62**
Dictyoglomi	1	1	**100**
Gemmatimonadetes	1	1	**100**
Lentisphaerae	1	0	0
Bacteroidetes	181	148	**82**
Firmicutes	312	289	**93**
Actinobacteria	238	228	**96**
Proteobacteria			
Alphaproteobacteria	235	188	**80**
Betaproteobacteria	143	131	**92**
Gammaproteobacteria	269	232	**86**
Deltaproteobacteria	84	76	**90**
Epsilonproteobacteria	15	12	**80**

a collection significantly determines the size and phylogenetic affiliations of individual holdings, and the expertise of curators is determined to a great extent by the history and tradition of collections (to name a few, the actinobacterial collection in the DSMZ and NBRC, Japan; the *Bacillus* and mycelium-forming actinomycetes holdings in the NRRL, USA; the *Lactobacillus* holdings in the CIP, or the *Vibrio*, *Pseudomonas*, or *Enterococcus* collections in the BCCM™/LMG). The history, including the research emphasis of the collection founders, also explains the strength in methods used in-house for authentication and characterization and the range of skills offered to the public, for example, providing identification service and training courses.

Based upon the range of genera and species covered, collections fall into one of several types. One type, represented by, for example, DSMZ, ATCC™, or NBRC, covers in-depth genera and type strains. Members of a second type do not, or do not only, concentrate on phylogenetic diversity, habitat, metabolism, or ecology but are also specifically strong in holdings of intra-generic and often intraspecies diversity, representing either a pathogenic potential (to humans, animals or plants), or organisms relevant to biotechnology (food, agriculture, pharmacy). The CIP, ATCC™, NCTC, or CCUG are well known for their holdings of pathogens, while the BCCM™/LMG collection, NRRL, and especially collections in subtropical and tropical regions maintain in-depth diversity of nitrogen-fixing bacteria and plant pathogens. The collections maintain a higher number of strains per taxon than those of the first category. The research collections usually cover a very narrow spectrum of genus diversity but in-depth coverage of intraspecies diversity. This is clearly shown by the example of the two INRA collections in which holdings are strains involved in either milk or cheese processing (CIRM-BIA) or in pathogenicity (CIRM-BP).

Non-type Strains as an Important Source of Biodiversity

The increasing deposition of non-type strains is due to several factors. Firstly, the strains can originate from a collection's own research, enlarging the holdings of those taxa which are in the prime focus of individual curators. Though often the number of isolates originating from environmental studies is too high to maintain the complete set of the strains isolated, the short intra-collection distances and the in-house availability of maintenance procedures favor rapid and competent deposition.

A second source of strains originates from research laboratories. These facilities may work independently or in a network of collaboration, including public collections. With the advent of molecular ecology and the growing awareness of the huge, yet unexplored biodiversity of microorganisms from the early 1990s on, increased funding has strongly supported research in genomics, metagenomic, and functional diversity. Microbiological research also benefitted from increased funding for sampling due to the awareness that linking sequences to function requires research on the organism itself and due to the inquisitiveness of scientists to isolate the organism for which the molecular data signaled phylogenetic uniqueness. The heydays of the recovery of extremophiles and the discovery and description of novel phyla and classes fall into the last two decades. While many of the more recently described novel taxa rarely embrace more than the type strain of the type species only, it can be assumed that more strains of these taxa are hidden in research collections. Normally, the result of the lack of proper identification and demanding circumscription protocols, not to mention the lack of funding for taxonomic research, which is not matched at all to the support for expensive sampling expeditions and isolation regimes. It is mostly up to the individual scientist to identify non-type strains that are worthwhile depositing, and this is done

Table 11.2

Comparison of genera and type strains described per phylum and holdings in EMbaRC collections, representing a regional network (Survey from 2009)

Phyla	Genera/species described[a]	DSMZ Germany	CIP France	BCCM™/LMG Belgium	CECT Spain	INRA CIRM-BP France	INRA CIRM-BIA France
Archaea	91/*379*						
Crenarchaeota	26/*56*	24/*52*	–	–	–	–	–
Euryarchaeota	65/*322*	58/*276*	–	–	12/*20*	–	–
Bacteria	1,595/*9,056*						
Aquificae	12/*27*	12/*25*	1/*1*	–	–	–	–
Thermotogae	6/*31*	5/*28*	2/*3*	–	–	–	–
Thermodesulfobacteria	4/*8*	4/*8*	1/*1*	1/*1*	–	–	–
Deinococcus/Thermus	6/*61*	6/*52*	2/*15*	3/*26*	1/*2*	–	–
Chrysiogenetes	1/*1*	1/1	–	–	–	–	–
Chlorobi	3/*15*	3/9	3/8	–	–	–	–
Chloroflexi	13/*20*	9/12	–	–	–	–	–
Thermomicrobia	1/*2*	1/1	–	–	–	–	–
Nitrospirae	3/10	2/6	1/1	–	–	–	–
Deferribacteres	6/9	4/6	2/2	–	–	–	–
Synergistetes	5/5	2/2	1/1	–	–	–	–
Planctomycetes	9/14	6/9	–	–	–	–	–
Fusobacteria	9/45	5/33	4/15	1/1	–	–	–
Chlamydiae	5/10	1/1	–	–	–	–	–
Spirochaetes	14/112	7/42	4/26	–	–	–	–
Fibrobacteres	1/4	–	–	–	–	–	–
Acidobacteria	6/7	5/6	–	–	–	–	–
Verrucomicrobia	13/31	5/7	3/3	–	–	–	–
Dictyoglomi	1/3	1/3	–	–	–	–	–
Gemmatimonadetes	1/1	1/1	–	–	–	–	–
Lentisphaera	1/1	–	–	–	–	–	–
Bacteroidetes	181/711	105/453	107/340	76/249	13//18	5/2	–
Firmicutes, Tenericutes	312/1,962	269/1.752	146/936	76/730	42/247	1/2	7/73
Actinobacteria	238/2,381	226/2.293	147/1.069	79/569	46/191	2/3	3/40
Proteobacteria	746/3,582	572/2.618	367/1.645	277/1.334	126/351	50/157	–
Total number of strains		19.735	22.452	16.505	2.658	2.239	3.082

[a]It should be noted that the total number of genera and species includes synonyms (see http://www.bacterio.cict.fr/number.html). This is due to the fact that the name of some species appears in two or more genera (synonyms), depending on the number of reclassifications (names once validly published or notified will remain valid irrespective of its present classification). The deviations from the actual numbers will remain uncorrected as the visualization of holdings in the individual collections will only be slightly affected

in an environment of underfunding of public collections. Generally, the number and kind of non-type strains to be deposited is a matter of negotiation between scientist and curator. Rarely are entire research collections transferred to public collections; in most cases, such collections consist of unique holdings which are at high risk of being discarded or transferred to a new facility with unknown long-term perspective. The WFCC established a specific task group in order to provide a focal point of call for any collection (industrial/private/academic) which itself considers to be endangered or in need of help or advice with respect to its future sustainability. One example for a successful rescue has been the post-emeritus transfer of the collection containing myxobacteria and cytophagas from Hans Reichenbach to the DSMZ or the Seeliger collection of *Listeria* strains transferred to Mark Achtman, Cork, Ireland.

Surprisingly, it is only recently that funding bodies have developed an interest in microbial collections established in the course of projects funded with taxpayer's money. Starting with the long-term and secure availability of taxon-affiliated data of mainly eukaryotes, such as the barcode of life sequences, environmental observatory data, remote sensing, or geographic atlas of plants and animals (to name only a few), microbiological

information was restricted to gene and genome sequences. But it is the living culture that is needed in order to verify data and to explore new scientific horizons on the basis of the deposited information. This situation is slowly changing as funding agencies place more emphasis on measures for appropriate maintenance of research collections and their evaluation; they foster collaboration with the expertise of curators working in acknowledged collections; they even financially support basic taxonomic groundwork in order to allow collection curators to objectively judge the novelty of strains which could complement their holdings with the goal to broaden the biodiversity of taxa for research in general. Only a small fraction of strains maintained in research collections will be deposited in public collections, unless a completely novel long-term storage strategy is developed. Here, the same criteria could be applied to those listed in ❯ *Box 11.1* for post-publication deposits. It should also be stressed that researchers should communicate specific experience on growth and maintenance for fastidious strains to collection curators prior to deposition in public collections. Usually, curators lack experience needed for members of mainly higher and novel taxa for which no strain had been deposited before, and they need to be informed before the arrival of such strains in order to optimally preserve them long-term. The number of isolates in research collections worldwide cannot be estimated, and, likewise, the percentage of novel strains worthwhile depositing cannot be assessed. The transfer of research isolates to the safe environment of a public collection will only be decided through an intense dialog between curators and researchers in academia.

The third source of strains is currently almost unavailable to the scientific public: namely, those strains which are included in the scientific literature. The instructions to authors of almost all peer-reviewed journals state that the editors expect that new and variant organisms, viruses, and vectors described in journals will be made available to all qualified members of the scientific community. Some journals even explicitly encourage authors to deposit important strains in publicly accessible culture collections and to refer to the collections and strain numbers in the text (e.g., FEMS journals). The Guide to Authors in Nature publications states that resources should be made available in order to allow others to "replicate and build upon the authors' published claims" (http://www.nature.com/nature/authors/gta/#a1.3), to check when aberrant results are discovered or to reevaluate the strains when new technologies are available.

Though the number of deposits of non-type strains indicated in ❯ *Fig. 11.1* over a period of 11 years sounds impressive, it is only a minute fraction of strains annually included in scientific studies. To give only two examples, in the first two issues of Volume 46 (2008) of the Journal of Clinical Microbiology, around 32,000 strains of mostly clinical origin were included, while about 20,000 strains were included in the publications of the 2008 volumes of ten European microbiology journals covering mostly applied and ecological topics (Stackebrandt 2010). However, hardly any of them were deposited in public collections for long-term availability. In the first example, only 0.03 % of strains investigated were deposited which is perhaps not surprising considering the taxonomic affiliation of these strains (mainly staphylococci, mycobacteria, Clostridia, enterobacteria, *Acinetobacter*, *Burkholderia*, *Chlamydia*) which accumulate rapidly in daily hospital routine and represent in almost every case, isolates of described species and few exhibiting new properties. In the second survey, only 0.94 % found their way into public collections. Release of material and/or deposition in public collections is left to the authors' discretion; although some journals may have a stricter implementation policy than others, enforcement mechanisms do not exist for those frequent cases where authors deny sharing the requested material.

During a recent EMbaRC meeting of editors, collection managers, and authors, it was confirmed that, though access to published material may work smoothly among scientists in certain disciplines and tightly knit scientific communities, access overall is dismal. Discussion on a strategy to enhance and facilitate access to microbial resources was done with awareness that deposition of *all* microbial strains is neither necessary nor achievable under the present funding system of public repositories. The rationale for recommending deposition in public collections was not based on the concern that authors are incapable of short-term handling of research strains; it was based on the fact that microbial resource centers have decades of experience in handling, safeguarding, and shipping a wide range of diverse materials that is otherwise prone to involuntary extinction by negligence or deliberate clearing of laboratory holdings. Against this background, a set of selection criteria were recommended that would allow all stakeholders to prioritize material for deposition (❯ *Box 11.1*).

Box 11.1 Post-publication Deposit of Microbial Strains to Underpin Good Practice in Science

Despite recommendations to release to the community microbial resources post-publication, the reality is far from satisfying. A recent workshop discussed the need for a coordinated and effective deposition policy and proposed a set of criteria to facilitate deposition into public service collections (Biological Resource Centers) of "key" prokaryotic strains.

The workshop participants decided against a mandatory post-publication deposition of microbial strains but agreed on a set of criteria based on the phylogenetic, metabolic, and genomic uniqueness of "key" strains worthy of deposit. The checklist would also contain the contact addresses of a range of public service collections together with their taxonomic priorities to facilitate contact between authors and curators. Completion of this checklist would be mandatory prior to manuscript submission. The definition of "key" strains should be seen as a first but not exclusive step to initiate the strain sharing strategy; environmental samples, including as-yet-uncultured microorganisms, metagenome libraries, and other material should also be considered medium-term. The following criteria were agreed upon:

- Uniqueness, based on a cutoff point of $\leq$98% of 16S rRNA gene sequence similarity to the most closely related species with a validated name. This sequence is currently the gold standard for phylogenetic affiliation of an isolate at the genus level.

- Metabolic uniqueness, based on the presence of a new pathway, modification of an existing pathway, metabolic differences compared to the type strain or the production of novel products.
- Genomic uniqueness, such as significant differences (≥20%) in genome size, genome architecture, or new regulatory mechanisms.
- Resources and parts thereof with fully sequenced genomes (prokaryotes, phages, plasmids).
- A second strain of those species or subspecies for which only the type strain has been deposited. For 79% of new species described in 2009, only the type strain is available.

A survey among scientists was carried out to determine the reasons for the lack of materials in public service collections and whether they felt improved access was needed. Of the 3,950 scientists in 49 countries who were asked to participate, 517 responded (13.1%). When asked if they had encountered problems in accessing strains, 76.8% indicated that they had encountered problems, frequently to always, when asking for strains. Around 50% indicated that they received no response at all, others were requested to pay for the strain and some were denied access because of patent issues. Almost 87% agreed there was a need to improve access to microbial resources and 79% agreed that journal publication guidelines should request that strains with particular properties, such as those listed above, must be deposited in public culture collections to maintain them for further research.

This response suggests that the responders believe that a behavioral change is necessary and that journals should request that strains associated with publications be deposited. The reasons given for lack of response or failure to receive strains were specifically indicated by about 100 scientists but are subject to conjecture, being a mixture of guesses and author citations. In approximately 39% of cases it is feared that researchers simply want to protect their research from exploitation by others. This appears to be the very opposite of the philosophy behind publication and dissemination of results and conflicts with accepted scientific principles and morals. About 31% referred to the authors response that strains were lost or were unavailable for nonspecified reasons; 25% referred to quarantine, customs, and biosafety regulations as severe obstacles for releasing strains, problems that would be better solved by international, experienced BRCs than by individual scientists. Additionally, to protect the investment made using public funds, the research funders must also consider whether they make similar deposit and availability requirements on material subject to their funded research. The workshop participants stressed that authors should make every reasonable effort to make material available, if they do not deposit material in public collections, it should be because their strains do not meet the above criteria; it was also considered important that journals and funding agencies police their policies and have a mechanism for accepting complaints where access to material is denied. Journals were recommended to introduce a mechanism for active agreement by authors to make material available when they submit an article.

The workshop did not address the financial consequences of enhanced deposition but, considering the urgency to act now, funding agencies need to reevaluate their responsibilities by providing long-term and increasing support for public repositories to allow these tasks to be performed (Emerson and Wilson 2009; Stackebrandt 2010, 2011)

E. Garay-Aubán[4] · P. Kämpfer[5] · Y. Lecuona[6] · J. Prosser[7] · R. Roséllo-Mora[8] · K.-H. Schleifer[9] · K. Smalla[10] · D. Smith[11] · E. Stackebrandt[12]
[4]CECT-Spanish Type Culture Collection, Parc Cientific University of Valencia, Paterna, Valencia, Spain
[5]Department for Applied Microbiology, Justus-Liebig University, Giessen, Germany
[6]EMbaRC, INRA, Rennes, France
[7]Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, UK
[8]Department of Microbiology, University of the Balearic Islands, Palma de Mallorca, Spain
[9]Department of Microbiology, Technical University Munich, Emil-Ramann-Str. 4, Freising, Germany
[10]Federal Research Center for Cultivated Crops, Institute for Epidemiology and Pathogen Diagnostics, Julius-Kühn Institut, Braunschweig, Germany
[11]CABI Egham, UK
[12]DSMZ- Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany

The Paradigm Shift: From Collections to Biological Resource Centers (BRCs)

Culture collections need to adopt new technologies and work toward providing what today's research community needs. BRCs need to work together to address these needs through coordinated and harmonized approaches and sharing tasks in a cost-effective and appropriate manner.

