Opinion

Diversification and focusing: strategies of microbial culture collections

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Implementation of quality measures, compliance with the Convention on Biological Diversity (CBD), and adoption of latest bioinformatics tools are among the main steps to be taken by microbial culture collections in order to provide resources for the emerging area of the knowledge-based bioeconomy. These measures have to be introduced side by side with the deposition of increasingly phylogenetically and physiologically diverse microbiological organisms. However, the necessary expansion of human resources and infrastructure is moving slowly, if at all. Furthermore, considering that the vast majority of microbial isolates do not find their way into public collections, a strategy should be devised to encourage researchers to deposit a higher fraction of strains. It appears obvious that in order to make available an even broader range of diversity to users and researchers, collections will have to decide whether to diversify on a broad taxon spectrum of the hierarchic system, holding a small number of representatives per species, or to follow the route of focusing on in-depth holdings of selected groups of organisms, depending on existing taxonomic expertise. These decisions require a worldwide coordinated activity with the outcome to be made transparent to users in an emerging global network.

The paradigm shift

Collections are multi-task facilities. Research embraces phylogenetic and taxonomic research on environmental DNA, genomes and living organisms, often in close cooperation with academic and industrial research laboratories. As providers of reference strains for molecular biology studies and for tests (e.g. specified in standard industrial and pharmacopoeia requirements), quality controls are high and demanding. Due to the conservative nature of taxonomy, characterization and authentication, collection scientists are under less pressure to follow immediately the most recent advances in molecular biology, and hence they maintain taxonomic expertise that otherwise would run the risk of being lost. It is therefore not surprising to find collections participating in more than 60% of descriptions of prokaryotic species – and this in turn reflects upon the professional capacity of the entire facility. Authentication of their holdings, using the most up-to-date chemical and molecular techniques, is applied to strains accessioned, maintained and released to users. Another consequence of quality management is the continuous professional development of staff, such as raising the awareness of potential biosafety and biosecurity risks, and shipping, import and export rules and regulations, as well as improving documentary practices and building strong customer service relationships, among other points. Whereas well-supported collections will have sufficient resources to employ the relevant technical expertise, less supported facilities will most likely pass these duties on to curators, leaving them in the uncomfortable situation of having to bridge the divide between their scientific interests and the economic interests of the facility.

The immense qualitative and quantitative differences among microbial collections in the public, private and

Glossary

Biological Resource Centres (BRCs): are both service providers and repositories of 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, and cDNAs), viable but not yet culturable organisms, cells and tissues, as well as databases containing molecular, physiological and structural information relevant to these collections and related bioinformatics (http://www.oecd.org/document/51/0,3343,fr_2649_37437_33791027_1_1_1_37437,00.html).

Global Biological Resource Centre Network (GBRCN): a network designed to accommodate the future needs of biotechnology and biomedicine (http://www.gbrcn.org/).

Convention on Biological Diversity (CBD): an international treaty (1992) to sustain the diversity of life on Earth (http://www.cbd.int/convention/).

World Data Centre for Microorganisms (WDCM): an electronic gateway to databases on microbes and cell lines and resources on biodiversity, molecular biology and genomes (http://www.wfcc.info/datacenter.html).

European Consortium of Microbial Resources Centres (EMbaRC): an EU-funded project that aims to improve, coordinate and validate microbial resource centre delivery to researchers from both public and private sectors. The EMbaRC project is a mixture of networking, access, training and research (http://www.embarc.eu/).

Organisation for Economic Cooperation and Development (OECD): an international organisation helping governments tackle the economic, social and governance challenges of a global economy. OECD Best Practice Guidelines for BRCs are given in Ref. [3].

World Federation for Culture Collections (WFCC): a federation within the International Union of Microbiological Societies (IUMS) concerned with the collection, authentication, maintenance and distribution of cultures of microorganisms and cultured cells (http://www.wfcc.info/datacenter.html).

Common Access to Biological Resources and Information (CABRI): a previously funded EU project aiming at providing biological resources and quality guidelines to users (http://www.cabri.org/).

European Culture Collections' Organisation (ECCO): a consortium of European collections to promote collaboration and exchange of ideas and information about all aspects of culture collection activity (http://www.eccosite.org/).

