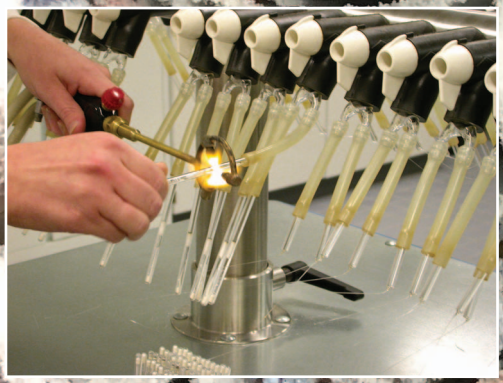
Microbiology AUSTRALIA

OFFICIAL JOURNAL OF THE AUSTRALIAN SOCIETY FOR MICROBIOLOGY INC.

Volume 40 Number 3 September 2019



Sustainable use and preservation of biological resources

BacPath 15

THE MOLECULAR BIOLOGY OF BACTERIAL PATHOGENS

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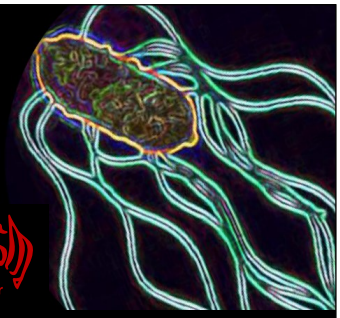

Invited Speakers

Susanne Gebhard, University of Bath
Ann Jerse, Uniformed Services University
Timothy Atkins, DSTL
Paal Skytt Andersen, Staten Serum Institute
Richard Titball, University of Exeter

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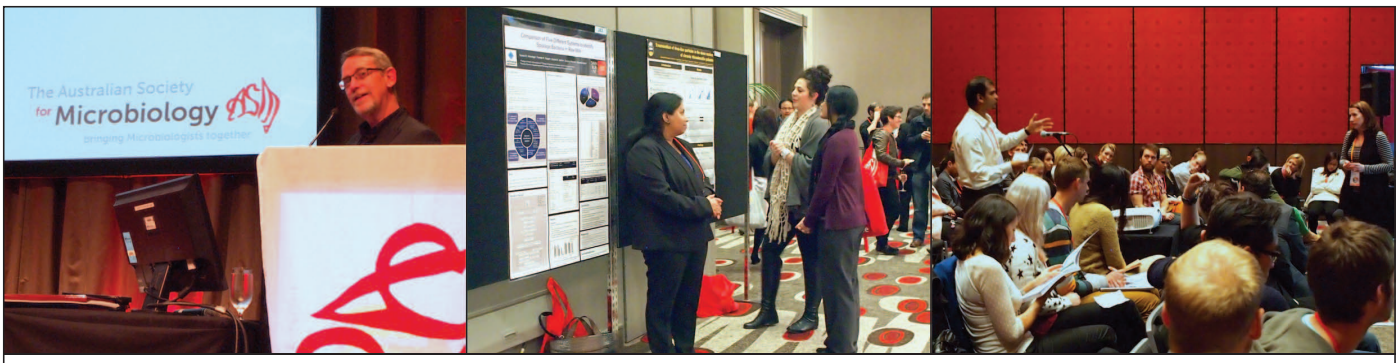
Committee Members
Charlene Kahler, Geoff Coombs, Mitali Sarkar-Tyson, Josh Ramsay, Dan Knight, Nicole Bzdyl



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Some benefits of ASM membership:

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Subscription rates
Current subscription rates are available from the ASM Melbourne office.

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Published four times a year in print and open access online by

CSIRO PUBLISHING
Unipark, Building 1, Level 1
195 Wellington Road, Clayton, Vic. 3168
http://microbiology.publish.csiro.au

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ISSN 1324-4272
eISSN 2201-9189

Contents

Cover image: Background image: National Animal Serum Bank straws at –80°C (courtesy of CSIRO AAHL); inset photos: storage of cultures (courtesy of Westerdijk Fungal Biodiversity Institute).

Dena Lyras
President of ASM

Climate change is one of the most important issues faced by our planet. As scientists, we seek to understand and find ways to prevent, and perhaps reverse, its effects. Although the impacts of climate change on the environment and extinction of animal and plant life are well studied, the consequences of climate change on microbes are largely unknown. We now understand that microbes underpin a healthy global ecosystem and that disruptions to microbial diversity often have devastating repercussions on the affected ecological niche. Indeed, changes in microbial diversity induced by climate change may therefore have important downstream consequences on the resilience of such ecosystems and their ability to withstand climate change.

The issue of microorganisms and climate change is the subject of an excellent and timely paper published in *Nature Reviews Microbiology* by one of our ASM members, Professor Rick Cavicchioli, and colleagues (Scientists' warning to humanity: microorganisms and climate change. Cavicchioli *et al*., *Nat Rev Microbiol.* 2019 Sep; 17(9): 569–586. doi:10.1038/s41579-019-0222-5). Many of you will already have read this paper; for those of you who have not, I urge you to do so. It describes what we know about the effects of microorganisms on climate change, from microbial climate-active processes and their drivers to the role of microbes in climate change mitigation. The effects of climate change on microorganisms are also described, as are the influences of climate change on microbial community compositions, physiological responses and adaptation.

This article is described as a 'Consensus Statement' and has been authored by 33 scientists from nine countries. It also serves as a microbiologists' call-to-action because of the importance of microbes to climate change biology. It encourages microbiologists to become more engaged in climate change studies, and alerts microbiologists and non-microbiologists to address the roles of microorganisms in accelerating or mitigating the impacts of climate change. The ASM has strongly endorsed this statement, which is described in more detail in a letter by Professor Cavicchioli, which follows this Vertical Transmission. We encourage our members to do the same by following the links in the article or by using the following link, and by signing the petition to show your support for this important aspect of climate change: https://www.babs.unsw.edu.au/research/microbiologists-warning-humanity.

I would like to extend our grateful thanks to the organisers of the national meeting for 2019 that was held in Adelaide. This was an excellent meeting, with over 500 delegates, and it was a tremendous success scientifically and socially. Of particular note, the public lecture speaker Wendy Jackson from PRIDA (Pacific Region Infectious Diseases Association) described the contribution scientists can make to improving health care in the Pacific region, which drew the interest of many of our members who are now planning on contributing to this effort. Our 2019 Rubbo Orator, Professor Tilman Ruff, was inspirational in describing the global imperative to eradicate nuclear weapons and showed us the power of individuals to tackle change and to promote peaceful outcomes. Congratulations and thanks to all members of the local organising committee especially the Conference Chair, Dr Stephen Kidd, and Co-Chair, Chris Ossowicz. Another excellent Annual Scientific Meeting is planned for Melbourne next year on 5–8 July 2020 – please add the dates to your diary.

As always, please visit our website www.theasm.org.au to access information regarding upcoming meetings and awards. Note our fresh new website, which is easier to navigate and currently showcases content created by our wonderful ASM Communication Ambassadors You may also like to follow, and contribute to ASM on Twitter, @AUSSOCMIC, or on Facebook to make sure you keep up with the latest news, trends and developments in Microbiology in Australia and around the world.

The Microbiologists' Warning: a Warning from All Microbiologists' to Humanity

Rick Cavicchioli

The Microbiologists' Warning is a Consensus Statement proclaiming that microorganisms are so critical to achieving an environmentally sustainable future that ignoring them risks the fate of Humanity. It aims to raise awareness of the microbial world and make a call to action for microbiologists to become increasingly engaged in, and microbial research to become increasingly infused into, the frameworks for addressing climate change. We must learn not just how microorganisms affect climate change (including production and consumption of greenhouse gases), but also how they will be affected by climate change and other human activities.

Anyone with microbiology training, professionals and students alike, are encouraged to become part of the Microbiologists' Warning by individually endorsing the Consensus Statement.

In addition to individuals, already more than 40 organisations representing national and international Academies and Societies have endorsed, including the Australian Society for Microbiology and the Australian Academy of Science.

The profile of the Consensus Statement has grown rapidly with the publisher website showing >65 000 accesses and an Altmetric score that is considerably higher than any other of the more than 2000 articles published by *Nature Reviews Microbiology*.

The Microbiologists' Warning is intended as vehicle for ALL microbiologists to motivate change in many and varied ways.

The Consensus Statement is Open Access and is intended to be freely distributed and used.

A powerpoint presentation is available for making presentations for conferences, teaching and outreach purposes and can be accessed via a shared Dropbox link – it is available for anyone wanting to make presentations about the Microbiologists' Warning; if the link no longer functions, contact Rick Cavicchioli.

Translations of the Consensus Statement are useful for allowing more scientists to read the article and are particularly valuable for enabling members of the media and general public to read and contemplate – even if the content is not fully comprehensible it will prompt questions to scientists and hence provide an important means of education and public understanding of the issues.

Currently, translations are being written in Chinese, Spanish, French, Portuguese, Greek, Russian and Turkish. A Word doc version of the publication to help translators is available – perhaps you or someone you know would like to translate into another language – if so, please contact me (r.cavicchioli@unsw.edu.au) to discuss and obtain the Word doc.

Things you can easily help with:

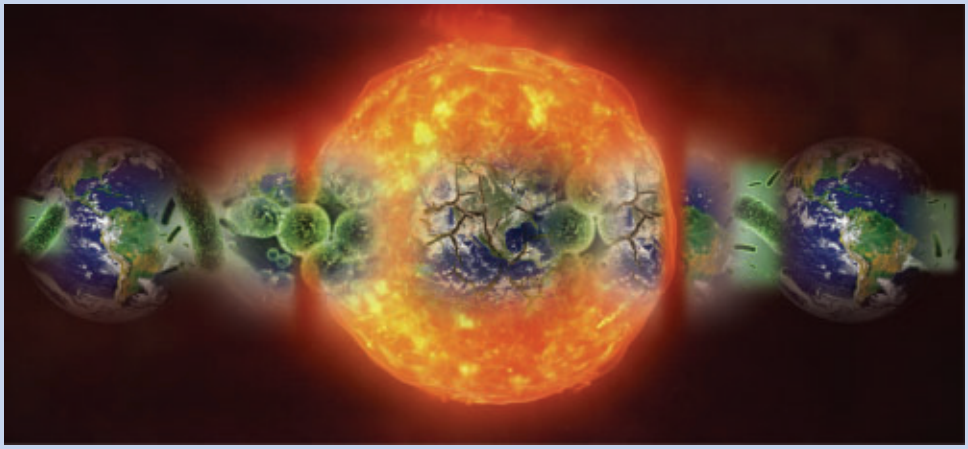
- Read the Consensus Statement
- Endorse individually. Endorsing as an individual is straightforward: click on the signatory site link, add a few details and submit.
- Request organisations you are a member of to endorse
- Distribute widely – amplify the message: email, Twitter, Facebook, LinkedIn

Things that will take a little more effort:

- Translate the Consensus Statement into another language
- Motivate the writing of additional statements emphasising a national focus

Food for thought:

- An urgency exists for improving understanding about the links between microbes and climate change, and also more generally for improving microbial literacy in society – the two go hand-in-hand. One avenue for achieving this is for funding agencies to enact schemes to specifically address the microbiology of climate change and microbial literacy. A priority of the scheme would be linkages to national (ideally) or international businesses/organisations that demonstrate tangible incorporation of microbiology into their 'thinking' and improved public understanding of microbes. Another priority would be interdisciplinary research (e.g. microbiologists with modellers and physical scientists) linking microbiology to non-microbiology disciplines so that the research collectively targets the microbial dimensions that are currently missing. Also see the Call to Action (box 2) in the Consensus Statement.



Sustainable use and preservation of biological resources



İpek Kurtböke, Wieland Meyer and Lindsay Sly

Microorganisms, microbially derived biotechnological applications and as causative agents of human, animal and plant disease are becoming increasingly significant in national economies. However, there remains a significant information gap on their species, ecological and genetic diversity[1,2]. Despite the recognition of their commercial value still little is known about their functional roles in sustaining global 'life support systems', such as in agriculture, forest, coastal and freshwater ecosystems as well as their detrimental roles in the environment. As a result, sustainable use, preservation of microbial resources and effective strategies to combat infections are of immense importance to mankind. Culture collections are thus the cornerstones of biotechnology, sustainable futures and infection control.

Many traditional culture collections (CCs) are now transitioning to operate as Biological Resource Centres since the Organisation for Economic Co-operation and Development (OECD) Task Force in 1999 developed the concept and guidelines for the BRCs (http://www.oecd.org/sti/emerging-tech/biologicalresourcecentres.htm). The BRCs play a crucial role in the preservation and provision of biological resources, research and development, conservation of biodiversity, and as repositories for the protection of intellectual property and resources for public information and policy formulation[1]. Meanwhile, The UN-Convention on Biological Diversity (CBD) was the first agreement to address all aspects of biological diversity: species, ecosystems and genetic resources. It marks the first time that genetic diversity is specifically covered in a binding global treaty (https://www.cbd.int/convention/). The subsequent Nagoya Protocol of the CBD ensures fair and equitable sharing of the benefits arising from the utilisation of genetic resources, including appropriate access to genetic resources and appropriate transfer of relevant technologies (https://www.cbd.int/abs/).

The need to efficiently capture, store, process and analyse large scale information related to the biological resources has also increased due to the advances in molecular biology. As a result, an Ad Hoc Technical Expert Group (AHTEG) on Digital Sequence Information on Genetic Resources was established at the CBD, followed by the actions to clarify terminology and concepts, and to assess the extent and the terms and conditions of the use of digital sequence information of genetic resources in the context of the Nagoya Protocol (https://www.cbd.int/abs/dsi-gr/2017-2018/default.shtml).