It is absolutely essential that any service industry moves with the times and the needs of its users. Culture collections are no different and have been adapting to change and increasing challenges. They have been providing a public service for over a century essentially collecting and distributing organisms. The core function of providing an authentically named strain has remained but broadened to characterization of their holdings to a greater extent. Such change is most often an independent decision dependent on the sector in which the collection or its host institution focuses with the consequence that public service collections have deviated in collection focus and service provided. Naturally, collections have learnt from one another in introducing new approaches and products that have worked for others as well as introducing their own innovations. More recently, certainly over the last four decades, change has been driven by consensus through communities such as the WFCC. The WFCC introduced guidelines for the establishment and operation of culture collections (http://www.wfcc.info/guideline.htm) to help collections to

provide the best service to the scientific community. A coordinated approach to microbial and cell culture resource has been fostered. New collections are created while others disappear. Over 980 WDCM registration numbers have been issued, but only 592 collections remain (Box 11.3). This has been attributed to several causes, the retirement of individuals who maintained them, a change of focus of the scientist or institution or the loss of funding. In the late 1980s, the Japanese government listened to their scientific community and challenged the OECD to address sustainability and development of culture collections. The OECD responded with the Biological Resource Center Initiative which now describes the modern-day culture collection as a Biological Resource Center and defines them as follows:

> Biological Resource Centers are an essential part of the infrastructure underpinning biotechnology. They consist of service providers and repositories of the living cells, genomes of organisms, and information relating to heredity and the functions of biological systems. BRCs contain collections of culturable organisms (e.g., microorganisms, plant, animal and human cells), replicable parts of these (e.g., genomes, plasmids, viruses, cDNAs), viable but not yet culturable organisms cells and tissues, as well as data bases containing molecular, physiological and structural information relevant to these collections and related bioinformatics" (Definition based on the one adopted at the 1999 Tokyo Workshop on Biological Resource Centers, where the concept of BRCs as an outgrowth of conventional pre-genomics ex situ collections of biological materials was developed – and incorporating scientific developments since 1999.) BRCs must meet the high standards of quality and expertise demanded by the international community of scientists and industry for the delivery of biological information and materials. They should provide access to biological resources on which R&D in the life sciences and the advancement of biotechnology depends. (http://oecdpublications.gfi-nb.com/cgi-bin/oecdbookshop.storefront)

Meeting the Challenges

The OECD report (2001) 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, bio-informational 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

The development of BRCs to make available high-quality biological materials for research and development was considered necessary to underpin biotechnology. BRCs must focus on adding value to their biological materials and link more intimately to the life sciences and bio-industry to help deliver the developing bio-economy. The OECD report "The bio-economy to 2030: designing a policy agenda" (2011) emphasizes that the biological sciences are adding value to a host of products and services, supporting what some have labeled the "bio-economy." The report explains that from a broad economic perspective, the bio-economy refers to the set of economic activities relating to the invention, development, production, and use of biological products and processes. If it continues on course, the bio-economy could make major socioeconomic contributions in OECD and non-OECD countries. These benefits are expected to improve health outcomes, boost the productivity of agriculture and industrial processes, and enhance environmental sustainability. The bio-economy's success is not, however, guaranteed: harnessing its potential will require coordinated policy action by governments to reap the benefits of the biotechnology revolution. The plethora of uses of microorganisms, not least their use as reference strains in taxonomy or use in standards, demands access to expertise, technologies, and data analysis.

The change in how science research is conducted today, utilizing new technologies and information, requires culture collections to adapt in order to provide the resources in a way that will facilitate their use and enable an accelerated discovery chain. The transition to the OECD envisaged BRC is the first step. The modern-day BRC can support countries by establishing a means to release the potential of their microbial resources to provide solutions to national economic, environmental, food, and healthcare problems and consequently contribute to achieving the United Nations Millennium Development Goals. This ambitious agenda for reducing poverty and improving lives can be partially delivered by better management and utilization of biological resources:

- Improve livelihoods (Millennium Goal – MG 1).
- Provide new sources of food and reduce agricultural losses (MG1).
- Lead to discovery of new drugs and treatments of disease to reduce child mortality and improve maternal health (MG 4, 5, and 6).
- Understand and contribute to environmental stability (MG7).
- Develop a global partnership in the conservation and utilization of microbial resources for development (MG8).

- Resources created from the above can be mobilized to promote gender equality and empower women (MG3) and achieve universal primary education.

The OECD BRC Initiative took into consideration that the growing worldwide demand for biological resources provides good reasons for greatly increasing the number and quality of culture collections. Only a very few large national centers are able to perform a comprehensive role. A higher number and a broader geographic coverage of high-quality public service collections are needed to reach this goal. The development, expansion, and survival of these face many challenges. These include those posed by 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 (OECD 2001).

Introduction of Quality Management Systems, Accreditation, OECD Best Practice Guidelines

The modern-day collection or BRC is an entity compliant with appropriate national law, regulations and policies, and operates to internationally validated criteria. The impact of legislation on the collection, handling and distribution is enormous. Keeping pace with new and changing legislation is absolutely essential and places an additional burden on the culture collection. To demonstrate that a BRC is implementing best practice, a third-party independent assessment process is needed. The OECD agreed best practice guidance to enable the delivery of high-quality materials ensuring they are authentic, preserved by "state-of-the-art" technology and that all associated information is validated. The OECD best practice guidelines for BRCs (OECD 2007) outline a process for certification or accreditation of BRCs. The BRC must apply for accreditation through a process approved by national governments *but* either through an accreditation body recognized by government *or* through a transparent accreditation procedure recognized by government *or* directly by government. There are a number of ways this might be achieved, but it is considered that the process should be based upon existing systems. Many collections although implementing best practices may not wish to go this far. The most appropriate model is one that sets the baseline for authentic, well-preserved, and validated strains and requires development in excellence (see below). The BRC seeks to add value to its holdings by further research on the characterization of the strains held to enable improvement in their public service role. It is envisaged that not all culture collections will become BRCs, but best practice should be implemented nevertheless.

The OECD BRC Task Force considered that the establishment of a common quality standard was a key issue in the development of BRCs. There are several examples of existing guidelines for microbial and cell culture collections available:

- The WFCC *Guidelines for the establishment and operation of collections of microorganisms* (http://www.cabri.org)
- The Microbial Information Network for Europe (MINE) project standards for the member collections (Hawksworth and Schipper 1989; Stalpers et al. 1990)
- Common Access to Biological Resources and Information (CABRI) guidelines (http://www.cabri.org)

There are also a number of general nonspecific standards and norms that can be applied to microbial laboratories, such as

- Good Laboratory Practice (GLP)
- ISO 9001 quality management systems
- ISO 17025 general requirements for the competence of testing and calibration laboratories
- ISO Guide 34 general requirements for the competence of reference material producers

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, a quality management system must go further and set minimum standards (Smith et al. 2001). The CABRI electronic catalogue project made available a set of guidelines to aid collections to put in place best practice (CABRI 2002). These cover critical elements in the handling, storage, characterization, and distribution of microorganisms and cell cultures and the handling of associated information. The EU project (QLRT-2000-00221) European Biological Resource Centers Network (EBRCN) ran in parallel to the OECD Task Force. The EBRCN consortium supported the work of the OECD Task Force by drawing together the key elements of the above-mentioned guidelines and standards to form the basis of the OECD best practice guidelines for BRCs (OECD 2007). This was formulated at two levels, the general criteria that can be applied to all BRCs and secondly, organism domain specific criteria that are applied to BRCs based on the biological materials they hold. Currently, two sets of general guidelines ("general best practice guidelines for all BRCs" and "Best Practice Guidelines on Biosecurity for BRCs") exist in parallel to two sets of domain-specific guidelines ("best practice guidelines for the microorganism domain" and "best practice guidelines for human-derived material").

- *The general best practice guidelines for all BRCs cover*
  - Organizational requirements
  - Equipment use, calibration, testing, and maintenance records
  - Documentation management
  - Data management, processing, and publication
  - Preparation of media and reagents
  - Accession of deposits to the BRC
  - Preservation and maintenance
  - Supply
  - Quality audit and quality review

- *The best practice guidelines for the microorganism domain cover*
  - Staff qualifications and training
  - Hygiene and biosafety
  - Equipment use, calibration, testing, and maintenance records
  - Preparation of samples
  - Information provided with the biological material supplied

  Additionally, specific guidance was prepared to cover potential dual-use organisms and to ensure BRCs implemented practice to ensure biosecurity. Dual-use is a term to refer to any technology or material which can be used for peaceful and military aims. Thus, the exploitation of biological material and biotechnology is an essential part of the international dual-use regulations with regard to bioterrorism and bioweapons.
- *The best practice guidelines on biosecurity for BRCs cover*
  - Assessing biosecurity risks of biological material
  - New acquisitions/reassessment of inventory
  - Biosecurity risk management practices
  - Physical security of BRCs
  - Security management of personnel and visitors
  - Incident response plan
  - Material control and accountability
  - Supply and transport security

Over 20 WDCM registered collections have some form of certification or accreditation to demonstrate the provision of quality services and material. The OECD best practice guidelines extend such certification criteria to address BRC operations more specifically setting a benchmark for culture collections worldwide. Mechanisms to ensure collections adopt these standards to deliver high quality should be put in place (OECD 2001). Although the OECD BRC Task Force wished to see high quality proven through an independent third-party auditing process, for example, certification, it wishes to base it on existing systems and internationally accepted scientifically based quality criteria.

The actual system of international standards is focusing the complementary aspects of management, technical skills, product conformity, and process stability. The OECD best practice guidelines added the aspect of regulatory affairs to this complementary system. Each standard is specialized to enhance the compliance of an organization to a single aspect (e.g., ISO 9001 –> management). Are the OECD guidelines really a new approach going further than the already existing standards, establishing a complementary system with an integrative character covering all aspects? Can the OECD guidelines in addition answer both the Task Force and the OECD demands? These main questions can be answered by identifying the issues, which impact significantly on the implementation and maintenance of quality management in culture collections and BRCs. These issues reflect a variety of managerial operations and perspectives including continuous improvement, organizational behavior, human resources management, customer relations, and the core processes in the laboratories. But in addition to these traditional key issues of quality management, mainly covered and endorsed by the global standards ISO 9001 or ISO 17025, modern culture collections are faced with far-reaching issues in their shift toward the modern BRC as defined by the OECD. These issues include social and socioeconomic tasks, sustainable financial management, balancing of commercial and scientific interests as equitable stakeholders, linkage to innovative information technologies, and realization of governmental and cross-national legislation in the fields of biotechnology and security interests. The transformational change from a national though networked repository for biological material toward a multitask facility being part of a global infrastructure for the emerging knowledge-based bio-economy requires not only an enlargement of managerial requirements but also a new mutual standard in quality management. Taking up the necessity to standardize and systemize the core activities of a BRC within a special tailored guideline covering most of the key issues, the OECD best practice guidelines have not only a high coverage of all requirements in one single standard, but in addition, they cover a broad spectrum of the requirements delivered by other standards. In fact, the new guidelines for BRCs offer an integrated approach to support a culture collection in their own development as well as in reaching a high compliance level in many normative aspects.

But, having the new set of guidelines for BRCs demands a new approach for third-party assessment. Especially in consideration that the transition of the guidelines into one's own organization, the handling of increasing requirements coeval to diminishing financial support and the exposure to regulatory compliance is an unsolved problem left to the individual BRC and their quality managers. Thus, the internationally organized, German Federal Ministry of Education and Research funded project, "GBRCN – Global Biological Resource Center Network" – is working on an assessment model based on the excellence principle. Implementing the OECD guidelines brings a multidimensional capability into an organization; the principle of the GBRCN of sharing and continuously improving these capabilities among all BRCs and culture collections is opening the way toward an excellence model in performance and in the delivery of the OECD requirements. The excellence approach would resolve the restrictive regime of a standard and its full compliance assessment by offering a stepwise development in accordance to the available resources and demands. Self-evaluation and third-party audits will still be the important instruments to gain confidence in the system and recognition of the delivered results. However, the new assessment model is not propagating the golden way; it shows that many approaches will lead to excellent quality in services, material, and science.

Currently, the discussion is ongoing, whether the OECD best practice guidelines will remain a part of the so-called GxP world, for example, Good Laboratory Practice or if they should become an ISO standard, thus broadening the existing set of standards with the special requirements for culture collections and their living biological material.

Quality and Certification: Costs and Benefits

Whatever system is selected, there will be costs associated with the achievement and implementation of the standards, and it is therefore important that the benefits to users and the BRCs themselves are clear. Some of these needs and benefits are outlined in the OECD report (OECD 2001). If the user benefits from the certification or accreditation of BRCs through better access to authentic and reproducible materials in a transparent and traceable way, how does the BRC benefit? There is an ever-increasing demand for authentic 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 BRCs strategy for long-term sustainability, but it is also an increasing requirement to satisfy the funders of research who seek high-quality science and solutions.

It is imperative that organisms utilized in biotechnology are maintained in a way that will ensure that they retain their full capacity. BRCs must ensure a high-quality product that will give reproducible results. To achieve this, BRCs must apply quality control and assurance measures to maintain these standards, taking into account the needs of users and of the facilities and resources available. The need for common standards is evident as the task of maintaining representative samples of microbial diversity cannot be achieved by one collection alone. Therefore, it is essential that a worldwide 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.

Standards also provide a useful target for new collections to achieve, but it must be remembered that standards should become part of the operations and not be a set of rules implemented separately. Their aim is to ensure good quality and traceability and encourage improvement and further development. Standards must fit the operation and not add excessive unnecessary burden. Implementing standards for operation allows collections to convince investors to establish the facilities, skills, and mechanisms needed to participate in international activities. However, it is not sufficient to set the standard and then forget about it. A process for review and update must be put in place to ensure that new technologies can be brought in to improve the standard.

The advantages of certified or accredited BRCs forming a network can be split into two groups, those that give benefits to the users and those that benefit the BRC itself although several could fall in both categories.

User Benefits

- A one-stop shop where both high-quality biological materials and the information associated with them can be found
- Conformity of both quality and authenticity of biological materials but also of processes and procedures to access them
- Confidence that the materials are fit for purpose
- Assurance that national law, policies, and procedures have been followed

BRC Benefits

- Recognition that they operate to international scientifically based quality criteria
- An international mark of quality
- Raised profile
- Sharing of tasks
- Common policies and procedures
- Competitive edge
- Level playing field
- Common access to data enabling links to be made to other international initiatives without duplication of effort
- Common approach to data access, sharing, and interoperability
- Improved data usage
- Collaborative research and development

Inevitably, introducing the requirements of the standard and accreditation or certification procedures to the collections to achieve the status of a BRC will be costly. However, used correctly, it can attract investment in the development of BRCs, and the outcome will be beneficial to all concerned.

Financial Sustainability of Public Service Collections and BRCs and Networks in Both Developed and Developing Nations

Implementing common standards and improving operations have additional costs. Despite there not being one model for the operational and financial sustainability of a collection, we can learn from the experience of existing culture collections. Studies by the OECD BRC Task Force and the EMbaRC consortium provide working models for BRCs. Although culture collections or BRCs have similar activities and objectives, they can be quite different in size, scope, and function. They may be described as either specialist or generalist collections, be small, based around an individual researcher or research team, or be large public service collections and a multitude of structures in between with differing financial models supporting each. Culture collection revenues traditionally come from supply, preservation, and various services associated with these, such as identification, characterization, or specialist consultancies. Culture collections also participate in research or service contracts, but most rely on some form of governmental or host institutional funding. A variety of activities relate directly to quality control, collection development, and operation that may include opportunities for some additional cost-recovery activities. Among several potential new sources of revenue is the generation of genomics and proteomics data that complement and add value to the biological materials themselves. The degree to which such activities may

actually provide support, sufficient to ensure financial sustainability of a BRC, is unproven. There are a few centers only that purport to declare themselves self financially sustainable.

The OECD model of BRCs includes considerable diversity of funding mechanisms for individual centers. However, it is to be expected that most BRCs will require some degree of commitment to core funding by their respective national governments. Other kinds of funding sources include support from industry, grants from agencies that support research, cost recovery through fees-for-service, development of databases, and other tools that complement the core role of BRCs, for example, even funding from charitable sources, especially those associated with public health or sustainable development. Furthermore, BRCs should be encouraged to coordinate their pricing policies and other activities to best serve their essential functions in response to the needs of sectors that depend on their biological resources.

The different approaches do not only rely on the expertise and function of the different collection hosts; but additionally, the needs and capacities of individual countries vary. Specifically, the needs of developing countries must be understood and accommodated. The OECD BRC Task Force advocated that national governments should identify collections and centers already capable of being designated as BRCs or forming a network and build upon and improve these rather than starting up new BRCs, especially in developing countries where resources are limited. Similarly, partnerships must be developed among BRCs and appropriate existing agencies, identifying their capacities and interests in terms of support for BRCs. A survey carried out by Stromberg et al. (2012) of 103 WFCC affiliated member collections, comprising mainly public service collections, demonstrated quite different balances between revenues and public support. Fifteen percent receive no public funding; 11 % received 1–40 % funding; 8 % receive 41–60 %; 13 % are 61–80 % funded; 54 % receive more than 80 % public funding. These differ widely from the total statistics for the WDCM which shows that the majority of the remaining 450 plus collections are not publicly funded and overall only about half the registered collections receive governmental support.

It is evident that at the outset when a collection is being considered, and before it is established, the financial plan and its sustainability must be designed. The WFCC guidelines (Anon 2010) state that the long-term support needed to enable collections to provide professional services must be considered, including appropriate operational facilities, the staffing levels to allow operation at a high standard and the training level of staff with research expertise related to the aims of the collection. The WFCC guidance presents funding as a key consideration. Administration and funding arrangements for collections require a long-term commitment from the parent organization. Support solely in the form of short-term contracts or without any allocation of core funding is inappropriate for service collections, aiming to provide long-term storage and supply services. Even the establishment of small in-house collections requires an ongoing source of direct, or indirect, financial support from a parent body. It is important to consider the level of funding, both now, and what it is likely to be in the future. This must be adequate to provide the range of services being planned and at a standard that users would expect. If secure resources are limited, in general, it is preferable to restrict the primary objectives of the collection to those which it has a strong probability of maintaining in the long-term. The financial models provided by existing culture collections of various types are well recognized and include

- The "General Collection" – often a national/regional facility.
  - "Popular" items for distribution can guarantee income.
  - Archive function requires subsidy.
- The "Specialist Collection" – usually more localized.
- The "Institutional Collection" can provide internal institutional service or wider external community/network service.
- The "Research Collection" provides a service relevant to one or more research interest.

