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Understanding patterns of use and scientific opportunities in the emerging global microbial commons

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Abstract

Rapidly growing global networking has induced and supported an increased interest in the life sciences in such general issues as health, climate change, food security and biodiversity. Therefore, the need to address and share research data and materials in a systematic way emerged almost simultaneously. This movement has been described as the so-called global research commons. Also in microbiology, where the sharing of microbiological materials is a key issue, microbial commons is attracting attention. Microbiology is currently facing great challenges with the advances of high throughput screening and next-generation whole genome sequencing. Furthermore, the exploration and use of microorganisms in agriculture and food production are increasing so as to safeguard global food and feed production. Further to several meetings on the subject, a special issue of *Research in Microbiology* is dedicated to Microbial Research Commons with a series of reviews elaborating its major pay-offs and needs in basic and applied microbiology. This paper gives an introduction to these articles covering a range of topics. These include the role of public culture collections and biological resource centers and legal aspects in the exchange of materials, microbial classification, an internet-based platform for data-sharing, applications in agriculture and food production, and challenges in metagenomics and extremophile research. © 2010 Elsevier Masson SAS. All rights reserved.

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1. Introduction

As scientists and user groups become better connected with each other, particularly through the internet, and as research focuses on issues of global importance, such as human health, climate change, food security and biodiversity, there is a growing need to systematically address sharing of and access to research data and materials beyond national jurisdictions, and thereby create greater value from international cooperation. This movement has been described in the literature as the emergence of so-called global commons, which have been defined as resources that are shared by groups of people

participating in decisions about how those resources should be managed and used (Hess and Ostrom, 2006). This also applies to research in microbiology, where the need to access reference materials for identification purposes, the specialization amongst collections of microbial materials, and collaborative efforts for increasing our understanding of the biodiversity of microorganisms result in interdependency amongst research communities on the global scale.

Despite considerable interest in specific areas regarding microorganisms and the existence of substantial and diverse public culture collections and research collections, the breadth of the subject has meant that the sector has so far received little systematic attention. Moreover, at present, this situation of exchange of biological materials within a global commons, is facing a set of important challenges, mainly related to the high cost of appropriate quality management (OECD, 2001) and the change in the international legal environment, which

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may hamper some of the most promising new scientific opportunities made possible by current advances in high throughput screening and increasing availability of full genome sequencing of entire microorganisms (Reichman, J. H., Dedeurwaerdere, T., and Uhler, P.F., unpublished data). For all these reasons, microbial genetic resources deserve to be analyzed in a systematic way, focusing on global trends in gene flow and uses and their possible benefits to life science research.

The first international meeting of the microbial commons initiative was held on the 7th and 8th of July 2005 in Brussels, Belgium (Dawyndt et al., 2006). This initiative was followed by two other international meetings into the questions of the legal, institutional and technical design of the microbial commons, one in Ghent on the 12th and 13th of June 2008, and a second in Washington DC on the 8th and 9th of October 2009 at the US National Academies. A major monograph on the legal, institutional and organizational design of the microbial commons is also in preparation, early versions of which have been presented and discussed at both the Ghent and Washington meetings (Reichman, J.H., Dedeurwaerdere, T., and Uhler, P.F., unpublished data). In the context of this work on the emerging microbial commons, however, a more systematic analysis of the existing patterns of exchange and the scientific pay-offs that can be expected from a more fully organized microbial commons is needed. In 2009, two international workshops on “Analyzing Patterns of Exchange and Use in the Global Microbial Commons” with microbial scientists and culture collection managers were organized to address these issues (18th and 19th of February and 25th and 26th of March, Brussels 2009). At the outcome of the workshops, a set of papers were commissioned which provide a systematic overview of the incipient global microbial research commons and present some in-depth case studies illustrating the socio-economic benefits of use and exchange of microorganisms, both regionally and internationally. The papers presented in the special issue on “Microbial Research Commons” in *Research in Microbiology* are the result of that effort.