The value of any BRC is directly linked to the diversity and quality of the cultures held and the extent and quality of the associated data with the preserved material. In addition, BRCs are the bodies that the public and the policy-makers can call on for objective help in developing regulations and guidelines for the safe and ethical use of biological resources while ensuring compliance with the three key objectives of the CBD. The World Federation for Culture Collections (WFCC; http://www.wfcc.info/) is the largest independent global organisation that represents microbial culture collections concerned with the collection, authentication, maintenance and distribution of cultures of microorganisms and cultured cells. The Federation has developed synergistic capacity with leading organisations (e.g. CBD, GBIF, WIPO, ISO, ECCO) and contributes toward the increase of knowledge on microbial diversity and in turn

Table 1. Major microbial collections currently active in Australia.

Culture collection	Year established	Institute	Collection curator	Microorganisms stored	Preservation method	Access	Website
Australian National Algae Culture Collection (ANACC)	1967	CSIRO	Ian Jameson ian.jameson@csiro.au 03 6232 5117	A	FT, O	PA, CA	https://www.csiro.au/en/Research/Collections/ANACC
Australian National Reference Laboratory in Medical Mycology (AMMRL)	1949	Royal North Shore Hospital, NSW Health Pathology	Kerry Weeks kerry.weeks@health.nsw.gov.au 02 9926 4319	B, FF, YF, A	RT	OA	
AWRI Wine Microorganism Culture Collection (AWRI)	1939	The Australian Wine Research Institute	Angus Forgan culture@awri.com.au 08 8313 6600	B, FF, YF	FR	PA, CA, SS	https://www.awri.com.au/research_and_development/wine-microorganism/
CSIRO Manufacturing Collection of Biocatalytic Microorganisms	2001	CSIRO	Dr Geoff Dumsday geoff.dumsday@csiro.au 03 9545 2344	B, FF, YF	FR	CA	
CSIRO Manufacturing Wood Inhabiting Fungi Collection	~1930	CSIRO	Dr Geoff Dumsday geoff.dumsday@csiro.au 03 9545 2344	FF	O	CA	
DAFWA Plant Pathology Culture Collection (WAC)	1960	Department of Agriculture & Food Western Australia	Nuccia Eyres neyres@agric.wa.gov.au 08 9368 3929	B, V, FF, YF	FD, FR	OA, CA	
FRR Culture Collection (FRR)	1970	CSIRO	Mark Wilson mark.wilson@csiro.au 02 9490 8315	FF, YF	FD	OA	www.foodscience.csiro.au/fcc
IFM Culture Collection	1997	IFM Quality Services Pty. Ltd.	Ingrid Flemming ingridflemming@ifmqs.com.au 02 9618 3311	B, FF, YF	FT, RT, RS, FD, FR	CA	
Murdoch University Algal Culture Collection	1983	Murdoch University	Associate Professor Navid Moheimani n.moheimani@murdoch.edu.au 08 9360 2333	A	FT, RT, O	PA	
National Mycology Reference Centre (ACH)	1962	SA Pathology	Dr Sarah Kidd Sarah.kidd@sa.gov.au 08 8222 3544	FF, YF	RT, FR	OA, CA	
Neurospora crassa Mutants Collection	1971	Flinders University	Professor David Catcheside david.catcheside@flinders.edu.au 08 8201 2335	FF	O	OA, CA	
NSW Plant Pathology & Mycology Herbarium (DAR)	1960	Orange Agricultural Institute	Dr Jordan Bailey Jordan.bailey@dpi.nsw.gov.au 02 6391 3985	B, V, FF	RS, FD. FR	OA, PA	http://www.dpi.nsw.gov.au/about-us/services/collections/herbarium
Queensland Plant Pathology Herbarium Culture Collection (BRIP)	1966	Queensland Plant Pathology Herbarium	Dr Roger Shivas roger.shivas@daf.qld.gov.au 04 0976 1956	B, FF, YF	FR	OA	collections.daff.qld.gov.au
PathWest Queen Elizabeth II Medical Culture Collection (DMPMC)	1966	Department of Microbiology, Queen Elizabeth II Medical Centre	Ian Arthur ian.arthur@health.wa.gov.au 08 6383 4531	B, FF, YF	RT, FR	CA, SS	
University of the Sunshine Coast Microbial Library (USC-ML)	2001	University of the Sunshine Coast	Dr Ipek Kurtböke ikurbok@usc.edu.au 07 5430 2819	B, BP	O	CA, SS	
UWA *Helicobacter pylori* Culture Collection	1997	University of Western Australia	Barry Marshall barry.marshall@uwa.edu.au 08 6457 4815	B	FR	CA	
Victorian Plant Pathogen Herbarium (VPRI)	~1890	Agriculture Victoria	Dr Jacqueline Edwards jacky.edwards@agriculture.vic.gov.au 03 9032 7330	B, V, FF	RT, RS, FD	PA, CA, O	
Westmead Medical Mycology Collection (WM)	1980	Westmead Institute For Medical Research	Professor Wieland Meyer wieland.meyer@sydney.edu.au 02 8627 3430	FF, YF	RS, FD, FR	OA, CA	
Wine Microbiology Culture Collection	2008	Waite Campus, University of Adelaide	Professor Vladimir Jiranek vladimir.jiranek@adelaide.edu.au 08 8313 6651	B, YF	FR	OA	

A, algae; B, bacteria; BP, bacteriophage; CA, collaborative access; FD, freeze dried and stored at 4–10°C; FF, filamentous fungi; FR, stored at –80°C; FT, frequent transfer; GP, stored in liquid nitrogen (gas phase); LP, stored in liquid nitrogen (liquid phase); O, other; OA, open access; P, protozoa; PA, purchased access; RS, refrigerated storage; RT, room temperature; SS, study-specific access; V, virus; YF, yeast fungi.

facilitates the strengthening of the roles of microbial culture collections as tools for research in biodiversity and biosystematics.

In light of the above developments, this special issue of *Microbiology Australia* is dedicated to the sustainable use and preservation of microbial resources. Beatriz Gómez-Castro and Regina Kipper from the CBD provide an overview of the *Nagoya Protocol on Access and Benefit-Sharing*, followed by two examples from Germany and Brazil. Manuela da Silva from Brazil contributes with a view from a non-Nagoya Protocol country, whereas Andrey Yurkov, Hilke Marie Püschner and Amber Hartman Scholz from the DSMZ, Germany, inform us about the European Union's first Registered Collection under the Nagoya Protocol. Tim Fitzgerald provides expert information on the biological deposits for patenting purposes under the Budapest Treaty. David Smith and Matthew Ryan visit the International Postal, Quarantine and Safety regulations. Juncai Ma, Linhuan Wu and İpek Kurtböke provide an update on the 2019 status of the World Data Centre for Microorganisms (WDCM) Global Catalogue of Microorganisms. William B Whitman, Hans-Peter Klenk, David R Arahal, Rosa Aznar, George Garrity, Michael Pester, and Philip Hugenholtz provide information on their collaborative initiative entitled *Genomic Encyclopedia of Bacteria and Archaea (GEBA) VI*: *learning from type strains*. Wieland Meyer, Ian Arthur, David Ellis, Alex Kan, Sarah Kidd, Krystina Maszewska and Kerry Weeks provide an overview of the major medical mycology culture collections in Australia. Roger Shivas, Dean Beasley, Kaylene Bransgrove, Yu Pei Tan and Geoff Bulow inform us on *Biodiscovery and the Queensland Plant Pathology Herbarium*. Lynda Wright provides information on the Australian Biobanks for serum and cells of human and animals. Lindsay Sly reviews historical aspects of Australian culture collections of microorganisms and explores new roles and opportunities for a network of *Australian Collections of Microorganisms* in the microbiome era.

The Australian Microbial Resources Research Network (AMRRN) was a nationwide initiative with the aim of developing a world class research network to discover and exploit Australian microbial resources and to make these resources and associated information available for applications in science, research, industry and education. In this special issue, we the guest editors, revisit the concept and call for a renewed nationwide effort by the Council of Heads of Australian Collections of Microorganisms (CHACM) to take the AMRRN concept further to fulfilment (see article by Sly), and to realise Skerman's WDCM vision of fully cataloguing microbial collections and making this information freely accessible. A list of major currently active microbial collections in Australia is provided in Table 1.

References

1. Kurtböke, D.İ. (Ed.) (2017) *Microbial resources: from functional existence in nature to applications*. Elsevier, Academic Press.
2. Kurtböke, D.İ. and Swings, J. (Eds.) (2004) *Microbial Genetic Resources and Biodiscovery*. Queensland Complete Printing Services, Australia.

Do you know...

Finding penicillin to treat infections wasn't the only major scientific discovery that shaped the amazing career of Percival (Val) Landon Bazeley. In January 1951, having overseen CSL commencing large-scale penicillin production, and completing a medical degree, Bazeley became interested in polio research. Again Bill Keogh saw the importance of local production of a crucial treatment for infectious disease. With the assistance of Sir Macfarlane Burnet, he approached the Prime Minister and arranged for Bazeley to be sent to Jonas Salk's laboratory in Pittsburgh. Bazeley worked with Salk and was responsible for the production of the lots of vaccine used in the first clinical trials in the US. Bazeley returned to Australia in 1955 to set up production of Salk vaccine at CSL. By June 1956 he had arranged for the production of 390 000 doses of vaccine, which rose to five million doses per annum in the next two years. To achieve all this he worked a seven-day week and could be found in the laboratories on public holidays and on one occasion, on Christmas Day.

Val Bazeley was truly a great Australian, a man of action and a man of great vision. The Bazeley oration ensures his name will be honoured as long as ASM continues bringing microbiologists together.

Taken from notes provided by Professor Ian Gust.

Nagoya Protocol on Access and Benefit-Sharing

Beatriz Gómez-Castro

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The Nagoya Protocol advances one of the three objectives of the Convention on Biological Diversity (CBD), namely 'the fair and equitable sharing of the benefits arising from the utilization of genetic resources'. The Protocol promotes equity in the sharing of benefits from the use of genetic resources and encourages the reinvestment of benefits into the conservation and sustainable use of biodiversity and ecosystems. Binding obligations established under the Protocol aim at creating greater legal certainty and transparency as well as more equitable partnerships between users and providers of genetic resources and associated traditional knowledge. The Protocol has the potential to leverage tangible impacts in provider countries and foster sustainable development for present and future generations.

Why are we talking about access and benefit-sharing?

The entry into force of the Convention on Biological Diversity (CBD) in 1993 marks a fundamental shift in how genetic resources are considered by the international community. Prior to the CBD, free access to genetic resources had prevailed. However, developments in biotechnology since the mid-1970s made the search for new and unforeseen uses for genetic resources possible. This attracted greater attention to the value of these resources. Like many other resources in the world, genetic resources are not evenly distributed. In the early 1980s, several countries started restricting access to the genetic resources under their jurisdiction and calling for increased control over their genetic resources[1].

During the negotiations of the CBD, many developing countries wanted the value and contribution of genetic resources to be recognised and the benefits resulting from their use to be shared more fairly and equitably. The fair and equitable sharing of benefits arising out of the utilisation of genetic resources came to be one of the three objectives of the CBD, together with the conservation of biological diversity, and the sustainable use of its components. The concept aimed at re-directing benefit flows back to countries providing genetic resources, while creating incentives for the conservation and sustainable use of biological diversity.

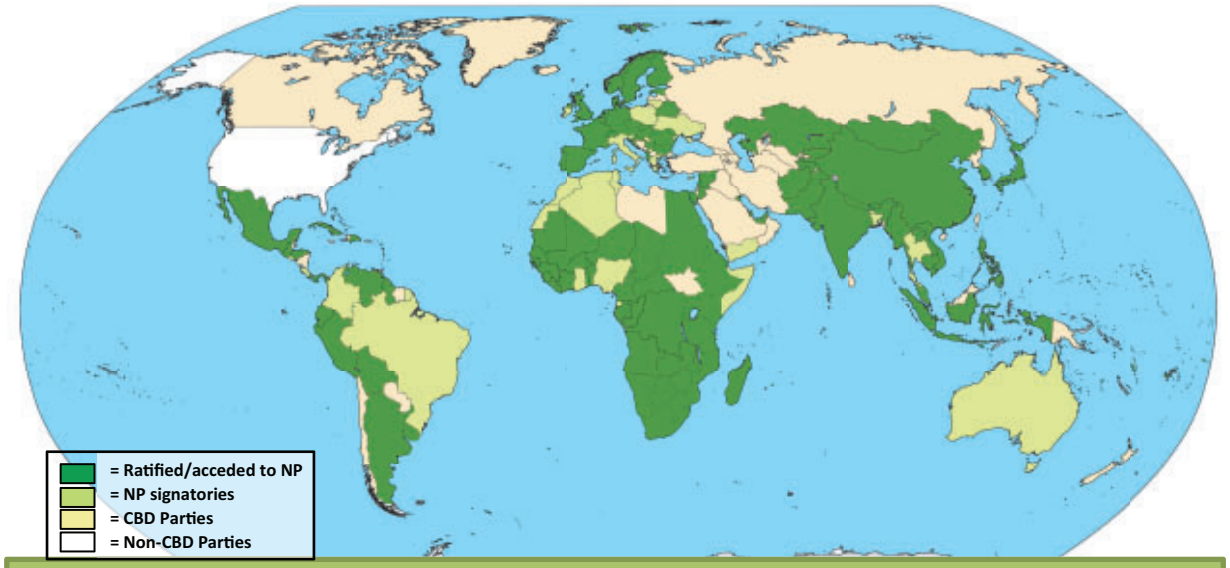
What are the access and benefit-sharing principles of the CBD?

The CBD established the concept of access and benefit-sharing (ABS), which refers to how genetic resources may be accessed, and how the benefits resulting from their use are shared between the people, institutions or countries using the resources (users) and the people or countries that provide them (providers)[2].