These models vary considerably in the proportion of income derived from the various sources defined below. It must be emphasized, however, that the larger the archiving function carried out for strategic reasons rather than supply, the greater is the need for public and private subsidy.

Financial Models for Biological Resource Centers

The diversification of activities in the transition from the "Culture Collection" to a BRC anticipates additional sources of revenue, both from existing activities and projects related to new technology-based partnerships. Two types of income streams are recognized. "Existing" income streams are those that support existing models of culture collections. "Anticipated" income streams which represent activities in which BRCs will or may participate in and that may generate recoverable income from stakeholders.

Existing Income Streams for BRCs

- Government support
- Private industrial support for participation in the functioning of BRCs
- Private industrial support for internal restricted BRC activities
- Public and private foundation support
- Public fundraising
- Fees for supply of biological resources and technical materials
- Provision of specialist services and technical consulting expertise
- Research income (grants and contracts)
- Fees for repository service (safe deposits and patent strain maintenance)
- Provision of technical courses
- Exploitation of and adding value to genetic resources

The understanding of current income lines for culture collections was assisted by the survey carried out by Stromberg et al. (2012) where they report that 67 % of affiliated member collections of the WFCC charge supply fees for strains provided. They go on to report that on average, 23 % of the recipients of cultures are

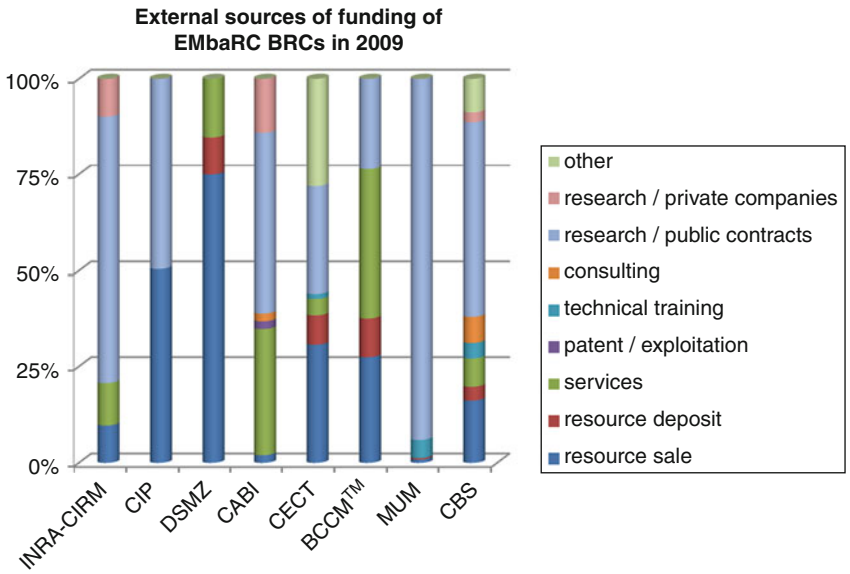

Fig. 11.3
Funding sources of EMbaRC BRCs compared for 2009 (see Abbreviations)

in industry, whereas 60 % are in academia or hospitals. They also report that there is a relatively small overlap in strains held, with between 1 % and 17 % of their holdings sourced from other collections. They report that 38 % are received with some sort of MTA, providing terms and conditions of supply. There is, however, an imbalance on where these collections are, with 82 % of them being in OECD countries and 10 % in the USA. Individually, although having overlap in the most popular reference strains, they have limited coverage of biodiversity, but together in a network, they could offer good coverage. Inevitably, there is an element of competition for the market, hence the determination of collections to store the "best sellers."

Potential or "Anticipated" Income Streams for BRCs from, for example, the supply of services for

- cDNA libraries, genomic libraries, filter sets, clones, plates, and PCR products
- Microarrays and reagents
- RNAi resources
- Accreditation/standardization-added value products and services
- Data storage and retrieval
- Software development/collaborations – data mining tools
- Technology development/collaborations – LIMS/robotics
- Sequence database annotation/phenotypic analysis
- Linking genomics databases to proteomics
- MLST (multilocus sequence typing) and population studies
- Product discovery, manufacture, and supply (potentially spun out to independent companies)

However, it is debatable whether all these activities have a market and offer worthwhile returns. Often such services are offered by specialist organizations, and the competition can be quite tough.

Culture Collection Funding

There should be a balance between governmental support, commercial, and other income lines to provide support for collections. There are several collections that are supported by governments but rarely are they fully supported. The government supports 235 of the 592 culture collections registered with the WDCM, a further 56 are semi-governmental, 218 are supported by university, 17 are supported by industry, and 25 are private. It can be argued that governmental funding is essential and appropriate but even long-term stability of such funds may eventually be under threat. Culture collections perform many functions for governments not least helping them meet their obligations to the Convention on Biological Diversity and making available biological resources to underpin science, education, and the economy. Such government funding is usually balanced against the income received for the various services and products offered by the collection. This leaves very little for investment and to enable the collections to improve their coverage and incorporate new and advancing technologies. Collections need sound and innovative business plans to allow them to keep pace with the ever-increasing demands of their users.

The EMbaRC project is examining sustainability of BRCs and has compared the revenue lines of the partner collections. These collections are in the main well-established public service collections with a long history of providing products and services. They show that they have common products and services, but the balance on how important each individual line is to each collection is significantly different.

The funding sources of some major West European collections (EMbaRC) for the year 2009 are shown in ❯ *Fig. 11.3*.

The level of public funding was mainly in the range of 65–92 % but with the exception of CABI which has no specific national funding but which has overall member country contribution of around 3 % with more invested in the collection maintenance activity. Other funding sources for the EMbaRC collections in 2009 were

- Maintenance and bench fees – 0–16 %
- Resource supply – 2–75 %
- Resource deposit – 0–10 %
- Services – 0–39 %
- Technical training – 0–1 %
- Consulting – 0–7 %
- Research/public contracts – 0–94 %
- Research/private companies – 0–14 %

Developing Income Lines

Not only do collections need to find novel ways of funding but also need to keep abreast and harness new technologies to produce information on the strains they hold, adding value with the aim to provide today's users with the information they need. It is not always possible to establish these technologies in-house, but it is possible to establish partnerships with manufacturers, other collections, or institutions with the expertise and facilities. Bioinformatics is of increasing importance to the operation of collections, and new ways of collecting, storing, analyzing, presenting, and interrogating information are required to make best use of biodiversity information. Molecular techniques are increasing in use to differentiate between strains and identification. Collections should be adopting such techniques to offer as services to users to counter the costs of utilizing these techniques for checking stability and authenticity of the strains they supply.

There are a number of ways BRCs can develop their individual business plans. However, it is crucial that BRCs do not become commercial entities; they must not compromise their public service role. Having said that they must do all they can do to reduce their public cost, a delicate balance is required. Some avenues that can be explored are outlined below.

Commercial

- Development and ownership of spin-off biotechnology companies, generally through partnerships, sale of products, and services as well as consultancy

Research Program Funding

- A series of projects to meet donor requirements, engaging research program funders to protect their investments by paying for deposits in collections

Government Department Support

- Provision of services to governments to help them achieve their conservation and utilization of biodiversity commitments, their environmental policies, and their commitment to poverty alleviation

Sponsorship

- Attracting donations to cover costs of biological resource provision, establishing a consortium of research program funders and sponsors

Other Financial Aspects of Operating a BRC Network (as Identified by the OECD Workshop on Funding Models)

While a uniform structure of funding is not necessarily critical, many BRCs will require a significant component of government funding. Some guarantee of ongoing funding is necessary to ensure that their essential functions remain reliable for R&D and support of biotechnology. Collections will be put at risk if a BRC network operates at the expense of individual BRC funding resulting in the individual BRC's folding for lack of support. The following points were derived from the SWOT (**S**trengths, **W**eaknesses, **O**pportunities, and **T**hreats) analysis of financial models of BRCs and a proposed model for BRC development.

Strategic Implications

- A prerequisite for any network is that it is built upon standards and accreditation. This defines the network. This may require further investment.
- A national strategy that provides core financial support for a national BRC (or BRCs) should be viewed as a prerequisite for participation in the international BRC network, to ensure that the network is sustainable.
- The international BRC network will be built upon national initiatives that in turn will evolve from existing activities (including culture collections). These activities are already based upon a range of income streams with varying levels of government support.
- Governments will be fundamental partners in the creation of national BRCs contributing to the international network, regardless of the level of financial support.
- Many existing culture collections will not wish to participate in the BRC network if this is inappropriate to their aims or goals, or if this is not justifiable given the level of investment required to raise/alter standards. Links to enable BRCs to draw resources from such centers will need to be created.
- Governments need to recognize that BRCs will take a regulated role in the supply and maintenance of

dangerous/pathogenic organisms. This important core aspect of BRCs provides a controlled framework for the availability of these sensitive resources. In turn, fulfilling this role requires a level of financial commitment.
- BRCs must use the opportunity of establishing an international network to seek sponsorship from a variety of new sources of support (national, international, public, private, and industry).

Operational Implications

- BRCs have to take a prominent role in capacity building and ensure a link between research-based collections and the BRC and the ultimate user.
- BRCs need to function as a strategic, national repository for key academic and industrial research resources, which will in turn provide an income stream. This is unlikely to operate on the basis of full cost recovery from supply income.
- Governments and their funding agencies must ensure that products derived from publicly funded research programs are deposited in BRCs as part of the conditions attached to any award. (This could result in a small element of the grant allocated to this task as appropriate – see below.)
- BRCs need to provide greater support to research-based collections in terms of training and advice on standards, quality control and integrate more with the national activities in key-related priority research areas (e.g., model organism research consortia).
- Governments must ensure that infrastructure aspects of the support for research are funded through relevant research programs.
- BRCs must create partnerships with centers of excellence and developing new technologies and databases to ensure that linkage is possible between these leading edge aspects of research and the physical resources held in BRCs.

It is anticipated that all of these strategic and operational changes relevant to the national role of BRCs will enhance their position in providing services of benefit to the scientific community and thus in turn benefit them by maximizing the potential for financial support.

A key element for discussion, however, remains the degree to which BRCs may benefit from the direct commercial exploitation of the resources that they hold. "Ownership" as a concept has, to a large degree, been avoided in the past with the BRC acting as a "custodian" of the resource. Widespread introduction of Material Transfer Agreements and implications that IPR and reach-through are requirements for access to resources would fundamentally alter the relationship between depositor, user, and the BRC. National mechanisms for implementing the Nagoya ABS protocol (CBD 2011) could impact heavily here.

Collections and Their Users: The Need to Know Each Other Better

Though not commonly encountered, collections are encouraged to conduct market studies and carry out regular surveys on customer satisfaction and buying behavior. Collections requiring a substantial proportion of their budget by generating revenues usually have in place a dedicated user-oriented management plan which, according to a functioning quality management, must be improved constantly. Knowledge about user demands and requests is indispensible for strengthening their market position. Therefore, collections should have access to certain basic information, such as

- Who is the user?
  For example, where are they working: in academia, bio-industry, food, or clinical sector or in public health or schools?
- What are the needs of the user? Are curators aware of them and is the management in a position to react quickly to satisfy user demands? Examples are as follows:
  Post-order communication
  Quality and type of packaging
  Modalities of shipment
  Correctness of delivered goods, such as authenticity and product information on safety aspects and handling
  Option to establish contacts
  Amicability and qualification of collections' contact person
  Goodwill policy in case of replacement shipment
  Handling of complaints
  Lead and delivery times
  Internet accessibility on information to
    Cultivation conditions
    Spectrum of services
    Databases
    New resources and products and new developments

The necessity to develop a more intense communication between collections and users is driven by the need to establish long-term and repeated use of the collection and its services. Once satisfied with some basic principles, such as high quality, short delivery time, and correct and timely information, the user will more likely as not become a loyal customer, independent of the fees for resources and services. The collection should develop a specific affiliation with the user (worldwide highly recognized brands have achieved this goal), and the collection should facilitate this relationship by documenting the advantages to be linked to justify this very resource center. Some are as follows: contact to a nationally/internationally leading collection; process reliability, such as provision of non-contaminated resources allowing reproducible results; range of a defined (either broad or specific) selection of products and services; close scientific support and consultancy, guaranteeing quality in products and processes; and long experience in taxonomy and identification, handling of recalcitrant, pathogenic and other delicate material, as well as expert knowledge in shipping packaging and import and export rules and regulations.

As compared to the collection-user relationship of 10 years ago, significant changes have already been introduced, for example, by establishment of a quality management (QM) system; one of the central features of a functioning QM system is the continuous process of improvement. This not only includes the strengthening of the collection-user relationship as mentioned above but also offers some of the advantages the customer is used to receive from well-managed online shops in other market segments. Top priority of online shopping is the option to pay by credit card. The latter issue is still a moot point in Europe as, in contrast to the situation in North America and a few other countries, most organizations do not allow payment by this means or do not provide credit cards to their employers. Another sector with room for improvement is the need for the collection to accompany the customer through the ordering and delivery process. Once an order is launched, basic information on entry date, confirmation of an order, order status, and date of dispatch should be provided. It must be the goal to have the material shipped within a few days – if not, the user should at least be informed about possible delays. Such requirements are clearly described in the OECD best practice guidelines (OECD 2007).

We are aware that online shopping for consumer goods cannot be fully compared with the provision of living organisms, which require a lengthy process of customer authorization, and administrative effort on export, import, and shipping regulations, not to mention delays of shipment of active cultures. It is, however, not overstated to indicate that most public service collections have not attempted to assess the satisfaction level of their users. Here, one can learn from commercial resource centers with which noncommercial collections compete in the same market. Collections should not hesitate to learn from the best cases of other organizations, and they should learn to react quickly to customer needs and demands. This requires the establishment of a client-led marketing policy, most efficiently executed by a professional marketing unit. Public service collections must recognize the need to make themselves more attractive through regular press releases of collection-related scientific headlines, by increased publications in international peer-reviewed journals, attractive training courses, and involvement in teaching and public lectures. These activities are already followed by larger public collections but continuously necessary to accompany measures of the core mandate and motive, that is, the provision of high-quality and non-contaminated biological references to support scientists in their goal to obtain reproducible data at the highest scientific level.

Scientific-Technical Cooperation Among Microbial Culture Collections

Although culture collection organizations have existed for many decades, they or their modern-day versions, the BRCs, have never been fully networked. National, regional and global organizations have endeavored to help promote collections and have coordinated some efforts. They have brought together metadata on their members to central points and have helped keep members up to date with the progress of science, changing legislation, and collaborative opportunities through newsletters, conferences, and workshops. However, coordinated strategies for ensuring comprehensive coverage of species and the diversity within them are yet to be put in place. Projects and individual initiatives have made some progress, but consolidating the many initiatives that are working toward this goal is crucial to establish a systematic and networked approach. This would bring advantages to both the users and the collections themselves but importantly provide an infrastructure to underpin research and development, enabling the harnessing of microbial and cell diversity to contribute toward providing solutions to the world's big challenges.

The WFCC has been promoting the activities of culture collections for over four decades and has done a tremendous job to help establish a sound operational basis (Box 11.2). It was first to try and establish minimum standards through their guidelines (Anon 2010), common standards form the platform on which networking is based. The WFCC, as are most culture collection organizations, is a community that exchanges views and ideas. Often, this results in the uptake of common approaches, but the organization has no mandate to affect institutional changes in policies and practices. This impedes the introduction of coordinated approaches. At the regional level organizations such as the European (ECCO, Box 11.4) and Asian (ACM, Box 11.5) networks work on behalf of collections. They have been very successful in bringing project consortia together to seek project funding to solve common operational problems or address common research issues. There are over 20 national federations that do similar things at the country level. However, a lot of work still needs to be done both by collections and governments if the goal to harness the power of microbial diversity is to be realized. We need to harness the properties and products of microorganisms more efficiently if we are to tackle the big global challenges of today in poverty alleviation, food security, healthcare, climate change, and the environment.

The OECD emphasizes that biological resources, such as microorganisms and their derivatives, are the essential raw material for the advancement of biotechnology (OECD 2001). However, they go on; scientific progress and the resulting growth of the knowledge-based bio-economy will depend on the facilitated and safe access to ex-situ held living biological material and its availability in an adequate and comparable quality worldwide. It is understood that this, in turn, requires putting in place coordinated policy actions by all stakeholders involved. To meet the increasing demands of the scientific community for comprehensive, up-to-date, and easy to access living biological material available from microbiological culture collections and related information, a series of coordinated activities were initiated in different regions worldwide, leading to network activities to foster communication and research among collections for the benefits of users and science.

Box 11.2 World Federation for Culture Collections

The World Federation for Culture Collections (WFCC) is a key global organization originated from an IAMS "section on Culture Collections" formed in 1963, which was reorganized as the World Federation for Culture Collections in 1970. From 1973, it was recognized as 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. 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 Micro-organisms (WDCM, *Box 11.3*). A congress is held every 3 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 working programmes addressing patent depositions, biosafety and biosecurity, safeguard of endangered collections, capacity building, and quality standards. Since 1986, the WFCC has overseen the activities of the WDCM which is now the data center for the WFCC and Microbial Resource Centers (MIRCENs) Network.

