Chapter 4
Microbial Resource Centers Contribute to Bioprospecting of Bacteria and Filamentous Microfungi

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Abstract The immense diversity of microorganisms has remained largely untapped, especially with regard to bioprospecting. Through their isolation, microbes attain a substantial monetary value which microbial domain Biological Resource Centers (mBRCs) preserve in a highly cost-effective manner. Typically, mBRCs are publicly funded in order to provide quality-controlled, and well-characterized microbial resources and data, at low cost to researchers. The present chapter outlines the preconditions and scenarios for mBRCs to expand their traditional tasks and enter the field of bioprospecting. It appears most promising to generate information on the biosynthetic potential of novel types of microorganisms through extended characterization, metabolic profiling, and genome analyses. Particular challenges are an improved access to the vast uncharted microbial biodiversity, the compliance with new legal requirements, and the efficient linking to private industry as a novel stakeholder. A business plan is developed herein that proposes to join the expertise of different mBRCs to create a platform that provides a "one-stop-shop" with restricted access to a large number of well-characterized, pre-screened microbial resources in a legally compliant manner. As a typical and inherent weakness, the limited public funding of mBRCs often will not permit an expansion of tasks through the existing funding alone. Revenues generated from sales of even high value microbial resources to bioindustry rarely will cover costs. Therefore, alternative funding could be sought from the government agencies in charge of the bioeconomy that traditionally are not stakeholders of mBRC, and through the participation of mBRCs in dedicated funding programs for bioprospecting.

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4.1 Introduction

Culture collections have been undertaking research with microorganisms for over 100 years. During this time, the public access to live cultures of microorganisms has played a pivotal role for progress in basic microbiological sciences in a number of ways: In microbial systematics, novel isolates need to be compared to existing strains to determine biochemical or physiological characteristics that serve to delineate and define previously unreported taxa (Rosselló-Móra and Amann 2015). Microbial strains also permit the testing of biochemical features sometimes predicted by the analysis of genome sequences, and allow the elucidation of novel metabolic pathways. In microbial ecology, isolated strains serve as models for microorganisms of the same phylotype that occur in complex environments. Isolated microbial strains have been deposited in public collections to ensure their unrestricted availability for subsequent scientific studies for these and other purposes. Live microbial strains also provide numerous opportunities for applications in agriculture, food processing, catalyses, environmental protection, and public health. The potential for application of microbial resources is particularly prominent in the pharmaceutical sector, especially given the problems of resistance in pathogenic bacteria to antibiotics and the lack of novel leads to treat the diseases as discussed elsewhere in this book.

Microbial resource centers (more precisely, microbial domain Biological Resource Centers *sensu* OECD, mBRCs) provide products and services that go far beyond the maintenance and distribution of microbial resources offered by typical culture collections (OECD 2004). In particular, mBRCs rely on specific and approved quality assurance procedures, provide profound expertise in microbial systematics, and maintain legal expertise with regard to property rights, biosafety, and biosecurity. They also deliver the associated data and maintain the expert knowledge essential for cultivation and physiological analysis of microbial strains. Whereas mBRCs traditionally support basic research, their holdings and competencies have so far been exploited only rarely for natural compound discovery and the bioeconomy.

The present chapter analyses the current state of mBRCs as they apply to bacteria and filamentous microfungi. The larger macrofungi such as mushrooms are considered elsewhere (www.iucnredlist.org). It features the (a) type and value of their holdings, (b) currently changing legal framework for their acquisition and distribution policies, and (c) future challenges and potential novel functions. A business plan is developed that intends to support mBRCs to cope with these future demands.

4.2 Microbial Resources

A bacterial strain represents the progeny of a single isolated cell and constitutes the basis for subsequent studies. Microbial strains that are authenticated, taxonomically defined, physiologically characterized, quality controlled, and also well-documented,

are designated 'microbial resources', in order to distinguish them from mere laboratory isolates that may lack the respective information. The worldwide inventory of microbial strains that are registered in the Culture Collections Information Worldwide (CCINFO) system of the World Data Centre for Microorganisms (WDCM 2015) currently amounts to 2.5 million strains. Of these, 1.05 million, 727,000 and 38,000 are bacteria, fungi and viruses respectively maintained in 692 culture collections from 71 countries. Despite these impressive numbers, however, they represent the existing microbial diversity rather poorly since most, or all, bacterial isolates belong to only 10,693 bacterial and archaeal species that are recognized to date (LPSN 2015), or to the 100,000 fungal species (Kirk et al. 2008). In contrast, current estimates of total prokaryotic diversity range between 10^7 and 10^9 species (Dykhuizen 1998; Curtis et al. 2002). The described number of species thus may represent only 0.1–0.001 % of the total prokaryotic diversity. Consequently, the worldwide holdings mostly consist of multiple strains of each bacterial species and hence represent an extensive phylogenetic redundancy. Furthermore, most of the currently available bacterial isolates belong to just four of the approximately 90 phyla that are presently recognized (i.e., the *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*) (Overmann 2012). By comparison, representatives of 60 bacterial phyla have rarely or never been isolated to date (Baker and Dick 2013).

Recent estimates based on high-throughput sequencing methods suggest that as many as 5.1 million fungal species exist (Blackwell 2011). Considering that there are currently around 100,000 species described, the majority of fungi are also yet to be discovered and cultured. At least in part, the failure to recover phylogenetically novel microbial lineages of bacteria and fungi can be attributed to current isolation methodology that is often found to be inadequate (Overmann 2012).

Since replication-competent cultures of bacterial type strains (those strains that follow the description and characteristics of novel species) constitute the basis for follow-up systematic comparisons, the deposit of a type strain in two public collections (preferably in two different countries) is mandatory for its valid description as a novel species (Lapage et al. 1992). Accordingly, the acquisition, maintenance and distribution of type strains have been major tasks of bacterial mBRCs. However, unlike the bacterial type strains that are almost completely secured in public collections, only 25.5 % of type strains from the 100,000 recognized fungal species are publicly available. In addition, mBRCs also maintain strains that are deposited in association with the publication of patents. The World Intellectual Property Organization (WIPO) statistics report that over 4500 organisms were deposited in 2012 (WIPO 2015), which allows researchers to fulfil this legal prerequisite for new patent applications.

From the high ratio between the total number of strains and the few type strains hosted by mBRCs and culture collections worldwide, and from the small overlap in the holdings of type strains in different culture collections, it can be deduced that the majority of the worldwide inventory of microbial resources represent non-type strains that were deposited for purposes other than systematics (Overmann 2015). Indeed, of the strains in the WDCM Global Catalogue of Microorganisms that had a registered origin, 48 % were genetically engineered fungi and 25 % were bacteria

of human origin (GCM 2015). Thus, isolates obtained through biotechnological projects or isolated during medical studies may represent a considerable fraction of the microbial resources that are currently deposited in mBRCs worldwide. Notwithstanding the rather high numbers of non-type strains, the holdings of mBRCs still represent only a small fraction of the isolates that are actually generated in microbiological laboratories worldwide: in contrast to the bacterial type strains that are almost all accessible, less than 1 % of the microbial isolates obtained through publicly funded research are deposited in mBRCs and hence run a higher risk of being lost before their potential has been explored further (see Sect. 4.5).

Because the uncharted proportion of microbial diversity is vast, it has been suggested that novel biochemical features and biosynthetic pathways are likely to occur in the underexplored bacterial or fungal lineages, which therefore may provide novel solutions for agriculture, biotechnology and public health. This hypothesis has been substantiated by the discovery of natural compound synthesis gene clusters and novel types of natural compounds in several phylogenetic lineages such as members of the candidate bacterial phylum 'Tectomicrobia' that occur in marine sponges (Wilson et al. 2014). Also, they occur in *Acidobacteria* (Quaiser et al. 2003), *Chloroflexi* (Nett et al. 2006), and *Planctomycetes* (Jeske et al. 2013). Recent studies report sampling fungal diversity by applying new isolation techniques and suggest that many species new to science are being discovered in unexplored environments (Blackwell 2011). As much as 13% of the tropical fungi yielded active compounds when screened for antifungal, antibacterial, antiviral, insecticidal, anti-helminthic, anti-cancer, anti-diabetes melitus, anti-inflammatory and pro-endocrinological leads in one large screening program (Bills et al. 2002).