Using genetic resources, whether from plants, animals or micro-organisms, refers to the process of researching their beneficial properties and using them to increase scientific knowledge and understanding, or to develop commercial products. Users of genetic resources are required to obtain permission, the prior informed consent (PIC), of the country providing access to the genetic resource and negotiating and agreeing on the terms and conditions of access and utilisation of this resource through the establishment of mutually agreed terms (MAT). This agreement is also to address the sharing of benefits arising from the utilisation of the resource.

Why is the Nagoya Protocol important?

Access to genetic resources can lead to benefits for both users and providers. ABS ensures that the way in which genetic resources, and associated traditional knowledge, are accessed and used,



119 Countries that of July 2019 have ratified/acceded to the Nagoya Protocol: Afghanistan, Albania, Angola, Antigua and Barbuda, Argentina, Austria, Belarus, Belgium, Benin, Bhutan, Bolivia, Botswana, Bulgaria, Burkina Faso, Burundi, Cambodia, Cameroon, Central African Republic, Chad, China, Comoros, Congo, Côte D'Ivoire, Croatia, Cuba, Czech Republic, Democratic Republic of the Congo, Denmark, Djibouti, Dominican Republic, Ecuador, Egypt, Eritrea, Estonia, Eswatini (Kingdom of), Ethiopia, European Union, Fiji, Finland, France, Gabon, Gambia, Germany, Guatemala, Guinea, Guinea Bissau, Guyana, Honduras, Hungary, India, Indonesia, Japan, Jordan, Kazakhstan, Kenya, Kuwait, Kyrgyzstan, Lao PDR, Lebanon, Lesotho, Liberia, Luxembourg, Madagascar, Malawi, Malaysia, Maldives, Mali, Malta, Marshall Islands, Mauritania, Mauritius, Mexico, the Federated States of Micronesia, Mongolia, Mozambique, Myanmar, Namibia, Nepal, Netherlands, Niger, Norway, Pakistan, Palau, Panama, Peru, Philippines, Portugal, Qatar, Republic of Korea, Republic of Moldova, Romania, Rwanda, Saint Kitts and Nevis, Samoa, Sao Tome and Principe, Senegal, Serbia, Seychelles, Sierra Leone, Slovakia, South Africa, Spain, Sudan, Sweden, Switzerland, the Syrian Arab Republic, Tajikistan, Togo, Tuvalu, Uganda, United Arab Emirates, United Kingdom of Great Britain and Northern Ireland, United Republic of Tanzania, Uruguay, Vanuatu, Venezuela (Bolivarian Republic of), Viet Nam, Zambia and Zimbabwe

DISCLAIMER: The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Secretariat of the Convention on Biological Diversity concerning the legal status of any country, territory, city or area of its authorities, or concerning the delimitation of its frontiers or boundaries.

Figure 1. World map of ratifications/accessions to the Nagoya Protocol.

maximises the benefits for users, providers and the communities where those resources are found.

In 2010, the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilisation was adopted as a supplementary agreement to the Convention to advance its third objective, building on the provisions of the CBD.

The Nagoya Protocol establishes an international, legally binding framework to promote the transparent and effective implementation of access and benefit-sharing at the national level. The Protocol helps establishing more predictable conditions for access to genetic resources while also helping to ensure benefit-sharing when genetic resources leave the country providing the genetic resources. Thus, the Nagoya Protocol helps to create trust between users and providers of genetic resources by providing a clear transparent framework for access and benefit-sharing and further legal certainty.

The Nagoya Protocol entered into force in October 2014, after ratification by 50 countries. As of 9 July 2019, 119 Parties [once a country ratifies an intergovernmental treaty, they become a Party to the treaty] to the CBD had ratified the Protocol (Figure 1)[3]. The Protocol receives broad support from both developed and developing countries in different regions. Ratification of the Nagoya Protocol enables countries to shape future priorities and guidance for the implementation of the Nagoya Protocol and also helps to build trust between users and providers.

What does the Nagoya Protocol provide for?

The Nagoya Protocol covers genetic resources, including microorganisms, plants, and animals, as well as traditional knowledge associated with genetic resources. It sets out a new regime with core obligations for its contracting Parties to take measures in relation to access, benefit-sharing and compliance. The compliance obligations are one of the main innovations of the Protocol. The Protocol also establishes an ABS Clearing-House to facilitate the exchange of information.

Access

Access to genetic resources is subject to the PIC of the provider country unless otherwise determined by that country. Parties that require PIC must establish clear and transparent procedures for accessing genetic resources and are to issue a permit when access is granted. The Protocol also regulates access to genetic resources for their utilisation. The term 'utilisation of genetic resources' is defined to mean 'to conduct research and development on the genetic and/or biochemical composition of genetic resources,

including through the application of biotechnology as defined in Article 2 of the Convention'[4].

Benefit-sharing

Under the Nagoya Protocol, benefits arising from the utilisation of genetic resources as well as subsequent applications and commercialisation are to be shared in a fair and equitable way with the provider country. This sharing is upon mutually agreed terms. The benefits to be shared may be monetary or non-monetary, such as training and education, transfer of technology, or sharing of research results [the Nagoya Protocol includes an annex with an indicative list of types of benefits that can be shared].

Compliance

With a view to support benefit-sharing once genetic resources have left the provider country and are being utilised by scientific or research institutions or industries in another country, the Protocol contains obligations to support compliance with the ABS requirements of the provider country and with the contractual obligations between users and providers of genetic resources reflected in mutually agreed terms. In addition, the Protocol puts in place a system to monitor the utilisation of genetic resources, based on permits (internationally recognised certificates of compliance) and checkpoints. Parties are to establish at least one checkpoint in order to monitor the utilisation of genetic resources by users within their jurisdiction.

ABS Clearing-House

The Nagoya Protocol establishes the ABS Clearing-House as a platform for exchanging information on ABS[5]. The Clearing-House is a key tool for facilitating implementation of the Protocol. Parties must publish information on their access and benefit-sharing procedures and the permits they issue in the ABS Clearing-House, so they become internationally recognised certificates of compliance. The certificate serves as evidence that a user has accessed a genetic resource legally and in accordance with the ABS measures of the provider country [more information on internationally recognised certificates of compliance and the monitoring system established by the Protocol is available at: https://vimeo.com/263320356/513f748f8a].

How does the Nagoya Protocol address non-commercial research?

During the negotiations of the Nagoya Protocol, the scientific community expressed concerns about the impact that ABS provisions could have on non-commercial research. The Protocol requires Parties to create conditions to promote and encourage research that contributes to the first and second objective of the CBD – that is conservation and sustainable use of biological diversity[6].

What does the Nagoya Protocol mean for users of genetic resources?

The Nagoya Protocol, in order to be operational, must be implemented by Parties through national legislative, administrative and/or policy ABS measures. National ABS requirements vary from country to country due to different national priorities and circumstances. It is the countries through their national ABS measures that determine the specific application of the Nagoya Protocol in their country and define which activities are covered by their ABS legislation.

Users of genetic resources need to follow the ABS requirements of: (1) the country providing the genetic resource; and (2) the country where utilisation of the genetic resource (i.e. research and development) takes place. Users from a country that is not a Party to the Nagoya Protocol still need to comply with the legislation of the country providing the genetic resources. The most convenient way to find national information on those ABS requirements is through the country profiles on the ABS Clearing-House[7]. Consulting the relevant ABS measures can help users to understand how to comply with ABS requirements. However, many countries are in various stages of developing their legal and institutional frameworks for ABS and making that information available on the ABS Clearing-House. To find more information on the requirements that would apply to a specific case or activity, users of genetic resources are encouraged to contact the ABS national focal point or the competent national authority(ies) in a country. The contact details as well as information on each country's ABS requirements are available in the country profile page on the ABS Clearing-House.

The publication 'ABS is genetic resources for sustainable development'[8] provides some specific examples of biodiscoveries as well as ABS measures and approaches in 27 countries

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This research did not receive any specific funding.

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Biographies

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Brazil, example of a non-Nagoya Protocol country

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Brazil was one of the first countries to regulate access to genetic resources, and to associate traditional knowledge and benefit sharing through Provisional Act 2186-16 of 23 August 2001 for purposes of scientific research, bioprospecting, and technological development. After almost 15 years of many criticisms and demands from civil society and other sectors, Law 13,123 was sanctioned on 20 May 2015[1] and entered into force on 17 November 2015, revoking Provisional Act 2.186.

The Law, known as the Biodiversity Law, regulates Article 1, Article 8(j), Article 10(c), Article 15, and Article 16, items 3 and 4 of the Convention on Biological Diversity (CBD), besides regulating part of Article 225 of the Brazilian Federal Constitution. It provides for access to genetic resource (known in Brazil as genetic heritage), for protection and access to associated traditional knowledge, and for benefit-sharing for conservation and sustainable use of biodiversity and creates the Genetic Heritage Management Council (CGen), the Brazilian National Competent Authority for ABS. Therefore, despite the fact that Brazil has not yet ratified the Nagoya Protocol (NP) on Access to Genetic Resources and the Fair and Equitable Distribution of Access and Benefit Sharing (ABS), the Law 13,123 is aligned with this international agreement.

The construction process of this new legislation was complex, considering the different interests and points of view of the various sectors of civil society, represented by academia, business sector, and holders of associated traditional knowledge, as well as those of the different ministries. The Law is regulated through Decree No. 8,772 of 11 May 2016 and to enable compliance with the legislation, the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen) was developed by the Ministry of Environment.

The Law 13,123 has a broader scope than the previous legislation and involves research, technological development, and economic exploitation of products arising from access to genetic resources (GR) and associated traditional knowledge (ATK). Due to the new definitions of GR [genetic information from plants, animals, and microbial species, or any other species, including substances originating from the metabolism of these living organisms], access to GR [research or technological development carried out on genetic heritage samples] and research [experimental or theoretical activity carried out on genetic heritage or associated traditional knowledge with the objective of building new knowledge by means of a systematic process that creates and tests hypothesis, describes and interprets fundamentals of observed phenomena and facts], the Law includes activities such as basic research related to taxonomy, phylogeny, epidemiology, and ecology, among others, as well as the obtention of genetic sequence from GR and their use.

Brazil set a precedent when it included genetic information in the scope of its ABS legislation, taking into account that in the last two meetings of the Conference of the Parties to the CBD (COP 13 and COP 14) and of the Parties to the NP (COP-MOP 2 and COP-MOP 3) the issue of Digital Sequence Information (DSI) was discussed

regarding its potential implications on the three objectives of CBD and the objective of the NP, which will be resumed at the next COP meeting. Brazil's position is to defend that if there is a product resulting from the use of DSI that benefit-sharing with the countries of origin of the genetic resource from which the DSI was generated is required. In cases of DSI originating from genetic resources coming from several countries, the proposal is for the monetary benefits to be deposited in a multilateral fund.

Based on the experience with the former legislation and to avoid uncertainties and questions relating to microorganism's origin, about whether they are native or exotic, the Law brings the determination that any microorganism isolated in Brazil is a Brazilian genetic resource. In this context, medical researchers should take into consideration that research involving pathogenic microorganisms, isolated or not isolated, present in human samples (e.g. blood, urine, tissues), shall also meet the requirements of the Law.

The prior informed consent (PIC) for accessing genetic resources was replaced by a self-declaratory registration in SisGen, which may occur during the phase of research and technological development. However, the registration must be conducted prior to shipment of the genetic heritage; application for intellectual property rights; marketing of intermediate product; dissemination of results (final or partial); or even notification of product developed from an access. Prior authorisation will also be required for cases involving foreigners, in which access takes place in the border area and Brazilian jurisdictional waters, on the continental shelf, and in the exclusive economic zone. Upon completing the SisGen electronic form, the registration receipt will automatically be issued. This document demonstrates that the user has provided the required information.

When there is a product derived from access to GR or ATK, notification in SisGen prior to economic exploitation is required. Benefit sharing only occurs when there is a product to be marketed and beneficiaries are the Federal Government or the ATK holders. Thus, the National Benefit Sharing Fund (FNRB) was established to receive money from the monetary benefit sharing and fines resulting from non-compliance with the legislation. These financial resources will be used to promote the conservation of biological diversity; recovery, creation and maintenance of *ex situ* collections; prospecting and training of human resources associated with the use and conservation of GR or ATK; among other initiatives. In the case of access to ATK the holders of this knowledge will be the beneficiaries and negotiate with the user. The Law also establishes that when monetary resources deposited in the FNRB are derived from the economic exploitation of products obtained from access to GR coming from the *ex situ* collections that are registered in SisGen, part of this resource will be shared with them. Another option of benefit sharing is the non-monetary benefit sharing, which can be by means of implementing projects for conservation and protection of biodiversity and ATK. Non-monetary benefit sharing may be more advantageous in some cases than the transfer of resources to the FNRB.

Regarding shipment of genetic resources abroad, it is necessary to sign a Material Transfer Agreement (MTA) between sender and recipient and to register the shipment in SisGen. A new resolution (CGen Resolution 12 of 09/18/2018), allows a single MTA to be used for all shipments with the same foreign institution, which may be valid for up to 10 years and can be renewed. Once the material under the MTA is shipped to an institution, it becomes its property for an indefinite period. Additionally, a shipment invoice is filled with the description of the GR to be transferred, which will be sent with a copy of the MTA and the shipment registration receipt together with the GR. The Law authorises the transfer of GR to third parties, provided that the accompanying MTA contains the same provisions of the original MTA, which should occur for all subsequent transfers. This is a breakthrough, especially when GR is a microorganism and has to be deposited in an international culture collection, considering that the former legislation required that the new users of the deposited microorganisms had to negotiate a new MTA with the original depositor, which made the process very difficult.