The WFCC is the largest independent global organisation 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; encouraging the development and implementation of quality and security procedures and the use of common standards and regulations; representing member interests in international organizations and fora; and promoting the establishment of culture collections and their perpetuation.

There are over 120 culture collections affiliated to the WFCC who have agreed to implement the WFCC guidelines (*Guidelines for the Establishment and Operation of Culture Collections*- Anon 2010) and who contribute to the delivery of its objectives. In the growing 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. The WFCC have been helping collections in this respect for over 4 decades. It is a goal that strains of organisms be supplied from member collections with traceability, conforming to national and international regulatory requirements, and that are preserved in such a way as to retain their full potential.

David Smith
CABI, Egham, UK

Box 11.3 The World Data Center for Microorganisms

In 1982, the World Data Center on Microorganisms hosted by the University of Queensland in Australia issued the World Directory of Collections of Cultures of Microorganisms (*Fig. 11.1*). The editors of the directory, Vicki F. McGowan and V. B. D. Skerman, articulated the roles of culture collections and the data center, "Culture collections occupy a central position in microbiology because effective research demands adequate and reliable sources of properly preserve cultures. As a result of their function as repositories of living organisms, culture collections promote microbiological research. Increased demands for historical information and strain data have created a need for easily accessible and up-to-date files of important information on the location and characteristics of cultures. Such needs can be met by the development of an adaptable system for storing, retrieving and exchanging information which can be used by all microbiologists."

The WDCM relocated in 1986 to RIKEN, Saitama, Japan, and then again in 1999 to the National Institute of Genetics, Japan, and introduced an online database and website to capture and diffuse information on culture collections and their holding. In the meantime, the number of culture collections registered in WDCM has increased year by year. However, it is to be noted that WDCM has issued 983 IDs to culture collections, that is, the community has lost about 300 culture collections since the first was registered. The WDCM collections hold in excess of 1.7 million strains: 44% are fungi, 43% bacteria, 2% viruses, 1% live cells, and 10% others (including plasmids, plant, animal cells, and algae).

In 1999, WDCM organized a symposium with the title of "Microbial Resources Centers in 21st Century – New Paradigm" back to back with the 1st OECD Meeting on Culture Collections. This was the moment when the concept of Biological Resource Centers was born. The participants of the two meetings recognized the impacts of biodiversity, genomics, and informatics on culture collections and agreed that culture collections had to evolve to become BRCs to meet their needs and those of users.

The online database of the world directory named CCINFO includes information on 592 culture collections in 68 countries as of March 2011; 235 of them are supported by the government, 56 of them are semi-governmental, 218 of them are supported by university, 17 of them are supported by industry, and 25 of them are private; 226 collections produce catalogues of holdings and there are 3,051 people working in them. These culture collections preserve 1,751,439 microbes. WDCM functions as an information hub of culture collections and their customers. The WDCM is now hosted by the Chinese Academy of Science Institute of Microbiology since April 2011 and it is expected that the functions of WDCM will be expanded to cover aspects of biodiversity, genomics, and advanced information and communication technologies (ICT). The URL addresses of the websites of WFCC and WDCM stay as they are, namely, http://www.wfcc.info/ and http://www.wdcm.org/, after the relocation of WDCM.

Hideaki Sugawara
DNA Data Bank of Japan, National Institute of Genetics, Mishima, Japan

Such activities are not new for regional networks (*Boxes 11.4* and *11.5*) or national networks, for example, the Brazilian initiative (*Box 11.6*).

Box 11.4 European Culture Collections' Organization and its Networking Activities

The European culture collections have collaborated since 1982 when the European Culture Collection Curators' Organisation was established to bring together the managers of the major public service collections in Europe to discuss common policy, exchange technologies, and seek collaborative projects. The organization opened itself to staff and users of microorganisms and is now named the European Culture Collections' Organisation (ECCO). There are currently >65 members, including 57 collections holding approximately 350,000 strains. The members have been involved in producing practical approaches to international rules and regulations. An initiative led by the Belgian Coordinated Collections of Microorganisms (BCCM^{TM}) produced a code of practice for collections to operate within the Budapest Treaty and the EU project Microorganisms, sustainable access and use, International Code of Conduct (MOSAICC) provided model guidelines for the operation within the spirit of the Convention on Biological Diversity. Several collaborative projects originated through discussions between ECCO members that have placed the European Collections at the cutting edge of culture collection activities and research. The most recent initiative is the EMbaRC project. They have resulted in technical guidelines and focused information documents covering requirements with which modern-day microbial collections are challenged. Substantial input was given by ECCO to the BRC initiative and the recent demonstration project for a Global Biological Resource Center Network (GBRCN). On a global level, the latter project aims to build a structured long-lasting network which will pave the way for collections to meet user needs. It addresses technical, legal, and administrative challenges presented in this globalized, fast-developing world.

Dagmar Fritze
DSMZ- Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany

Box 11.5 The Asian Consortium for Conservation and Sustainable Utilization of Microbial Resources (ACM)

The Asian Consortium for the Conservation and Sustainable Use of Microbial Resources was established by the consensus of participants of 12 Asian countries during the 10th International Congress of Culture Collection (ICCC-10) held at Tsukuba in 2004. Heads of culture collections and government officers were involved in the meeting as well as research microbiologists. The objective of the consortium is to promote collaboration among Government or public organizations in Asian countries for the purposes of enhancing conservation and suitable use of microbial resources in Asia. Currently the members are from Cambodia, China, Indonesia, Japan, Korea, Laos, Malaysia, Mongolia, Myanmar, Philippines, Thailand, and Vietnam.

The ongoing activities of the consortium are:

1. Exchange of views and information of the current policy of the Asian countries for science, technology, and the related matters
2. Establishment and management of the network of culture collections including a common database
3. Enhancement of public awareness on the consortium's activities for the conservation and sustainable use of microbial resources in consideration of the Convention of Biological Diversity
4. Development of human resources for handling of microbial resources technically and legally
5. Promotion of research and development on microbial resources and their application in industrial and other uses
6. Establishment of a common scheme for international transfer of microbial resources
7. Scientific meetings (seminars, workshops, training courses, etc.) and other related activities

The General Assembly Meeting of the ACM has been held annually since 2004 in different countries. Task Forces for Bioresource Information Management, for Human Resource Development and for Management of Material Transfer are set up. These activities will be of value for the standardization and authorization of international transfer of microbial resources. Many Asian microbiologists are eager to study the microbiological diversity in the nature of various natural environments. ACM is also expected to achieve the rule of International Code of Prokaryote Nomenclature in compliance with the laws and regulations relevant to the Convention on Biological Diversity.

The 7th ACM meeting was held in Japan again in 2010, the International Year of Biodiversity, and adopted the Kazusa Statement on 15th October as follows:

- Kazusa Statement
  In the 7th ACM Meeting at Department of Biotechnology, National Institute of Technology and Evaluation (NITE) in Kazusa, Chiba Prefecture in Japan, members of the Asian Consortium for the Conservation and Sustainable Use of Microbial Resources (ACM) recognize that:
  Microorganisms such as filamentous fungi, yeasts, mushrooms, bacteria, archaea, and microalgae play important roles in the global ecosystem either directly or indirectly
  The diversity of isolated microbes only account for less than 10% of the total species, which means that many novel and yet-to be-discovered microbes inhabit the earth

The diversity of microbes is endangered by global climate changes, habitat changes, over exploitation, and ecosystem destruction

Long-term laboratory preservation of microbes is technically well attainable

Microorganisms are crucial biological resources to academia, biotechnology, and bio-industries contributing to technology, economy and social developments

They have also reached the following agreements:

1. Prompt action of each country toward ex situ conservation of microbes is imperative.
2. For effective ex situ conservation of microbes, international research cooperation is essential.
3. Active international research cooperation needs to be promoted by establishing a scheme to facilitate international transfer of microbial resources, further provision of technical cooperation, and capacity building in full compliance with the principles of the Convention on Biological Diversity.
4. For clarification of endangered microbes and conservation areas, a list of domestic microbes should be created.
5. Microbial taxonomists should take an initiative on the creation of such a list with the support of international research cooperation.
6. Demand for and importance of microbial taxonomists should therefore be well recognized in each country, so that having training programs in place for microbial taxonomists who can keep inter-generational continuity seems imperative.

To achieve the intention of this Kazusa Statement, the establishment of a Microbial Resource Center (MRC) in each country is necessary. By establishing the MRC, the training program for microbial taxonomists, legal management of microbial resources, and the creation of the list of domestic microbes through exploration, characterization, conservation, and sustainable utilization of these microbial resources can be carried out. Furthermore, the MRC can make a significant contribution to the development of the bio-industry by providing scientific and technical services to various users. The MRCs in countries must endeavor to maintain close coordination with each other and dedicate to exploration and promotion of utilization of microbes.

Ken-ichiro Suzuki
Biological Resource Center, National Institute of Technology and Evaluation, Chiba Pref, Japan

Box 11.6 The Brazilian Network

The increasing demand for high-quality biological material and information as a consequence of the growth of the Brazilian bio-based economy is requesting the implementation of strategies and funding mechanisms to enhance and consolidate an integrated infrastructure of distributed biological resource centers. The goal is not only to underpin the actual needs for biological material from industry and academia, but to foster innovation in biotechnology, addressing issues related to emerging legal, technical, and sanitary barriers associated with the access of biological material and genetic resources to the global market.

History of the Brazilian Resource Centers Network

The need for consolidating a network of microbial collections in Brazil was discussed for the first time at the Second International Congress of Culture Collections held in São Paulo in 1973. The recommendations from this congress organized by the Brazilian Society of Microbiology (SBM) in collaboration with the WFCC influenced the 1980s and 1990s strategies for development of biotechnology in Brazil. A key enabling instrument for improving microbial resource centers in Brazil was the implementation of the Program for Human Resources in Strategic Areas (*Programa de Recursos Humanos em Áreas Estratégicas -RHAE*) funded by the Ministry of Science and Technology (MCT). The RHAE program developed in collaboration with WFCC promoted the training of a number of experts in international collections associated with the organization of nearly 50 training courses and seminars focused on issues related to collections management, preservation techniques, and microbial taxonomy.

In the late 1990s the effort carried out by the Organization for Economic Cooperation and Development (OECD) to discuss the critical role of microbial collections as infrastructure to underpin biotechnological innovation was crucial to renew the discussion on the need to improve the bio-collections infrastructure in Brazil. The OECD report on "Biological Resource Centers: underpinning the future of life sciences and biotechnology" (http://www.oecd.org/dataoecd/55/48/2487422.pdf) published in 2001 was the catalyst factor for the establishment of a Task Force to discuss the conformity assessment of biological material. The result of the work carried out by the Task Force is summarized in the document *Sistema de Avaliação da Conformidade de Material Biológico* (MCT 2002, System for the Conformity Assessment of Biological Material) (http://www.ctnbio.gov.br/upd_blob/0000/10.pdf). This timely report summarizes the state of the art of Brazilian collections within the framework of the international scenario and discusses the challenges and opportunities associated with the implementation of a conformity assessment system for biological material in Brazil. The assessment of the local legal framework compared to international norms and guidelines, associated with a proposal for capacity building was key to guide the participation of Brazilian experts at the OECD Biological Resource Center Task Force that resulted in the publication of the "OECD Best Practice Guidelines for Biological Resource Centers" (http://www.gbrcn.org/fileadmin/gbrcn/media/OECD_guidelines_for_brc.pdf) and the "OECD Best Practice Guidelines for on Biosecurity for BRCs" (http://www.oecd.org/dataoecd/6/27/38778261.pdf) in 2007. The Brazilian document on conformity assessment and the OECD guidelines were incorporated as appropriate in the

MCT capacity building strategy and discussed at biannual events organized by the Brazilian Society of Microbiology (SBM). The importance of SBM support to Brazilian microbial collections in this decade was reviewed by Canhos et al. (2007).

Based on the recommendations of the ''System for the Conformity Assessment of Biological Material,'' the MCT launched a capacity building program to improve quality management in selected service collections. The institutional arrangements and the effort to reorganize the institutional systems of collections in Brazil were reviewed by Canhos et al. (2009).

The need to develop strategies focused on the reorganization and consolidation of the infrastructure to support biotechnological innovation in Brazil was addressed by the Presidential Decree 604 (http://www.planalto.gov.br/ccivil_03/_ato2007-2010/2007/decreto/d6041.htm) signed on 8 February 2007. The decree establishes the National Policy for the Development of Biotechnology and makes specific recommendations for the modernization of microbial collections as a key step in the implementation of the Brazilian Network of Biological Resource Centers (Br-BRCN).

Networking Model and Institutional Arrangements

As opposed to establishing a large national center to provide a wide range of biological materials and specialized services, the Brazilian strategy is focused on the consolidation of a distributed network of specialized resource centers to meet the growing demands of the user community. The reorganization and quality management enhancement of networked collections in organizations such as Fiocruz (*Fundação Oswaldo Cruz*) (http://www.fiocruz.br//cgi/cgilua.exe/sys/start.htm?infoid=5574&sid=17) and Embrapa (Empresa Brasileira de Agropecuária) (http://www.embrapa.br), and specialized collections like CBMAI, the Brazilian Collection of Environmental and Industrial Microorganisms (http://webdrm.cpqba.unicamp.br/cbmai/english/index.php) represent important starters in the strategy for the implementation of the Br-BRCN.

Fiocruz coordinates one of the best-structured networks of epidemiological control and public health in the world and hosts several microbial collections with holdings ranging from archaea, bacteria, and fungi to protozoa. For the last 5 years, Fiocruz has been working on the harmonization of procedures and protocols, focusing on quality management based on ISO/IEC 17025/05 and OECD Best Practice Guidelines for Biological Resource Centers. This program is supported by the installation of the information management software, which is at the moment integrating data from 11 collections at Fiocruz with the System for Collections of Biotechnological Interest (SICol*Net*) (http://sicol.splink.org.br/). The Fiocruz *Leishmania* Collection (CLIOC) (http://clioc.fiocruz.br/index?) a Reference Collection of the World Health Organization (WHO) which is being prepared to be the *core* collection of the Fiocruz BRC. Its experience will be replicated to the other culture collections at the institution. CLIOC has a specialized holding with more than 1,000 *Leishmania* strains, mainly from the New World. CLIOC's mission is dedicated to preservation, storage, distribution, taxonomic characterization, and identification of *Leishmania* and associated information. CLIOC services meet the needs of public research and educational institutions, industry in general, offering assistance and technical and scientific consultancy, training and development of specific research projects.

The MCT's capacity building program focused on quality management in selected microbial collections as candidates to acquire the status of Biological Resource Center (BRC). It allowed the participation of CBMAI and CLIOC in the Demonstration project for a Global Biological Resource Center Network (GBRCN) (http://www.gbrcn.org/). This project is supported by the German Federal Ministry of Research and Education (BMBF) following work in the OECD to improve access to high-quality biological resources and information to support research and biotechnology as a platform for a knowledge-based bioeconomy.

MCT's program aiming at the establishment of the BR-BRCN is being implemented in close coordination with the activities sponsored by Brazilian Ministry of Development; Industry and Foreign Trade (MDIC) focused on the establishment of a Depository Authority for patent purposes at the National Institute of Metrology, Standardization, and Industrial Quality (INMETRO) in association with the National Institute for Industrial Property (INPI); and the implementation of the INMETRO program for certification and/or accreditation of Biological Resource Centers in Brazil.

Information System Architecture

To support the consolidation of the Brazilian network of resource centers the MCT is funding the development of the μSICol software and implementation of SICol*Net*.

The μSICol is a collection management software to support digital documentation and traceability of all processes associated with day-to-day management of microbial collections, including methods and procedures for strain authentication, preservation techniques, stock control, quality management procedures, and distribution of strains and biological reagents. The software is a multiplatform system, designed to be compatible with different data management systems. It has multi-user and multi-language capability and supports the installation of multiple collections and sub-collections. It is designed to document specific fields of importance to microbial collections based on the WFCC Guidelines for Operation and Management of Collections of Cultures of Microorganisms (Second Edition, 1999) (http://www.wfcc.nig.ac.jp/GuideFinal.html), the Common Access on Biological Resource and Information (CABRI) Guidelines (http://www.cabri.org/guidelines.html), and the OECD Guidelines for Quality Management and is fully compatible with the DarwinCore extension for microbial strains (http://rs.tdwg.org/dwc/). The database provides a specific view that allows the exchange of data using TDWG Access Protocol for information Retrieval (Tapir) (http://www.tdwg.org/dav/subgroups/tapir/1.0/docs/tdwg_tapir_specification_2010-05-05.htm) in a simple and immediate way. The system is being

continuously developed to accommodate new features and requirements, including reports of daily activities and indicators of the collection holdings including taxonomic profile, geographic distribution of deposits, clients, and services provided.