European Biological Resource Centers Network (EBRCN): a previously funded EU project dealing with issues raised by the OECD Initiative on BRCs (http://www.cabri.org/FAQ/faq.html).

International Code of Nomenclature of Prokaryotes: governs the scientific names for prokaryotes and the rules for naming taxa of bacteria (http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=icnb&part=A185).

Knowledge-based bioeconomy: a concept that transforms life-sciences knowledge into new, sustainable, eco-efficient and competitive products (http://cordis.europa.eu/fp7/kbbe/about-kbbe_en.html)

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academic sectors, which is not always immediately visible to users, had been the starting point of a Japan–OECD (Organisation for Economic Cooperation and Development; Glossary) initiative at the end of 1990s, that aimed to enhance the quality biological resources in an appropriate legal framework, thus underpinning and driving life sciences research. Over the following decade the OECD, together with collections and stakeholders, worked towards the definition of Biological Resource Centers (BRCs) as quality-driven facilities to provide a sound and professional basis for highest sustainable economic growth [1,2]. The establishment of a BRC secretariat has been recommended to oversee the implementation of quality assurance procedures and to guide collections in their attempt to reach the BRC seal of quality. The secretariat has reached the consolidation stage, aiming at a Global Biological Resource Center Network (GBRCN). In order to avoid discrimination or favoritism of the term 'Culture Collection' or 'BRC' the plain term 'collection' will be used in this article whenever both facilities are addressed or concerned. In addition, the broad range of possible microbial organisms will be primarily restricted to prokaryotes here. In order to significantly increase awareness, and make public invaluable microbiological resources available to scientists, this article will stress the necessity for collections to intensify the dialog among themselves and to became organized in a global network, to actively cooperate with funding bodies for research and collections, and to seek agreements with editors and authors for mandatory deposition of strains. With the successful implementation of binding rules not only will the collections raise their profile and long-term sustainability, but research and the bioeconomy in general will benefit from the availability of a broad range of biological material that would otherwise be irretrievably lost.

Collections and their role in science

In 1993, shortly after becoming the head of the German Collection of Microorganisms and Cell Cultures (DSMZ), a collection supported by the government, a senior scientist of the responsible ministry indicated to me that it was out of the question that collections could ever maintain the full range of microbial diversity. His opinion partly derived from the emerging knowledge about the vast prokaryotic diversity based upon gene sequence analysis (from 1990 on), and was partly designed to make me aware of the pointlessness of seeking substantial support for an expansion of the collection. It is unlikely that this comment was made as a reaction to the then previously published text of the Convention on Biological Diversity (CBD; in particular Articles 9a and 9b), which requested signatory governments to establish and maintain facilities for the *ex situ* conservation of components of biological diversity, including microorganisms, and preferably in the country of origin of such components. In the 15 years after the CBD came into force (29 December 1993) national agencies were absorbed by the major imperative to implement strategic plans, and therefore concentrated on issues that only peripherally touched upon microorganisms. The collections themselves, at first uncoordinated but later networking at the regional level, took the initiative to comply with some of the Convention's duties and responsibilities [3].

Collection of microorganisms has come a long way, from basic beginnings in the 1890s with Kral's collection in Prague and the collection at the Institute Pasteur, Paris, to the resource centers of 2010 driven by quality excellence. Only a few collections established in the early era of microbiology survived the politically and scientifically turbulent 20th century, such as the American Type Culture Collection (ATCC, USA), the Centre de Ressources Biologiques de l'Institut Pasteur (CRBIP, France), or the Centraalbureau voor Schimmelcultures (CBS, The Netherlands). No one, however, can give even an approximate number of microbial collections, their founding and their shut-down dates over the 120 year history of microbial holdings. No one can estimate the number of once properly maintained, irrecoverable valuable strains that were discarded due to the abandonment of facilities. The World Data Centre for Microorganisms (WDCM) currently lists 574 collections, some public, others academic or commercial, in 68 countries, but this apparently impressive number does not reflect the ongoing struggle of the vast majority of collections, listed or not, for at least medium-term funding, the constant search for taxonomic excellence, and often the lack of appropriate recognition by either peers or hosting organizations.