Unfortunately, mBRCs (a) have not succeeded in securing a large fraction of the microbial isolates that are generated worldwide, (b) could make only limited contributions towards cultivating uncultured microbial diversity and (c) have played only a small role in attempts to access and explore microorganisms for future applications and these situations needs to change for a more effective bioeconomy.

4.3 Monetary Value of Microbial Resources

mBRCs represent an investment largely made by the public, often through governmental funding over long time periods. As such, it is important to understand what these investments produce in concrete terms and to assess future possibilities for exploitation of these investments for social purposes. There are several ways of assigning a financial value to the existing holdings of mBRCs through the: (1) cost for isolating a microbial strain from a complex sample, (2) cost of acquisition and curation, (3) supply fee, and (4) potential of microbial resources to yield high value marketable products. The following calculations do not consider non-monetary value, such as the scientific, cultural, and educational significance, as no methods exist to quantify them.

Obtaining novel types of microbes from environmental samples involves a series of labour-intensive steps for enrichment and isolation. Microorganisms that grow rapidly in aerobic, high nutrient, predefined complex media require a rather limited effort for cultivation that corresponds to a value of 450 € (Smith 2012). This cost represents an average of the 47 WFCC affiliated collections. In general, slow growing organisms such as filamentous fungi, which may take 7 days to grow fully, will cost more than fast growing bacteria, which may take 24 h. This is from increased (electrical) power requirements, the need for different equipment, greater obsolescence of equipment because they take longer to grow, and more person hours per organism. On the other hand, bacteria may require higher powered and more expensive microscopes because they are smaller.

However, strain characterisation was not included in the above estimations. Recent cost for an accession of a fungus to the CABI collection is ca. € 900 (cf. € 918 for DSMZ, see below). This value is modest (compared to the much higher estimate of the full monetary value of a more fastidious bacterial strain described in the following paragraphs) and does not consider the fact that many isolates occur only occasionally in other mBRCs worldwide. For example, of the 25,611 names of fungi listed in the World Data Centre for Microorganisms (WDCM 2015), almost 50 % are present in only one of the fungal collections listed (Sugawara et al. 1993). While these numbers are inflated as they represent names listed in the individual collections that also include synonyms, anamorph names, and spelling variants, they are still indicative and are supported by the data from the CABI collections. Here, 3360 of the 4541 species of filamentous fungi (i.e. 56 %) are represented by only one strain and if such a unique strain was lost, the recovery of an isolate with similar characteristics from its natural environment may be difficult and would incur substantially higher costs. This would inevitably render strains with specific and particularly desirable phenotypic properties much more valuable than the value of 450 € given above.

Significantly, the readily-culturable bacteria and filamentous fungi that are still being obtained through low-cost cultivation approaches, typically show little biological novelty (Singh et al. 2013). On the other hand, phenotypically novel bacteria often have unknown growth requirements, and are highly fastidious: These require substantially more person hours for enrichment and isolation as exemplified by *Myxobacteria*, *Acidobacteria* and *Dehalococcoides ethenogenes* (Foesel et al. 2013; Maymó-Gatell et al. 1997; Sanford et al. 2002). Based on the detailed compilation of all actual costs that are associated with the isolation and characterization of a more fastidious bacterial strain, a monetary value of 9836 € through cultivation can be obtained (Overmann 2015). This estimate encompasses all work steps and materials comprising of costs for personnel, consumables, and the depreciation of the equipment, but not the costs for sampling itself. However, in countries with an emerging economy (e.g., India), the monetary value attained would be somewhat lower due to lower wages (e.g. 5042 €; Overmann 2015).

In contrast to these high values, the costs for deposit and curation of microbial resources are modest. The acquisition of a bacterial isolate by mBRCs requires the cultivation and initial preservation (liquid nitrogen and freeze-drying) and identifi-

cation with extensive quality control (e.g., by 16S rRNA gene sequencing, MALDI-TOF, biochemical testing, fatty acid analysis and microscopy). Acquisition of filamentous microfungi would be similarly laborious (in this case, sequencing of the internal transcribed spacers is used instead of 16 S rRNA gene sequencing). Based on recent estimates, these activities incur costs of 918 € at the Leibniz-Institut DSMZ (Overmann 2015) and 900 € at CABI (see above), which is in the same order of magnitude as previous estimates for other mBRCs (Smith 2012). Costs for the curation, i.e. the long-term preservation and maintenance of live cultures, are even lower, with numbers ranging from 7.45 € (CABI) to 3.60 € (DSMZ) (both per strain and year). These expenses for the long-term storage of microbial resources amount to only 0.03–0.07 % of their overall monetary value. Evidently, mBRCs provide a very cost-effective way of preserving the monetary value of isolated strains.

Supply fees (retail prices) for microbial resources are even lower than the costs for acquisition and curation, and vary considerably between individual mBRCs. For example, CABI charges a fee of 209 € per freeze-dried ampoule and 278 € for an active culture, whereas corresponding prices at the DSMZ currently range between 75 and 175 €. Those of the American Type Culture Collection (ATCC) are between 350 and 418 € for most bacterial strains. Based on the feedback of DSMZ customers, researchers at public institutions often do not have the funds to afford the more expensive microbial resources. It becomes evident then, that the revenues generated through microbial resources are not sufficient to cover the concomitant expenditures of strain acquisition by the mBRCs. Thus, mBRCs could not support research and development in a sustainable manner without substantial public funding.

Finally, a monetary value can be assigned to microbial resources through their potential to yield high value marketable products. Annual global sales of pharmaceutical drugs amounted to about 956 billion US$ in 2011 (IMS Health Market Prognosis 2012). Of every 5000 to 10,000 natural products that generate a hit in initial screens, only one eventually will become an approved drug (ten Kate and Laird 1999; PhRMA 2012). If only uncharacterized microbial isolates are available for bioprospecting, they need to be screened entirely in an untargeted approach which typically yields only a low percentage of “talented” strains (strains capable of synthesizing natural compounds) of approximately 10 % (Hindra Huang et al. 2014; Weissmann and Müller 2010; Xie et al. 2014). Taken together, about 100,000 uncharacterized strains would be needed to statistically yield just one single pharmaceutical product that would reach the market place. Consequently, even the entire holdings of the largest mBRCs (≤ 165,000; WDCM 2015) are too small to arouse the interest of the pharmaceutical industry. Equally, providing microbial strains for untargeted screening is economically unattractive for mBRCs, since the market prices of microbial resources used by pharmaceutical companies has been calculated to range between US$ 2–60 per strain and hence barely reached the retail prices of mBRCs (Miyazaki 2006).

Based on the above considerations, mBRCs could strengthen their role in the bioeconomy and public health by providing more attractive microbial resources for research and development. This can be achieved through (1) an increase in holdings of strains from underexplored phylogenetic groups (recovered through their own

research projects or a suitable accession policy; see Sect. 4.2), (2) an in-depth characterization of microbial strains and growth experiments that systematically vary growth parameters to release a greater potential for natural compound synthesis or other applications, (3) collecting high quality genomic information that permits genome mining, and (4) providing sufficient documentation of genetic and metabolic properties of microbial strains that would permit a concise, targeted, and sector-specific bioprospecting by the users. Based on the numbers given above, improving the chances of novel discoveries by these measures would increase the monetary value of microbial resources by up to tenfold.

4.4 Current Functions of Microbial Domain Biological Resource Centers

Functions of a mBRC include the *ex situ* conservation of microorganisms, strain identification services, training opportunities and consultancy (WDCM 2015). mBRCs thus represent custodians of national resources providing the living materials to underpin the science base. So far, mostly strains isolated in the course of studies of microbial systematic and diversity and other projects of basic research, are deposited in mBRCs during the publication process. In addition to their public collections, many mBRCs carry out safety deposit (confidential holdings) and patent deposit services for researchers.

The role of mBRCs for basic research extends far beyond microbial systematics and the provision of type strains (see Sect. 4.1). This is exemplified by the fact that two thirds of the 11,020 scientific publications referring to strains of the DSMZ over the past 30 years appeared in journals outside of microbial systematics (Overmann 2015). Scientific articles based on deposited strains are cited more than twice as often as publications on strains that are not publicly available (Furman and Stern 2011). These data emphasize that public collections of mBRCs are a key to future scientific discoveries.