SICol*Net* allows the dynamic integration of strain data available in Brazilian collections with relevant information sources ranging from molecular to ecosystems databases. Alignment with emerging technologies and adoption of internationally agreed standards and protocols to secure systems interoperability are key features of SICol*Net* architecture. The Virtual Catalogue of Strains based on the architecture developed for *species*Link (http://splink.cria.org.br) allows the dynamic integration of strain data with information on host organisms (botanical and zoological information). Using a simple system of mapping and filtering of sensitive data, the system allows data providers to have full control over the data served to the network, with an appropriate crediting system. Each collection determines what data is restricted and what is public. Through a web interface, users may search and retrieve nonsensitive data in different formats, may rapidly and efficiently visualize species occurrence data on maps, and also have access to a number of indicators. The system also provides reports on each collection's profile, based on metadata and on the analysis of online data and reports on data quality.

Future Developments

It is expected that, in 2012, the Depository Authority for patent purposes at the National Institute of Metrology, Standardization, and Industrial Quality (INMETRO) will be in operation and that the regulatory framework for the accreditation of resource centers candidates to acquire the BRC status will be in place in Brazil. Fiocruz is working on the implementation of a large-scale/long-term effort to establish a Biological Resource Center for Health (BRC - Health) – unique in the world –focused on the study, preservation and distribution of microorganisms and biological materials relevant to neglected diseases; innovation in epidemiology surveillance; as well as the development and production of bio-compounds directed to diagnosis, vaccines, and drugs.

V. Canhos[17] · E. Cupolillo[18] · M. da Silva[19] · C. Pirmez[19]
[17]Reference Center on Environmental Information (CRIA), Brazil, UK
[18]Fiocruz Leishmania Collection (CLIOC), Rio de Janeiro, Brazil
[19]Fiocruz Vice-Presidency for Research and Reference Laboratories Rio de Janeiro-RJ, Brazil

Similarly, individual collections cooperated in other global initiatives or in those of other communities. All of these initiatives have common goals: that the enormous amount and range of both the living biological material itself and the data pertaining to this material are placed at the rapid disposal of researchers in academia and industry under full legal compliance and curated in a pure and authentic form.

Examples for Joint Activities and Global Cooperation

EU Projects Tackling Quality Issues of Data and the Biological Material

The Microbial Information Europe (MINE) project was an early ambitious, taxonomy-based start with a clear user-driven data selection. The main goals were the harmonization and digitization of data on over 150,000 catalogue strains, standardization of formats, and contents of fields of databases, as well as common thesauri (Gams et al. 1988; Stalpers et al. 1990).

On this important base, the Common Access to Biological Material and Information (CABRI) project was built to offer online access to data across various collection databases in Europe with search options through individual and combined catalogues of collections. The great merit of this project was to develop guidelines for two of the service aspects of culture collections: (1) for the handling of the biological material and (2) for the handling of the related data. The laboratory side of the guidelines covered aspects of accession, authentication, maintenance, storage, and supply for such kinds of biological material as bacteria, archaea, fungi, yeasts, animal, human, and plant cells, and genomic material. A compilation of model in-house procedures was added. On the data side, minimal data sets (MDS) and recommended data sets (RDS) were agreed outlining the minimum amount of data that should accompany a particular strain or culture when it is put into a publicly accessible catalogue. Both types of guidelines aimed at raising scientific and technical quality of holdings and data to better support modern research and application. CABRI was later incorporated as a core activity into the EBRCN project, and CABRI guidelines are still available today.

A project with minor microbial participation was the ENBI a regional complementation of GBIF (see below). It formed an intermediate level between national GBIF activities and the global GBIF level. One interesting outcome of this work was a monograph on digital imaging of biological specimens from the zoological, botanical, and microbiological areas. This work aimed at setting and publishing standards for improved quality photography. For the microbial side, among other items, the microscopical mounting method of "agar slides" was presented (Fritze 2005).

Providers and users of biological material worked together in the MOSAICC project on the development of a system for appropriate management of access to and transfer of microbiological resources (see report on http://bccm.belspo.be/projects/). The goal was in particular to help implement the provisions of the CBD concerning Prior Informed Consent (PIC) and mutually agreed terms (MAT) in the context of monitoring transborder movements of biological material.

Within the European Biological Resource Centers' Network project (EBRCN, 2001–2004), emphasis was laid on the development of information documents concerning the various regulatory issues around collection work, such as classification of microorganisms on the basis of risk, Convention on Biological Diversity (CBD), intellectual property rights, regulations

governing packaging and shipping (2010 update available) and control of distribution of possibly dangerous microorganisms (http://www.ebrcn.eu/).

Recent EU Activities

The ongoing European Consortium of Microbial Resources Centers (EMbaRC; 2009–2012) project includes work on improving protocols for the authentication and preservation of cultures and combines the additional aspects of training and research activities. Within the area of compliance with regulatory requirements, emphasis is laid on the development of a Biosecurity Code of conduct. It aims to improve, coordinate, and validate microbial resource center delivery to European and international researchers from both public and private sectors. The EMbaRC project is a mixture of networking, access, training, and research (http://www.embarc.eu).

MIRRI: A New Initiative Within the EU Strategy Level of ESFRI

A new initiative to strengthen the European innovation capacities was developed by the European Strategy Forum for Research Infrastructures. The microbiology collection community led by the GBRCN secretariat (see below) and supported by ECCO and EMbaRC worked with ESFRI state representatives to place the Microbial Resources Research Infrastructure (MIRRI) on the ESFRI road map and as such is a priority for research funding. The resultant high-quality global platform will be designed to accommodate the future needs of biotechnology and biomedicine.

MIRRI will bring together European microbial resource collections with stakeholders (their users, policy makers, potential funders and the plethora of microbial research efforts) aiming at improving access to enhanced quality microbial resources in an appropriate legal framework, thus underpinning and driving life sciences research. Emphasis will be laid on the conservation of biodiversity, on services for research and application, as well as CBD-ABS, biosafety, biosecurity, and bio-risk matters. The overall aim is to support research, development, and bio-economy by improving access to and use of the microbial material. On this platform, strong interaction of all kinds of stakeholder working with microbiological material will be enabled, ranging from scientific and industrial providers and users to collections and policy makers, as well as to regulatory bodies and others. Non-European participation is strongly encouraged. MIRRI will integrate services and resources, bridging the gap between the organism and provision of innovative solutions. MIRRI will as well provide coherence in the application of quality standards, homogeneity in data storage and management, and workload sharing to help release the hidden potential of microorganisms. All 57 microbial resource center members of ECCO in the 26 European countries are invited to join the initial consortium of collaborators in this initiative.

Toward a Global Network

To deliver their services, BRCs preserve their holdings using long-term storage techniques such as cryopreservation and lyophilization depending upon organism type. Quite often, these techniques require optimization to enable not only survival but also retention of properties. Seldom can a single collection invest in preservation research, and often the improvement and testing of new techniques is done through projects. Networks can support each other to carry out research. Not every collection has the ability to handle every strain they are offered, and networks can share the burden with organisms being deposited in BRCs which have the expertise and facilities to handle them. There is extensive legislation that impacts upon access to, the safe handling, distribution, and use of biological resources (Fritze and Weihs 2000; Smith and Rohde 2007, 2008). A number of culture collection organizations exist to help collections keep up to date in a constantly changing legal framework notably biosecurity, shipping regulations and ethical access and use, common information resources can be established and common procedures implemented across the network to ensure compliance. Therefore, networks can increase single BRC capacity.

The work of the culture collection organizations has been invaluable and has only been limited by their voluntary nature, relying on input of dedicated people as and when they can contribute. Collections need to increase the availability of biological material for the verification of experimental data and the authenticity of reference material used in research. Deplorably, the scientific literature is full of data which cannot be verified because the material is either no longer available and/or the material once used to generate the data has changed or deteriorated. This challenge needs to be met with a coordinated approach requiring an infrastructure to support it. Such strategies cannot be achieved by projects with a defined lifespan. At a global level, the GBRCN aims at bringing together regional efforts such as those of ECCO help disseminate the outputs of projects such as EMbaRC as well as the Asian initiatives (*Box 11.5*) and national activities such as those in Brazil (*Box 11.6*) will play a key role.

The GBRCN demonstration project emanates from an OECD Working Party on biotechnology initiative. For boosting the activities, a small central secretariat is presently supported by the German Ministry of Research and Education (BMBF) to coordinate activities to deliver improved support to the life sciences. High-quality research in the life sciences and innovative solutions to global problems requires access to high-quality biological materials and associated information. No one single entity can provide the necessary coverage of organisms and data; therefore, the enormous task of maintaining biodiversity must be shared. Although the goal of the GBRCN would be to bring together BRCs from all four domains, animal, plants, human-derived material and microorganisms, the project focus was on microorganisms.

The GBRCN demonstration project secretariat coordinates some activities of candidate microbial domain BRCs in 15 countries in order to deliver:

- The establishment of a network differentiated from existing organizations
- The implementation of OECD best practice in BRCs (OECD 2007) assessed by independent third parties
- A strategy for the full GBRCN defining its infrastructure and governance mechanisms, its secretariat's structure, and function with a program of activities

A GBRCN will also help the science community address the maintenance of biodiversity range and magnitude, meeting biosecurity requirements, bridging gaps in our knowledge and protecting investments in research. A GBRCN will support the BRCs keeping abreast of modern scientific developments, meeting quality needs for research, supplying authentic cultures, supplying standardized biological material for testing and quality control, developing comparable methodology and harmonizing procedures and reconciling research and development demands with compliance with regulations.

Exploitation of biological materials must be in compliance with conventions, treaties, and law, for example, the CBD. The CBD requires that Prior Informed Consent (PIC) be obtained in the country where organisms are to be collected. Terms, on which any benefits will be shared, must be agreed. The benefits may be monetary but could be nonmonetary such as information, technology transfer or training. If the organism is passed on to a third party, it must be under terms agreed by the country of origin. This will entail the use of material transfer agreements between supplier and recipient to ensure benefit sharing with, at least, the country of origin. Access and benefit sharing rules must be followed by those countries having signed the Nagoya ABS Protocol (CBD 2011). The national implementation of the protocol may well impede access and exchange of materials and information. In this context, the collection community will have to work toward a mutually beneficial multilateral operational framework to facilitate science and the discovery process.

Biosecurity (Box 11.7) impacts heavily on the operations of public service microbial domain Biological Resource Centers, hence the activities of the WFCC and GBRCN. The GBRCN and the European EMbaRC project promote the implementation of OECD BRC best practice which includes the biosecurity guidance as well as aspects of biosafety, particularly in regard to implementation of national legislation. Concerns exist on financial constraints of BRCs/culture collections to implement best practice regarding biosecurity, particularly with the requirement of risk assessment. Another key concern is the lack of easy access to regulations and other information regarding national rules and regulations governing the movement of materials. It is evident that culture collections adopt compliant procedures firstly governed by national laws but specifically compliant with the Biological and Toxin Weapons Convention (BTWC). They must endeavor to reduce the potential for misuse of biological agents, toxins, or associated information or technologies. To this end, the GBRCN and EMbaRC projects have designed a Biosecurity Code of conduct for BRCs which, when finalized, will be (morally) binding for GBRCN members. The Biosecurity Code of conduct for BRCs sets out an undertaking by microbial BRCs to tackle their responsibilities and provides a baseline for their operation.

Box 11.7 Biosecurity

Research on biological material and the resulting knowledge have benefitted mankind in many respects, ranging from basic science to applied agriculture to medicine and biotechnology. However, as so often, scientific results can also be used for malicious purposes – the dual use potential. This possibility includes not only information, but also access to the biological material itself. At first the political accent was on biological warfare. Bioweapons are attractive because they are relatively cheap, leave the infrastructure intact, are self-perpetuating but may allow immunization, and have a delayed onset. Hence political activities concentrated on arms control, resulting in the Biological and Toxic Weapons Convention (1972, BTWC) with the aim to prohibit the development, possession, and use of biological weapons, and in the Australia group, which intends to prevent the supply of harmful organisms to malafide third parties.

When Ivins in 2001 sent a series of letters with contents contaminated with *Bacillus anthrax* spores, the risk of misuse of microorganisms suddenly became apparent; he changed the microbial world. Although the number of victims was limited (22 infected, 7 deceased), the consequences were severe as the public was shocked. The horror scenario of a mad scientist threatening society had suddenly become reality. The trust in a world containing only scientific institutions with sufficient instruments of self-control had been shattered. Biosecurity issues became a major concern for politicians, who immediately reacted by increasing the budget for biological warfare research and regionally by radiating imported parcels, thus jeopardizing the sharing of all biological material. The latter was remedied quickly, but the need for well-executed and transparent biosecurity regulations and the raising of public awareness remained. The task to restore the trust was taken up by both international organizations and the scientific community, and two major contributions were made to provide clear and reliable guidance: the OECD Best Practice guidelines for BRCs (2007), including the OECD Best Practice guidelines on Biosecurity for BRCs, and the Laboratory Bio-risk Management Standard, CWA 15793 (2008).

In analogy with biosafety, biosecurity also recognizes four risk categories. They are labeled negligible, low, moderate, and high risk, and by necessity the definitions for these categories do not allow unambiguous classification. Moreover, they are based on the biosafety classification, and hence focused on threats against humans, not crops.

The international standardization of lists dealing with the organism content of biosecurity risk groups, or at least with those directly affecting humans, would be advantageous, but

the political impediments are considerable and have not been solved yet. With regards to plant pathogens, the situation is even worse, because national legislation only concentrates on national interests, e.g., in the absence of a host in the respective country, a pathogen for that host is not considered to represent a risk. However, potential abusers may obtain such material from research groups or BRCs in those countries where such an organism is not on the quarantine list. This must be prevented by all means.

In order to decide on the necessary biosecurity measurements for a specific organism, a risk assessment has to be performed. As the potential targets for dual use are not only humans but also crops, life stock, or the human environment in general; these elements have also to be considered, and the biosecurity classification cannot be a simple translation or adaptation of an existing biosafety classification. Although for the human aspects there may be a considerable similarity, there will also be significant differences. For example, the highest biosafety category contains only viruses, while the highest biosecurity category contains also *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*, next to the toxin producer *Clostridium botulinum*. Especially where risks for crops are concerned, the role of fungi (which with a single possible exception does not qualify for the highest biosecurity level with regard to humans) becomes important.

The requirements for a risk assessment in compliance with the OECD Best Practice guidelines are high. When sources of potential harm have been identified, the following elements have to be considered to assess the potential for misuse:

- Availability of the organism in nature
- Requirements (necessary skills and knowledge) for isolation and reproduction
- Environmental viability (survival chances)
- Conditions for dispersal (air or contact)

In case of virulence, knowledge on the infective dose, pathogenicity, lethality, incubation time, and transmissibility is required, as is information on the presence of effective countermeasures.

It will be clear that due to the high demand, many organizations and institutions cannot fully comply with these requirements, and ways have to be found to remedy this. In practice, bio-risk assessment is performed by comparison, which includes the biological material, its interaction with the substrate, dispersal system, knowledge of properties of taxonomic relatives, even tests on the organism itself. Data on virulence are usually absent, or scattered in the literature, and a tedious search for such properties need to be performed. In practice it has worked well as far as the author is aware, but in order to comply with the guidelines, collaboration with other facilities and access to specialist knowledge has to be established. In this scenario, the outreach of international societies could play an essential role.

Within an organization having to deal with biosecurity issues, any respective measures need to be implemented as part of the quality management system and regulated and supported by the senior management. It is their task to integrate bio-risk management throughout the organization, provide adequate resources and identify opportunities for improvement and prevention. They are responsible for the appointment of qualified staff and for subsequent training to maintain the desired quality. They have to convince the funding bodies of the necessity for good biosafety and biosecurity management and to provide the personnel with a supportive environment, involving working space and equipment. They also are responsible for compliance with legal requirements, communication to staff and relevant third parties, and for a reliable and appropriate risk assessment. Finally they are responsible for screening the outgoing information on potential dual use. In practice they should appoint a Biosecurity Officer to ensure internal compliance with the adopted regulations.

In order to obtain maximal collaboration of staff, it is essential that the awareness level be high. It is necessary to devote specific and sufficient attention to the education and additional training of all staff to the risks of misuse of biological material, information and life sciences research and the requirements of regulations in this context. This requires not only training in, but also auditing of, knowledge and practices with regards to biosecurity. Moreover it is also the responsibility of a biological resource collection (BRC) or research group to inform involved third parties on their responsibilities, for example, when high-risk biological material is shipped to authorized users.

Within an organization, accountability is an essential element. Both management and staff should be aware of the presence, location, and risk of the organism they are using. BRCs should maintain and update inventories of the biological material in their custody. Any finding or suspicion of misuse of biological material, information, or technology has to be reported immediately and directly to competent persons or commissions within the organization. To maintain trust in these institutions, persons reporting on misuse have to be protected. They must not suffer any adverse effects from their actions.

Restriction to the accessibility of potential dual use biological material is a vital element in biosecurity management. Depending on the appropriate biosecurity risk level resulting from the biosecurity risk assessment, physical security has to be selected. For a low level, a generally secure area is sufficient, but for a moderate or high-risk level, respectively, a restricted area or a high-security area, with different degrees of containment, are necessary. Staff and visitors have to be screened before access is allowed to areas in which potential dual-use biological materials are stored or used.