Foreign researchers will be able to access native biodiversity only if they are associated with public or private Brazilian scientific and technological research institutions, which must take responsibility for registering the activity in SisGen. This requirement also applies to access Brazilian GR deposited in *ex situ* collections or to genetic sequences deposited in public databases, which were obtained from Brazilian GR. Since this requirement of association is also for basic research, there is a discussion for facilitating the process in these cases.

In this context it should be considered that the term access is interpreted in different ways by different countries. For Brazil, access is the same as the use of GR, that is, research and technological development involving these resources. While for the vast majority of other countries access is the collection or isolation of GR. Therefore, when using Brazilian GR deposited in *ex situ* collections, even before 2014 when Nagoya Protocol came into force, the Brazilian legislation must be complied with.

There were improvements regarding the former legislation, however many adjustments are still needed regarding the operational rules and SisGen. Within CGen, the Sectorial Chamber of the

Academy, which is an open forum and the main entities representing the Brazilian academy take part, was created to debate, elaborate and propose solutions to be further submitted for approval at CGen. The Chamber has been very active and have already proposed many resolutions and technical orientations which have been approved by CGen that have improved the process. The Chamber is also involved with discussions and proposals for the new version of SisGen that is being proposed to ameliorate and correct the problems identified during the use of the current system since the end of 2017. The expectation is that the process will be improved and will allow the compliance of the legislation in a more optimised and efficient way.

Conflicts of interest

The author declares no conflicts of interest.

Acknowledgements

This research did not receive any specific funding.

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Biography

Dr Manuela da Silva received her PhD degree in Food Science (Microbiology) at the State University of Campinas (UNICAMP) in 2002, part of which was conducted at the Food and Drug Administration (FDA-NCTR) in USA. In 2002 Dr da Silva joined the Oswaldo Cruz Foundation (Fiocruz), institution of science and technology under the Brazilian Ministry of Health, and currently works as Director of the Biological Collections from Fiocruz. She is a member of the Executive Board of the World Federation of Culture Collection (WFCC) and of the Executive Committee of the Global Genome Biodiversity Network (GGBN). She was member of the Brazilian National Competent Authority for ABS (CGen), from 2011 until 2015. At the moment she is the Coordinator of the Sectorial Chamber of the Academy of CGen.

DSMZ: the European Union's first Registered Collection under the Nagoya Protocol



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The Convention on Biological Diversity and the Nagoya Protocol have created new challenges for international microbiological research. With the implementation of the Nagoya Protocol in 2014, the European Union created a new voluntary legal mechanism, the Register of Collections, to help users of collections, including culture collections, have an easier path to Nagoya Protocol compliance by using a so-called 'registered collection'. The Leibniz Institute DSMZ is the first, and so far only, collection to successfully be entered into the Register. The challenges and lessons

learned during this process can be informative for culture collections and users of microbial resources beyond the EU and indeed around the world.

The 1992 UN Convention on Biological Diversity (CBD) and its supplementary agreement, the 2010 Nagoya Protocol (NP), aim to promote biodiversity conservation, ensure sustainable use of biodiversity, and enable fair and equitable benefit sharing from use of biodiversity. The NP entered into force on 12 October 2014 and has changed biological research by creating a new and complex set of administrative and legal hurdles for researchers studying preserved *ex situ* as well as isolating new living organisms from their natural habitats, known in CBD/NP-language as 'genetic resources' or GR, from a country that claims sovereign rights over its GR. The CBD and NP do not distinguish between basic and applied research or commercial or non-commercial and, as such, nearly all microbiological research dependent on environmentally-sourced organisms or samples, including identification, taxonomic classification, deposition, distribution and further utilisation, can be covered by both CBD and NP depending on the national legislation where and when access (sampling) took place.

In the European Union (EU), user compliance with the NP is regulated by Regulation No. 511/2014 of the European Parliament and establishes due diligence measures for users of GR in the EU, which basically means a user must prove they followed the laws in place when conducting research and sampling abroad. This regulation applies to GR collected in a country that is a Party to the NP after the entry into force of the NP for the EU (12 October 2014) or after the country implements national legislation[1]. The European law is not retroactive and does not apply to 'old' GR although the legal definition of access is important. In practice it makes a difference whether GR is newly isolated from an environment or acquired from an *ex-situ* collection and different countries handle this in different ways (see the discussion on the definition of Access to GR below). The law does not require collections to comply with non-Nagoya CBD obligations (i.e. GR that is out of NP) although the DSMZ is diligent about ensuring access and benefit sharing (ABS) obligations are fulfilled and requests documentation from depositors accordingly.

The role of culture collections as major suppliers of GR was recognised in the EU regulation with the establishment of a 'Register of Collections' in which a collection voluntarily fulfils part of the due diligence obligations of the user by seeking NP-related 'permits' before distributing GR. In order to do this systematically, a collection must demonstrate that their management practices enable them to comply with national NP regimes for all incoming material. Only collections that fall within EU jurisdiction can apply for a 'registered' status, although their products continue to be available for users around the world and the NP/CBD information they provide is undoubtedly still helpful in fulfilling non-EU due diligence obligations.

In March 2018, the Leibniz Institute German Collection of Microorganisms and Cell Cultures GmbH (DSMZ) became the first collection in the EU register. The DSMZ submitted a 14-page application (along with 11 supporting documents) to the German Agency for Nature Conservation (BfN) in November 2017, and was officially approved by BfN on 18 March 2018. The application took around four months to prepare by a two-person science-legal team (AHS and HMP) and was a high priority for the DSMZ Scientific Director, Professor Jörg Overmann. There were significant personnel investments from quality management, scientific, and administrative staff with costs estimated around €200 000. The application was also supported in a close partnership with the BfN. Below we share our experiences of obtaining the registered collection status, and challenges and opportunities that arose during this process.

Compliance with the Nagoya Protocol

Many microbial collections collect up-front scientific and administrative information through accession forms, but the CBD and NP extend the information required and can complicate the deposit process. Here are the steps we took towards NP compliance in our collection:

(1) Geographical scope

The first step was to review our collection catalog and confirm that all strains collected in 2014 and beyond (NP entry into force) had a country of origin associated with them. For strains that pre-dated the CBD we accepted, if necessary, 'country of origin unknown' accompanied by evidence from our records or the literature that the collection date was pre-1992.

We then edited and standardised records with 'imprecise' countries of origin: states which no longer exist (e.g. Czechoslovakia), large geographic areas (e.g. Europe), islands (e.g. Hawaii), and dependent territories (e.g. Puerto Rico), duplicates (e.g. Burma and Myanmar), and spelling errors. We applied the ISO 3166 code for the representation of names of countries and their subdivisions and corrected our records accordingly to only include recognised sovereign states. With this list in hand, for all future deposits, we instituted the use of a drop down menu in our online accession form and mandatory geographical coordinates to avoid uncertainty. We added to the ISO list, areas not regulated by the

Nagoya Protocol: Antarctica and the high seas (international waters), both regulated by separate UN treaties.

(2) Temporal scope

Whether a new strain is in or out of CBD/NP scope is dependent on geography and time. As such, sampling date is essential to determine whether access to GR is regulated. We reviewed all strains in the collection that had no sampling date information and an accession date after 1992. We used information from the deposit form, literature, and internal records to determine whether a sampling date could be determined. When no collection date was available, we substituted the accession date (when a strain was deposited) which is a more conservative value and added 'sampled before + accession date'.

(3) Legal scope (PIC and MAT)

The CBD and NP invented a new vocabulary for granting access to GR – 'prior informed consent' (PIC) and 'mutually agreed terms' (MAT). These can be none, one, or two or more documents depending on how the national legislation works and they can carry different names such as 'research permit' or 'material transfer agreement' although their content must reflect PIC/MAT and be issued by the appropriate CBD/NP authority (see ABS-CH discussion below). At the beginning of our compliance check, very few strains in our collection had PIC/MAT documents, meaning they were 'in scope'. But, recognising that the 'in scope' strains and, thus, their associated documents would grow over time, we established both a new standard operating procedure and IT system to enable transparent review of PIC/MAT.

This decision tree for determining legal compliance is outlined in Figure 1 and is partially automated by receiving legal scope information from the ABS-Clearinghouse (ABS-CH) API (application programming interface) – specifically NP/CBD status (is the country a Party or not) and the date of entry into force. Based on this information from the ABS-CH, it can be determined whether PIC/MAT is required. Then the manual 'legal check' begins and the legal team verifies with the provider country (by directly contacting the national focal point, NFP) that the documentation received is adequate and sufficient. If necessary, an unofficial translation of the documents is also requested. If so, the strain is accepted; otherwise it is rejected. Besides the list of required documents, the legal team checks with the NFP which national authority is allowed to issue the documents. This procedure ensures that our holdings are legally compliant and we can pass this certainty on to our customers.

(4) Depositor due diligence

In order to help our depositors understand the CBD/NP legal complexity, we published several websites[2–4] including 'Deposit of biological material at the DSMZ: Compliance with the Nagoya Protocol'[2] and explained important terms and restrictions and provided links to resources which help to determine territorial waters, Exclusive Economic Zones (EEZ) in the Sea, and locate

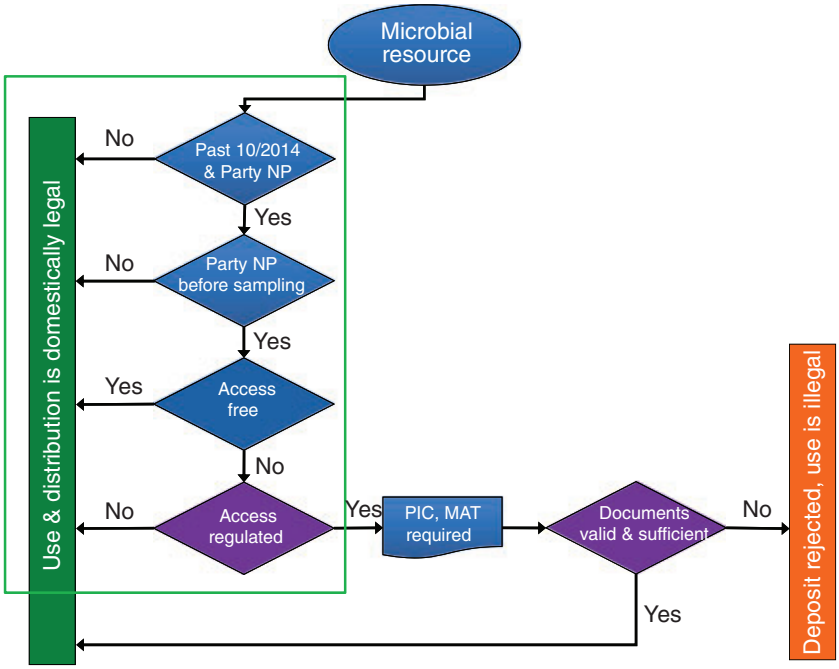

Figure 1. The legal decison tree for receiving new microbial deposits (GR) into the DSMZ public collection. The blue trapezoids indicate decision points that are either automated by connecting to the ABS-CH or through an internal database on free access. All deposits in the large green box would lead to an entry in the DSMZ public catalog, 'There are NO known Nagoya restrictions for this strain'. All remaining strains would be accepted with a Nagoya restrictions box and a link to a download of the PIC/MAT documents. Figure adapted and translated from Overmann, 2017 *BioSpektrum.*

national focal points. We prepared an infographic to explain the minimal requirements on information applied to a deposited strain[3].

(5) User due diligence

The DSMZ online catalog offers customers an overview of each bioresource's relevant scientific information and, since Registration, the country of origin, sampling date, and any associated documentation (PIC/MAT or any other MTA). The documents can be downloaded and saved by the users ensuring the chain of custody required by the NP. If there are no known Nagoya restrictions this is also explicitly posted in the catalog (Figure 2). Strains accompanied with confidential documents cannot be accepted for deposit in the open collection and the depositor is informed during the deposit process that the documents will be published on our website and agrees to this as a condition of acceptance.

DSMZ also began using a Material Transfer Agreement (and accompanying Terms & Conditions) that explicitly require users to: (1) use bioresources for non-commercial research purposes only; (2) not distribute strains to third parties; and (3) to adhere to the terms listed in the Nagoya Restrictions section of the catalogue. With their signature the customer commits to the observance of these regulations. The first two restrictions have been in place in DSMZ before the admittance to the Register of collections. If we become aware of infringements, we report them to the responsible authorities, e.g. the regulatory authorities (like BfN) or other competent national authorities (CNA) which are responsible for ensuring that those users are compliant with EU Regulation 511/2014 or other national legislation.

The advantage of purchasing from a Registered Collection is that a European user can simply report the DSMZ and the strain number in their due diligence reports along with other required information creating a simpler path to compliance than gathering the information and PIC/MAT from the original depositor.

(6) Nagoya and the Bacteriological Code of Nomenclature

Strains deposited for the purpose of describing a new species of prokaryote must be deposited in the open collection. Furthermore, according to the International Code of Nomenclature of Prokaryotes (ICNP), type strains and the associated information must be available from at least two culture collections *without restrictions*. Strains from a country with strong NP/CBD restrictions can be disqualified from becoming a type strain of a new species under the ICNP because it is not possible to distribute these strains without restrictions. The Certificate of Availability for prospective type strains issued by the DSMZ follows the principle of unrestricted access to proposed type strains and the DSMZ does not accept type strains from countries with restrictions on distribution[3].