2. Public collections of microorganisms, spiders in the web

Many microbiologists will occasionally need particular strains as taxonomic reference, but also as test material, and they order these from a public microbiological culture collection. These culture collections (biological resource centers, or BRCs) serve an essential infrastructure function by making available biological materials and information of guaranteed identity and quality, for scientific investigation and development in an international context. As described by Janssens et al. (2010), the global distribution and exchange of microorganisms is organized on a formal basis by the network of over 500 public culture collections which are members of the World Federation of Culture Collections (WFCC). It is their historical mission to organize the collection, authentication, maintenance and distribution of cultures of

microorganisms and cultured cells. Through the culture collection network, cultures are distributed and made available for research and development with marginal distribution costs, often with the possibility of further distributing the cultures to qualified third parties and with major benefits for the development of downstream applications.

The situation of the public culture collections is characterized by a high level of interdependency (Dedeurwaerdere et al., 2009). The largest public culture collection, with approximately 25,000 strains, holds less than 2% of the total strain holdings of the WFCC members and only a minor fraction of currently known microbial biodiversity. Intense collaboration and exchange amongst culture collections is a necessary consequence of this situation. Furthermore, scientists may also exchange strains in an informal way between their in-house research collections where the bulk of microbial research is done. These research collections play an important role in the overall research cycle, because this is where the first selection and screening of reference materials is undertaken. On average, deposits from these research collections represent 30% of the yearly accession of new holdings in the public culture collections, while both academia (58%) and the private sector (23%) are important recipients from cultures of the public culture collections (Dedeurwaerdere, 2010a).

Many of the services provided by BRCs and knowledge accumulated in these institutions are relatively unknown to the applied microbiologist or scientist. Yet these centers are essential infrastructures for life sciences and biotechnology. Therefore, researchers should be encouraged to deposit important microorganisms with these collections. Editorial boards of scientific journals along with funding bodies should support this by demanding these deposits as part of their review process and research output.

3. Standardized electronic information exchange, the StrainInfo initiative

Many BRCs have built up their collections over decades. Due to exchange policies, BRCs have in their holdings subcultures of so-called original strains. Only during the past decade have digital databases with information on the holdings of the individual BRCs become available. Many of these include, apart from cataloguing of their biological material, additional strain information (meta-information) that is in most cases not overlapping and also provided under different formats between the BRCs for mutual subcultures. Various attempts have been made to standardize the information in such databases to allow easy linking (e.g. Stalpers et al., 1990). A recent platform, StrainInfo (<http://www.straininfo.net>) (Dawyndt et al., 2005) is becoming the reference for information and meta-information on microorganisms at the strain level. It integrates the information from various BRCs as well as related information retrieved from other databases and makes it available in a common electronic format. To realize this, a microbial common language (MCL) for the exchange of microbial information has been developed (Verslyppe et al., 2010). As a result, strain designations, historic information

on their deposit in BRCs, growth characteristics, genomics and other data, and relevant publications are shown to the user in an integrated way. This system has the potential to evolve, to some extent comparable to the Wikipedia-information network, into a global microbiological data-network of which the data can be directly used in scientific research, for example in combination with genomics for comparative studies.

4. Taxonomy and its implications in microbial commons

In clinical or environmental microbiology, it is a frequent task to determine the species to which an isolate recovered from a sample belongs. This process of identification has practical implications since, by inductive generalization, it is assumed that the isolated organism has the same features as other members of this species. Consequently, identification of a bacterium from a patient's sputum as *Mycobacterium tuberculosis* or from a general infection as methicillin-resistant *Staphylococcus aureus* (the so-called MRSA) has severe implications in the sense of treatment and/or infection control. Thus, the determination of the name of the species to which an isolate belongs is an important act in applied microbiology.

It happens that an isolate cannot be assigned to an already known species and that it is hence a representative of a newly discovered species. Description of new species and nomenclatural changes are part of the specialized field regarded as microbial taxonomy, and which encompasses three successive elements, characterization, classification and nomenclature. Recent reviews on bacterial taxonomy have been given by Tindall et al. (2010) and by Moore et al. (2010). Reviews on virological taxonomy have been given by Fauquet et al. (2005) (see also <http://www.ictvdb.org/>), on the taxonomy of fungi by Hibbett et al. (2007) and Lutzoni et al. (2004) (see also <http://www.indexfungorum.org/>), and of yeasts by Kurtzman et al. (in press). Here we will focus on some aspects of bacterial taxonomy.