The World Data Centre for Microorganisms (WDCM, http://www.wfcc.info/datacenter.html) also reflects the wide range of expertise and materials offered by collections. Less obvious is the range of funding agencies, and no information is provided on the individual collections' clientele spectrum, the users from academia and agricultural, food, healthcare and biotechnological sectors with their different expectations on demand and quality towards resources, consultation and delivery time.

In some countries, predominantly located in East Asia, India and South America, new state-of-the-art national collections were established that benefited from large national biodiversity initiatives. Against expectations, several renowned collections in the USA, UK and Australia struggle for public support, with the consequence that they must increasingly follow either the commercial route, concentrating on best-selling resources, or face being limited in their desire to expand both holdings and expertise. There are a few exceptions, notably in Belgium (Belgian Coordinated Collections of Microorganisms; BCCM), Germany (DSMZ), Japan (Japan Collection of Microorganisms; JCM), The Netherlands (CBS), and increasingly, in Brazil (Brazilian Collection of Microorganisms from the Environment and Industry; CBMAI), Korea (Korean Culture Center of Microorganisms; KCM) and China (Culture Collection of Beijing Agriculture University; CCBAU), countries with appreciable government support for microbial resource centers – whereas in other countries with established holdings of excellent worldwide reputation, such as the UK and the USA (to name only two), government expenditure for their support is diminishing.

In order to move towards excellence and to maintain a cutting-edge, there is a need for improved quality management, for the establishment of research components, and to satisfy national and international rules

and regulations (e.g. biosafety and security). As a consequence of this development, resource centers are likely to gain higher recognition by stakeholders but this will not necessarily improve the situation regarding their basic needs. Given that support rarely matches the demands for maintaining even the newly-described type and reference strains, it appears that collections are in a tight spot. Expensive quality-associated activities must accompany, although not detract, from the genuine collection activities that are less obvious for funding agencies than research output. Their cautionary admonishment to avoid duplications of holdings and to focus on added-value and user-desired products are well received, but this does not release governmental funding bodies from their responsibility according to Article 9 of the CBD.

A survey of some European collections

Currently, a small group of well-established European collections are working together in a European Union (EU)-sponsored project, the European Consortium of Microbial Resource Centres (EMbaRC), aiming to improve, coordinate and validate collection delivery to European and international researchers. The goals are to ensure harmonization of the quality of microbial collections, taking the current OECD best-practice guidelines [4] and emerging national quality standards for microbial resource centres to the international level. The consortium agrees that coordinated action is needed to store all relevant strains resulting from European research and that they need to work together to provide the capacity and expertise.

The following section summarizes data acquired in 2009 through a survey of four major European BRCs with prokaryotic holdings including BCCM/LMG (Belgian Coordinated Collections of Microorganisms/LMG Bacteria Collection, Laboratorium voor Microbiologie, Universiteit Gent, Belgium); DSMZ in Braunschweig, Germany; CRBIP in Paris, France, CECT (Colección Española De Cultivos Tipo; Spanish Type Culture Collection) in Valencia, Spain, and two INRA (Institut National de Recherche Agronomique) research collections, in Rennes and Tours, France. Together, these facilities provide strains from 25 of the 27 described prokaryotic phyla, >75% of all described genera and >85% of all described type strains (http://www.cabri.org/). The majority of holdings are within the four phyla of the domain Bacteria, namely Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, where historical collection emphasis has evolved into a specific strength of BRCs (i.e. CRBIP, medical; BCCM/LMG and INRA collections, biotechnology and agriculture; DSMZ and CECT, taxonomic and ecological diversity) and to which the majority of newly described species are associated. Some overlap exists for the approximately 8000 different type of strains maintained, mainly in the four phyla listed above, but hardly any overlap exists for the about 51 000 non-type strains maintained in these collections. These impressive prokaryote numbers, covering almost the complete spectrum of cultured prokaryotic diversity at the taxon level of species and above, are maintained by as few as 35 curators working in the six collections evaluated.