The complex NP legal 'potpourri'

All Parties to the CBD are required under international law to provide information on their national legislation and contact information. However, in practice, the ABS-CH is not legally binding

Stenotrophobacter roseus
DSM 29891

BACTERIA	How to read the following data (Example)
Name:	***Stenotrophobacter roseus*** Pascual et al. 2016
DSM No.:	**29891, Type strain**
Strain designation:	Ac_15_C4
Other collection no. or WDCM no.:	LMG 28889
Isolated from:	semi-arid old flood plain fallow soil
Country:	Namibia Kavango region, Mashare (17° 53' 37.9" S, 20° 14' 50.7" E)
Date of sampling:	26.03.2011
Nagoya Protocol Restrictions:	Documentation related to the Convention on Biological Diversity and the Nagoya Protocol. Users must download, read, and adhere to the terms listed in the document(s) listed here. Users are legally required to maintain records of these document(s) for 20 years after the last use of the resource. **Genome sequencing is not permitted on this strain without prior written approval from the Namibian competent national authority, NBRI.** File Download

Paeniglutamicibacter antarcticus
DSM 29880

BACTERIA	How to read the following data (Example)
Name:	***Paeniglutamicibacter antarcticus*** (Pindi et al. 2010) Busse 2016
Synonym(s):	*Arthrobacter antarcticus* Pindi et al. 2010
DSM No.:	**29880, Type strain**
Strain designation:	SPC26
Other collection no. or WDCM no.:	LMG 24542, NCCB 100228
Isolated from:	spade core sediment
Country:	Antarctica Larsemann Hill
Date of sampling:	before 2008
Nagoya Protocol Restrictions:	There are NO known Nagoya Protocol restrictions for this strain.

Figure 2. Two entries from the DSMZ public catalog with and without CBD/NP restrictions.

for users although the NP countries are technically required to use it, which leads to a great deal of uncertainty. Over the course of two weeks in September 2017, we (HMP) wrote to all 198 countries under the contact email address found in the ABS-CH (either NFP or CNA) asking what would need to be done in order to accept a microorganism from their country for deposit in our collection. The results were discouraging: 30 (16%) replied with information, 33 (18%) replied and promised to provide a detailed answer later, and the majority 120 (64%) did not reply. Over the next year, we continued to reach out to national focal points as new deposits came into the collection, which only slightly improved the response rate: informative replies were received from 63 (34%) countries and 28 (15%) are still expected to provide us the NP-relevant information. No answer was received from 83 (44%) countries. Astoundingly, despite the legal requirement in international law to use the ABS-CH, 14 (7%) countries either do not have contact information in the ABS-CH system or the emails bounced. A further complication is that national legislation and laws are not always translated into English and country representatives do not respond because of language barriers. These data taken together show that, in some cases, a legal deposit will be nearly impossible.

As our experience with the NP grows, we continue to be astonished by the intricacies and diversity of the national regulations implementing the NP as well the grand challenge of gathering information from provider countries. Even in the EU the situation is not easy as there are a wide variety of access regimes in place: some countries have granted free access (e.g. Germany), while others have restricted access (e.g. France and Spain), others do not have any legislation in place (e.g. Italy), or have proposed different rules for different regions within the country (e.g. Belgium). Legislation between mainland and overseas territories may differ as well. For example, Denmark's ratification of the Nagoya Protocol does not apply to Greenland.

There is much room for clarification of definitions of 'access' and 'utilisation', as well as key dates from which a national legislation applies. The original EU regulation refers to date of access. A few countries have defined it as 'date of sampling' others as 'whenever you have access for the first time to the sample from the country of origin', so that old material sampled before the ratification of NP (and potentially CBD) and preserved in an *ex-situ* collection would fall under the EU regulation. Likewise, there is no common position regarding the difference between utilisation of GR, basic research on GR and quality control (QC) of a strain deposited in a culture collection. This problem is amplified in light of the ongoing debate on the regulation of Digital Sequence Information (DSI).

The NP provides a legal framework for access and benefit sharing among Parties and the EU Register of Collections is restricted to NP Parties. However, it is important to remember that a non-NP-Party may have national laws that govern access to their resources. Brazil is probably the most well-known example of a NP non-party country which has developed its own national legislation regulating access to GR[5]. Despite its non-Party status, the DSMZ is doing its best to follow Brazilian national laws.

Outlook

In a time of increasing legal overheads, it is important that microbial culture collections continuously exchange information and share their experience with competent authorities, national and foreign, and with other collections and colleagues through professional associations like the World Federation for Culture Collections, and regional networks such as the European Culture Collections' Organization (ECCO), the Asian Consortium for the Conservation and Sustainable Use of Microbial Resources, and the Latin American Federation of Culture Collections.

But even with good exchange and networking, the process of strain deposition is simply becoming more complex. Several authors have pointed out the negative impact of the NP regulations on biodiversity assessments and conservation efforts[6,7]. The situation in microbiological research is not much different. Long and complicated applications for sampling permits discourage many scientists (e.g. [8–10]) and additional problems can be expected when a strain needs to be sent out of the country of origin and deposited in an open collection. The number of strains deposited in the DSMZ for the purpose of taxonomic description has rapidly dropped down from nearly half of deposits (48% and 46% in 2014 and 2015, respectively) to 16% in 2018. We also have observed a trend towards growing numbers of strains originating from countries who declared free access to their GR or from the U.S., a country that is not a Party to the CBD. Another interesting trend is the growing interest on the deposition of 'old' material, strains isolated before the NP and CBD, as a part of safeguarding collections from retiring scientists.

Obtaining the Registered collection status is a long-term investment which is aimed to provide additional service and value for our customers, who would otherwise be responsible for obtaining and checking the NP documents themselves and to demonstrate good will and competence to provider countries. To date, the registered collection status has not led to an increase in sales, but we have heard from colleagues that they appreciate the additional quality control and legal clarity. Over the past year, there has been growing interest from our customers in the strain-related information regarding the NP, perhaps due to the year-old EU due diligence declaration[4]. Overall, we feel strongly that the Registered

Collection was the right decision on balance and look forward to working with European and international colleagues in assisting microbiologists with the legal overhead that is now our daily reality.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We acknowledge the leadership and commitment of Jörg Overmann in deciding to undertake the registered collection process. We also thank the ABS staff of the German Agency for Nature Conservation, especially Thomas Greiber and Ellen Frederichs, for their collegial exchange and support navigating a new legal mechanism for the first time together. We are deeply appreciative of the DSMZ curators that did the individual checks of hundreds of strains and remain on the daily front lines of Nagoya compliance. This research did not receive any specific funding.

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Biographies

Andrey Yurkov studied soil sciences and microbiology at the Lomonosov Moscow State University in 1998-2002 and completed his doctorate thesis in 2006. Since 2012, he is the curator for fungi and yeasts at the DSMZ. He is a member of executive boards of the World Federation for Culture Collections (WFCC) and the International Mycological Association (IMA).

Hilke Marie Püschner studied in Hannover and Göttingen and completed her doctorate in medical law. She finished her education in Braunschweig, among others at the Higher Regional Court Braunschweig. Since 2017 she is a lawyer at the DSMZ and among other things responsible for the observance of the Nagoya Protocol.

Dr Amber Hartman Scholz is the Deputy to the Director at the Leibniz Institute DSMZ, the German Collection for Microorganisms and Cell Cultures, in Braunschweig, Germany. She headed the team that led to the DSMZ becoming the first Registered Collection under the Nagoya Protocol in the European Union, demonstrating the collection's voluntary and stringent compliance with EU Regulation 511/2014. Her broader work at the DSMZ focuses on internationalization, strategic development, and science policy. Dr Scholz has broad experience in science and policy through her work in the United States at the White House Office of Science and Technology Policy (OSTP) as Executive Director to the President's Council of Advisors on Science and Technology from, the National Cancer Institute as a Policy Advisor, and as a Science Fellow to the California State Senate Environmental Quality Committee. She received her PhD in Biology with a focus on the human intestinal microbiome and bioinformatics methods development in 2009 from the Johns Hopkins University.

Do you know...

The Bazeley Oration, first given in 1992, is sponsored by Commonwealth Serum Laboratories (CSL) to honour the achievements of a former director, Percival (Val) Landon Bazeley, who was one of Australia's most distinguished microbiologists. One of these achievements was spearheading the production of penicillin by CSL.

Bazeley was born in Orbost in Victoria in 1909. He studied veterinary science at Melbourne University and after graduation joined CSL as an assistant veterinary research officer in 1938. At the outbreak of WW2 he enlisted but continued to work at CSL until his posting to Papua New Guinea in 1941. However, shortly before he left, the Oxford group published their seminal paper on the therapeutic use of penicillin. Another great Australian, Bill Keogh, recognised the significance of this work and convinced the War Cabinet that Australia needed to be self-sufficient and that Bazeley was the man to make this happen. Bazeley was ordered home and promptly dispatched to the USA where he spent the next three months visiting US manufacturers. On his return Bazeley set himself the target of producing penicillin within six weeks. By February 1944, 10 weeks after his return, a sizeable quantity of material had been produced and by April, CSL was able to provide penicillin to civilians, the first country in the world to do so. A truly amazing feat for an extraordinary man who became a national hero and in 1956 Director of CSL.

Taken from notes provided by Professor Ian Gust.

Biological deposits for patenting purposes under the Budapest Treaty

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A patent confers a limited-term right to exclude other parties from using an invention, in exchange for a comprehensive description of the invention. The granted claims of a patent define the scope of the right that is conferred.

Patents are jurisdiction-specific – a patent granted in one jurisdiction does not (at least directly) confer rights in other jurisdictions. However, a number of international agreements establish provisions for patent protection, including the Paris Convention for the Protection of Industrial Property (Paris Convention)[1], the Patent Cooperation Treaty (PCT)[2], and the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS)[3]. There is variation in the particular requirements for obtaining patent protection among jurisdictions, even those that are signatories to the same international agreements. Nevertheless, in the great majority of jurisdictions, for a patent to be granted its claims must be considered novel and inventive over earlier publicly available information, and supported by disclosure associated with the patent.

With regard to support requirements for patent protection, generally, it is considered necessary that the associated disclosure is sufficient to enable a legally defined 'skilled person' to perform the invention across the full scope of the granted claims. Typically, disclosure that can be relied on for support purposes is in the form of written description presented in the patent specification and figures. However, for inventions involving biological material, it may be difficult to adequately characterise the biological material using written description alone.

Budapest Treaty deposits for patent support

Factors associated with the eligibility of particular forms of biotechnology for patent protection can be complex, and vary substantially among jurisdictions. That topic is not dealt with in detail here – the reader is directed to Sigareva and O'Donnell[4], Fitzgerald *et al*.[5,6] and Kimura and Burton[7] for exemplary articles dealing with patent eligibility of biotechnology. Nevertheless, in many jurisdictions it is permissible to obtain patent protection for naturally occurring biological material, including microorganisms, when isolated from the natural environment[8]. In others, notably the United States, although isolated naturally occurring biological material cannot be patented *per se*, inventions involving naturally occurring biological material (for example, the use of a microorganism for an industrial purpose) can be patent eligible, as can engineered material differing in a meaningful way from its natural counterpart[8].

Questions of patent eligibility aside, where patent protection is sought for a biological invention there may be concerns around the degree to which written description of biological material required for the invention is adequate for support purposes[9]. The Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (Budapest Treaty)[10] was developed in response to this issue. The Budapest Treaty is an international patent law treaty that was originally signed on 28 April 1977, and first entered into force on 9 August 1980. The World Intellectual Property Organization (WIPO) is responsible for administering the treaty. As of August 2019, there are 82 contracting parties to the Budapest Treaty.

In general terms, the Budapest Treaty establishes that a sample of a 'microorganism' deposited at a designated International Depositary Authority (IDA) may be relied on for a patent application[10]. This enables a patent applicant to provide a deposit to meet support requirements, where associated written description alone is deemed insufficient to characterise the sample. Relevantly, the Budapest Treaty does not include any definition of the term microorganism. This has resulted in general practice that a deposit under the Treaty can be used to facilitate support for any biological material that an IDA will accept[11]. Biological material that may be deposited includes, for example, cell cultures of unicellular and multicellular organisms, including bacterial, fungal, plant, animal, and human cells; viruses; nucleic acids and proteins; and embryos and seeds[11]. Individual IDA's generally have internal policies regarding sample types that will be accepted. A useful summary of this information is maintained by WIPO[11].

The Budapest Treaty includes provisions on conditions that must be followed for deposit and storage of biological material. Among these provisions, the treaty requires all IDAs to accept deposits on

the same terms for all depositors, to furnish samples of deposits to all entitled parties (and only these parties), and to store deposits for a minimum period. More particularly, the treaty specifies that parties entitled to receive a sample of a deposit include the depositor, anyone with the depositor's written authorisation, and any jurisdiction's intellectual property office that declares that it is handling a patent application associated with the deposit. Entitlement of other parties to samples of deposits may be defined by jurisdictional laws. For example, in the US any person can obtain a sample once the patent is granted, with the depository notifying the inventor when someone makes such a request. Additionally, the treaty requires deposits to be stored for at least thirty years, and at least five years from the most recent request for a sample[11].

Importantly, however, the Budapest Treaty does not include provisions as to when a deposit must be made in order for it to be relied on by a patent application; rather, timing requirements are dictated by jurisdictional law. Requirements can also vary among individual jurisdictions as to when and how the jurisdiction's patent office is to be alerted of a deposit associated with a patent application. Jurisdictional laws may further include provisions specifying circumstances in which a deposited biological sample should or must be provided to support a patent application[10].

Practical considerations for Budapest Treaty deposits

Given the potentially critical role of Budapest Treaty deposits in providing patent support in relation to biological material, it is important for patent applicants and practitioners to consider whether a deposit should be made when pursuing patent protection for inventions involving biological material. It is also important to ensure that formal requirements are met to enable all relevant patent applications to validly rely on any biological deposit that is made.