Although the bacterial species concept is man-made and subjective, microbiologists nowadays generally accept a phylogenetic species concept (Stackebrandt et al., 2002; Wayne et al., 1987) in which overall DNA relatedness (measured by DNA:DNA hybridization) is crucial for species delineation that must be supported by phenotypic differences measured e.g. by a polyphasic study (Vandamme et al., 1996). Although alternatives for this concept have been proposed (e.g. by Staley, 2006), overall genome sequencing followed by comparative analysis taking into account the common genome content seems to support the bacterial species concept (Goris et al., 2007; Konstantinidis and Tiedje, 2005).

An overview of all currently described bacterial species can be found on the Internet (Euzeby: <http://www.bacterio.cict.fr/>). New species names must be published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM) before being valid. Over the past years, the number of described species has increased enormously (Moore et al., 2010), mainly due to the description of species encompassing one strain only. This development is a matter of debate since the diversity of

those species is unknown, which makes it difficult to identify isolates among them (Christensen et al., 2001).

5. Exchange of microbial materials among scientists

Outside BRCs, much greater numbers of strains are kept in non-public collections, including those at universities and other research institutes, companies and reference centers like public health institutes. These collections have been set up for special purposes and their existence depends on local policies and considerations of the institutions that keep them, including research targets of the institutions, resources and facilities, or employment of scientists with particular interest in certain microorganisms. Since these policies and considerations may change over time, the long-term maintenance of these collections is uncertain. The non-public collections are usually part of a particular project or purpose, as can be the case for reference institutes. Database management, storage conditions and quality control measures of these collections can differ considerably from those of BRCs. Awareness of the existence of such collections follows from publications, conferences, personal contacts, etc. Materials from these collections can be requested by scientists wishing to verify published results of these organisms or to perform other research on these organisms. As discussed by Staley et al. (2010), free exchange of materials by scientists has long been practiced and is important both from a scientific and ethical point of view. However, free exchange may not always happen. For example, requests can be ignored or the corresponding author replies that he/she no longer has direct access to the material. Furthermore, the logistics and required administration can make it difficult or impossible to comply with requests for exchange. Apart from this, certain organisms with important value for future publications or known or likely commercial value (Reichman, J.H., Dedeurwaerdere, T., and Uhler, P.F., unpublished data) will not always be exchanged freely due to institutional policies or demands from contributing scientists and/or collections who expect to have a fair share of the benefits of commercial uses in recognition of their contribution to the research on the organisms. Their disposal will require formal material transfer agreements (MTAs) (Staley et al., 2010).

To improve the accessibility of these special culture collections and protect them against loss, several measures could be taken:

- Holders (curators) of these collections should be stimulated to report the existence of the collections at a given (internet-based) platform, for example, set up with support from national or international scientific societies.
- Holders (curators) of the collections (for example, the scientists who brought the material together) should be trained to comply as much as possible with those of formal public culture collections. Initiatives like those supported by the FP7 EU program (e.g. the EMbaRC – European Consortium of Microbial Resource Centers – <http://www.embarc.eu/>) should be strengthened and national public

culture collections might play a role in giving advice and training.

- Governments, institutions and funding agencies should be convinced of the importance of particular special purpose collections and provide finances and conditions to maintain them.
- Standard MTAs should be designed by scientific agencies and policymakers in order to decrease the administrative costs for the scientists and culture collection managers in dealing with case by case MTAs, and to provide sufficient legal guarantees for maximum availability of the organisms for further downstream research use (Reichman, J.H., Dedeurwaerdere, T., and Uhlir, P.F., unpublished data).

Altogether, a great effort needs to be made in the safeguarding of research or other special purpose collections which are not formal BRCs.