The current demand for microbial strains (including archea, bacteria, filamentous fungi, microalgae, viruses and yeasts) provided by 13 European mBRCs organized within the Microbial Resource Research Infrastructure (MIRRI) (MIRRI 2015) has been determined in the course of preparing the MIRRI business plan. The mBRCs distributed about 198,000 individual strains between 2010 and 2012. These were provided equally on national and international bases; 60 % of the strains were shipped to users located in the non-profit sector and 40 % to for-profit organizations (in the latter case often for non-commercial research, however). MIRRI estimates that microorganisms are utilised by around 1000 institutions in Europe, constituting about 400 universities and 600 research institutes which encompass at least one biological department.

The 13 MIRRI-mBRCs represent less than 10 % of the worldwide holdings and less than 2 % of the collections registered with the WDCM. Half of all CCINFO

registered microbial strains are kept in just 10 % of the countries registered, in particular the United States, Brazil, Japan, China, the Republic of Korea, and India (WDCM 2015). Obviously, the global demand for microbial strains is much higher than that in Europe alone and are approximately 0.5 million per year for the WFCC affiliate collections (Smith 2012). Since the strains are distributed by less than half (308 of 692) of the registered culture collections (WDCM 2015), the global average amounts to about 1600 cultures which are supplied per culture collection per year. Larger mBRCs distribute much higher numbers (e.g., the DMSZ provides 37,000 different microbial resources per year).

mBRCs have been little involved in bioprospecting because of their public mission. In the past, large pharmaceutical companies typically established their in-house, proprietary collections through bilateral agreements for sampling and exploitation with partner countries (e.g., Salazar et al. 2002). However, as the pharmaceutical and biotech industries have gone through significant streamlining and cost-cutting measure in the recent decade and especially in the recent global economic crisis, many natural product departments have closed or been downsized. Furthermore, small to medium sized companies often are unable to maintain a significant in-house capacity. Thus, in principle, there is a market niche here that is not currently fully serviced. Through their decades of successful operation, mBRCs have established cultivation and preservation skills, as well as profound knowledge on microbial biochemistry and physiology, which will be required to access a larger fraction of the uncharted microbial diversity and which are particularly important for fastidious or slow growing microbial strains, that require specific cultivation techniques for isolation. It is particularly attractive to participate in the search for, and retrieval of, novel types of microorganisms and to unlock their potential for future research and applications to facilitate the future development of mBRCs.

4.5 Future Demands for Microbial Resources and Microbial Domain Biological Resource Center Services in the Bioeconomy and Biotechnology

A recent analysis of 835 articles of eight European microbiology journals revealed that less than 1 % of the bacterial isolates cited had been secured in public mBRCs (Stackebrandt 2010). By accessing, maintaining and professionally distributing a higher fraction than just 1 % of the microorganisms used in microbiological research, mBRCs could become instrumental in unlocking their potential for basic and applied research. Although many of the existing microbial strains may represent novel isolates of already described species, phylogenetically closely related strains can still exhibit a distinct genetic potential and novel, unknown phenotypic properties (Jaspers and Overmann 2004). Therefore, at least some of these not-yet-publicly-available isolates are of potential relevance for future scientific discovery. The so-called ‘key strain’ concept has recently been established (Stackebrandt et al. 2014) and subsequently amended (Overmann 2015) to aid the prioritization of

suitable strains that should be deposited and secured in mBRCs. The substantial monetary value of microbial resources (see Sect. 4.2) could be preserved and future scientific work with these strains promoted if this concept was consistently applied. A similar situation would exist if scientific journals or research funders required the deposit of the strains in mBRCs as a pre-condition to publication or continued funding (as is the case for the deposit of nucleotide and protein sequences in public databases). The present financial budgets of most mBRCs severely constrain their capacities for additional curation, storage and distribution work. Tight networking, moderate increases in budgets, and concerted action of mBRCs would provide a highly cost-effective way to accommodate a significantly larger amount of microbial resources (see Sect. 4.8).

However, future demands for support by mBRCs are likely to extend far beyond securing the microbial strains isolated in academic research laboratories. In particular the profound competence of mBRCs to cultivate fastidious and novel types of microorganisms and thereby render the uncharted fraction of microbial diversity accessible to others, is likely to become increasingly important. One potential area where the existing strengths of mBRCs could be directly coupled to societal needs is in the development of new antibiotics. Indeed, the number of new anti-infective compound classes developed over the past 40 years has been decreasing steadily, while resistance problems caused by current antibiotics treatments have been increasing concomitantly. This increasing gap in anti-infective innovation has rendered the discovery of novel lead compounds a highly pressing issue (Cooper and Shlaes 2011). The present lack of novel lead compounds has been attributed to the small share of bacterial (mostly streptomycetes and myxobacteria) and fungal diversity that have been explored for secondary metabolites (Bérdy 2005; Scannell et al. 2012). Past screening programs by the pharmaceutical industry that aimed at the discovery of novel natural compounds yielded microbial isolates that frequently produced already-known antibiotics and antibiotics classes (Baltz 2006). However, biochemically and physiologically novel bacteria are typically found in underexplored phylogenetic lineages (Wu et al. 2009) in which novel natural compounds have already been detected. The wide swath of uncharted microbial diversity provides mBRCs with huge targets for novel acquisitions.

Approximately, 60 % of all marketed pharmaceuticals are either developed directly from natural compounds or from chemically modified derivatives (Newman and Cragg 2012). More than half of prescriptions filled in the USA in 1993 contained at least one major active compound derived from, or modelled on, natural compounds and 42 % of the sales of the 25 top-selling drugs worldwide are either biologicals, natural products or entities derived from natural products (ten Kate and Laird 1999). Also, the screening and chemical analysis of natural products continues to provide novel chemical scaffolds for the development of novel drugs (Butler 2005; Chin et al. 2006). Given the current decline in expenditures for R&D by biotechnology companies (Ernst and Young 2014) that in part may be attributed to the limited access to novel biotechnological products and processes (EuropaBio 2014), a proprietary biodiscovery strategy of mBRCs would provide specific and novel opportunities for mBRCs and fill a need from bioindustry. Simultaneously, the vast

microbial diversity makes it essential to coordinate efforts and focus detailed studies on those microbial resources that have the highest potential for application. As an example, although about 20,000 marine bioactive compounds were discovered since the mid-1960s through successful bioprospecting programs, only about ten have reached the market (Mayer et al. 2010; Rocha et al. 2011). Here, major bottlenecks have been difficulties of synthesizing larger amounts of natural compounds either through their producer organisms or by chemical synthesis (Glaser and Mayer 2009). Therefore, it may be rewarding for mBRCs to (1) develop procedures for selecting suitable microbial resources early during the isolation or acquisition stage, (2) generate the necessary information on biosynthetic pathways and the underlying gene clusters on the induction conditions of non-constitutively expressed biosynthesis pathways, and (3) provide upscaling data on large scale cultivation of suitable producers.

Finally, a growing future demand for scientific services that are technologically demanding and/or require specialized scientific knowledge. With the recent advances in and widespread availability of, sequencing technologies and bioinformatics, standardized and high quality genomic DNA for purchase will become an increasingly attractive resource for the customers of mBRCs, since it alleviates the users of the burden to cultivate fastidious or slow-growing microbial strains. Microbial sequence information is expanding exponentially and large sequencing programs (e.g., the GEBA project of the DOE Joint Genome Institute, CA, USA; Wu et al. 2009) are devoted to generating a large, phylogenetically diverse database of genome sequences for future in-depth studies of bacterial strains. This means that results from comparative genomics will replace the experimental services traditionally offered by mBRCS, such as DNA/DNA hybridization, and determination of the GC-content. Other services, such as taxonomic identification or chemotaxonomic analysis based on phospholipid fatty acid profiles, will probably continue to be requested from mBRCs since the necessary equipment and expertise is not maintained by many research laboratories. Furthermore, isolation of difficult-to-grow pure cultures, optimization of growth conditions, phenotypic characterization, and unique environmental sampling most likely represent services that will be requested at an increased frequency in the future. An integrated spectrum of such expensive and labour-intensive services can be offered most cost-effectively through combining the complementary expertise and instrumentation of individual mBRCs by means of networking.