In times of economic stagnation and decreasing public support for collections of microorganisms, collections must be asked about their own initiative to raise their profile and portfolio, and hence revenues. At the management level the past five years have witnessed the introduction of a quality management system, mostly adopting the ISO 9000 series certification. Other relevant possible standards that can be applied to microbiology laboratories have been extensively elaborated by Smith *et al.* [5]. The network of collections and resource centres at the global level: the Global Biological Resource Centre Network (GBCRN) and the World Federation for Culture Collections (WFCC)]; at the regional level, such as CABRI (Common Access to Biological Resources and Information), ECCO (European Culture Collections' Organisation) and ACM (the Asian Consortium for Conservation and Sustainable Use of Microbial Resources); and at the national level (e.g. the Brazilian Program for Biological Resource Centers), are all excellent examples of cooperation between such facilities in their search for common policies and practices. Despite national emphasis and competition for the same biotechnology clientele, partners of the EU projects CABRI and the European Resource Centers Network (EBRCN) manage an internet one-stop shop of their holdings which is mirrored by China and Brazil. In addition, several larger facilities have expanded their holdings by including plasmids, phages, and isolated DNA; they offer state-of-the-art taxonomic expertise in chemotaxonomic, molecular and phylogenetic analysis, they initiate cooperation for biotechnological exploitation of their holdings, and do not tire of investing in the education of young taxonomists – a task previously mainly taken care of by academic institutions.

Holdings and deposition of type and non-type material

Where then is the space for the original task of a microbial collection, namely the accession, maintenance and provision of biodiversity? In the following the issues of type and non-type strains are discussed separately.

Type strains are the holy grail of prokaryotic nomenclature because they constitute the name-bearing reference strains of a species. No other strain is so well protected by rules and for no other strain are the deposition standards as strict. A new type strain must be deposited in at least two public collections in at least two different countries in order to have the name (and therefore the species) validated; (for details see *International Code of Nomenclature of Prokaryotes*: http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=icnb&part=A185 and http://www.bacterio.cict.fr). Failure to do so will lead to the species having no standing in systematics even if the entry is published. This policy guarantees (i) that no strain involved in patent issues will receive the status of a type strain (a line of a newly isolated strain could be made available for exploitation and inclusion in a patent if it is deposited as a different strain: http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=icnb&part=A185) and (ii) that type strains are available worldwide as reference strains. Whereas between 1980–1994 and 1995–2000 the number of type strains ranged only between 100–200 and 200–300 annually, respectively, it steadily increased in the following years to reach 663 in 2009. In this context it is advisable

to remember that the estimates for the number of species per gram of diverse soil samples range between 2000 and 10^7 [6,7]. The huge discrepancy between the described novelty and potential novelty not only sheds light on the problems of cultivating novel strains but also circumscribes the future avalanche of novel geno- and phenotypes to be maintained.

Usually authors deposit type strains in not more than two to three different collections, but it is not uncommon for curators to exchange type material. This duplication is desired because references should be as widely accessible as possible in a minimum of time and at low expense (i.e. shipping and custom charges). Whereas large public collections, such as DSMZ or JCM which house a high number of phyla, are likely to archive between 500 and 600 type strains annually, the number will be smaller for those collections concentrating on specific groups of prokaryotes. The reception of type material is done under the most careful consideration of authenticity and this requires close contact between depositor and collection staff. In addition, intense testing of optimal long-term storage procedures, viability tests and documentation at the early stage of deposition consume so much time that acquisition of additional strains is decreasing in prominence. Even a well-equipped and well-staffed collection does not acquire and maintain more than between 900 and 1100 strains annually. It is worth noting that between 1990 and 2000 the number of species based upon characterization of only a single isolate remained at about 40% [8] whereas in 2009 it was a high as 79% based on a survey of volume 59 of the *International Journal of Systematic and Evolutionary Microbiology*. In other words, about 520 of the 660 new prokaryotic species were defined by the type strain only. Deposition of additional strains of these single-strain species is highly desirable because it would broaden the range the genetic and epigenetic diversity of these taxa. Because the maintenance protocol has already been developed, their inclusion in collections should be less troublesome.