6. A global microbial commons in practice

In the preceding paragraphs, we have discussed the concept of a microbial commons and the required infrastructure in terms of BRCs and ‘private’ culture collections, an internet-based platform for exchange of data (Straininfo) and well-defined taxonomies of the organisms involved to realize its aims. In this section, we will discuss a number of examples in agriculture and food microbiology in which a microbial commons can play or is already playing an important role.

6.1. Rhizobial symbiotic nitrogen fixation

Symbiotic nitrogen fixation is the main route for sustainable input of nitrogen into agro-ecosystems. In agriculture, inoculation of legume crops soil with suitable rhizobia can improve nitrogen fixation. Soybeans and forage legumes are important nitrogen-fixing crops. Knowledge of the biodiversity at large and of local populations of rhizobia is important for the development of successful inoculation strategies. In a review, Lindström et al. (2010) discuss inoculation practices, the biodiversity and classification of rhizobia and recent insights gained from genome analysis of these organisms. Taxonomically important strains (i.e. type strains and other taxonomically relevant strains) are deposited in BRCs and can be easily obtained. However, inoculant strains for agriculture are not regularly deposited in culture collections, but are maintained by companies or research institutes and not listed in public databases which make them less accessible for exchange. Furthermore, strains studied by taxonomists, molecular biologists and those used for inoculation may differ because of different importance for the users. A combined effort to combine knowledge on biological nitrogen fixation (BNF), taxonomy, population biology and genomics of rhizobia might contribute to further development of the rhizobial commons (Lindström et al., 2010).

6.2. Biological control in agriculture

Artificial introduction of living microorganisms into the environment to control a pathogen is another application of microorganisms in sustainable agricultural systems. Species belonging to different bacterial genera i.e. *Bacillus*, *Pseudomonas*, *Agrobacterium* and *Streptomyces*, have been registered in the United States as biocontrol agents (Fravel, 2005). A review of the role of fluorescent pseudomonads in biocontrol is given by Höfte and Altier (2010). These organisms can increase plant growth and improve plant health, and occur in soil and plant hosts worldwide. The genus *Pseudomonas* is very diverse, with 128 species including the fluorescent pseudomonads. There is no general congruence between biocontrol mechanisms and phylogeny of strains. Screening of isolates for biocontrol activity is usually done by testing large sets of strains effective on local crops under local environmental conditions. Reference strains from BRCs are required as control strains to assess the taxonomic and phylogenetic position of new field isolates and to test the specificity of detection of pathogenic and beneficial pseudomonads.

Although there are dedicated BRCs mainly dealing with plant pathogenic microorganisms and nitrogen-fixing organisms, there are no culture collections exclusively specialized in bacterial biocontrol agents. Altogether, both public and private collections are important in the application and basic studies of biocontrol agents.

6.3. Networks for the diagnosis of plant pathogens

Plant diseases are caused by a variety of microorganisms including, amongst others, nematodes, viruses, bacteria and fungi. These diseases can spread over wide geographic regions with great consequences for national and international food production and trade. Control methods can be performed to prevent introduction, establishment or spread of the pathogens and pests. In this context, the so-called quarantine organisms are most important. In Europe, the European and Mediterranean Plant Protection Organization (EPPO) is an intergovernmental organization responsible for European cooperation in plant health (see <http://www.eppo.org/>).

The diagnosis of the cause of the diseases is difficult and requires extensive expertise and availability of a variety of rapid and reliable detection methods. The problems and challenges in this field are presented by Barba et al. (2010) and illustrated by two examples (on *Fusarium* wilt of banana and the sharka disease of stone fruits by the *Plum pox virus*). Collections of plant pathogens are important to preserve the variability of species in terms of pathogenicity, virulence or metabolite and/or toxin production. Plant pathogen collections focused on plant health management (should) provide a number of activities (Barba et al., 2010): (1) provide reference material for diagnostics; (2) optimize and validate protocols to detect plant pathogens; (3) screen crop for traits that confer resistance to pathogens (here a role for national reference centers seems more appropriate) using strains endemic to the region; (4) perform analysis of the risk posed

by pathogenic strains. Identification of plant pathogens relies first of all on national reference laboratories. If diagnosis cannot be performed, collaboration with more advanced laboratories should be sought. It is important that collections house in their services advanced knowledge and expertise to characterize plant pathogens and population variability and develop appropriate tools for diagnosis and detection, to link science to practical demands (Miller et al., 2009). Links between collections are important for acquiring insight into the variations of pathogens and develop appropriate tools for their identification on a global scale.