4.6 Structured Information on Microbial Resources and the Key Role of Databases

In order to harness bacteria for new applications, and as discussed in the preceding sections of this chapter, a sufficient amount of strain-associated (meta)data will be needed for future use and application of the available microbial resources.

As a first step toward structuring information on microbial resources, the World Data Centre for Microorganisms was established and went online in 1997. Of the 692 public culture collections registered in the WDCM CCINFO system to date, only 110 have published their strain holdings online, or in a digitalized catalogue (WDCM 2015). Accordingly, the WDCM Global Catalogue of Microorganisms (GCM) has been established to improve the access to strain-related information and to promote scientific and industrial use of the public microbial resources (Wu et al. 2013). The GCM database links strain catalogue information as provided by individual collections to the nucleotide and protein sequences of the strains stored in molecular databases. At the time of writing, this information had become available for about 335,000 strains, including 108,000 bacteria and 171,000 fungi, from 68 culture collections (GCM 2015). The WDCM minimum dataset comprises 15 items, most importantly, the name and number of the individual strains, their source, history of deposit, geographic origin, and growth media or temperature. This information can be searched interactively in the database which also provides a homology and keyword search for the literature linked to the strain.

As a second initiative, the StrainInfo Bioportal (Dawyndt et al. 2005) offers comprehensive aggregated information on the numbers, taxonomic names, and International Nucleotide Sequence Database Collaboration (INSDC) accession numbers of deposited strains and dynamically compiles their exchange history by integrating the catalogues of many culture collections worldwide. In addition, StrainInfo links to literature connected to the strains. StrainInfo thus constitutes an important resource to elucidate the history of the deposit and assist in the exchange of bacterial strains by the different culture collections.

Despite these efforts, the wealth of biochemical, physiological and ecological data available for each microbial strain had remained largely inaccessible until recently and was not systematically searchable, since the strain-associated (meta) data were typically dispersed across a considerable number of publications. In order to overcome the fragmented nature of available information on bacterial strains, Bac*Dive* – the Bacterial Diversity Metadatabase was recently established by the Leibniz-Institute DSMZ (Söhngen et al. 2014). Bac*Dive* covers data on the taxonomy, morphology, physiology, cultivation, geographic origin, application, biological interactions, and the appropriate sources of supply for each strain together with its genome and 16S rRNA sequences deposited at the INSDC. The relational database behind BacDive was constructed by defining more than 400 data fields for each strain. Besides the relevant primary scientific literature, sources for the annotation include detailed internal descriptions of culture collections and expert-compiled compendia on strains which are not publicly available. In Bac*Dive*, the majority of the data is manually annotated and curated. Importantly, the portal offers powerful advanced search functionalities that allow the combination of more than 30 search fields for text and numerical data. Bac*Dive* will provide quick and complete access to information about the cultured microbial biodiversity which is currently not available in any other database; this innovative tool also enables the user to filter the information for all strains according to particular attributes.

An important precondition for improving the usability of microbial resources is the central accessibility and comparability of all existing strain metadata and molecular data. A central, integrated portal providing this combined information based on common standards and quality criteria would constitute a highly desirable resource for academia and industry. This would facilitate locating a particular microbial resource for the customer and is, indeed, one of the core goals of the MIRRI network. By offering additional information on natural products (e.g. antibiotics), genomics, and biosecurity, the integrated portal could also serve as an essential resource for bioindustry and the development of the bioeconomy. An integrated portal would provide a major incentive for participation of individual mBRCs, since it would increase their visibility. The most practical solution to the challenge could be to link the data of the already existing databases (e.g., StrainInfo Bioportal, Bac*Dive*, Silva; Quast et al. 2013). In fact, StrainInfo and Bac*Dive* already have established standards and data fields according to the methods developed in collaboration with the Genomic Standards Consortium (Tuama et al. 2012; Yilmaz et al. 2011). Individual mBRCs will need further improvements to provide their data more dynamically, in addition to providing the data exchange standards. Accordingly, an integrated portal could be realized in a short time period (estimated 2 years) and, importantly, would capitalize on existing expertise and human resources to ensure the future availability of the data.

4.7 Ownership and Legal Constraints for the Exploitation of Microbial Resources

Through the Convention on Biological Diversity (CBD) that entered into force on 29 December 1993, the sovereign rights over genetic resources were transferred to the country of origin (CBD 2015; Article 15.1), instead of being considered a common heritage of mankind. The CBD aims at the (a) conservation of biological diversity, (b) sustainable use of its components, and (c) fair and equitable sharing of benefits (both monetary and non-monetary) arising from the utilisation of genetic resources. Since the latter is expected to provide incentives to provider countries for the conservation of biological diversity within the states of origin, these goals are interdependent.

In the case of microorganisms, which constitute a significant, if not the largest, fraction of biological diversity on the planet ((a) 10^7 to 10^9 and (b) 5.1×10^6 species of (a) bacteria and (b) fungi respectively; Sect. 4.2), the first (conservation) and second (sustainable use) aim of the CBD are less relevant for four biological reasons (Overmann 2015):

1. Microorganisms, especially bacteria, are highly unlikely to become extinct because of the large size of their populations;
2. Microbial field sampling of microscopic organisms is not as invasive or destructive compared to larger organisms since small environmental samples are normally required;

3. Most species of bacteria do not show a definable biogeography due to an efficient dispersal across and between continents;
4. Furthermore, different bacterial and fungal species show a considerable functional redundancy in metabolic functions and may also synthesize identical natural compounds.

Based on the missing threat of extinction of bacteria and also many other microorganisms, it is understandable, that pharmaceutical companies have not been willing to finance the conservation of areas with high natural biodiversity (Simpson 1997). However, it must be pointed out that environmental conditions impact on microbial chemistry and strains from different locations can have different properties, hence preserving these areas could be important.

Thus, the inherent ecological assumptions of the CBD with regard to conservation and sustainable use, do not fit our understanding of microbial ecology and therefore the policy implications of the CBD are misaligned for microbial research. We exclude the larger fungi here, some appearing on the International Union for Conservation of Nature Red List of Threatened Species (www.iucnredlist.org) and the current authors refer generally to the microfungi. By contrast, the third goal of the CBD (Access and Benefit Sharing, ABS) is of much higher relevance for the future work with microbial resources, particularly since the large potential for natural product discovery (Sect. 4.5) renders microorganisms prime targets for exploitation and future utilization.

With the aim to provide the necessary legal framework for the effective implementation of Access and Benefit Sharing (ABS), The *Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity* (the 'Nagoya Protocol'; CBD 2015) was adopted on 29 October 2010 in Nagoya, Japan and entered into force on 12 October 2014, 90 days after the deposit of the fiftieth instrument of ratification and has been ratified by 59 parties (although the U.S., Canada, Russia, and China are notably absent). In Europe, the implementing EU Regulation No 511/2014 on ABS (EU 2014) was enacted simultaneously. In Europe, individual EU member states will now implement the necessary individual national legislation. Globally, each country signatory to the CBD and the Nagoya Protocol will implement its own controls, ensuring compliance with its requirements.

The Nagoya Protocol addresses all activities related to the utilization of genetic resources in the broadest sense. Besides research and development directly targeting future applications, e.g., through screening of microbial strains for useful natural products, or for the control of pests (including invertebrates, vertebrates, weeds and diseases), the Nagoya Protocol also covers basic research that is not intended to commercially exploit microbial resources, for example biodiversity surveys, or the export for taxonomic identification. However, article 8 does offer countries the opportunity to make special considerations for non-commercial use. The objective is the fair and equitable sharing of benefits arising from the utilization of genetic resources, their biochemical composition, and from traditional knowledge associated with these genetic resources, through the implementation of suitable mechanisms and by balancing regulatory controls and due diligence. Utilization of a

genetic resource must be traceable along the value chain of research, development, innovation, pre-commercialization and commercialization. Benefits are not only monetary but also non-monetary, such as the sharing of research results, technology transfer, and training opportunities.

