Biodiversity and the Role of Microbial Resource Centres

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Micro-organisms were the first forms of life on earth and have evolved into the most ecologically, genetically and metabolically diverse species known. Micro-organisms belong to all three Domains of life: The Bacteria, Archaea and Eukarya as well as the Viruses. They have shaped the evolution of the planet and continue to nurture and sustain the environment, plants and animals on which human society depends. While we continue to face difficulties posed by emerging animal, plant and human pathogens, most micro-organisms are beneficial. Exploitation of microbial genetic diversity has been fundamental to advances made in biodiscovery and biotechnology. Micro-organisms are major sources of important pharmaceutical and industrial products for world-wide community benefits in health, agriculture and industry. Cultures of micro-organisms have been essential for the production of enzymes, fermentation products and metabolites. With advances in molecular biology, genes of micro-organisms and whole natural communities are being exploited and fuelling accelerated interest in biodiscovery. The OECD is strongly promoting that biological resource centres are essential to underpin advances in biotechnology, the life sciences and the bioeconomy. Microbial resource centres are more than collections. They work within the Convention on Biological Diversity (CBD) that was implemented to support the conservation and utilisation of biodiversity and recognises the principles of fair and equitable benefit sharing. They preserve and provide authenticated, genetically stable microbial and cell cultures, provide access to information on cultures and their characteristics, and undertake identification and description of new species. In Australia, the Council of Heads of Australian Collections of Micro-organisms is collaborating with the NCRIS Atlas of Living Australia project to develop the Australian Microbial Resources Information Network (AMRiN) integrated collections database to provide access to information on Australian microbial cultures for use in research, industry, government and education.

EMERITUS PROFESSOR LINDSAY SLY was Professor of Microbial Systematics and Microbial Ecology, Director of the Centre for Bacterial Diversity and Identification, and Curator of the Australian Collection of Micro-organisms at the University of Queensland where he undertook research and teaching of the biodiversity, physiology, metabolism and ecology of bacteria from natural and industrial environments. He has made major contributions to knowledge of microbial diversity, to the understanding of phylogentic relationships amongst species in diverse bacterial and archaeal divisions, and to the development of molecular tools for the identification of bacteria. In 2001 his contributions were recognised with the prestigious international Bergey Award. He was president of the World Federation for Culture Collections from 1996 to2000 and is currently Chair, Council of Heads of Australian Collections of Micro-organisms.

Importance of microbial diversity

Micro-organisms were the first forms of life on earth and have evolved into the most ecologically, genetically and metabolically diverse species known. Micro-organisms belong to all three Domains of life: the Bacteria, Archaea and Eukarya (algae, fungi, yeasts and protozoa), as well as the Viruses. They have shaped the evolution of the planet and continue to nurture and sustain the environment, plants and animals on which human society depends. I have previously reviewed the importance of microbial diversity and the role of microbial resource centres (Sly 1998a) and the following highlights the important issues raised in that publication.

Micro-organisms are an essential component of biological diversity, without which there can be no sustainable ecosystems (Hawksworth 1991, 1992; Hawksworth and Colwell 1992; Sly 1994; Center for Microbial Ecology 1995; Staley *et al.* 1997). It is estimated that at least 50% of the living biomass on the planet is microbial (Center for Microbial Ecology 1995) yet probably < 0.1% have been characterised. Micro-organisms provide a major source of genetic information for molecular biology and biotechnology (Bull *et al.* 1992; Nisbet 1992; Center for Microbial Ecology 1995; Staley *et al.* 1997). While some micro-organisms are serious pathogens of humans, animals and plants and pose a threat to health, food security and food safety, most are beneficial and sustain the environment and soil health. Plants and animals depend on microbes to grow and perform optimally.

Most advances in knowledge of the function and role of micro-organisms have been derived from pure culture studies, while most advances on the ecology and interactions of micro-organisms are likely to come from the application of molecular studies using signature DNA and rRNA probes. These studies have highlighted many important functions of micro-organisms in relation to agricultural processes and food security. However, until we have more complete knowledge of microbial species and functional diversity, decisions about the role of micro-organisms and their influence on sustainable ecosystems are being made on the basis of very incomplete information. Without a thorough knowledge of microbial diversity and ecology, decisions concerned with sustainability are likely to be flawed.