6.4. *Lactobacilli* in food and feed production

Lactic acid bacteria form a heterogeneous group of bacteria of great metabolic versatility which can be found in a variety of ecological niches and which have been used for millennia in food production. An overview of their taxonomy, diversity and biotechnological applications is given by Giraffa et al. (2010). Members of the genus *Lactobacillus*, the largest group of lactobacilli, produce lactic acid by fermentation which causes a rapid pH drop in raw material. Furthermore, they have proteolytic activity and produce aroma compounds, bacteriocins and exopolysaccharides. Therefore, these organisms have many applications in food preservation, as starters for dairy fish, meat and vegetable fermentation, and in the production of wine, beer and silage. They have also been proposed as probiotics and microbial cell factories for the production of so-called nutraceuticals, i.e. food or food products that provide both health and medical benefits. Particular species, subspecies or strains are used for specific purposes. For example, *Lactobacillus helveticus* is used along with thermophilic lactic acid bacteria in cheese manufacturing, while *Lactobacillus plantarum* is used as silage inoculant. Genetic modification and metabolic engineering are tools to obtain strains to be used for special purposes (Giraffa et al., 2010). Exchange of isolates between culture collections in different geographic areas may extend the spectrum of useful strains for application in biotechnology. Already, 25 sequenced genomes of *Lactobacillus* species are in public databases, allowing functional genomics studies to obtain insight into the metabolic potential of lactobacilli. Results from these approaches are expected to reveal characters of biotechnological importance. Next, strain collections can be screened for relevant markers and, hence, strains selected for biotechnological applications (Siezen et al., 2004).

6.5. The preservation and distribution of yeasts

Like lactobacilli, the application of yeasts in fermentation is related to the history of mankind (McGovern et al., 2004). The applications of yeasts in the processing of food are numerous and, globally, there is a wide diversity of yeasts involved in these processes. Well-known examples of frequent use of yeast fermentation are in bakeries, beverages, dairy products and e.g. as protein, amino acid and vitamin sources in animal feed (Daniel and Prasad, 2010). The global diversity of yeasts in fermentation is largely unexplored, in particular in

Africa, Asia and Latin America, where fermentations are part of traditional practices. Furthermore, many cultures used in published studies of processing of staple food bases like cassava have not been deposited with BRCs and are not available to the biotechnical and scientific community (Daniel and Prasad, 2010). Furthermore, a relatively small number of well-documented species are widely used in food production, as food additives and in agriculture. The role of BRCs to serve as a repository and distribution center for yeasts is illustrated by Daniel and Prasad (2010) who made an inventory of two yeasts, the widely used *Saccharomyces cerevisiae* and a specific beer brand using *Dekkera bruxellensis*. The high number of cultures of *S. cerevisiae* in specialized and general BRCs (~50,000 including 28,000 deletion mutants) emphasizes the biotechnological and economic importance of this organism. The authors noted wide temporal gaps between isolation, property description and eventual application of strains, and they recommended long-term depositories.