Accordingly, the Nagoya Protocol imposes the following obligations on all users (in the broadest sense) of genetic resources (compare Article 4 of EU Regulation No 511/2014):

- all materials collected or received by the users must have relevant permits or Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT) that include sharing of benefits, if applicable,
- the user is required to provide information on the source of genetic material, and the associated PIC and MAT to the national checkpoint, including available unique identifiers. For materials received from other sources, the user needs to obtain the information relevant to ABS,
- the user is required to transfer to subsequent users all information relevant to ABS,
- records of all the activities related to the use of a genetic resource must be maintained by the user for 20 years after termination of use.

On the level of signatory states, the following specific measures to implement the Nagoya Protocol are currently considered. The ABS Clearing House (ABSCH) represents a key tool for facilitating the implementation of the Nagoya Protocol (ABSCH 2015). The ABSCH is a platform for exchanging information on access and benefit-sharing where each Party to the Nagoya Protocol is required to make available (a) its legislative, administrative and policy measures on ABS, (b) information on the national focal point and competent national authority or authorities, and (c) information on permits or their equivalent, that were issued at the time of access. The National Authorities may choose to issue an Internationally Recognized Certificate of Compliance (IRCC), a record created nationally when a permit or equivalent (PIC and MAT) is made available to the ABS Clearing House in order to facilitate the monitoring of the utilization of genetic resources along the value chain, that extends from (a) isolation, characterization, screening and application trials to (b) commercialization of products. Although 197 party and non-party states have filed information on their national focal points, no single country has yet registered any IRCC (ABSCH 2015). Based on the current legal situation described above, mBRC now face numerous challenges:

1. Information on existing PIC has so far been supplied for only 26 of the ~25,000 bacterial strains deposited in the DSMZ. This very low incidence strongly suggests that many depositors were not aware of the legal restrictions that have been in place since 1993. It is foreseeable that the implementation of the Nagoya Protocol will result in a significantly increased demand of mBRCs customers for legal support and expertise at all stages of microbiological work, starting at the planning phase of research projects.

2. Notably, the Nagoya Protocol clearly states (in Article 8) the need for simplified measures on access for non-commercial research purposes for developing countries. Countries of origin can very efficiently facilitate utilization and capitalize of their microbial resources by rapidly implementing the concept. By making access mechanisms straight-forward, whilst implementing best practice in tracking genetic resource use by commercial companies, it will encourage the use of microbial resources and increase the chances of discovery and long-term benefits. This will have a lasting effect on the future development of science in and the bioeconomy of a country. A delayed implementation of the ABS clearing house will result in users remaining unaware of relevant procedures. Delayed implementation or over complicated and demanding systems for the legal mechanisms, may result in a significant competitive disadvantage for domestic science, research and development. In particular, (a) novel types of microorganisms (due to their presence in other countries) may be isolated, characterized and published by competing researchers, (b) domestic researchers cannot participate in bi- and multilateral research projects and hence will be isolated from technology transfer and capacity building, and (c) identical natural compounds may be isolated more rapidly by competitors in other countries (Overmann 2015).
3. The 115,000 strains in the WDCM Global Catalogue of Microorganisms with known geographic origin records were isolated from 164 different countries. Most of these characterized strains originated in Europe (36 %) and Asia (25 %) (Wu et al. 2013). For the rest of the WDCM registered strains, information is unavailable or has not been entered into the GCM database. Nevertheless, the available data indicate that microbial diversity of the entire African and South American continents is severely underrepresented within culture collections. These regions could unlock the potential of their microbial diversity through implementation of appropriate ABS tools and networking with existing mBRCs and research centres.
4. The workload for scientific users to comply with the Nagoya Protocol can be significantly reduced by sourcing materials from mBRCs that have implemented community best practice and utilizing clusters of expertise and competent institutions. As a suitable tool, EU Regulation No 511/2014 identifies, and stipulates that ‘Union trusted collections’ or ‘Registered collections’ be established to apply standardized procedures for (a) exchanging and supplying microbial resources, (b) providing the necessary documentation of compliance with the Nagoya Protocol, (c) monitoring the transfer of microbial resources, and (d) keeping the necessary records. The fact that only 12,147 (i.e., 4 %) of the strains in the GCM have detailed information on their history (Wu et al. 2013), clearly demonstrates the magnitude of the additional effort that mBRCs need to establish the database entries that are now needed. This includes source, geographic coordinates of sampling location and information on the isolator, depositor and ownership.
5. The microbial resources in public collections are used only rarely for commercial applications. Using the Leibniz-Institute DSMZ as an example, 37,000

resources (mostly bacterial, but also fungi, human cell lines, plant viruses, and genomic DNA) are delivered annually, of which only one (i.e., 0.003 %) was used for commercialization. Interestingly, DSMZ each year receives 100 enquiries for commercialization. This means that almost all potential customers eventually abstain from commercial applications because of the public accessibility of the resource and difficulties in clarifying the legal requirements for their use. It is evident that legal certainty combined with an exclusive access to microbial resources, will be critical to their future commercial exploitation. This will require negotiations of appropriate ABS measures with the view to bioprospecting. However, for countries interested in advancing their bioeconomy sector, the fact that most microorganisms do not occur exclusively in their territory, will constitute a particular incentive to participate in multilateral biodiscovery programs. These will enable a rapid and efficiently translation of the results to biotechnological or medical applications.

4.8 Role of Networking Between Microbial Domain Biological Resource Centers

Dedicated, long-term research infrastructures provide the most effective means to (a) preserve the monetary value of living microbial resources, (b) gather and maintain the professional knowledge and data associated with them, and (c) offer the essential scientific services (in particular identification, chemotaxonomy, and physiological characterization). Through these characteristics, mBRCs underpin innovation and discovery and are key to the realisation of the targets for sustainable and inclusive growth in the bioeconomy. However, single mBRCs alone are not capable of covering the vast, so far unexplored molecular and physiological diversity, including the supporting services and data that are of interest to future bioprospecting projects. Furthermore, the current fragmentation of individual holdings, services, strategies and policies result in duplication and gaps in what can be offered. These inherent weaknesses are costly in times of public budget constraints, major demographic changes and increasing global competition. Networking of mBRCs could provide a practical solution to these challenges.

There are various forms in which culture collection networking has taken place in the past (Smith et al. 2013, 2014); efforts to coordinate and develop mBRCs have been undertaken at national, regional and global levels. An example is when collections collaborate in project consortia to answer research programme calls which address specific microbial problems or research areas. These are often short lived, and are usually dictated by the term of the funding: Hence they are insufficient to maintain new structures and competences after termination of the project. Another is the formation of national, regional and international federations to (a) further the activities of collections, (b) facilitate access to their materials, and (c) improve operations. Networking at the national level is quite frequent. Most are loose federations bringing together collection staff and users to discuss common

issues and share information. The Belgium Co-ordinated Collections of Microorganisms (BCCM™) is an example of a more defined infrastructure where policy and strategy are set through the Belgian Science Policy Office which coordinates operations, research and development. The U.S. Culture Collection Network (USCCN) is funded by the National Science Foundation and facilitates scientists working with laboratory based collections of microbes. Overall, there are 17 such national federations (Smith 2014).

At the international level, the oldest and largest federation of collections is the World Federation for Culture Collections (WFCC) which was founded in 1968 and currently has over 120 affiliated collections. It promotes and supports the establishment of culture collections and related services. Information networks are established between the collections and their users, while workshops and conferences are organised resulting in publications and newsletters (WFCC 2015). In particular, this effort has resulted in the World Data Centre for Microorganisms with 692 registered collections (WDCM 2015). The European Culture Collections' Organisation (ECCO 2015) followed in 1981 and joins 61 member collections from 22 European countries. ECCO has been an incubator for pan-European activities and has driven the development of collections. It was instrumental in coordinating activities through joint initiatives and projects, e.g., within recent European Community Framework Programmes, including the Common Access to Biological Resources and Information (CABRI – http://www.cabri.org), European Biological Resource Centres Network EBRCN – http://www.ebrcn.net) and the European Consortium of Microbial Resource Centres (EMbaRC – http://www.embarc.eu).

Federations, like the majority of other regional and national networks, are mostly run on a volunteer basis and rely on individuals to carry out the operations using small amounts of funding, often from membership fees. Federations typically have no mandate to change the institutional policy and strategy of their members, although they are more enduring than project consortia. However, to meet modern day challenges, mBRCs must more actively share tasks and strategically coordinate their activities. They need to (a) work under common quality management systems to deliver consistently high quality materials, (b) coordinate their accession policies to arrive at complementary holdings, (c) share facilities, technologies, and expertise and (d) train young researchers for higher cost efficiency.