Recent advances in molecular methods (Woese 1987; Ward *et al.* 1990, 2008; Liesack and Stackebrandt 1992; Stackebrandt *et al.* 1993; Olsen *et al.* 1994; Amann *et al.* 1995; Hugenholtz and Pace 1998) have revealed the inability of traditional culturing methods to fully show the diversity of bacteria and other micro-organisms, and have shown that the species diversity in most terrestrial and aquatic environments is far greater than expected. The vast majority of microbial diversity (> 90%) remains to be discovered and its function determined as there are no or few cultured representatives in an increasing number of phylogenetic lineages. Consequently, actions which lead to loss of microbial diversity are likely to result in the loss of valuable knowledge of our natural microbial resources and understanding of sustainability drivers.

Micro-organisms occupy important niches in all ecosystems and are responsible for much of the recycling of the elements in nature, and are important components of food webs. Micro-organisms often have unique functions (e.g. nitrogen fixation, nitrification, denitrification, chemolithoautotrophic carbon dioxide fixation, methane formation and sulfate reduction) in the biogeochemical cycles, in soil formation and in climate regulation, and influence atmospheric composition (including greenhouse gases—Rogers and Whitman 1991). The first micro-organisms evolved over 3.8 billion years ago and consequently exhibit the greatest breadth of genetic and metabolic diversity on the planet, far greater than that of the plants and animals combined (Center for Microbial Ecology 1995; Staley *et al.* 1997). Some are able to grow under extreme conditions, and also in anaerobic environments that cannot sustain plant or animal life. Micro-organisms often exhibit symbiotic relationships with plants (e.g. *Rhizobium*, *Frankia* and mycorrhizal fungi in plant roots) and with animals (e.g. tube worms and mussels). Animals depend on micro-organisms in their intestinal tracts for digestion and for the production of nutrients and essential vitamins.

The genetic and metabolic diversity of micro-organisms has been exploited for many years in biotechnological applications such as antibiotic production, food, food processing, alcoholic beverages, fermented foods and waste treatment. Micro-organisms are the major sources of antimicrobial agents and also produce other important pharmaceutical and therapeutic compounds including antihelminthics, antitumour agents, insecticides, immunosuppressants, immunomodulators and vitamins worth $35–50 billion annually in global sales (Center for Microbial Ecology 1995).

The scientific benefits of microbial diversity research include a better understanding of the role and function of microbial communities in various terrestrial, marine and aquatic environments; a better understanding of the sustainable ecology of plants and animals; improved capacity to maintain soil fertility and water quality; and a better understanding of the full consequences of animal and plant extinctio; and of perturbations on ecosystems. The economic and strategic benefits are the discovery of micro-organisms for exploitation in biotechnological processes for new antibiotic and therapeutic agents; probiotics; novel fine chemicals, enzymes and polymers for use in industrial and scientific applications; for bioremediation of polluted environments and bioleaching and recovery of minerals; as well as preparedness against exotic and emerging pathogens of humans, animals and plants.

The signing of the Convention on Biological Diversity in 1992 (CBD) (UNEP 1992) focussed attention on the value of micro-organisms as sources of genetic information (Bull *et al.* 1992; Nisbet 1992; Center for Microbial Ecology 1995; Staley *et al.* 1997). At about the same time the development of molecular methods for detecting micro-organisms in the environment revealed the poor state of knowledge of both cultured and non-cultured microbial diversity (e.g. Ward *et al.* 1990; Liesack and Stackebrandt 1992; Stackebrandt *et al.* 1993; Amann *et al.* 1995; Hugenholtz and Pace 1996). Subsequently, extensive research effort and substantial research funding has been directed to the areas of microbial diversity, microbial ecology and biotechnology in Asia, the European Community and the USA (Clutter 1995; Staley *et al.* 1997). North–South attention has also been directed towards collaborations with developing countries in the tropical ‘megadiversity’ regions. In addition, many developing countries have become appreciative of the need to explore, protect and exploit their own microbial resources. Australia is in a unique position to take advantage of the wide range of ecological habitats of microbial diversity within its own boundaries and in the Asia–Pacific region through collaboration (Hawksworth 1994). Access to microbial resources and sovereign rights with respect to micro-organisms in environmental samples and cultures in collections have been the focus of international meetings (Kirsop and Hawksworth 1994; Sands 1994; WFCC 1996) Australia, like most signatory countries to the CBD, has been developing national and international protocols for access and benefit sharing.