7. New scientific challenges

7.1. Analysis of the microbial diversity in soil, a metagenomics approach

The ocean and soil harbor the greatest microbial diversity on the planet. It is generally estimated that less than 1% of the living organisms on earth can be cultivated by current methods. The yet unknown reservoir of microorganisms is expected to encompass huge genetic and metabolic diversity. Analysis of this global diversity with application of the most recent genomic and bioinformatic approaches is a tremendous challenge. Benedetti and Mocali (2010) have focused on the use of metagenomics to study the entire genome (the so-called metagenome) of soil biota to discover novel species, genes and molecules relevant for biotechnology and agricultural applications. An overview of the most recent approaches in metagenomics is provided. The advent of next-generation sequencing tools has considerably increased the potential of metagenomics (Simon and Daniel, 2009). Nevertheless, a number of obstacles have to be dealt with, including the complexity of the microbial communities and the difficulty in managing the large amount of genomic data (Benedetti and Mocali, 2010). In response to the need to integrate massive data sets of metagenomic information with biological information, international genomic databases have been set up which provide standardized data formats for simple retrieval and computation by users. Although these initiatives are important steps, Benedetti and Mocali (2010) plead for a combination of scientific, technical, legal, institutional and normative efforts to achieve an overall infrastructure linking microbial research with genetic resources to explore the diversity and metabolic potential of the soil biome.

7.2. Advances in extremophile research

Extremophilic organisms can inhabit ecosystems that are, from a human perspective, extreme in the sense that these systems have a very high or low pH, high or low temperature,

high salinity, high pressure or combinations of these conditions. These organisms can be found in the three domains of life, Archaea, Bacteria and Eukarya, and are not only of interest for their ability to live in particular extreme environments, like hot springs, but also for their biotechnological potential. A well-documented example is the discovery of Taq polymerase, the enzyme recovered from *Thermus aquaticus* in a thermal spring (see also Staley et al., 2010) and so successfully applied in the polymerase chain reaction. Isolation of extremophiles and exploration of their biochemistry, genetics and regulation of their metabolism to gain a better understanding of their extremophilic life style is an important area of research. Averhoff and Müller (2010) discuss two examples of adaptations of extremophiles to their environment. One example focuses on the molecular (regulatory) basis of salt adaptation in halophiles upon exposure to increasing osmolality, the other on the role of horizontal DNA transfer as a means to adapt to extreme environments. The authors argue that their findings have been obtained with pure cultures and planktonic cells. Recent studies have revealed that the organisms in nature live in biofilms, and gene expression in biofilm-encapsulated cells is distinct from planktonic cells. Further research will be focused on the behavior of extremophiles and their interaction with the biotic and abiotic environment. Altogether, research on extremophilic organisms, including their diversity and life style, is a challenging field and, from the microbial commons and biodiversity point of view, preservation of areas harbouring these organisms is of great importance.

8. Final conclusions

There has been a dramatic increase in interest in commons in the last 10–15 years, from traditional commons managing the use of exhaustible natural resources by fixed numbers of people within natural borders, to global information commons, dealing with non-rival, non-excludible goods by a potentially limitless number of unknown users. The emerging global genetic resource commons fits somewhere in between, shifting in the direction of information commons as digital-information infrastructures which enable physically distributed commons to be networked in virtual global pools.

As has been shown in the above review, the field of microbial genetic resources is characterized by a strong and lively commons-based innovation sector which has recently been empowered by new digital means for distributed collaborative research. Networking pools of genetic resources in a global commons is needed to generate sufficient investment in the vast quantities of genetic resources that are neglected because of their still unknown scientific and/or unlikely commercial value. These neglected resources are the building blocks for future scientific research and have enormous value for sustaining biodiversity and local livelihoods.

However, the range of obstacles to full realization of the new opportunities offered by global networking of genetic resources is vast. This review discusses a set of measures that are called for from a purely scientific perspective, such as

deposits upon publication or the deepening of common quality management. Other major obstacles to be overcome are of a legal, institutional and economic nature and are analyzed systematically elsewhere (Dedeurwaerdere, 2006, 2010b; Reichman, J.H., Dedeurwaerdere, T., and Uhler, P.F., unpublished data). The breadth of these obstacles shows the need for appropriate organizational forms, legal arrangements and social practices. These can help to better secure the global microbial research community's need to address issues of common concern, such as global food security, global health, biodiversity conservation and climate change. As discussed in this paper, in response to these obstacles, governments, non-profit organizations, global research communities and culture collections have developed a range of initiatives for the exchange of materials and information which have already delivered important outcomes. The key issue is how to build upon these incipient global microbial genetic resource commons and place them on a solid scientific and institutional basis.

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