The Organisation for Economic Cooperation and Development (OECD) Biological Resource Centre initiative (1999–2006) provided the framework for best practice and biological resource networking. It proposed a Global Biological Resource Centre Network (GBRCN) to (a) integrate services and resources, (b) encourage innovative solutions, (c) provide coherence in the application of quality standards, (d) allow homogeneity in data storage and management, and (e) facilitate workload sharing. It recommended that the new generation culture collections (i.e. mBRCs) undertake a proof of concept for the GBRCN that would enhance microbial resource availability and quality. As a consequence, the demonstration project for a GBRCN (http://www.gbrcn.org) commenced in 2008. The German Government, through the German Federal Ministry of Research and Education (Bundesministerium für Bildung und Forschung, BMBF), supported a small

Secretariat to draw national efforts together in developing tools for the establishment of the GBRCN. National and regional efforts have been initiated to establish the GBRCN with the aim to build a structured, long-lasting global network, enabling collections to meet user needs since the project report was published (Fritze et al. 2010). Unnecessary competition between regional networks with similar goals can be reduced if they (a) become partners of GBRCN, (b) become signatories to a membership agreement that will establish a common operational framework, and (c) participate in the decision making processes of the GBRCN. Currently, GBRCN has partners in North- and South America, Africa, Asia and a strong base in Europe.

The success of the GBCRN demonstration project and collaboration with EMbaRC and ECCO, led to the pan-European initiative MIRRI being placed on the European Strategy Forum for Research Infrastructures (ESFRI) road map. This distributed infrastructure currently interlinks 43 public collections and research institutes from 19 countries holding more than 360,000 microbial resources. MIRRI brings together European microbial resource collections and their stakeholders (users, policy makers, potential funders and the plethora of microbial research efforts) and links them to non-European country partners with the aim to add value through:

- a coordinated approach to the implementation of best practice and coherent application of quality standards,
- a coordinated strategy to provide a broader, less redundant coverage of microbiological resources and services,
- a distributed platform for microbial taxonomy to ensure best use of the remaining expertise and to put in place a human resource development program,
- common policies across international boundaries facilitating legitimate access,
- establishing facilities and resources in countries or regions rich in microbial diversity, but without resources and facilities to utilize them in research,
- homogeneity in data storage and management, enabling data mining and targeting of specific microbial resources for specific tasks, through the MIRRI information portal,
- a business model where access to all microbial resources can be found through contact with a single mBRC (Schüngel et al. 2013).

Finally, the Asian Consortium for the Conservation and Sustainable Utilization of Microbial Resources (ACM) has been established (Ando et al. 2014). Based on the principles of the Nagoya Protocol, ACM has established a Network of International Exchange of Microbes under ACM (NIEMA; http://www.acm-mrc.asia/am/acm10.html) which facilitates movement from a single collection (the registering collection) to another network member, but without distribution to outside of the network without permission. Strains exchanged under the NIEMA scheme can only be used for non-commercial purposes whereas a facilitated exchange of microbes for commercial use is not addressed. Thus this particular network scheme requires additional mechanisms to link with other networks for a broader exchange of biomaterials, and cannot legally provide them for a broader use in research and development. mBRC networks have to develop novel mechanisms that conform to

existing laws to allow a broader application of bioresources, while limiting additional administrative burdens. Given the persistent growth of the bioeconomical sector and the very limited funding available for taxonomic work, mBRCs are expected to make a much more visible contribution towards the bioeconomy than previously.

4.9 A Business Plan for Microbial Domain Biological Resource Centers

As outlined in Sect. 4.3, mBRCs cannot operate in a cost-covering manner if their mission is to support publicly funded scientific research with high quality, and well characterized, microbial resources. Indeed, the majority of all CCINFO registered culture collections are affiliated to universities or are governmental (WDCM 2015) and the level of public funding of mBRCs is in the range of 65–92 % (Smith et al. 2013). Nevertheless, user needs dictate the structure, activities and future planning of mBRCs which is similar to the private enterprise situation. In this respect, mBRCs are distinct from other scientific institutions in that they are not primarily founded on the success and originality of their scientific research, but need to provide key microbial resources, information, and expertise for research and development. These particular features have to be considered when developing business plans that integrate additional tasks of mBRCs related to bioprospecting.

Traditionally, the main stakeholders of mBRCs are (a) the depositors, academic users, national funding bodies, and umbrella organisations to which mBRCs are affiliated (scientific associations and WFCC), and (b) intergovernmental organisations including OECD. The (a) necessity to safeguard key strains (Sect. 4.5), (b) need for novel types of microbial resources and (meta)data (Sects. 4.5 and 4.6), (c) recent developments regarding the national implementation of the Nagoya Protocol (Sect. 4.7), and (d) necessity to network (Sect. 4.8), will call additional stakeholders into action. In particular, these comprise (a) scientific journals, (b) other mBRCs and collaborating scientific institutes that act as network partners, (c) industrial users such as small and medium enterprises (SMEs) and industrial organizations, and (d) national regulatory authorities (Fig. 4.1). Individual mBRCs will be able to identify the requirements of additional and specific stakeholders through a more tailored stakeholder analysis.

Identifying the most influential and reliable stakeholders, together with raising their interest and engaging their support, will be key issues for mBRCs if they are to enter the field of bioprospecting in a sustainable manner. The establishment of small biotechnology companies can play a decisive role in the initial discovery of, for example, promising marine bioactive compounds, as these enterprises will work closely with academics and governmental agencies when performing the initial steps in the discovery of new natural products (Genilloud 2014). Collaboration between private companies and public institutions will be of paramount importance for financial support in the discovery process. Crude extracts and pure compounds