Microbial resource centres

Microbial resource centres have an extremely important role underpinning the conservation of microbial biodiversity and enabling advances in agriculture, food security, biotechnology and education. They constitute essential scientific infrastructure that maintains collections of cultures of micro-organisms—living libraries of our natural scientific heritage. Depending on their research roles, services provided and quality systems, they are also referred to as Microbial Genetic Resource Centres, and more recently as Biological Resource Centres (OECD 2001, 2007). Microbial resource centres are more than collections. They work within the CBD that was implemented to support the conservation and utilisation of biodiversity and recognises the principles of fair and equitable benefit sharing. They preserve and provide authenticated, genetically stable microbial and cell cultures, provide access to information on cultures and their characteristics, and undertake identification and description of new species. The fundamental role is the ex-situ conservation and supply of viable and genetically stable cultures and genes for scientific research and testing. Important cultures resulting from research are accessioned, studied and conserved. These functions enable and add value to research for applications in industry and biotechnology. Microbial resource centres also maintain extensive databases and thus provide access to information on cultures, their characteristics, literature and DNA sequences, for example. They also are centres of taxonomic expertise for identification and characterisation of micro-organisms and provide training in taxonomy and preservation. With the decline in the teaching and research training in microbial taxonomy in universities, microbial resource centres are likely to have an increasingly important role in taxonomy research training that needs to be recognised and funded accordingly.

Maintaining living microbial cultures requires specific conservation skills and quality assurance to ensure genetic stability. It is essential that microbial cultures are considered as a global resource for the orderly progress of science and technology. However, such a strategy necessitates that each country meets its obligations wherever possible. There are strategic advantages for the ex-situ conservation of micro-organisms within the country of origin. Worldwide there are almost 600 culture collections of micro-organisms in 68 countries registered with the WFCC World Data Centre on Micro-organisms (http://www.wfcc.info/datacenter.html). These collections hold 1.5 million cultures of micro-organisms and cultured cell lines, with by far the majority being held in Europe, North America and Asia. It is concerning that only 11 collections are listed for Africa and none in the Pacific region. Currently there are 35 collections listed for Australia, down from 50 in 1998 (Sly 1998b). In 1998, these microbial resource centres in Australia held 65 000 cultures and it is important that the microbial diversity in these collections is protected and the on-going loss stabilised. Culture collections in Australia primarily have institutional roles and the host institutions are usually universities, CSIRO and government laboratories, together with a few industries (DEST 1966). Most cultures are bacteria and fungi with minor holdings of protozoa, algae, viruses, plasmids and vectors, and animal cell lines. A number of collections are engaged in plant pathology, taxonomy, mycorrhizal microbiology, insect microbiology, forest microbiology, food science and ecology, as well as plant breeding and biodeterioration of significance to agriculture and food security.

Key functions in agriculture and food

Microbial resource centres have played a key role in agricultural and food research over many decades. The Food and Agriculture Organization (FAO) recognises the important role of microbial genetic resources and microbial resource centres for productive agriculture and food security (FAO 2009) and also in understanding the consequences of climate change (Fujisaka *et al.* 2009). Examples of areas where microbial resource centres contribute to agriculture and food security are shown in Table 1. Table 2 lists examples of significant international collections that have had a long-term involvement in the conservation and supply of cultures for research and regulatory compliance. Not only do these collections provide valuable resources and expertise; the cultures from this research are available for extension of this research. The intersection of existing knowledge and resources with often new independent discoveries frequently leads to accelerated innovation (e.g. development of PCR). Table 3 lists examples of significant Australian collections which continue to contribute to Australian microbial resources for agriculture, food microbiology, plant pathology, quarantine and trade.