Scientists and BRCs should undertake an information risk assessment to determine what information presents a potential biosecurity risk and steps need to be taken to protect such information that could be used to locate the material and facilitate theft. During assessment or application procedures and during the execution of research projects on potential dual-use aspects, emerging threads have to be considered. Any risk that publication of results on potential dual-use organisms will contribute to misuse of that knowledge has to be minimized.

Access of unauthorized persons to internal and external e-mails, post, telephone calls, and data storage concerning

information about potential dual-use research or potential dual-use materials has to be prevented and communication of sensitive information has to be regulated.

Transport of biological material classified as moderate or high requires special conditions, both inside and outside the organization. Inside the organization, transport containers are required and high-risk material may never be unattended outside the high-security area. In case of transport to third parties, both these parties and the transporters have to be screened for both their capacity to deal with the material and their intentions to prevent dual use, in consultation with the relevant authorities and parties. Export control has to be performed in accordance with applicable regulations.

For packaging, the WHO guidelines on International Regulations for the Packaging and Transport of Infectious Substances and the International Air Transport Association (IATA) Dangerous Goods Regulations (DGR), or other applicable regulations, for example, for road transport regulations in various countries, should be utilized.

International projects and organizations are now working on a Code of Conduct for BRCs, combined with an inventory of problems occurring in practice when implementing the guidelines. They are also involved in the setting up of a database, which should allow fast and reliable information on legislation and regulations per country, for example, import and export regulations for microorganisms, transport regulations, quarantine organisms, biosafety and biosecurity regulations, classification lists for human pathogens, animal pathogens and plant pathogens. It should also contain a list of experts that could advise on biosecurity items.

Joost Stalpers
CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

Capacity Building

The paucity of national BRCs and the challenges outlined above necessitate an efficient coordinated program for capacity building. Again, a task that is better organized and delivered by a network. The major regions or countries of the world are serviced by collections providing whole organisms, but there are gaps particularly in countries rich in diversity but poor in resources. There are about 60 major collections supplying cultures in Europe while there are very few in Africa. It is necessary to establish expertise and facilities to access conserve and utilize microbial diversity. Capacity building is one of GBRCN's pillars for the sustainable development of Biological Resource Centers. Through the transfer of knowledge as well as skills and abilities, GBRCN supports BRC candidates as well as acknowledged BRCs and accompanies them through the development process. The focus of the capacity building is

- To help countries to establish BRCs
- To enable BRC candidates to become BRCs and GBRCN members
- To assist approved BRCs to gain higher levels of compliance to the OECD best practice guidelines (BPG)
- To support political and socioeconomic decision-making processes in the fields of BRC related bio-economy

Authenticity Check of Microorganisms

One of the most crucial tasks of public collections is the delivery of authentic material that matches most closely material originally accepted by the collection. Changes can occur through human error during routine maintenance steps of subcultivation or intrinsically due to one of the laws of biology, namely, populations of organisms change genetically and irreversibly through time (http://hunstem.uhd.edu/ABOUT/). Especially in microorganisms, detection of such changes is challenging as short generation times and high mutation rates unavoidably lead to the formation of heterogeneous cultures. Diversification occurs at the genetic and epigenetic level during growth and repeated cultivation as has been observed for decades and experimentally proven more recently (Lenski and Travisano 1994; Rainey and Travisano 1998). As pointed out by Arber (2003) using *Escherichia coli* as an example, spontaneous genetic mutations occur in single cells at a rate of 0.1–1 % of cells per generation during exponential growth and also in the stationary phase. If cells were singled out, the genetic variation would be subjected to natural selection and consequently to reproductive and geographic isolation. The observation of ecotypes reported for strain-rich species, differing in physiology, genome content, and ecology (Cohan and Perry 2007), already starts at the strain level.

Collection curators are aware of the risk of increased microevolution by repeated sub-cultivation, most obvious by the loss of plasmids and changes in colony morphology, and have reacted by keeping the numbers of culture transfers to a minimum. As minute changes may occur during growth and sudden environmental changes such as refrigeration and freezing, most public service collections in their terms of delivery include a clause in the paragraph concerning the quality of products phrases such as the one used by the DSMZ: "Customer is aware of the products and services being biological material and therefore subject to changes of quality beyond the control of DSMZ. DSMZ's high quality standard supports availability of pure, viable and authentic biological material. DSMZ shall not be deemed to have guaranteed certain properties of the products and services except if it has expressly confirmed such guarantee in writing."

In order to ensure as far as possible the originality of the cultures, strains deposited are preserved with a minimum number of transfers. The type of preservation method depends on the organism, but freezing a few straws or alternatively glass capillaries with material from the original culture received in liquid nitrogen is good practice (see http://www.cabri.org/guidelines/micro-organisms/M204.html). Depending on the estimated number of requests by users, this master stock should contain between ~6 and ~50 straws/capillaries, one of which is used for

the production of lyophilized cultures. The number of replicates of this stock, too, depends upon the estimated user demand.

Authentication differs from the characterization of a strain in goals and effort. Characterization attempts to circumscribe a novel organism by as many properties of taxonomic value as possible to allow subsequent unambiguous identification. The range of properties to be determined is usually set by the properties defined to be useful for members of taxa (usually species and genera) described previously. Authentication may only use a subset of such properties or, especially today, molecular traits that had not been included in the original description. In both cases, characterization and authentication, purity checks of the culture under investigation, is mandatory. In the pre-molecular era, authenticity checks relied upon microscopy, culture characteristics (e.g., pigmentation or colony characteristics) and basic physiological tests. As the latter requires time for growth, confirmation of identity took at least a few days. The introduction of semiautomated commercial substrate utilization and enzymatic reaction panels shortened the time for authentication. These tests work well for those taxa which were among the hundreds of strains used for the construction of associated databases, such as those used in the food and pharmaceutical industry and on species found routinely in the clinical environment. As databases are not available for strains of new species, the use of such identification systems often delivers disappointing identification results and misidentifications. Also, as nowadays descriptions of newly named species embrace a single strain only, the type strain, the spectrum of reactions is usually too narrow to unambiguously identify additional strains of such species which may deviate in their physiological reaction from the type strain. Nevertheless, species descriptions published over the past 20 years rely on commercial kits for circumscribing substrate utilization patterns, acid production and the activity of enzymes and only rarely manually prepared media for checking such properties (e.g., Smibert and Krieg 1994). Consequently, in order to obtain more reliable test results an ad hoc committee for the reevaluation of the species definition in bacteriology (Stackebrandt et al. 2002), and more recently Tindall et al. (2010) strongly encourage authors of new descriptions to refrain from commercial kits for studying metabolic reactions.

For culture collections, the molecular era started with the introduction of one-dimensional protein patterns (Kersters and De Ley 1975), restriction enzyme and PCR-based techniques and 16S rRNA gene sequence analyses (Fischer et al. 1983). Although the latter method has the advantage of being highly reproducible and allows the generation of a cumulative database, the highly conservative primary structure does not allow discrimination at the strain level. Two other molecular techniques, DNA-DNA hybridization (DDH) (Johnson and Ordal 1968) and DNA-rRNA hybridization (DeLey and De Smedt 1975), have been used for classification at the family and genus level, respectively. However, they have failed to authenticate at the strain level. Even the DDH method lacks reproducibility to affiliate isolates to strains. Several molecular typing methods (PFGE, AFLP, RAPD, RFLP, and more) are more reliable for strain authentication though the lack of a portable database somewhat restricts the applicability of these techniques to the level of individual laboratories. Molecular methods can differ widely in their ability to differentiate strains, and the user should be aware of the strength and restrictions of the individual methods. An excellent summary of early techniques available in the 1990s, their intra- and interlaboratory reproducibility, equipment needed, costs, and duration, has been given by Olive and Bean (1999).

Among the typing methods, the ribotyping (Grimont and Grimont 1986) approach has been successfully established in several public and industrial collections, executed either manually or by the automated Riboprinter microbial characterization system (E. I. du Pont de Nemours & Co., Inc.). It has especially facilitated strain identification over the past 10 years through the ease and reproducibility of the robot functionality. The advantage of ribotyping over the amplified rDNA restriction analysis (ARDRA) (Pukall et al. 1998) approach lies in the use of the entire rrn operons rather than of individual 16S rRNA genes. In that restriction sites also occur within structural genes, the spacer regions including tRNAs and the flanking DNA regions, the diversity of fragments hybridizing to a fluorescently labeled rrn probe is much higher than those of ARDRA.

The recent introduction of the MALDI-TOF approach is a major step forward in a fast and cheap, though highly reliable, authentication at the strain level. While riboprinting works on the gene level, the highly amplified ribosomal proteins are the main targets for the MALDI-TOF spectrometric method. The resolution power of this method is significantly higher than that of comparative 16S rRNA gene sequence, often being suitable for affiliation of strains to the subspecies level. However, as the species as defined today does not necessarily reflect genomic coherency, the range of intraspecies dissimilarities determined for strains may vary between different taxa. A linear TOF mass spectrometer operates on the principle that when a group of ions of differing mass/charge (m/z) ratios move through a region of constant electric field, they will traverse this region in a time which depends upon their m/z ratios (for more details and the principle of a reflection TOF-MS, see http://www.abrf.org/abrfnews/1997/june1997/jun97lennon.html). As not all proteins occurring in a cell will be charged, only a small fraction of protein masses are detected, usually in the mass range between 2,000 and 12,000 m/z. The advantages of working with this approach, especially for the identification of those species for which a high number of strains (mainly of clinical origin), have been analyzed, and spectra deposited in databases have been outlined in Chapter X of Konstantinidis and Stackebrandt (2011). Public service collections usually cover a broad range of diverse microorganisms for which commercial MALDI-TOF databases are less well suited. In this case, the authentication laboratory has to generate tailor-made in-house databases for specific taxa. The method has been used to verify the authenticity of many type strains of different taxa maintained in different EMbaRC collections (Schumann, Bizet, Arahal, and de Vos, unpublished). The majority of these strains showed highly similar MALDI-TOF protein profiles, indicating that microevolution does not affect the masses of housekeeping proteins and ribosomal proteins; the few strains identified as being

misclassified or mislabeled have been discarded and exchanged with authentic ones. MALDI-TOF appears to be the method of choice for routine identity checks of strains in long-term storage and for authenticity checks before shipment.

Access to Microbial Biodiversity Data

A wealth of invaluable information for academic and industrial users as well as regulatory bodies is available in resource collections, and research data are scattered in hundreds of thousands of publications since the beginning of microbiology. To put this treasure at hand, individual approaches will not suffice any more to meet the global needs. Modern information technology developments will offer increasingly easier access to data, while working toward interoperability of databases will make searching through countless databases all over the world practicable. In order to archive a comprehensive overview on published data and to offer these data to the user, standardization of data production, data recording, and data presentation should be harmonized, at least to allow text mining software to efficiently extract data. The unstructured recording of phenotypic data in current species descriptions of prokaryotes is a simple example of the present-day failure to cope with strain-associated data. It is, however, promising to see recent attempts to standardize the electronic exchange of meta-information about microorganisms which led to the definition of the Microbiological Common Language (MCL) (Verslyppe et al. 2010) within the framework of the Bacterial Commons (Dawyndt et al. 2006).

CODATA, MINE, CABRI, and GBIF: Examples of Past and Ongoing Activities

A number of initiatives aiming at the coordination and standardization of activities to give access to information available at culture collections have been set up. Among them, WDCM (see *Box 11.3*), CODATA, MINE, and CABRI have set baselines and provided model roles.

CODATA, the Committee on Data for Science and Technology, is an interdisciplinary Scientific Committee of the International Council for Science (ICSU) founded over 30 years ago. Its main objective is to foster and advance science and technology through developing and sharing knowledge about data and the activities that work with data (www.codata.org).

MINE (see for details further above) was designed to harmonize and computerize data on >150,000 strains of microorganisms in European culture collections and to develop a common database on microorganisms held in culture collections. Requirements for efficient data recording and computerization were established by the 12 participating European countries together with details of the data structure used, the hard- and software configurations, data entry procedures and online access. The main merit of MINE has been the development of an internationally agreed format: 135 fields for fungi and yeasts and 145 fields for bacteria which cover the most important aspects of microbial taxonomy, ecology, physiology, and biochemistry, also including data pertaining to the practical applications of microorganisms. The disadvantage is that entries need to be added manually, thus prone to errors and omissions.

The Common Access to Biological Resources and Information (CABRI, see further details above) has its roots directly in the previous MINE project from which it inherited the dedicated taxonomic data structure. Its main goals are to increase awareness of the scientific user community of the quality and variety of European culture collections and to ease access to information and material. In order to reach this objective, the project has implemented a unified access to culture collection catalogues of participating collections, by also guaranteeing a common level of quality of material and related information. The final achievement of the project has been the development of an online "one-stop-shop" for biological resources (www.cabri.org) which allows the user to check on the availability of a particular item, interrogating one or more catalogues at the same time, and to pre-order the required biological resources, once located. CABRI membership is open to any recognized European BRC, willing and able to work at the CABRI quality levels.

Participating resource centers agree to find consensus for data fields and content type, harmonize procedures and agreeing on equivalent methods and procedures and producing guidelines for each type of biological material. CABRI can be seen as a pioneer model for an integrated, while distributed database which is searchable through a common gate. CABRI is currently available through the main website (see http://www.cabri.org/) and four web mirrors (see http://www.it.cabri.org/, http://www.be.cabri.org/, http://www.fr.cabri.org/, http://www.cn.cabri.org/).

Three distinct data sets were defined for each biological material. The minimum data set (MDS) consists of mandatory information needed to identify a unique strain or cell line: strains for which this information is not available cannot be inserted into the catalogue since they lack essential data. The recommended data set (RDS) includes useful information for an improved description of the material. These data should always be included in the catalogue, when available. The Full Data Set (FDS) provides all remaining information that is available at the collection for a strain or cell line. Since the individual CABRI catalogues are independently built, each collection can have its own FDS, although information which is available in the FDS undergoes a harmonization effort (http://www.cabri.org/guidelines/catalogue/CPexport.html).

At an international level, major attention has recently been devoted to bioinformatics for biodiversity. Discussions initiated through the OECD Working Group on Biological Informatics 1996–1999 have led to the Global Biodiversity Information Facility (GBIF). GBIF aims to provide a worldwide network of interlinked biodiversity databases so that the worlds' scientific biodiversity data can be made freely available. It is based on an implementing agreement signed by governments and interested organizations. The initial focus of GBIF is on species- and specimen-level data. It works in close contact with existing and ongoing activities with similar or complementary goals on

a national and international level. Implementation of GBIF is nationally and/or regionally driven (see http://www.gbif.org/). However, microbial data are still today represented only on a smaller scale. The extended Darwin Core (as developed by the Brazilian group) reflects better the needs of microbiology than the ABCD Schema and will be the basis for the envisioned future information resource within GBRCN.

The OECD Best Practice Guidelines

In the OECD BRC best practice guidelines, detailed requirements are formulated for the processing of biodiversity data. Clear reference is taken to previous works such as CABRI, GBIF and WDCM. The following is an excerpt from OECD best practice guidelines – general (DSTI/STP/BIO(2007)9/FINAL), heading Data and Informatics which defines the responsibilities of resource centers and depositors concerning quality assurance:

Box 11.8 Data and informatics

39. The BRC should manage and store data and produce electronic catalogues based on authenticated and validated information.

Data Management

40. Depositors are responsible for assuring the quality of data associated with the biological material. The BRC may require evidence to assure the validity of the data.

41. The authentication of data may differ from center to center, but a BRC should:

- Provide traceability of data through a history of modifications (dates and signatures of inputs, validations, modifications and deletions).
- Give signature for data entry, validation, modification or deletion.

42. The BRC should use a standard terminology and formats for data management and exchange and standard protocols for data transmission to networks (domain, regional or global networks):

1. Select data format, data representation and data transportation taking into consideration existing standards for data processing, e.g. DarwinCore/DiGIR and ABCD schema/BioCASE for strain data, CCINFO for the organizational information of BRCs.
2. Check vocabulary against standard reference lists or thesauri.
3. Keep consistency among BRCs for searching and retrieving of information from catalogues and databases:
   - Each biological material record should contain a Minimum Data Set, a Recommended Data Set, and/or a Full Data Set in accordance with domain specific criteria.
   - Spell checking for every field should be a basic requirement.
   - International English should be chosen as a preferred language of data (in addition to local language if different).
   - A standardised approach should be adopted to certain scientific symbols (to avoid any errors due to incorrect reading of a character set, standard ASCII alternatives to symbols should be used (examples follow):

43. BRCs should adopt procedures to detect errors in data to improve their quality and consistency. This is an essential part of information management and should be both applied to the input of new data as well as to preexisting information in current databases:

- For existing data, a series of checks should be carried out to ascertain their validity and completeness. As more BRCs become associated, more searches should be made for common classes of error to allow more efficient error correction.
- For new data, wherever possible, inputting should be checked against authorized lists of not only scientific names but also thesaurus/ontology to prevent errors such as mistyping.
- BRCs should present evidence that they have applied a recognised protocol appropriate for each data element (A comprehensive treatment of Data Cleaning can be found in Chapman, A.D., *Principles and Methods of Data Cleaning – Primary Species and Species-Occurrence Data*, Version 1.0, Publisher - Global Biodiversity Information Facility (GBIF), 2005.