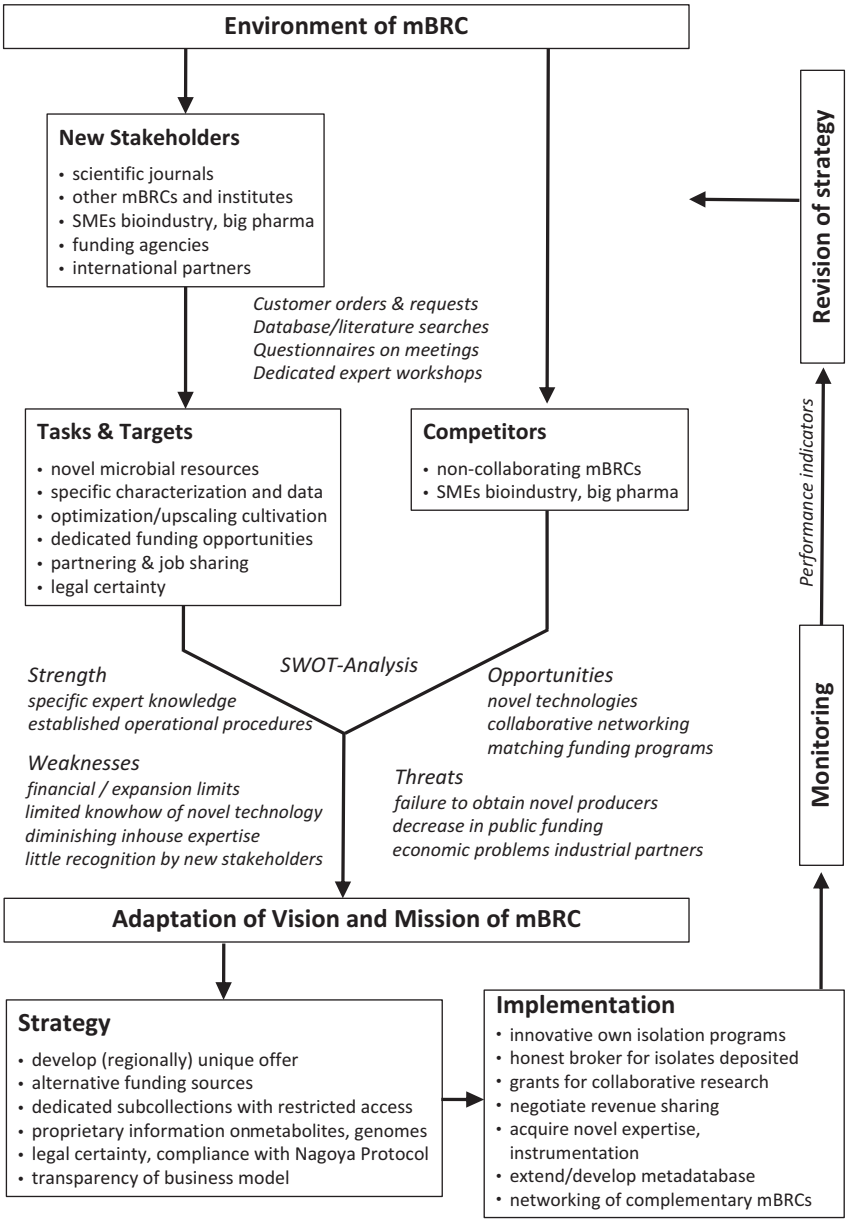

Fig. 4.1 Elements with key aspects (*boxes*) and specific approaches (*in italics*) that constitute a possible business plan for the future development of an mBRCs considering to enter the field of bioprospecting. The envisioned time frame is 5 years

produced by academic laboratories can be used for diverse bioassays as part of broader collaboration programs with private biotech companies worldwide. One challenge for universities is to devise mechanisms that protect intellectual property and simultaneously encourage partnerships with the private sector, by recognizing that the chances of a major commercial pay-off are small, especially if drug discovery is pursued by a single institution (Rocha et al. 2011). In this respect, mBRCs can also support universities by providing the necessary expertise on the true costs and time requirements of isolation, cultivation, upscaling and characterization procedures.

The development of the downstream elements of the business plan is largely determined by the actual needs of depositors and users that are involved in bioprospecting. In contrast, public funders of mBRCs determine the overall mission of most mBRCs, but typically have less specific expectations and directives. Based on the public relations experience of the DSMZ, the analysis of novel customer needs must be performed proactively by the mBRC itself and cannot solely be based on (rather sporadic) requests filed by individual customers. The crucial information on depositor and user needs can be gained through (i) an analysis of customer orders and (ii) the requests for missing materials, (iii) database and literature searches revealing the potential type and number of future deposits and the associated information required, (iv) questionnaires to interrogate delegates of the appropriate scientific meetings or bioindustry events, and (v) expert workshops organized by mBRCs to discuss needs and solutions with international experts (Fig. 4.1).

Traditional products must be complemented by the accession of novel products to meet the needs of new users. Many mBRCs make available DNA, enzymes, secondary metabolites and other derivatives from authenticated strains, or curated databases linked to genome sequences, either as the standard inventory, or on a case-by-case basis. mBRCs can move beyond their traditional services by developing commercial products through the provision of biotechnological solutions to problems, active compounds, and contract research services. These can be funded through public-private investment and spin-off companies.

The non-governmental organization CABI, has been moving in this direction since the 1990's after direct UK Government funding ceased. For example, it identified the need for a rapid test kit to detect fungal contamination in kerosene used as plane fuel because previously available detection methods required as long as 3–10 days for a test result. The company Conidia Bioscience (http://www.conidia.com) was established to develop the FUELSTAT™ detection kit that is changing paradigms for detection of contaminants in fuel and which is now recommended in the Boeing Aircraft Maintenance Manual (http://samtheta.org/id-docs/b/boeing-aircraft-maintenance-manual-pdf-39871.pdf). This demonstrates that culture collection staff can devise solutions to current microbial problems and establish companies. At CABI, the profits are partly used to support biosystematics, biological collections and fundamental research. CABI has also been involved in developing biocontrol agents and one particular success, in collaboration with partners, has been Green Muscle, a fungal product used for control of African Locust (Lomes et al. 2001). The profits fund biodiversity initiatives in Africa. Although neither of these examples are related to bioprospecting per se, the future will reveal whether

establishing spin-off companies with proprietary products are as beneficial as mBRCs acting directly as brokers between owners and their commercial users of microbial resources.

In conclusion, mBRCs planning to make a significant contribution towards bioprospecting, need to gain suitable information on the (a) required microbial resources and associated (meta)data, (b) additional expertise, (c) funding programs that might support additional activities of mBRCs, (d) legal requirements, (e) possible partners, and (f) potential competitors. In this analysis, the internal strengths and weaknesses as well as external opportunities and threats must be considered, e.g. by undertaking a SWOT-analysis (Fig. 4.1). This will support the development of a specific product and an appropriate marketing strategy that promote the unique selling points of the individual mBRCs.

It is likely that individual mBRCs will choose to focus on those groups of organisms for which they already possess the essential skills and operational procedures, given the tremendous taxonomic, biochemical and physiological diversity that remains undiscovered. As an inherent weakness, the limited public funding of mBRCs often will not permit an expansion of tasks into novel diversity. It has to be emphasized that revenues generated through sales of even high value microbial resources and associated metabolomic and genomic data to bioindustry will not cover costs. Therefore, alternative funding requires to be sought from the government agencies in charge of the bioeconomy that traditionally are not stakeholders of mBRC, and through participation in dedicated funding programs for bioprospecting such as the EU Horizon 2020 INFRADEV provide numerous opportunities.

Suitable performance indicators have to be developed and applied when monitoring mBRCs for the success of novel strategies and their implementation (Fig. 4.1). One indicator is the rate of increase in the number of specific target strains deposited (Fig. 4.2). The success of data provision can be monitored through analysis of online visitors to (meta)databases maintained by an mBRC. In addition, the third-party funds acquired need to be considered. Publications relating to biotechnology and bioprospecting are another suitable measure of impact. Standard evaluation procedures ascribe to first or corresponding authorships of scientific publications have a significantly higher value than co-authorships. In the case of mBRCs, however, a large number of co-authorships by curators, or other workers in mBRCs, should be valued highly since it clearly documents that the work and expertise is enabling the scientific success of institutions other than mBRCs.

Even if numerous novel types of microorganisms can be efficiently recovered in the future, an untargeted screening approach to the bioprospecting of thousands of novel isolates would be far too costly (i.e. between 0.5 and 1 billion Euros per approved drug (Overmann 2015). Instead, promising isolates for the future development of pharmaceuticals, or for biotechnological applications could be identified, based on a sufficient knowledge of their biochemistry and physiology, which can be obtained by mBRCs as partners and brokers. Therefore, one key to a successful entry of mBRCs into the field of bioprospecting will be to generate information on the biosynthetic potential of novel types of microorganisms. This can be achieved by extended characterization, metabolic profiling, and genome and transcriptome

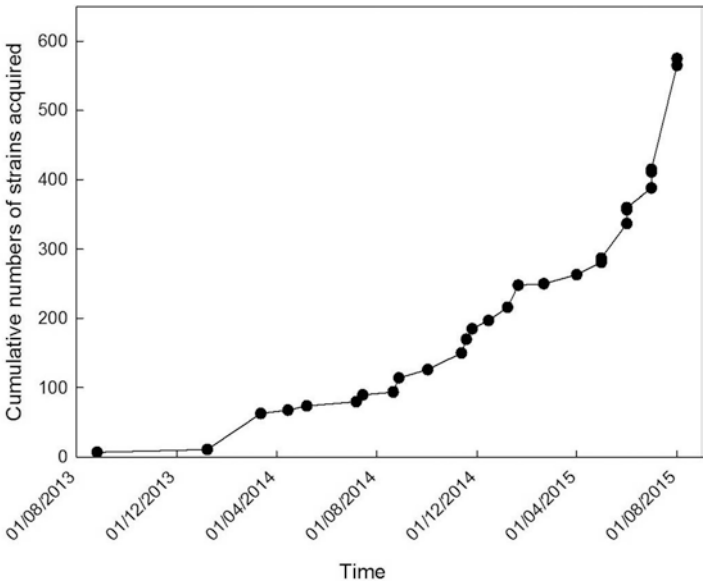

Fig. 4.2 Example for the monitoring of the successful acquisition of novel target microbial resources by the Leibniz-Institute DSMZ. The time course of the increase in strains of pathogenic bacteria deposited in the DSMZ sub-collection "Central Pathogen Repository of the German Centre of Infection Research" is depicted

analyses. Furthermore, information on mass cultivation in bioreactors would be advantagenous as mentioned previously. Pre-screening will probably yield information to substantially increase the rate of discovery of novel compounds and significantly increasing the value of the individual microorganism. Most mBRCs do not possess state-of-the-art genomic and metabolomic technology which enable the generation of the essential high throughput data. However, this could be gained through collaboration with external partners.