A determinative issue for deposit under the Budapest Treaty is, of course, whether patent protection is to be sought in any jurisdiction that is a party to the treaty. Many jurisdictions of major economic significance are signatories to the Budapest Treaty, including Canada, China, France, Germany, India, Italy, Japan, the UK, and the USA. Accordingly, patent protection will frequently be sought in one or more jurisdictions in which it is appropriate to use the Budapest Treaty for biological sample deposit. While not the focus of this article, it is noted that in jurisdictions yet to accede to the Budapest Treaty, other jurisdiction-specific arrangements may be available for biological sample deposit.

Assuming that patent protection for a biological invention is to be sought in a Budapest Treaty jurisdiction, it remains necessary to decide, in all of the circumstances, whether submission of a biological sample is required or at least desirable. For the majority of signatory jurisdictions, legislation regarding the provision of biological samples simply specifies that a biological deposit may be used to assist with support of an application, where written description associated with the application is insufficient to enable the skilled person to carry out the invention. Relevantly, however, some jurisdictions have more prescriptive requirements for the availability of biological samples. For example, Australian legislation dictates that deposit of a culture of a microorganism is required 'if the invention is a microorganism' or 'if the invention involves the use, modification or cultivation of a microorganism which is not reasonably available to a person skilled in the art and if, without a sample of such microorganism, such person could not reasonably be expected to be able to perform the invention'.

Even in the absence of prescriptive requirements in a jurisdiction of interest, where biological material that is not otherwise well-characterised or accessible is required to perform an invention, the safest approach from a support perspective would typically be to arrange for submission of a sample of this material, to rely on if necessary. However, an important practical consideration for the deposit of biological samples is the associated expense. In this regard, IDA fees of ~US$2000 per sample are typical for sample storage, as at the time of writing. Accordingly, the cost of sample deposit may represent a barrier to submission, particularly where large numbers of biological specimens are involved.

It will also be appreciated that where historically it may have been either impossible or unduly prohibitive in terms of time or cost to perform detailed analysis of biological material for patent support purposes, this may no longer be the case. With current technology, abundant biological information (e.g. nucleic acid and protein sequences; metabolite profiling; gene expression information) can be obtained comparatively quickly and cheaply, such that substantial characterisation of a range of biological samples may be feasible. Accordingly, in some cases, it may be desirable to perform detailed analysis to facilitate comprehensive written description of biological material for support purposes, rather than attend to sample submission. Notably, if an invention for which protection is to be sought has been developed in an academic research context, characterisation of associated biological samples may be desirable from a research publication perspective.

Where a decision is ultimately made to submit a biological deposit under the Budapest Treaty, timing issues will also need to be

appropriately managed. In most jurisdictions, for a biological sample to be relied on for a patent application a sample must have been submitted by either the filing date of the application itself (e.g. Canada; the UK), or the earliest priority date of the application (e.g. China; Germany). However, different requirements apply in some jurisdictions. For example, in the US the deadline for deposit of the biological material is at payment of the patent issue fee (although the US patent office strongly encourages the deposit to be made on or before the filing date of the application and any deposit made after the filing date can be subject to a requirement for evidence that what was deposited is what was disclosed and claimed). Furthermore, as noted previously, some jurisdictions specify other deadlines, such as for inclusion of filing receipt details obtained from an IDA in the patent application, or provision of these details to the jurisdiction's intellectual property office[11]. Although specific formal requirements for Budapest Treaty submissions in signatory jurisdictions are helpfully summarised by WIPO[11], variation in these requirements substantially increases the complexity of ensuring that patent applications in all jurisdictions can validly rely on corresponding samples. It is therefore notable that greater harmonisation of formal requirements for reliance on Budapest Treaty deposits has recently been formally proposed (see AIPPI Standing Committee on Pharma and Biotechnology[12]). Such harmonisation could be advantageous for patent applicants and practitioners if successfully implemented.

Conclusions

A core principle of the patent system is the provision of exclusive rights to an invention for a limited term, in exchange for public disclosure that allows the invention to be performed. The deposit of biological samples under the Budapest Treaty provides a mechanism to disclose biological material that is required for an invention, where it may be difficult or impossible to characterise the material using written description alone. Although a substantial number of jurisdictions are signatories to the Budapest Treaty, including most of the world's major economies, many others are yet to accede to the treaty. Furthermore, specific requirements for reliance on Budapest Treaty deposits vary among signatories. When pursuing patent protection of biological inventions, applicants and practitioners should carefully consider whether sample deposit under the Budapest Treaty should be performed, and ensure that formal requirements are met for all applications to allow for any sample that is deposited to be validly relied on.

Conflicts of interest

The author declares no conflicts of interest.

Acknowledgements

This research did not receive any specific funding.

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Biography

Tim Fitzgerald is a senior biotechnology patent attorney at IP Gateway, located in Brisbane, Australia. Tim is passionate about helping innovators protect their intellectual property, and has experience working with diverse clients – from those with extensive experience in IP matters and large commercial portfolios, through to those exploring IP protection for the first time. Before joining the IP profession, Tim spent 10 years as a research scientist, undertaking Honours and a PhD in plant molecular biology, then working for CSIRO. Tim's research was industry focussed, exploring quality and drought resistance traits in rice, and disease resistance in wheat. From his time as a researcher, Tim has published 20 peer-reviewed journal articles and book chapters, and is a named patent inventor himself.

International postal, quarantine and safety regulations

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There are numerous legislative regulations that impact on microbiology, microbial Biological Resource Centres (mBRCs) and culture collections, with which all microbiologists must comply. These affect access for collection, handling, distribution/shipping and utilisation of microbial resources. Areas where regulations are triggered are international post, quarantine and safety. The legislation and supporting documents are often difficult to find and understand, therefore the World Federation for Culture Collections (WFCC) has a long history in providing advice and guidance to help compliance with such legislation. A visit to the WFCC library (http://www.wfcc.info/wfcc_library/) will provide information on postal requirements shipping dangerous goods and on control measures in place for biosecurity to control access to dangerous pathogens. This paper will update such communications and provide relevant information on: Health and Safety (H&S); Quarantine regulations; and Postal Regulations and Safety. Other papers in this special issue will address elements that impact on distribution and use of microorganisms for example in packaging, legislation on the proliferation, distribution and misuse of dangerous pathogens, export licensing measures, the Convention on Biological Diversity and the Nagoya Protocol, ownership of Intellectual Property Rights (IPR) and the provision of safety information to the recipient of microorganisms. The advice is generic and users are advised to refer to their own National guidance and implementation acts to ensure they are compliant. The work was compiled from authors' efforts in their management of an mBRC and most recently contributions to the EMBRIC project (http://www.embric.eu/) in particular Deliverable 6.1 'Microbial pipeline from environment to active compounds' (http://www.embric.eu/deliverables).

Overview

Work with microorganisms involves sampling, isolation, characterisation and may extend to generation of extracts, purification and assaying of nucleic acids, proteins and potential bioactive compounds, with the goal to place products on the market. Table 1 lists some of the relevant regulations and guidance on international postal, quarantine and safety regulations and provides links to the source; community best practice is listed in Table 2.

The reach of a laboratory's H&S procedures extend beyond the laboratory where the work is carried out to cover all those who may come in contact with substances and products from that laboratory. A microorganism in transit will put postal staff, freight operators and recipients at risk. Some organisms are relatively low hazard whilst others are quite dangerous. It is essential that safety and shipping regulations are followed to ensure safe transit and that H&S requirements, quarantine regulations and postal regulations are met. It is critical that biological resource centres operate to high standards[1,2] and currently there are guidelines available for adoption and use (Table 2).

Health and safety (H&S)

There are numerous national requirements for H&S; e.g. in the EU, H&S is covered by Directive 89/391/EEC, which implements measures to improve H&S at work. It is designed to encourage improvement in occupational H&S in all sectors, both public and private; promote workers' rights to make proposals relating to H&S, to appeal to the competent authority and to stop work in the event of serious danger, seeking to adequately protect workers and ensure that they return home in good health at the end of the working day (http://ec.europa.eu/social/main.jsp?catId=148). The EU legal framework in the area of occupational safety and health

Table 1. Regulations and guidance governing microbiological activities.

Regulatory area	Guidance document	URL
Hygiene and Bio-safety	Organisation for Economic Co-operation and Development (OECD)	https://www.oecd.org/env/ehs/aboutchemicalsafetyandbiosafety.htm
	United Nations Industrial Development Organisation (UNIDO) Bio-safety Information Network and Advisory Service (BINAS)	http://binas.unido.org/ebiosafety/
	United States Department of Agriculture Animal and Plant Health Inspection Service	https://www.aphis.usda.gov/aphis/home/
	US Food and Drug Administration (FDA)	http://www.fda.gov/
	World Health Organization (WHO) Biorisk management	https://www.who.int/ihr/biosafety/en/
	Centers for Disease Control and Prevention (CDC) and United States Department of Agriculture (USDA) select agent program	https://www.selectagents.gov/
	Centre for Food Safety and Applied Nutrition (CFSAN)	https://www.fda.gov/AboutFDA/centersoffices/officeoffoods/CFSAN/default.htm
	Belgian Bio-safety Server	www.biosafety.be
	The Dutch Genetically Modified Organism Bureau	https://www.loc.gov/law/help/restrictions-on-gmos/netherlands.php
	UK Advisory Committee on Releases into the Environment (ACRE)	https://www.gov.uk/government/organisations/advisory-committee-on-releases-to-the-environment/about/our-governance
	National Chemical Emergency Response UK	https://the-ncec.com/
	American Biological Safety Association (ABSA)	http://www.absa.org
	European Biosafety Association (EBSA)	http://www.ebsaweb.eu
	Advisory Committee on Dangerous Pathogens (ACDP) (UK)	http://www.hse.gov.uk/aboutus/meetings/committees/acdp/index.htm
	Health and safety advice in UK	https://www.hse.gov.uk/
	UK Control of Substances Hazardous to Health (COSHH)	http://www.hse.gov.uk/nanotechnology/coshh.htm
	UK Management of Health and Safety at Work	http://www.hse.gov.uk/managing/index.htm
	The management and operation of microbiological containment laboratories	http://www.hse.gov.uk/biosafety/management-containment-labs.pdf
	EC Directive 2000/54/EC - biological agents at work	https://osha.europa.eu/en/legislation/directives/exposure-to-biological-agents/77
	Directive 2001/18/ on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC	https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32001L0018
Transport and shipping	First generation guidelines for NCI-Supported Biorepositories – Federal register Vol. 71, Number 82, Page 25814, April 28, 2006.	https://www.federalregister.gov/documents/2006/06/02/06-5059/first-generation-guidelines-for-nci-supported-biorepositories
	Federal Register: Hazardous Materials: Harmonization with the United Nations	https://www.federalregister.gov/documents/2006/08/31/06-7200/hazardous-materials-harmonization-with-the-united-nations-recommendations-international-maritime
	Universal Postal Union	http://www.upu.int/en.html
	WHO Guidance on Regulations for the Transport on Infectious Substances	https://www.who.int/ihr/publications/WHO-WHE-CPI-2017.8/en/
	IATA Dangerous Goods Regulations (DGR)	https://www.iata.org/publications/dgr/Pages/index.aspx
	Accord Européen relatif au transport international des merchandises dangereuses par routes (ADR)	https://treaties.un.org/pages/ViewDetails.aspx?src=TREATY&mtdsg_no=XI-B-14&chapter=11&clang=_fr
	Export Licenses for dangerous organisms	https://www.gov.uk/guidance/export-and-import-licences-for-controlled-goods-and-trading-with-certain-countries
Quarantine regulations	For Europe see European and Mediterranean Plant Protection Organization (EPPO) website	http://www.eppo.int/QUARANTINE/quarantine.htm
	CDC import regulations US	https://www.cdc.gov/importation/laws-and-regulations/index.html
	WFCC: European Biological Resource Centre Network (EBRCN) Quarantine Regulations guidance document	http://www.wfcc.info/doc/September_draft-EBRCN_Quarantine_Regulations.doc

Table 2. Community best practice.

Source	Item	URL	Overview
Organisation for Economic Development and Co-operation (OECD)	OECD Best Practice Guidelines for Biological Resource Centres	http://www.oecd.org/sti/emerging-tech/38777417.pdf	Covers general regulation compliance
Common Access to Biological Resources and Information (CABRI)	CABRI Guidelines	http://www.cabri.org/guidelines.html	Guidelines for Collection & Quality Management
European Consortium of Microbial Resources Centres (EMbaRC)	Public Deliverables	http://www.embarc.eu/deliverables.html	Overview of existing legislation, guidelines
	A Biological Resource Centre (BRC) operational standard	http://www.embarc.eu/deliverables/EMbaRC_D.NA1.2.1_2.28_BRC_standard.pdf	Based on the OECD best practice guidelines for BRCs – a working draft for an ISO Standard
World Federation for Culture Collections (WFCC)	World Federation for Culture Collections (WFCC) Guidelines for the Establishment and Operation of Collections of Cultures of Microorganisms	http://www.wfcc.info/guidelines/	Basic quality management guidance for culture collections; Section 17 covers aspects in compliance with regulation
	WFCC Library provides an information resource from the EBRCN project	http://www.wfcc.info/index.php/wfcc_library/contribution/	The resource covers: Information resource on transport Quarantine Regulations Alternative Safety Data Sheet BRC Compliance with the CBD Health and Safety Requirements Resource Legislation

(OSH) is outlined in the OSH Strategic Framework 2014–2020 (http://ec.europa.eu/social/main.jsp?catId=151&langId=en).