Data Processing

44. The informatics system employed by BRCs should provide appropriate facilities for information management, linkage and exchange.

45. The databases should contain either information relating to strains held by a BRC (which at least, should be retained as long as a strain remains viable), or other relevant data items or composite data needed by the BRC (e.g. users records). On the loss of a strain the database record should be either printed and stored on file or copied to a digital archive before the entry is removed from the working database, placed in reserve or annotated to indicate that it is no longer available as living material.

46. The BRC should preferably choose standard data schema and protocols to make the databases distributed and interoperable. Confidential data should be clearly identified in relation with user authentication capability, encryption techniques and other related information security tools.

47. The informatics system should ensure regular data back-up. Off-site storage of data is desirable. Data archives should be maintained in accordance with the maintenance of the biological resource storage policy. The support of these archives should be regularly updated according to its physical characteristics (obsolescence) and to software compatibility.

48. BRCs should introduce appropriate measures (protocols, tools and standards) in their own informatics systems to assure reasonable security of information. There are existing systems, e.g. authentication by user ID and password, encryption, encryption of messages and restriction of IP addresses that may provide the basis for such measures. Backup-files should be stored in secure cabinets.

From: OECD Best Practice Guidelines –General (DSTI/STP/BIO (2007)9/FINAL)

Minimum and Recommended Data Sets

Based on the previous works in MINE and CABRI, the concept of minimum and recommended data sets was adopted by the OECD guidelines and slightly updated, in particular with a view to the necessary information of "country of origin" for implementing the requirements of the Convention on Biological Diversity. It should be noted, however, that these attempts defined data fields but have failed to set up a standardized, open infrastructure that allows electronic processing. *Table 11.3* shows as example the data sets for bacteria. Similar data sets are also available for filamentous fungi, yeasts, protozoa, cyanobacteria, archaea, virus, plasmids, phages, and cDNA/cDNA libraries.

Table 11.3
Minimum (MDS) and recommended data set (RDS) bacteria

Minimum data set	Recommended data set (in addition to MDS)
Accession number	Serovar
Other collection numbers	Other names
Name	Isolated from
Infrasubspecific names	Mutant
Organism type	Genotype
Restrictions on distribution	Literature
Status	
History of deposit	
Geographic origin	
Conditions for growth	
Form of supply	

Long-Term Storage of Microorganisms: At the Very Heart of Collection Activities and Responsibilities

Why Do We Need Long-Term Preservation?

Proper preservation methods suspend metabolic activities instantly while retaining viability and genetic and physiological stability of the specimen. This is the basis for the safe and long-term maintenance of strains of microorganisms of scientific or industrial interest, which is an inevitable prerequisite for continuous and efficient research and production. Availability of strains maintained in a genetic and physiological unchanged state must be guaranteed over years. For example, important production strains, reference strains for research and testing and other strains with valuable properties should be available for comparative examinations even after decades. Or, to give another example, sometimes years after a certain bacterial species has been described, it becomes clear that strains of this species have important, useful properties. It is then most helpful to have the reference strain available in the most original state and ready to be shipped. If such a strain is lost, time, information, and research funds are lost as well, and the re-isolation of strains with exactly the same properties as that of the type strain is highly unlikely.

Despite the fundamental significance of reliable availability of pure and stable cultures, quite often, only little attention is paid to the maintenance of these cultures in research laboratories. It is obvious that in terms of strain maintenance, these facilities have different requirements than those required in a public culture collection. As usually neither equipment nor staff experience is available in the research laboratories, some basic procedures for proper curation of strains and information should be implemented. Still today, bacterial cultures are often maintained over years through periodical transfer onto fresh media. However, this practice is not only considerably time and material consuming but presents risks of contamination, selection of mutants, loss, and mislabeling, and therefore the method should be avoided. Scientists should identify as early as possible those strains worth maintaining from their research. As has been outlined above (*Box 11.1*), scientists have the obligation to share with peers those strains included in scientific publications, meaning that subcultures of such strains need to be available and prepared in a manner that optimally preserves the properties of the original culture. It is our recommendation to contact public service collections to guarantee availability and dispatch according to national rules and regulations.

A number of reliable methods for short, medium, and long-term maintenance of microorganisms have been developed (Kirsop and Doyle 1991; Day and Stacey 2007). Some of the short-term methods are simple and may be performed in any laboratory but may not be successful for a broad range of organisms. For long-term methods, more sophisticated equipment is required; these, in turn, seem to be effective for most microorganisms. However, it must be stated that there is no universal preservation method for all microorganisms. Different taxonomical groups, even strains within a species, may react differently to the various preservation conditions. *Table 11.4* gives an overview of different long-term preservation methods.

Table 11.4
Comparison of some methods used for long-term preservation

Method	Costs for material and equipment	Working time consumed initially	Working time consumed over storage time	Working time consumed on supply	Survival of organisms	Genetic stability	Space needed	Suitability for shipping	Danger of contamination	Dependence from power supply
Periodic transfer	Low	Low	High	High	2–6 months[a]	Low	High	Active cultures acceptable	High	Cool room required
Drying over silica gel	Low	Medium	Medium	High	1/2–7 years[a]	High	Low	Difficult for individual replica; needs reactivation	High	Can be independent
Deep freezing −80 °C	Medium	Medium	Medium	High	1/2–7 years[a]	Medium	Medium	Needs reactivation or shipping on dry ice	Depending on method; high	Dependent
Freeze drying	High	High	Low	Low	> 40 years[b]	High[d]	Medium	Very good	Low	Can be independent
Cryo-storage in LN_2	High	Low	Low	High	> 40 years "indefinite"[b]	High	Low	Needs reactivation	Low	Can be independent

[a]Depending upon taxon and strain
[b]In service collections, experience indicates that organisms that survive the first ~5 years will survive "indefinitely" in all probability
[c]Experience shows that if organisms survive the initial freezing and, upon recovery, the thawing process, they will survive indefinitely
[d]If drying is performed too extensively, bound H_2O may be removed; this may result in DNA damage

Choice of Preservation Technique

Each of the different methods has its advantages and disadvantages which we need to know to ensure the right one is applied to meet the special conditions or needs in each circumstance. The selection should be decided upon after comparison of the conditioning factors of a given method, the available equipment and the needs of the user.

Some general aspects should be considered with any choice of methodology:

- Viability should be maintained as high as possible.
- Genetic changes should be avoided as far as possible (this is also most likely supported by a high survival rate).
- The risk of contaminating the preserved culture should be as low as possible.

Some technical aspects refer to effort/efficiency balance which is especially important when larger numbers of organisms need to be processed:

- The number of ampoules or replicas to be prepared should be considered according to the procedure chosen (expense of work, material or space for storage, authentication procedure, demand).
- If cultures are to be supplied to third parties, they must be present in an appropriate form; public collections almost always assume that strains will be requested.
- The present or envisaged market need for a given culture.
- The availability of space in the facility, its financial situation, and personnel expertise and availability.

1. Drying methods have the advantage that, once the culture has been successfully preserved, material can be stored independently from power supply. This can be done in the dark at ambient temperature, though storage at lower temperatures between ∼+10 °C and ∼−20 °C extends shelf life considerably. Dried specimen ("ampoules") can be used perfectly for shipping of cultures, and the method bears relative low costs for material.
2. Storage at ultralow temperatures (below −139 °C), for example, the procedure of preservation in liquid nitrogen (LN_2) at −196 °C ("cryo-storage") is much faster and more reliable than any other methods. As a drawback, costs for material are higher, and preserved cultures need to be revitalized and incubated before shipping.
3. Temperatures of −70° C to −90 °C as generated by electrical deep freezers or by solid CO_2 (below −78 °C) have been shown to give useful results for the preservation of some types of microorganisms. Freezing at −80°C is often applied especially in research groups. This can be an acceptable method for medium-term storage of cultures maintained for own, in-house use within a research group. However, these temperatures range within the margin where water migration into cells is possible, and therefore, cells have to be carefully protected by appropriate additives to obtain reasonable periods of storage.

As a conclusion, the safest storage for microorganisms to be preserved long-term is provided by liquid nitrogen. Nitrogen is also much safer than other liquefied gases as it does not burn, is not toxic, and is cheaper than other gases. Nevertheless, care must be taken to avoid suffocation due to displacement of oxygen. However, when considering continuous supply of cultures, drying, and storage of microorganisms under vacuum is the method of choice for preservation.

Factors Influencing the Survival Rate of Microorganisms During Freezing and Drying

Successful preservation of microorganisms not only depends on the application of an appropriate cooling, drying, thawing or rehydration regime. Other factors (*Table 11.5*) determined by the organism itself have been shown to be important, such as type and strain of the organism, growth conditions, nutritional status, and growth phase. Additionally, the diluting medium and growth medium used for reactivation and determination of viability will influence their recovery.

Nevertheless, for most procedures, basic factors determining survival of the preserved organisms are similar.

Protective Suspension Media for Freezing or (Freeze-) Drying

For the protection of cells against damage during drying, freezing, and freeze-drying as well as during storage, microorganisms have to be suspended in a protective suspension medium. The composition of these media may depend upon the type of organism to be preserved. Some examples of protective suspension media are given below. However, as far as possible, widely applicable routine methods should be established. Especially with larger collections, the laborious and time-consuming individual treatment of each culture cannot be afforded.

Protecting effects of compounds have been assigned to maintaining macromolecular structures (replacing H_2O molecules) (Suggett 1975), protecting against O_2 or oxygen radicals (Lion and Bergmann 1961), avoiding damage to membranes (Morichi 1970) or maintaining a certain level of residual moisture (Nei 1974; Danilova et al. 1980).

For drying processes, complex organic substances, for example, skimmed milk, serum, and peptones and also pure substances, for example, sugars, amino acids, and mixtures thereof have been proven supportive for keeping high viability.

Substances protecting living cells against freeze-thaw injury (cryoprotectants) can, on the one hand, be compounds with defined low molecular weight, such as glycerol, dimethylsulfoxide (DMSO), methanol, or sugars. On the other hand, these can be compounds with defined high molecular weight, such as starch, hydroxyethyl starch (HES), or polyvinylpyrrolidone (PVP) and undefined substances, such as proteins, malt extracts, or blood (Farrant 1969; Fry 1966; Fuller 2004; Heckly 1978).

Table 11.5
Factors influencing preservation

Factors		Comments
General	Kind of organism	Size, taxon, strain, suspendability
	Culture conditions	Nutritional state
	Age	Mid to late log phase
	Concentration of cells	The higher the better[a]
	Media for preparing the suspension	Protective media, stabilizing structure, replacing H_2O, protection against O_2, retention of residual moisture
Freezing	Freezing and storage temperature	Storage at as low as possible
	Freezing velocity	
	Cryoprotectant	Depending on the permeability of the membranes
Drying	Drying temperature	
	Drying velocity	
	Residual moisture	Influenced by length of the drying process, temperature of cold trap and final vacuum (ideally 10^{-1} to 10^{-2} mbar)
	Storage conditions like: Gas atmosphere Temperature	Best under vacuum (without O_2) 4–10 °C recommended
Method of reactivation	Medium	
	Temperature	
	Rehydration time	With freeze-drying: allow the material to rehydrate for 10 min
	Thawing velocity	With LN_2: plunge into 37 °C warm water

[a]Concerning the cell concentration mentioned above, it has been demonstrated that the survival rate is positively influenced by an initially higher concentration of the cell suspension. While for various organisms with an initial cell density between 10^7 and 10^8, a drop by two log levels was observed; no drop was observed when the initial cell density ranged between 10^{10} and 10^{11}

The effects of penetrating cryoprotectants are manifold, as they

- Partially replace intracellular water
- Thus prevent a too high increase of salt concentration
- May also replace water molecules for the stabilization of proteins and membranes
- Influence ice crystal formation
- Like DMSO (NOTE: toxic), increases the permeability of membranes

Non-penetrating cryoprotectants work differently as they

- Cause an osmotic dehydration of cells
- Reduce extracellular salt concentration
- Influence extracellular ice formation
- May stabilize membrane structures

An extensive compilation of protective suspension media suitable for the freezing and freeze-drying of microbial strains can be found on www.cabri.org (> guidelines > microorganisms > Part 3: Guidelines for maintaining deposits – Appendixes – M/1998/3.00 Appendix 3).

Simple Methods for In-House Purposes

Cultures may need to be maintained by simple methods, for example, as cultures on slants which are over-layered with sterile paraffin oil, in distilled water or by simple drying methods. In any case, periodical transfer onto fresh media should be avoided due to the fairly high danger of contamination and physiological and genetic changes (see above).

Cultures may be dried in earth, sand, pumice-stone, above silica gel, or on porcelain or glass beads; however, some facts should be kept in mind:

- A protective medium like skimmed milk or skimmed milk with myo-inositol, serum, or nutrient broth should be used.
- The amount to be dried should be as small as possible.
- The drying process should not take too long.
- The dried cultures should be stored in the cold, if possible under vacuum and dark.

It is recommended to use such preparations rather for in-house purposes than to use them for supply to third parties. The problem usually accompanied with these methods is that the storage receptacle needs to be opened many times to remove the required sample of the dried organism. This immediately presents the danger of contamination and negative impact on survival rates.

When drying over silica gel on glass or porcelain beads is chosen, the bacterial suspension is surface-dried but without direct contact with the drying agent. Silica gel develops considerable heat when taking up water, which may be harmful for the organisms attached. Silica gel with blue indicator (toxic $CoCl_2$) may be used, though today it is recommended to avoid $CoCl_2$ and silica gel with other indicators are available. The amount of drying agent should be sufficiently large so that only the smaller part of the silica gel will change in color during the drying process.

The method of drying over silica gel in gelatine disks, originally described by Stamp (1947), uses a protective medium containing peptone, meat extract, gelatin, and sodium ascorbate. The complex organic compounds are used for stabilizing macromolecular structures and/or to serve as physical barriers to maintain a certain residual water content. Na ascorbate is added as an oxygen radical trap, as it is suspected that cell damage occurs through oxygen radicals. During hardening and

drying of the gelatine drops, small disks are formed which may be stored in presterilized screw cap tubes containing dry silica gel.

Preferred Methodologies for Long-Term Storage: Freeze-Drying and Cryo-storage in Liquid Nitrogen

General Aspects of the Freeze-Drying Process

Preservation of microorganisms by drying under vacuum from the frozen state (through sublimation of ice) has been used for more than 60 years. Methods and equipment have been developed over the years and nowadays present a reliable and effective preservation method for most bacteria, fungi, and yeasts. During the freeze-drying process, wet material is frozen and the ice directly transferred into the gas phase. The ice sublimes without melting. The porous cake resulting from this has, in principle, the same size and shape as the original frozen mass. Through adding of water or culture medium, the original state is reconstituted. In general, freeze-dried material is highly soluble. However, it should be noted that freeze-dried organisms are extremely susceptible to oxygen. To exclude this negative effect, the cultures should be stored in glass ampoules sealed under vacuum.

Practical Aspects of the Freeze-Drying Process

Media for Cultivation

Microorganisms should be cultivated on media which allow good growth and from which they can be harvested easily. Incubation on agar slopes is preferred. In the case of liquid cultures, these must be centrifuged before suspending in the protective medium.

Age of Cultures

Fast-growing organisms are harvested generally after about 24 h of incubation. This is around the mid to late logarithmic phase. Slow growing organisms must be incubated adequately. Spore-forming bacteria and fungi are incubated until optimal spore formation.

Ampoules for Freeze-Drying

Within a small margin, the dimensions of the ampoules are of minor importance. However, with the "single-vial-method," vials may be constricted by hand if the inner diameter is around 6 mm. When preparing the "double-vial ampoule" (see CABRI guideline for more details), outer tubes with an inner diameter of about 14 mm are recommended. With these, using an ampoule constrictor machine is recommended.

Protective Medium

For many microorganisms, skimmed milk has been proven an effective protective agent. To avoid caramelization, skimmed milk should be autoclaved in small amounts at 115 °C for only 13 min. Thorough sterility testing is therefore necessary, particularly for the presence of heat-resistant spores of thermophilic organisms. Thus, testing should be performed at 30 °C as well as 55 °C.

Sterility

During preparation of freeze-dried cultures, both, the cultures as well as the personnel, must be protected from contamination or hazard. In parallel to the common safety precautions for microbiological work, it must be observed that during the drying process cell material may escape the ampoule in the form of fine particles and contaminate the vacuum chamber or the whole freeze-drying apparatus. To avoid this, the ampoules must be provided with a filtering closure, which, simultaneously, will avoid contamination of the culture when air is allowed to enter the vacuum chamber after the first or primary drying (when true freeze-drying is applied).

End Vacuum

The evacuation process must be monitored. Optimally, a final vacuum between 10^{-1} and 10^{-2} mbar should be reached to guarantee good survival rates over longer periods.

Note: The use of silica gel as moisture indicator as used with the double-vial ampoule is meant as an "optical help" only to indicate loss of vacuum during storage. The change in color of the indicator early on in the drying process does not mean that a sufficiently deep vacuum has already been reached.