Another key issue for a successful entry into the field of bioprospecting, is to establish sustainable strategies for gaining better access to the vast uncharted microbial biodiversity and better quality control, characterization and distribution to users. Particular challenges include (a) compliance with new legal requirements that threaten to impede efficient international collaboration, (b) the difficulties of individual mBRCs to collaborate with industry, (c) the need to increase the attractiveness of mBRCs to bioindustry, and (d) the need for developing countries to gain access to bioindustry.

One possible solution is to join the existing expertise of established mBRCs that attract sufficient funding, to rapidly create a platform providing a single place for a large number of well characterized, pre-screened microbial resources in a legally compliant manner (Fig. 4.3). For example, there are currently 45 major collections supplying cultures in Europe. No single collection would be able to compete with the coverage attained by the MIRRI consortium. Due to its public funding and

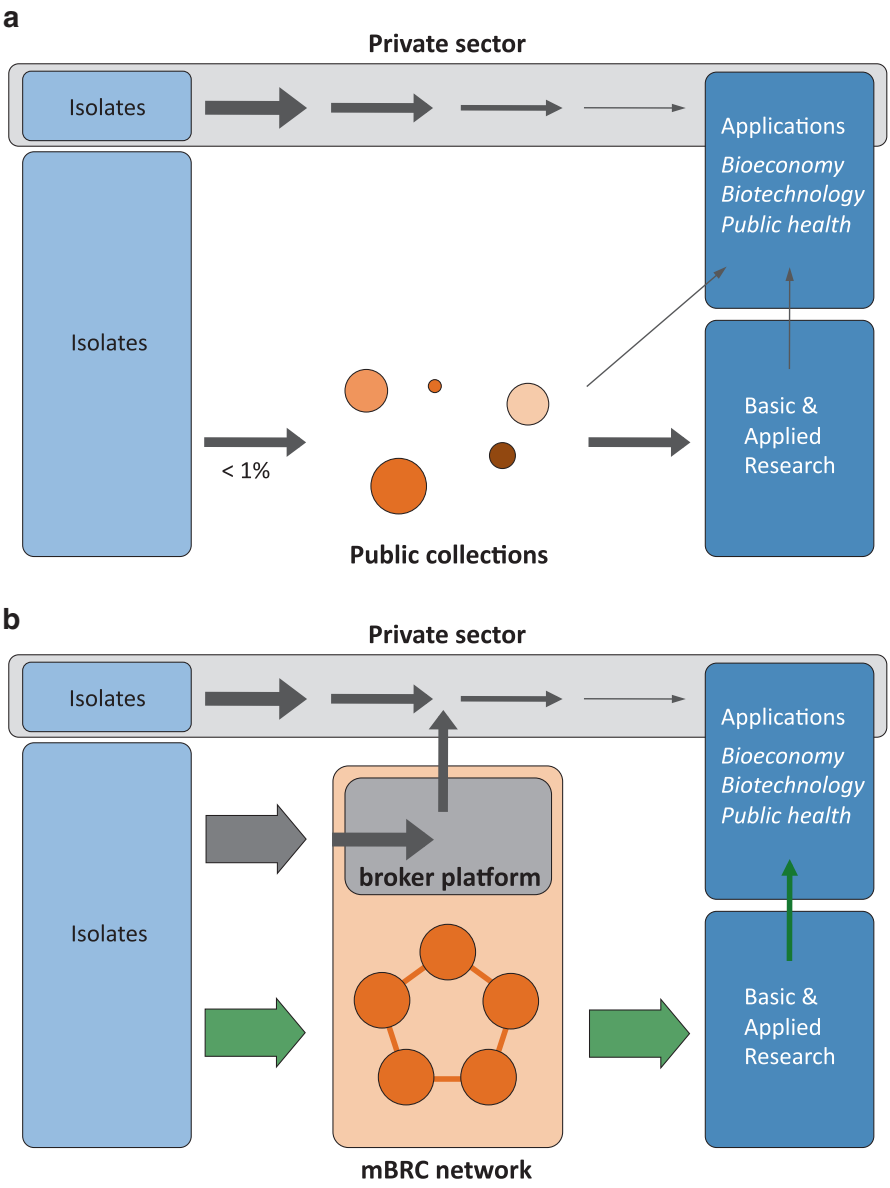

Fig. 4.3 (**a**) Present situation of culture collections and mBRCs that provide microbial isolates for basic research and application. Currently, culture collections and mBRCs acquire less than 1 % of isolates generated through public research. Since they act mostly in an uncoordinated and non-complementary manner, they cannot make major contributions towards bioprospecting. (**b**) Concept to enable mBRCs to make major contributions towards bioprospecting through formation of a lasting, tight network and establishing a platform specifically devoted to broker strains for bioindustry

hence financial independence, this network of mBRCs could act as financially independent, honest broker that would constitute a more visible and attractive partner for private companies than each individual mBRC. Furthermore, such a platform would provide an easy and reliable entry point for developing countries to supply microbial resources at different levels (undefined natural samples/enrichments, microbial isolates, pre-screened target strains with existing metadata), and offer them to bioindustry in geographic regions and markets that would otherwise be inaccessible. On the other hand, if bioindustry needs to be established in a developing country, it could use such a platform to gain access to a much broader choice of microbial resources than the individual country could provide individually. Microbial resources that are particularly enriched in a certain region, e.g. strains related to the bioremediation of specific mine tailings, specific xenobiotica contaminations, or plant-growth-promoting bacteria associated with endemic plants, could be offered most effectively and in a competitive manner by the respective country for future bioprospecting.

Finally, the management of those collections dedicated to bioprospecting has to consider the distinct legal requirements of bioindustry. Accordingly, such subcollections would only become attractive to a user from bioindustry if it was given exclusive access to the microbial resources. The would include the associated data, and if legal certainty is provided, e.g., by pre-negotiated contracts for each bioresource that fix the share of benefits for all partners involved (i.e. country of origin, scientists involved in the detection, isolation and characterization, and mBRCs providing storage, quality control, databases and the distribution system).

4.10 Conclusions

The immense diversity of bacteria and filamentous fungi remains largely untapped. Extending bioprospecting activities to these novel microorganisms with often unknown physiological properties requires specialized skills with respect to the isolation, cultivation and characterization of fastidious strains, dedicated (meta)databases, but also legal competencies with respect to international law. While many of these essential skills and infrastructures are maintained at mBRCs, the latter so far focus on preserving microbial resources for basic scientific studies and providing them to academic and industrial researchers. mBRCs could make a significant contribution towards bioprospecting by (a) improving access to uncharted microbial diversity, (b) extending the characterization, metabolic profiling, and genome analysis of novel microbial resources, (c) establishing proprietary databases with information relevant to bioprospecting, and (d) providing the means for exclusive exploitation of microbial resources. However, since public funding is limited and the revenues generated from sales of microbial resources rarely cost-covering, alternative funding sources are needed by mBRCs to enter the field of bioprospecting.

There is not one single business model that fits all. However, the mBRCs business plan (Fig. 4.1) developed here emphasizes the elements necessary to enter the field of bioprospecting. It goes beyond a simple strategic plan by including specific internal goals and measures for their implementation and providing tools for monitoring and decisions. The business plan would be useful for involving additional new stakeholders and help to efficiently acquire novel bioresources and develop services, improve database structure, novel funding schemes and thereby restructuring a mBRC.

However, for many mBRCs, their business plan will be an ongoing, cyclic process as user needs, technology and funding schemes change (Fig. 4.1). It provides a procedure to improve culture collections by linking them into a network to efficiently underpin the bioeconomy and ultimately make a lasting impact on bioprospecting (Fig. 4.3). Remote mBRCs in, for example, developing countries can also be included in this network. However, it is crucial that mBRCs do not become commercial entities - they must not compromise their public service role.

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