Non-type material is abundantly available for certain organisms, such as pathogens, food-processing and food-spoiling organisms, biotechnologically relevant strains, and bacteria involved in crop improvement. Because the majority of such strains have growth and maintenance requirements similar to those of the respective type strain, their long-term storage is less demanding. The emphasis on certain genera or physiological and phylogenetic groups is mainly due to the research activities of curators who often perpetuate the legacy of their founding professors. Whereas small and focused endangered holdings sometimes find a home in an established collection with relevant expertise, scientists rarely use the option to deposit strains. This is documented by a survey performed on the 2008 issues of eight European microbiology journals (journals covered were *Archives of Microbiology*, *Extremophiles*, *FEMS Microbiology Letters*, *Antonie van Leeuwenhoek*, *International Microbiology*, *Environmental Microbiology*, *Systematic and Applied Microbiology*, and *Microbiology (Reading, England)*]. This survey screened 835 articles for the number of isolates investigated (except for cloning vectors), the origin of the reference strains, and how many of the isolates found their way into public collections. Of the about 20 200 strains listed, only 190 strains (0.94%) were deposited. This staggering low number includes 154 strains originating from a single publication [9]. Of the 20 200 strains, the majority (68%) belonged to the Phylum Proteobacteria, especially Class Gammaproteobacteria (45%) and 17% to the phylum Firmicutes; these phyla are already well represented in collections. Nevertheless, the taxa investigated included a significant number of strains that warranted deposition and public availability. According to publication policy, many journals expect authors to make biological materials available to researchers. In a anonymous request to obtain strains from 100 randomly selected authors of the journals screened above, only 19% indicated their willingness to provide strains, and 5% confirmed deposition of these strains in public collections after publication; of the others contacted, 61% did not respond at all and 15% responded that the strains had either died or were included in patented processes, and hence were unavailable.

In addition to the 20 200 strains indicated above, about 5980 strains were used as reference strains in comparative studies. These strains carried official collection numbers or were named type strains without strain assignment. In a closer look, however, most of these strains were passed on by colleagues or taken from laboratory collections with unknown quality control. Only about 1372 (22.9%) of the reference strains used were actually obtained from public collections. Collections might worry about the loss of revenues and by being ignored in their principal task to serve scientists. Even so, the scientific community should be more worried about the fact that, in the absence of routine authenticity checks, the so-called reference strains might actually have deviated from their original material, thus serving as a false reference, threatening to weaken the foundations of the knowledge-based bioeconomy.

Towards considerate collection strategies

The situation examined for a few major European public collections can most likely be transferred to a global picture on collections. In summary, collections differ in breadth (diversification) and depth (focus) of phylogenetic and metabolic diversity of the taxa maintained. The size of individual holdings and the expertise of curators are determined partly by financial support, partly by the history of the collections. This history also explains the respective strengths both in methods used in-house for authentication and characterization as well as in offering skills to the public by providing identification services and training courses. Whereas individual collections differ in diversity at the species level, a broad range of prokaryotic biodiversity is maintained at all taxonomic levels at the regional level. Going forward there are a few considerations that, if addressed, could strengthen microbial collections. These include (i) collections that aim to receive BRC status will need substantial support for establishing and maintaining quality management and accreditation status, as well as for the expansion of research, training and bioinformatics. (ii) Any expansion of collection diversity, both in terms of phylogenetic diversity and in-depth coverage at the level of genus and species, will require increased expertise of curators and technical staff. (iii) A strategy needs to be

outlined which encourages authors to deposit a higher fraction of strains that are so far under-represented in public collections.

In regards to expanding collections, the diverse nature of funding schemes, the affiliation of collections to research facilities, and the varying, and changing collection focus all exclude the possibility that all collections might cover the same range of prokaryotes. Omitting type strains from this discussion (because their public availability is assured), the decision whether to follow diversification or in-depth focusing has already been made for some collections in the past, and other collections will also have to decide whether to follow this route. Research agencies should make it obligatory to record a minimal data set for isolates obtained by research grants as well as the deposition of selected isolates. The collection community must work with all microbiologists, collection users, editors and research programme funders to ensure that all key strains emanating from research work are preserved for future use and confirmation of results. This is necessary to ensure that investments in delivering outputs from publicly funded research are protected. It is essential that the primary biological materials upon which data in publications or in public databases are based are made available, and preserved as deposited, so that spurious or unusual findings can be further explored or to allow further work as new technologies arise. The EMbaRC project has taken the first solid steps towards the development of a coordinated strategy to ensure that authentic high-quality microbial resources are available to meet the demands of today's research programmes. The strategy to ensure adequate coverage of microbial resources, however, is a global one; it is foreseen that the EMbaRC initiative will be pursued in the establishment of a global approach, the GBRCN.

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