Sealing Off of Ampoules

Sealing off ampoules is done to maintain a vacuum. Care should be taken that the tips are perfectly sealed and rounded so that cracks or breakage during storage can be avoided. In practice, freeze-drying is performed in various ways adapted to specific needs.

True Freeze-Drying Process

With this method, the bacterial suspension is mixed with protective medium, then frozen and transferred to the vacuum chamber in the frozen state. Vacuum is applied before the suspension starts melting, and water is removed by sublimation.

A full description of the procedure can be found in www.cabri.org (Guidelines > "Click here to read the guidelines" > Microorganisms > Part 3: Maintaining deposits > Appendixes; edited and amended > M/1998/3.00 Appendix 5.08 'Preservation of Bacteria by Freeze – Drying (True Freeze-drying)). In M/1998/

3.00 Appendix 5.08.1, a flow chart of the freeze-drying procedure is shown. For recording each step of the preservation procedure and results of viability checks, protocol form M/1998/3.00 Appendix 5.08.2 is suggested.

Centrifugal Freezing

To shorten the exposure time to air/oxygen, the decreasing temperature in the freeze-drying chamber due to evaporation can be an alternative method for freezing, as the removal of water under vacuum results in a quick loss of about 10 % water in a relative short time. As this is an energy-consuming process, the residual suspension will freeze. To avoid the strong frothing, which would normally occur and which would expel some of the contents from the ampoules due to the release of gas, the samples are centrifuged during the evaporation process until the material is frozen.

A full description of the procedure can be found in www.cabri.org (Guidelines > "Click here to read the guidelines" > Microorganisms > Part 3: Maintaining deposits > Appendixes; edited and amended. Flow Diagram M/1998/3.00 Appendix 5.10.1 "Centrifugal Freeze-Drying").

The Double-Vial, Liquid-Drying Method, as Applied in the DSMZ for Bacteria and Fungi

This modified method, applied for example by the DSMZ for a wide spectrum of prokaryotes and fungi, includes a drying step from the liquid state. The advantage is less stressful for the cells and less water vapor developed. Due to the much smaller amount of water vapor, the drying process may be even run without a freezing chamber.

The principle includes the transfer of a small drop of a heavy suspension of organisms in fresh medium onto the porous cake of a pre-dried skim milk pellet. The proportion of the drop of suspension to dried skim milk is such that the amount of liquid is absorbed at once and totally. This method can then be combined with the "double-vial method," where the small, cotton plug stoppered vial, containing the dried skim milk and the drop of suspension, is inserted into a bigger tube. This tube is then constricted and connected to the manifold of the freeze-drying machine.

Note: As cells are under extreme stress, the time lapse between transfer of drops onto the milk cake and connection of the constricted vials to the manifold of the freeze-dryer should be as short as possible.

A full description of the method can be found under www.cabri.org (> guidelines > microorganisms. Part 3: Guidelines for maintaining deposits – Appendixes M/1998/3.00 Appendix 5.11).

Opening of Ampoules

When opening ampoules that had been sealed under vacuum, especially the one vial preparations, care should be taken to avoid the following hazards:

- Contamination of the culture through air entering the ampoule when opening
- Release of fine particles of the dried bacterial mass into the air (the sudden inrush of air when cracking an ampoule may result in a back surge of particle-loaded air), thus contaminating the air of the laboratory

Note: If cultures belong to hazard group 1, (freeze-) dried cultures in ampoules sealed under vacuum can be opened in an ordinary transfer cabinet. In other cases, ampoules should be opened in a biohazard safety cabinet of the appropriate level.

A full description of the methods can be found under www.cabri.org (> guidelines > microorganisms. Part 3: Guidelines for maintaining deposits – Appendixes M/1998/3.00 M/1998/3.00 Appendix 5.14).

Cryopreservation In or Above Liquid Nitrogen

General Aspects

Freezing of living cells or parts of them to very low temperatures and storage at these temperatures stops metabolic activities and retains viability and genetic stability of the specimens. Even molecular motions are significantly reduced at sufficiently low temperatures and cease below −139 °C. These characteristics make cryogenic storage very attractive for the long-term preservation of living cells.

Studies, as early as around 1900, have shown that microorganisms can withstand freezing down to ultralow temperatures (liquid air, liquid hydrogen). The discoveries of Polge et al. (1949) and of Lovelock and Bishop (1959), that glycerol and dimethylsulfoxide (DMSO) protect living cells against freezing damage, greatly influenced the further development of the technology in this field. Considerable progress has been made over subsequent decades with regard to the control of the freezing and thawing process to obtain optimal results. A broad range of living cells – from the small-sized prokaryotic to the larger sized eukaryotic cells (such as fungi; protozoa; algae; and plant, animal, and human cells and even tissues) – can be retained viable for long periods by low temperature storage (Reed 2008; Day and Stacey 2007).

The safest cryo-storage for both, the organisms to be preserved *and* the personnel, is that provided through liquid nitrogen: −196 °C. Compared with other liquefied gases, nitrogen is safer – it does not burn, is not toxic – and is cheaper than other more rare gases. Excellent storage containers and additional equipment is supplied by several manufacturers in many countries.

Living cells consist mainly of water, and in the protective or growth media, they are surrounded by water containing different amounts of electrolytes. Ice crystal formation occurring during freezing inside or outside the cells removes liquid water. This may impact negatively on cells which normally depend on a balanced ionic environment and hydration state of their macromolecules. Shrinkage of cells and ice crystals may be responsible for damage to the cytoplasmic membrane (Morris 1981). However, it should be borne in mind that ice crystal

formation not only occurs during freezing but also when cells are thawed slowly to subzero temperatures; therefore, rapid thawing is recommended.

To safeguard cells from freezing injuries, cryoprotectants are added to the freezing suspension. For this purpose, certain defined low molecular weight or high molecular weight (see further above) compounds or undefined complex substances are applied. A common characteristic of such compounds is that they are nonionic polar molecules with a pronounced ability to H-bonding. Those compounds penetrating the cell membrane should be applied in molar concentrations (0.5–1.5 M), while non-penetrating agents are used at much lower (around 0.01 M) concentrations.

A full description of the method can be found under www.cabri.org (> guidelines > microorganisms. Part 3: Guidelines for maintaining deposits – Appendixes M/1998/3.00 M/1998/3.00 Appendix 5.04).

Miniaturized Method for the Cryopreservation of Bacterial Cultures: The Glass Capillary Method

The limited capacity of storage containers, together with increasing costs for equipment and nitrogen, creates problems when larger numbers of different organisms, each in multiple replicas, have to be preserved. Miniaturized methods which have been developed by several investigators may help to overcome this problem (Hippe 1991). The basic idea of these methods is to reduce the volume of cell suspension to be preserved and of the unit holding it. Use of small plastic straws or glass capillary tubes replacing the common vials or ampoules is now standard.

A full description of the glass capillary method is given in www.cabri.org (> guidelines > "click here to read the guidelines" > microorganisms > part 3: maintaining deposits: appendixes; edited and amended > m/1998/3.00 appendix 5.01 Preservation of bacteria by freezing and low temperature storage in glass capillary tubes).

Often, filamentous bacteria and fungi cannot be suspended fine enough to apply the above capillary method. Especially the fastidious, non-sporulating strains need special attention. For such organisms, a smart procedure has been developed in which young mycelium together with the agar on which the organisms is growing is punched out using short pieces of PVC straws. These can be the larger common drinking straws or the thinner straws used for artificial insemination in cattle breeding. Before punching out growth and agar, the growth is covered with a 10 % glycerol solution (take care for thorough wetting of the growth), which is poured off after about 2 h. Several of such straws can then be assembled in a cryo-vial which is stored in the vapor phase of liquid nitrogen.

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References

Anon (2010) The WFCC Guidelines for the establishment and operation of culture collections. http://www.wfcc.info/guideline.html. Accessed 03 July 2011

Arber W (2003) Elements for a theory of molecular evolution. Gene 317:3–11

CABRI (2002) Common Access to Biological Resources and Information http//:www.cabri.org

Canhos VP, Sette LD, Cupolillo E, Tigano MS, Vazoller RF (2007) O papel da Sociedade Brasileira de Microbiologia no suporte à consolidação da Rede Brasileira de Coleções de Culturas de Microrganismos. Microbiologia in foco 2:40–48

Canhos VP, Ferraz de Souza RD, Ávila JPC (2009) Avanços na implementação da Rede Brasileira de Centros de Recursos Biológicos. Microbiologia in foco 9:46–47

CBD Nagoya Protocol (2011) https://www.cbd.int/abs/

Cohan FM, Perry EB (2007) A systematics for discovering the fundamental. Units of bacterial diversity. Curr Biol 17:R373–R386

Danilova MV, Nadirova JM, Emtseva TV (1980) The viability's dependence of lyophilized and longly stored bacteria on the quantity of residual humidity. Izv Akad Nauk SSSR, Ser Biol 3:449–451

Dawyndt P, Dedeurwaerdere T, Swings J (2006) Explorating and exploiting microbiological commons: contributions of bioinformatics and intellectual property rights in sharing biological information. Int Soc Sci J 188:249–258

Day JD, Stacey G (eds) (2007) Cryopreservation and freeze-drying protocols. In: Series: methods in molecular biology, 368, 2nd edn. Humana Press, ISBN 1-58829-377-7

DeLey J, De Smedt TJ (1975) Improvements of the membrane filter method for DNA: rRNA hybridization. Ant van Leeuwenh 41:287–307

Emerson D, Wilson W (2009) Giving microbial diversity a home. Nat Rev Microbiol 7:758

Farrant J (1969) Is there a common mechanism of protection of living cells by polyvinylpyrrolidone and glycerol during freezing? Nature 222:1175, London

Fischer A, Kroppenstedt RM, Stackebrandt E (1983) Molecular-genetic and chemotaxonomic studies on Actinomadura and Nocardiopsis. J Gen Microbiol 129:3433–3446

Fritze D (2005) Digital imaging of prokaryotes for taxonomic purposes. In: Häuser CL, Steiner A, Holstein J, Scoble MJ (eds) Digital imaging of biological type specimens. A manual of best practice. Results from a study of the european network for biodiversity information. Staatliches Museum für Naturkunde, Stuttgart, viii + 304 pp, ISBN 3-00-017240-8, pp 153–171

Fritze D (2010) A common basis for facilitated legitimate exchange of biological materials proposed by the European Culture Collections' Organisation. Int J Commons 4:507–527

Fritze D, Weihs V (2000) Systematics and legislation. In: Priest F, Goodfellow M (eds) Applied microbial systematics. Chapman and Hall, London, pp 447–469

Fry RM (1966) Freezing and drying of bacteria. In: Meryman HT (ed) Cryobiology. Academic, London/New York, pp 665–696

Fuller BJ (2004) Cryoprotectants: the essential antifreezes to protect life in the frozen state. CryoLetters 25:375–388

Gams W, Hennebert GL, Stalpers JA, Jansens D, Schipper MAA, Smith J, Yarrow D, Hawksworth DL (1988) Structuring strain data for the storage and retrieval of information on fungi and yeasts in MINE, Microbial Information Network Europe. J Gen Microbiol 134:1667–1689

Grimont F, Grimont PAD (1986) Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Ann Inst Pasteur/Microbiol 137B:165–175

Hawksworth DL, Schipper MAA (1989) Criteria for consideration in the accreditation of culture collections participating in MINE, the Microbial Information Network Europe. MIRCEN J 5:277–281

Heckly RJ (1978) Preservation of microorganisms. Adv Appl Microbiol 24:1–53

Hippe H (1991) Maintenance of methanogenic bacteria. In: Kirsop BE, Doyle A (eds) Maintenance of microorganisms and cultures cells, 2nd edn. Academic, London, pp 101–113

Johnson JL, Ordal EJ (1968) Deoxyribonucleic acid homology in bacterial taxonomy: effect of incubation temperature on reaction specificity. J Bacteriol 95:893–900

Kersters K, De Ley J (1975) Identification and grouping of bacteria by numerical analysis of their electrophoretic patterns. J Gen Microbiol 87:333–342

Kirsop BE, Doyle A (eds) (1991) Maintenance of microorganisms and cultured cells: a manual of laboratory methods. Academic, London, 308 pp

Konstantinidis K, Stackebrandt E (2011) Defining taxonomic ranks. In: Rosenberg E, DeLong EF, Thompson F, Lory S, Stackebrandt E (eds) The prokaryotes: a handbook on the biology of bacteria, 4th edn. Springer, New York

Lenski RE, Travisano M (1994) Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. Proc Natl Acad Sci USA 91:6808–6814

Lion MB, Bergmann ED (1961) The effect of oxygen on freeze-dried *Escherichia coli*. J Gen Microbiol 24:191–200

Lovelock JE, Bishop MWH (1959) Prevention of freezing damage to living cells by dimethyl sulfoxide. Nature 183:1394–1395

Morichi T (1970) Nature and action of protective solutes in freeze-drying of bacteria. In: Iizuka H, Hasegawa T (eds) Culture collections of microorganisms. University Park Press, Baltimore, pp 351–361

Morris GJ (1981) Cryopreservation: an introduction to cryopreservation in culture collections. Culture Centre of Algae and Protozoa, Cumbria

Nei T (1974) Some aspects of freezing and drying of microorganisms on the basis of cellular water. Cryobiology 10:403–408

OECD (2001) Biological resource centres – underpinning the future of life sciences and biotechnology. (http://oecdpublications.gfi-nb.com/cgi-bin/oecdbookshop.storefront)

OECD (2007) Best practice guidelines for biological resource centres (Online), http://www.oecd.org/dataoecd/7/13/GGTSPU-styx2.bba.de-7664-3281383-DAT/38777417.pdf Accessed 20 Jan 2011

OECD (2011) The bio-economy to 2030: designing a policy agenda. The Organization for Economic Cooperation and Development (OECD), Paris

Olive DM, Bean P (1999) Principles and applications of methods for DNA-based typing of microbial organisms. J Clin Microbiol 37:1661–1669

Polge C, Smith AU, Parkes S (1949) Revival of spermatozoa after dehydration at low temperatures. Nature 164:666

Pukall R, Brambilla E, Stackebrandt E (1998) Automated fragment length analysis of fluorescently-labeled 16S rDNA after digestion with 4-base cutting restriction enzymes. J Microbiol Meth 32:55–64

Rainey PB, Travisano M (1998) Adaptive radiation in a heterogeneous environment. Nature 394:69–72

Reed BM (2008) Plant cryopreservation – a practical guide. Springer, Heidelberg. ISBN 978-0-387-72275-7

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC, pp 607–655

Smith D, Rohde C (2007) Biological resource centres compliance with law. Society for Microbiology, UK. http://www.sgm.ac.uk/pubs/micro_today/pdf/0299brc.pdf

Smith D, Rohde C (2008) Safety in microbiology. Laboratory Manager Issue, vol 125. Croner, UK, pp 4–6

Smith D, Ryan MJ, Day JG (2001) The UKNCC biological resource: properties, maintenance and management. UKNCC Secretariat, Egham. ISBN 0954028503

Smith D, Ryan MJ, Stackebrandt E (2008) The *ex situ* conservation of microorganisms: aiming at a certified quality management. In: Doelle HW, DaSilva EJ (eds) Biotechnology. Encyclopedia of Life Support Systems (EOLSS). Developed under the Auspices of the UNESCO, Eolss, Oxford, UK. http://wwweolss.net

Stackebrandt E (2010) Diversification and focusing: strategies of microbial culture collections. Trends Microbiol 18:283–287

Stackebrandt E (2011) Letter to the editor. Int J Syst Evol Microbiol 61:479–481

Stackebrandt E, Frederiksen W, Garrity GM, Grimont PAD, Kämpfer P, Maiden MCJ, Nesme X, Rossello-Mora R, Swings J, Trüper HG, Vauterin L, Ward AC, Whitman WB (2002) Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. Int J Syst Evol Microbiol 52:1043–1047

Stalpers JA, Kracht M, Jansens D, De Ley J, Van der Toorn J, Smith J, Claus D, Hippe H (1990) Structuring strain data for storage and retrieval of information on bacteria in MINE, Microbial Information Network Europe. Syst Appl Microbiol 13:92–103

Stamp TC (1947) The preservation of bacteria by drying. J Gen Microbiol 1:251–265

Stromberg PM, Dedeurwaerdere T, Pascual U (2012) The contribution of public networks to knowledge accumulation: ex situ collections in microbial research (in press)

Suggett A (1975) Water-carbohydrate interactions. In: Duckworth RB (ed) Water relations of food. Food Science and Technology, a series of monographs. Academic Press, London, pp 573–586

Tindall P, Kämpfer P, Euzéby J, Oren A (2006) Valid publication of names of prokaryotes according to the rules of nomenclature: past history and current practice. Int J Syst Evol Microbiol 56:2715–2720

Tindall BJ, Rosselló-Móra R, Busse HJ, Ludwig W, Kämpfer P (2010) Notes on the characterization of prokaryote strains for taxonomic purposes. Int J Syst Evol Microbiol 60:249–266

Verslyppe B, Kottmann R, De Smet W, De Baets B, De Vos P, Dawyndt P (2010) Microbiological common language (MCL): a standard for electronic information exchange in the microbial commons. Res Microbiol 161:439–445