Table 1. Examples of areas where microbial resource centres contribute to agriculture and food security

Plant endosymbionts (e.g. *Rhizobium* for biological nitrogen fixation)
Plant growth-promoting bacteria
Biocontrol agents (e.g. pathogens of weeds, fungi, insects)
Inocula to restore soil health and nutrient release (e.g. phosphorus)
Source of genes for plant improvement (e.g. insect resistance)
Reference cultures for food safety testing, quarantine, trade
Reference cultures for animal and plant disease testing
Enzymes for food improvement and processing
Cultures for food fermentations and nutritional supplements
Innovative biodiscovery

Table 2. Examples of significant international collections

National Collection of Plant Pathogenic Bacteria (UK)
CABI Genetic Resource Collection (UK)
Centraal Bureau voor Schimmelcultures (Netherlands)
USDA ARS Culture Collections (USA)
American Type Culture Collection (USA)
Canadian Collection of Fungal Cultures (Canada)
Agricultural Culture Collection of China (China)
International Collection of Micro-organisms from Plants (New Zealand)

Global networking initiatives

The World Federation for Culture Collections (WFCC) is the peak international body that fosters culture collections, their documentation and networking. The WFCC and the OECD Directorate for Science, Technology and Industry, Committee for Scientific and Technological Policy (OECD 2001) recognise that biological resources in culture collections are a world resource that needs to be accessible across national boundaries for the orderly progress of science and biotechnology. UNESCO, through its Microbial Resource Centres Network (MIRCEN), has fostered and supported the development of collections in developing countries and the training of scientists in the management and maintenance of collections as well as long-term cryopreservation techniques. In 1972 the WFCC with the support of UNESCO and UNEP established the WFCC World Data Centre for Microorganisms (WDCM) at the University of Queensland to document the metadata and species holdings of the worlds' microbial culture collections. In 1986 the WDCM was transferred to the RIKEN in Japan and subsequently to the National Institute of Genetics, and will move to the Insti-

Table 3. Significant agricultural and food microbial collections in Australia

Rhizobium Research Collections, Sydney, Adelaide, Perth
Australian Legume Inoculants Research Unit, NSW Department of Primary Industries, Gosford
BRIP Plant Pathology Herbarium Collection, Queensland Primary Industries & Fisheries, Brisbane
CSIRO Food and Nutritional Sciences Collection, Sydney
IMVS Culture Collection, Institute of Medical and Veterinary Science, Adelaide
Department of Agriculture & Food Western Australia Plant Pathogen Collection, Perth
Phytoplasma DNA Collection, Charles Darwin University, Darwin
Australian Wine Research Institute, Adelaide
CSIRO Livestock Industries, Brisbane
Plant Pathology Herbarium Collections, New South Wales Agriculture, Orange
Australian Collection of Micro-organisms, University of Queensland, Brisbane

tute of Microbiology of the Chinese Academy of Science in 2011. The WDCM remains the authoritative record of the worlds' microbial resource centres and continues to adapt to new information technologies and develop to meet changing global needs.

The OECD has recognised the essential role of biological resource centres for the life sciences and biotechnology (OECD 2001) and has developed best-practice guidelines for biological resource centres (OECD 2007). To progress the development of standards to implement the best-practice guidelines, a demonstration project for a Global Biological Resource Centre Network (http://www.gbrcn.org/index.php) commenced in 2008 involving BRCs in 15 countries including Canada, United Kingdom, Netherlands, France, Belgium, Germany, Finland, Portugal, Spain, Italy, China, Japan, Brazil, Uganda and Kenya. In order to maximise Australia's global collaboration and to provide world-class microbial resource centre facilities, there is an urgent need to develop a network of OECD-compliant biological resource centres by establishment of purpose-built facilities and or up-grading of suitable microbial culture collections (Sly 2008). This infrastructure will enhance current and future progress in many areas of the life sciences, biotechnology, industry and education, and will allow Australia to join the emerging OECD Global Biological Resource Centre Network (GBRCN).

Australian Microbial Resources Research Network

The Australian Microbial Resources Research Network was established within the framework of the ARC Research Network Seed Funding Program in 2004. While not receiving further funding for the development of a full ARC Research Network, the seed funding played an important catalytic role. The Research Network aims to provide integrated electronic access to Australian collections of micro-organisms and to bioinformation databases to meet national strategic needs for microbiological resources and to support the competitive development of the life sciences and biotechnology industries in Australia. This objective is now being fulfilled through collaboration in the NCRIS 'Atlas of Living Australia' project. The network links researchers and fosters the discovery and exploitation of Australian microbial resources and associated information.