A risk assessment of handling and supply of organisms is required and should include an assessment of all hazards involved, not just infection, but others such as the production of toxic metabolites and the ability to cause allergic reactions. Organisms that produce volatile toxins or aerosols of spores or cells present a greater risk. It is the responsibility of the microbiologist to provide such assessment data to the recipient of a culture, to ensure its safe handling and containment. Adequate assessment of risks includes:

- Provision of adequate control measures
- Provision of H&S information
- Provision of appropriate training
- Establishment of record systems to allow safety audits to be undertaken
- Implementation of good working procedures

Good working practice requires assurance that correct procedures are actually being followed and this requires a sound and accountable safety policy (http://www.hse.gov.uk/pubns/hsc13.htm). This demands suitable and sufficient assessment of the risks to H&S to which any person, whether employed or not may be exposed through their work (http://www.hse.gov.uk/risk/). These assessments must be regularly reviewed, especially when changes in procedures or regulations dictate, and must be recorded. The distribution of microorganisms to others outside the workplace extends these duties to protect others. In Europe the protection of workers from risks related to exposure to biological agents at work is addressed by Directive 2000/54/EC – biological agents at work of the European Parliament and of the Council of 18 September 2000 (https://osha.europa.eu/en/legislation/directives/exposure-to-biological-agents/77). This Directive lays down minimum requirements for the H&S of workers exposed to biological agents at work.

Quarantine regulations

Clients who want to obtain cultures of non-indigenous plant pathogens must obtain a permit to import, handle and store from the appropriate Government Department. Under the terms of such a licence, the shipper is required to see a copy of the Ministry permit before such strains are supplied. The BRC must do its best to ensure that non-indigenous pathogens are not distributed unless the recipient has a current licence.

Quarantine legislation is in place in countries world-wide restricting the import of non-indigenous plant and animal pathogens. Those who want to import such organisms must hold the relevant import permit, which can be obtained from the relevant country authority, for example, Canada, UK and USA. Information on the transport of plant pathogens throughout Europe can be obtained from the European and Mediterranean Plant, Protection Organisation (EPPO; https://www.eppo.int/).

Postal regulations and safety

Countries have their own national regulations governing the packaging and transport of biological material in their domestic mail. International Postal Regulations covering postage of human and animal pathogens are very strict on account of the safety hazard they present. There are several organisations that set regulations controlling the international transfer of such material (Table 1). It is common practice to send microorganisms by post, as this is more convenient and less expensive than air freight. However, many countries prohibit the movement of biological substances through their postal services. The International Bureau of the UPU in Berne publishes all import and export restrictions for biological materials by national postal services. IATA Dangerous Goods Regulations (DGR) require that packaging used for the transport of hazard group 2, 3 or 4 must meet defined standards[3]. There are several other regulations that impose export restrictions on the distribution of microorganisms. These include control of distribution of agents that could be used in biological warfare; EU Council Regulation 3381/94/EEC on the control of export of dual-use goods (Official J. L 367, p1). There are several qualified courier services that can be engaged to ensure shipping regulations are followed. There is an obligation to have a certified shipper for class A organisms in the IATA guidelines and it is the responsibility of the microbiologist that organisms are labelled, packed and shipped correctly by such a certified shipper.

It is critical that microbiologists are aware of and follow legislation; see Guidance on regulations for the Transport of Infectious Substances 2007– 2008. WHO/CDS/EPR/2007.2 World Health Organization 2007 (https://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2007_2cc.pdf). Article III of the Biological Weapons Convention (BWC) obliges the States Parties not to transfer to any recipient whatsoever, directly or indirectly, and not in any way to assist, encourage, or induce any States, group of States or international organizations to manufacture or otherwise acquire any of the agents, toxins, weapons, equipment or means of delivery specified in article I of the Convention (https://www.un.org/disarmament/wmd/bio/). This is a legally binding obligation. A number of countries have implemented national export licensing measures as an effective means of implementing these obligations and to avoid the possibility of the inadvertent supply of any item that could be used in a BW program.

A safety data sheet must be despatched with an organism indicating which hazard group it belongs to and what containment and disposal procedures are necessary. Article 10 of the EU Directive 90/379/EEC regulates that manufacturers, importers, distributors and suppliers must provide safety data sheets in a prescribed format (https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A31990D0379).

Summary

All microbiologists must keep abreast of regulatory requirements. Although there are numerous publications on the operation of mBRCs[4] the requirements extend to all microbiological laboratories. Participation in microbiological and culture collection communities such as the WFCC will help ensure you are aware of your compliance needs.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

CABI is an international intergovernmental organisation, and we gratefully acknowledge the core financial support from our member countries (and lead agencies) including the United Kingdom (Department for International Development), China (Chinese Ministry of Agriculture), Australia (Australian Centre for International Agricultural Research), Canada (Agriculture and Agri-Food Canada), Netherlands (Directorate-General for International Cooperation), and Switzerland (Swiss Agency for Development and Cooperation). See https://www.cabi.org/about-cabi/who-we-work-with/key-donors/ for full details.

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Biographies

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Skerman and beyond: 2019 status of the Global Catalogue of Microorganisms



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The World Federation for Culture Collections (WFCC)-MIRCEN World Data Centre for Microorganisms (WDCM) was set up as a data centre of WFCC and UNESCO World Network of Microbiological Resources Centres (MIRCEN). The WDCM is a vehicle for networking microbial resource centres of various types of microorganisms. It also serves as an information resource for the customers of the microbial resource centres (http://www.wdcm.org/). The WDCM was established in 1966 by the late Professor V.B.D. Skerman in Australia, later moved to Japan in 1986 and since 2010 is based in China under the Directorship of Dr Juncai Ma. Current databases at the WDCM are the Culture Collections Information Worldwide (CCINFO), Global Catalogue of Microorganisms (GCM) and the WDCM Reference Strain Catalogue. In addition, Analyzer of Bio-resource citations (ABC) and Statistics on Patented Microorganisms are available (http://www.wdcm.org/databases.html). In this article the status of the GCM and its associated 10K type strain sequencing project that currently provides services to taxonomists for standard genome sequencing and annotation will be communicated.

In the 50-year history of the WDCM, the capabilities and uses of information technologies have expanded greatly, with high-throughput sequencing technology leading to an exponential increase in DNA sequence data. Microbiology and biotechnology are sciences that rely on DNA sequence data that is vital for determination of the genetic make-up and functional roles of microorganisms in nature[1]. Culture collections in this context have an important function as data and information repositories thus serving academia, industry and the public. As a result, the WDCM is now facilitating the application of cutting-edge information technology to improve the interoperability of microbial data, promote the access to and use of data and information, and to coordinate international cooperation between culture collections, scientists and other user communities. Curators and scientists from culture collections not only share data but also design and implement the generated data platforms that meet the changing requirements of microbiologists.

Culture Collections Information Worldwide (CCINFO) is a registration system and metadata archive for culture collections around the world. WFCC recommends that every culture collection register in the CCINFO database before providing public services. CCINFO serves as a metadata recorder. It provides a unique identifier for each culture collection and lists the species names of collections' holdings. Currently, 783 culture collections from 76 countries have registered with CCINFO and 131 of these collections have registered with WFCC (http://www.wfcc.info/) as affiliate members representing 49 countries. The foundational structure of the WDCM ensuring information transfer from key organisations is highlighted in Figure 1. Using the unique strain numbers and species names, WDCM developed ABC, a data mining tool to extract information from public resources such as Pubmed, WIPO, Genome Online database and NCBI nucleotide sequence database. After catalogue information is submitted from individual culture collection, WDCM automatically links this catalogue information with the available knowledge on each strain extracted by ABC, which is subsequently accessible to the public through the Global Catalogue of Microorganisms (GCM). Currently, 127 collections

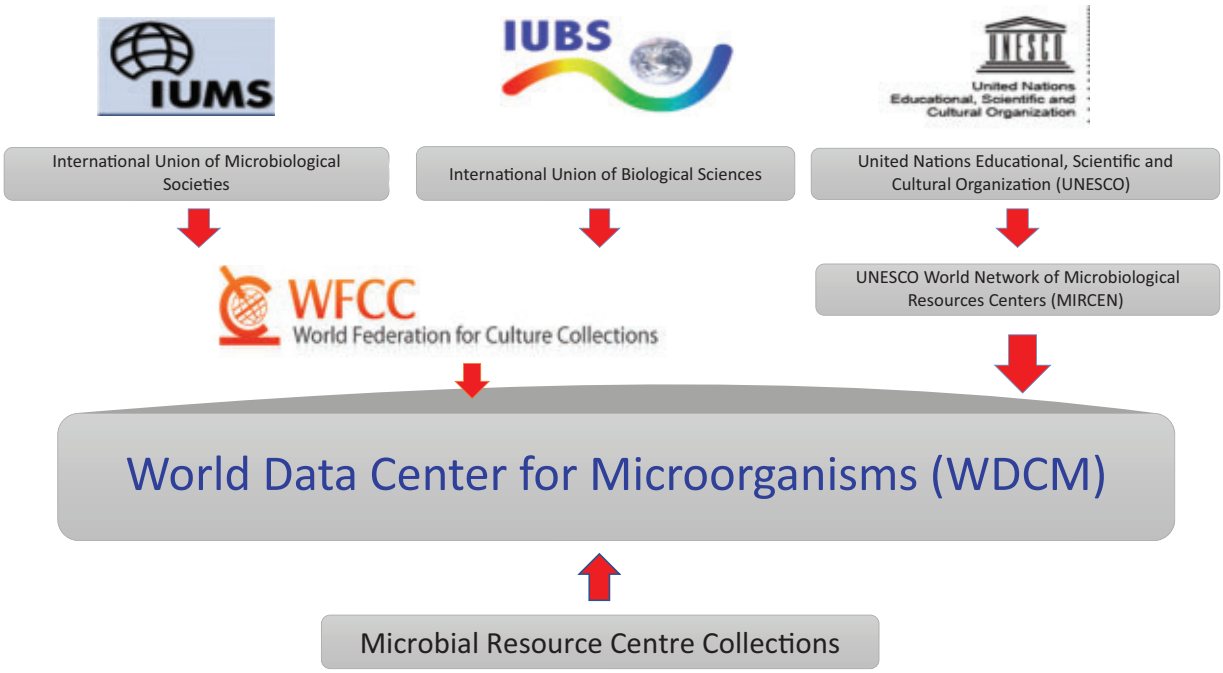

Figure 1. Key organisations feeding information into WDCM.

from 48 countries are part of the GCM and information on the 447 512 strains and 54 736 species is available. The original isolation places of the isolates are also listed: Asia, 44; Africa, 46; Europe, 42; North America, 17; Oceania, 9; and South America, 13. Such information is of importance for accessing, tracking, monitoring and benefit sharing, and compliance with the Nagoya Protocol (https://www.cbd.int/abs/). The GCM can aid culture collections in monitoring the utilisation of their microorganisms. GCM can also facilitate access to strains that might otherwise have been unavailable prior to becoming a part of GCM such as recent accessibility into the 1000 strains at the Vietnam Type Culture Collection. GCM has also provided support to 40 different culture collections to create their online catalogues (Figure 2). Moreover, regional organisations such as the Asian Network of Research Resource Centres

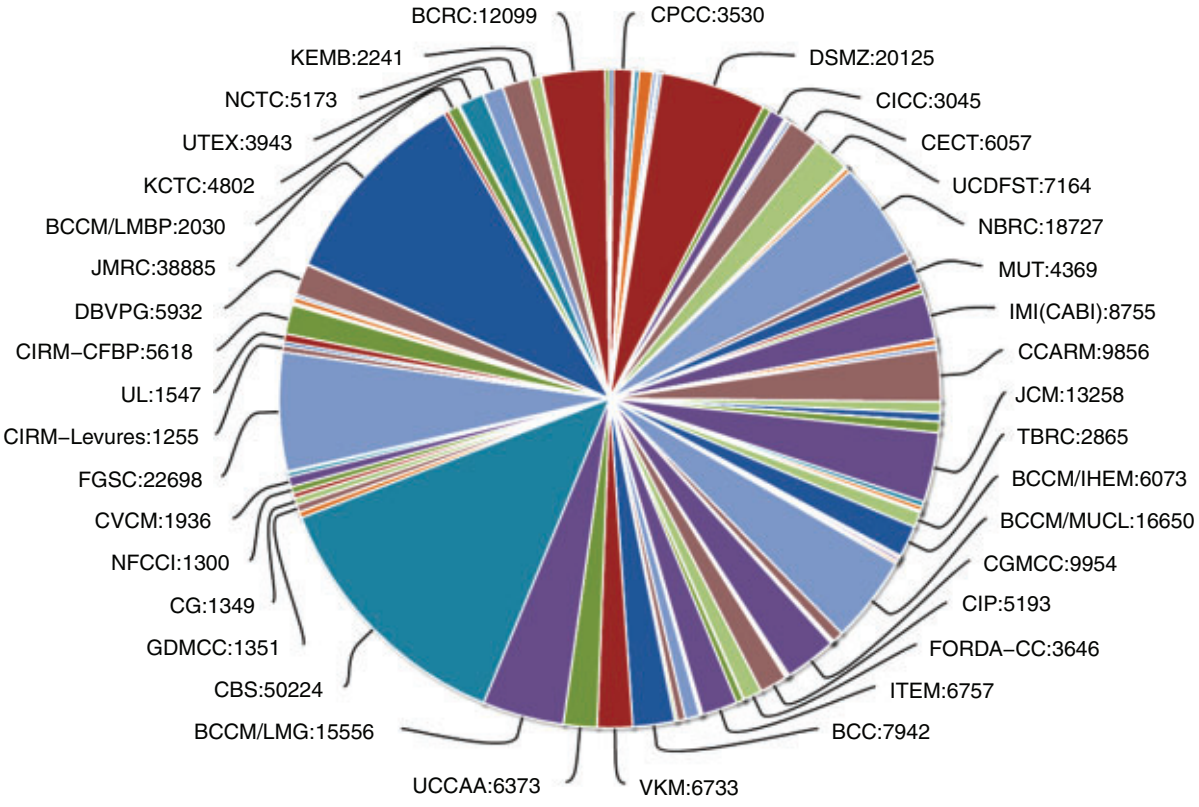

Figure 2. GCM guided online catalogue creation by different culture collections (for the acronyms please refer to http://gcm.wfcc.info/strains.jsp?strain_number=&strain_name=Bacillus%20subtilis&marklog=strainnamelist).