The Australian Microbial Resources Information Network (AMRIN) web site (http://www.amrin.org/Home.aspx) has been developed to improve access to information sources and to facilitate scientific advances and efficiency through collaboration. AMRiN will be further developed as a database hub within the Atlas of Living Australia to integrate the data within Australia's microbial culture collections. Recently, AMRRN established the Council of Heads of Australian Collections of Micro-organisms (CHACM) (http://www.chacm.org) as the peak body to foster and oversee the development of microbial culture collections and development of BRCs in Australia.

Atlas of Living Australia

The 'Atlas of Living Australia' (ALA) (http://www.ala.org.au/) is a very welcome outcome from the Australian National Collaborative Research Infrastructure Strategy (NCRIS) initiative to develop science infrastructure capacity for the future. NCRIS identified the need to have access to information in Australia's biological collections to foster research and help in government and community decision-making. The ALA is being developed in collaboration with the

Australian biological collections communities in museums, herbaria, government, research institutes and universities to provide integrated electronic access to data and biological material for research and other applications. The ALA project enables free access to Australian biodiversity information online with the first public release of information scheduled for October 2010. The atlas will provide online access to biodiversity information from museums, herbaria and biological collections, including information previously not available to the public, research literature, observations, maps and images.

The development of the ALA is evidence of government recognition of the importance of this information for innovative science. However, to maximise the impact, funding is also required for maintenance and expansion of collections. This is particularly so for microbial collections and biological resource centres seeking compliance with OECD GBRCN best practice guidelines for incorporation of quality systems for living material such as microbial and cell cultures.

Issues, challenges and recommendations

Microbial resource centres underpin the life sciences and enable advances in agriculture, food security, biotechnology and education, but their important role needs more recognition and support from government, funding agencies and host institutions. Apart from their fundamental role for the conservation of microbial biodiversity, they are necessary for the supply of reference control cultures essential for regulatory compliance for health and trade. Many government ministries and agencies support programs in agriculture, food, health, quarantine, industry, science and education which depend on taxonomic decisions and access to standard cultures for quality assurance and regulatory compliance. There is also a growing need to manage impediments limiting exchange of cultures between researchers due to quarantine requirements, commercial IP, biosecurity, and access and benefit-sharing protocols.

New long-term infrastructure funding mechanisms are needed to support microbial resource centres. Apart from the ex-situ conservation and supply of current microbial genetic resources, there needs to be more comprehensive accession of cultures used in research publications and publically funded research projects than is currently the case. In order to be internationally relevant and engaged in collaborative programs, collections will also need to meet emerging international OECD GBRCN best-practice standards that will in most cases require improvements in facilities and staffing levels. There is a need to improve the future security of microbial resource centres by inclusion in national and international infrastructure programs. This move will help reverse the loss of collections and the microbial biodiversity when researchers retire or institutes change direction and priorities. Exploitation of the untapped potential of microbial diversity and innovative biodiscovery will be fostered by the support of microbial resource centres.

Making biological collections eligible for long-term infrastructure funding will enable fulfillment of the recommendations to strengthen and support culture collections of micro-organisms made in *The National Strategy on the Conservation of Australia's Biological Diversity (1992)*, the House of Representatives Standing Committee on Primary Industries and Regional Services report on *Bioprospecting: Discoveries Changing the Future* (2001), and the report on the *Review of the Innovation System*, Department of Innovation, Industry, Science and Research (Cutler 2008).

There is an urgent need to train and mentor the next generation of taxonomists and curators of microbial resource centres. Many curators of collections are approaching retirement and many who have already retired are not being replaced. Likely global changes (e.g. climate change) will probably affect microbial ecology and species' ranges and affect demand on microbial resource centres and their expertise.

Various reports have called for accelerated research on microbial diversity. Such an initiative would expand knowledge and opportunities for biodiscovery and innovative agriculture and food industries. It would also help to reverse the decline in teaching and research training in microbial taxonomy and ecology in universities. One model which should be considered is the establishment of 'research centres of excellence' in microbial diversity, taxonomy and ecology in collaboration with collections. This would assist in training the next generation(s) of research scientists and academics in microbial biodiversity and taxonomy to meet future challenges. As taxonomic expertise has been run down across the biological disciplines, biological resource centres will become the centres of taxonomic expertise to manage these resources and will be important for providing high-level research training and careers in taxonomy and identification for young PhD and postdoctoral scientists.

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