(ANRRC) and the Asian Consortium for the conservation and Sustainable Use of Microbial Resources (ACM) are using the data stored in the GCM to create their online catalogues.

In the advance stage of the GCM 2 development the following points are targeted: (1) From strain data to 'omics' data and to improved database platform, (2) from database to knowledge base and to improved personalised services to microbiologists, (3) from data search to analysis to cloud-based analysis pipeline integrated.

In addition, the GCM has initiated an international project titled 'The global catalogue of microorganisms 10K type strain sequencing project' to close the genomic gaps for the validly published prokaryotic and fungi species[2]. This project has two core subprojects: (1) to sequence 10 000 bacterial and archaeal type strains; and (2) to sequence selected fungal type strains. The outcomes of the project will close currently existing large gaps in the available genomic sequence information published for bacterial and archaeal species. This gap is even larger for fungal type strains. The GCM led and internationally coordinated effort will facilitate the generation of a more comprehensive genomic information platform to be used for research *via* in-depth genome mining. Information to be generated on the taxonomic, phylogenetic, and functional genes of microorganisms will be of immense value for the advancement of biological sciences and biotechnology.

In this project, the genomes of 10 000 type strains will be sequenced (http://gcm.wdcm.org/typestrain/) with the WDCM covering the costs for sequencing services, database system and data analysis. Upon completion, raw data and analysed results will also be published online and made freely available. So far, 25 collections from 16 different countries have agreed to take part in the project (Table 1).

The project has established standard operational procedures for DNA extraction, sample submission, sequencing, and data processing to ensure that all genetic resources, data, and metadata associated with type strains are appropriately obtained, recorded, and stored. A project proposed by the WDCM, 'CD 20170: Specification on Data Integration and Publication in Microbial Resource Centers', is currently under development and will meet the standards of the International Organization for Standardization (ISO).

International Working Groups have been established to provide expert advice during the selection of type strains for genome sequencing. In addition, SOPs, database establishment and intellectual property rights and legal issues have been clarified. WDCM will only use strains and DNA samples provided by formal collaborators, for sequencing, data mining and integration into the data platform for microbial resources. Collaborative research agreements were also put into place.

All sequencing is currently being conducted at the BGI and IMCAS, which has the largest sequencing capacity in the world at >30 Tb/day with Sequencers (295+): BGISeq-500, Illumina/HiSeq, Illumina/MiSeq, AB/3730xi, Roche/454, PacBio RS, Sequel, Bionano Irys System, Life Tech/Ion Torrent. Out of the 719 type strains received to date, 465 of them have been genome sequenced.

Table 1. 10K genome sequencing project participant collections.

ATCC, USA
BCCM/IHEM, Belgium
BCRC, Chinese Taipei (Taiwan)
CAIM, Mexico
Westerdijk Institute (former CBS, The Netherlands)
CCM, Czech Republic
CCUG, Sweden
CECT, Spain
CICC, China
CIP, France
CGMCC, China
FGSC, USA
ICMP, The Netherlands
JCM, Japan
KCTC, Korea
KACC, Korea
KMM, Russian Federation
MUM, Portugal
NCTC, UK
NBRC, Japan
NCAIM, Portugal
PCU, Thailand
TBTR, Thailand
UCD-FST, USA
VKM, Russian Federation

The GCM type strain sequencing project encourages all culture collections to participate in this international collaborative project. Interested parties should be willing to provide DNA for type strains held in their collections. All microbiologists and institutions from related fields are welcome to submit subprojects for genomic data-related research questions. In addition, the WDCM has established a MOU with the *International Journal of Systematic and Evolutionary Microbiology* and the Bergey's Manual Trust in March 2019 and will provide free services for genome sequencing and annotation required for description of new species and publication[3] (Figure 3).

In the light of the above presented information, we now would like to bring the focus to Australia and ask the Council of Heads of

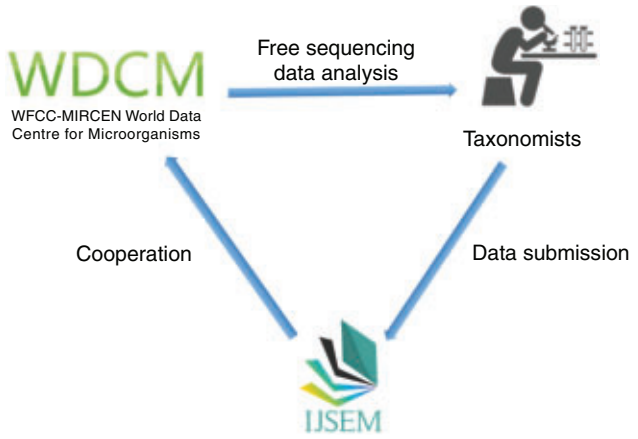

Figure 3. Genome sequencing and annotation services to be provided by the WDCM for the IJSEM.

Australian Collections of Microorganisms (CHACM), who were part of the original Australian Microbial Resources Information Network (AMRIN)[4], to work closely with the GCM to establish their online catalogues. We look forward to the fulfilment of Skerman's vision of the GCM and to revitalise it in Australia by following in the footsteps of Professor Lindsay Sly who created the AMRIN and CHACM as a vision for the future interlinked-development of microbial collections in Australia (also see article by Sly[5]).

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (grant XDA19050301), the Bureau of International Cooperation of the Chinese Academy of Sciences (grants 153211KYSB20160029 and 153211KYSB2015 0010), the National Key Research Program of China (grants 2017YFC1201202, 2016YFC1201303, and 2016YFC0901702), the 13th Five-year Informatization Plan of the Chinese Academy of Sciences (grant XXH13506), and the National Science Foundation for Young Scientists of China (grant 31701157).

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Biographies

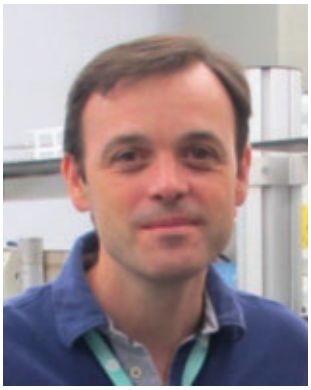
Dr Juncai Ma is director of the Center for Microbial Resource and Big Data, Institute of Microbiology, Chinese Academy of Sciences (CAS), director of World Data Center for Microorganisms (WDCM), executive member at the World Federation for Culture Collections (WFCC), chair of the Mirrors Working Group of International Barcode of Life Project (iBOL), and Convener of the Information Technology Committee, Asian Network of Research Resource Centers (ANRRC). Dr Ma has initiated the international cooperation project of the Global Catalogue of Microorganisms (GCM), which assists culture collections across the world in the managing, disseminating and sharing of information, and which greatly increases the visibility as well as the accessibility of microbial strains.

Dr Linhuan Wu is a young data scientist working at the Institute of Microbiology, Chinese Academy of Sciences and WDCM (WDS Regular Member). To improve the accuracy and efficiency of data sharing among the microbial community, Dr Wu has designed and established an international data standard system for microbial resources information management and data sharing. As the team leader of the Global Catalogue of Microorganisms (GCM), Dr Wu has implemented the first uniform database management system for culture collections worldwide. She also works as a principal scientist of WDCM and the secretary of the former CODATA Advancing Informatics for Microbiology Task Group.

Dr İpek Kurtböke has been working in the field of biodiscovery and has been an active member of the international actinomycete research community since 1982. She currently conducts research and teaches in the field of applied microbiology and biotechnology and is senior lecturer at the University of the Sunshine Coast (USC), Queensland. She has also been an active member of the World Federation for Culture Collections (WFCC) including serving as the Vice-President of the Federation (2010–2013) and currently is the President of the Federation (2017–2020).

Genomic Encyclopedia of Bacteria and Archaea (GEBA) VI: learning from type strains







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Type strains of species are one of the most valuable resources in microbiology. During the last decade, the Genomic Encyclopedia of Bacteria and Archaea (GEBA) projects at the US Department of Energy Joint Genome Institute (JGI) and their collaborators have worked towards sequencing the genome of all the type strains of prokaryotic species. A new project GEBA VI extends these efforts to functional genomics, including pangenome and transcriptome sequencing and exometabolite analyses. As part of this project, investigators with interests in specific groups of prokaryotes are invited to submit samples for analysis at JGI.

What are type strains? By definition, type strains are descendants of the original isolates that were the basis for species descriptions, as defined by the International Code of Nomenclature of Prokaryotes[1]. They exhibit all of the relevant phenotypic and genotypic properties cited in the original published taxonomic circumscriptions. Type strains are also deposited in public culture collections and are likely to remain available for the foreseeable future. Thus, the importance of type strains in nomenclature is only secondary to their value as a biological resource. By the rules of nomenclature, a type strain cannot be identical with any other type strain. Since 1987, the difference between type strains has generally been defined genetically[2]. In terms of genome sequences, this level of

diversity is equivalent to about 70% DNA : DNA hybridisation and 95% average nucleotide identity (ANI) among the conserved DNA[3,4]. In terms of phenotypic similarity, species generally possess S values as defined by numerical taxonomy of >70%, which is close to the limit of significance[5]. Thus, any two properly described type strains must be appreciably different. If these same criteria were applied to mammals most of the primates would be members of the same species[6].

During the last decade, the Genomic Encyclopedia of Bacteria and Archaea (GEBA) projects at the US Department of Energy Joint Genome Institute (JGI) and their collaborators have undertaken a program to sequence the genomes of all the type strains of prokaryotic species. As of February 2019, 16 232 validly named prokaryotic species have been formally described, most of which are represented by type strains. The genomes of 7647 of these have been sequenced, 3145 by the GEBA projects. Another 2300 genome sequencing projects are currently underway at JGI. In addition, the World Data Centre for Microorganisms (WDCM) of the World Federation of Culture Collections (WFCC) began an additional project called GCM2.0 to sequence the genomes of the remaining prokaryote type strains[7]. This project parallels the efforts of GEBA, and the projects are closely coordinated to ensure that there is no overlap in sequencing efforts. Currently, the WFCC has completed or has in progress more than 2600 genome sequences of type strains (www.gcm.wdcm.org).

Beginning in 2013, the GEBA project began sequencing prospective type strains during their formal description[8]. The WDCM also provides a similar service to make genome sequencing readily available to laboratories worldwide[9]. Moreover, all three of the major microbial systematics journals, *International Journal of Systematic and Evolutionary Microbiology*, *Systematics and Applied Microbiology* and *Antonie van Leeuwenhoek*, now either require or strongly recommend including genome sequences in the descriptions of novel species. Minimum standards for the use of genome data for taxonomy of prokaryotes have also been proposed[10]. For these reasons, we expect that genome sequences will be included in the description of most new species in the future. This change in policy insures that the number of type strains without sequences will no longer increase. The major challenge then is sequencing the genomes of the type strains that have been previously described without a sequence. However, given the abovementioned initiatives, it is now possible to expect that the remaining type strains will be sequenced in the near future. When this goal is realised, this biological resource can be fully utilised.

Why sequence type strains? In the larger context, only a small portion of prokaryotic diversity has ever been cultured. The genomes of all known type strains are estimated to represent no more than 15% of the total diversity[11]. However, given the enormity of prokaryotic diversity, this is not an insignificant fraction. Our knowledge of remaining organisms comes largely from metagenome sequencing of environmental DNA. Because the type strains are well characterised, their genome sequences provide the framework for inferring the biological properties of uncultured prokaryotes from their genome sequences. Specifically, it will address a number of complementary questions. How is gene content related to function? Is the presence of certain genes and combinations of genes a strong predictor of phenotype? What properties of prokaryotes are predicted from their phylogeny? In the absence of clear understanding of the functional annotations of genes, what properties can be predicted from those of their relatives? Can probabilistic models be developed to express the likelihood of organismal properties from gene content and phylogeny? Currently, the data to address these issues do not exist at a scale that will generalise to all prokaryotic life.

There is also a number of other, very different but equally valid reasons to sequence the genomes of microbial type strains. As more genomes become available for specific groups, the applications of genome-based systematics are revolutionising the classification of prokaryotes. Genomes provide more reliable and complete data, and allow formation of more meaningful groupings of higher taxa. For instance, genome sequences suggest that the NCBI taxonomy, which is based largely on 16S rRNA sequences, contains a large number of misclassifications within the *Clostridia* and *Bacteroidetes* (Figure 1). Already genome sequences collected in large part by the GEBA projects have led to major taxonomic revisions of the *Actinobacteria*, *Bacteroidetes*, *Epsilonproteobacteria*, *Geodermatophilaceae* and the *Rhodobacteraceae*[13–17].

Because they are phylogenetically diverse, the GEBA genomes are also very useful for identifying metagenome sequences from environmental DNA[18]. For instance, the first thousand GEBA genomes enabled classification of more than 25 million proteins from metagenomes. The same genomes led to a >10% increase in the known protein sequence diversity in prokaryotes[18,19]. Most type strains were described because of their environmental, medical or commercial importance. Their genomic sequences will contribute greatly to our knowledge of the processes in which these prokaryotes play fundamental roles. Identification of prokaryotes is still a major challenge that hinders many practical applications. Genomic sequencing of type strains provides tools that greatly facilitate identification and classification.

While continuing the efforts to sequence the genomes of type strains, the newest GEBA project, GEBA VI, will go beyond genome

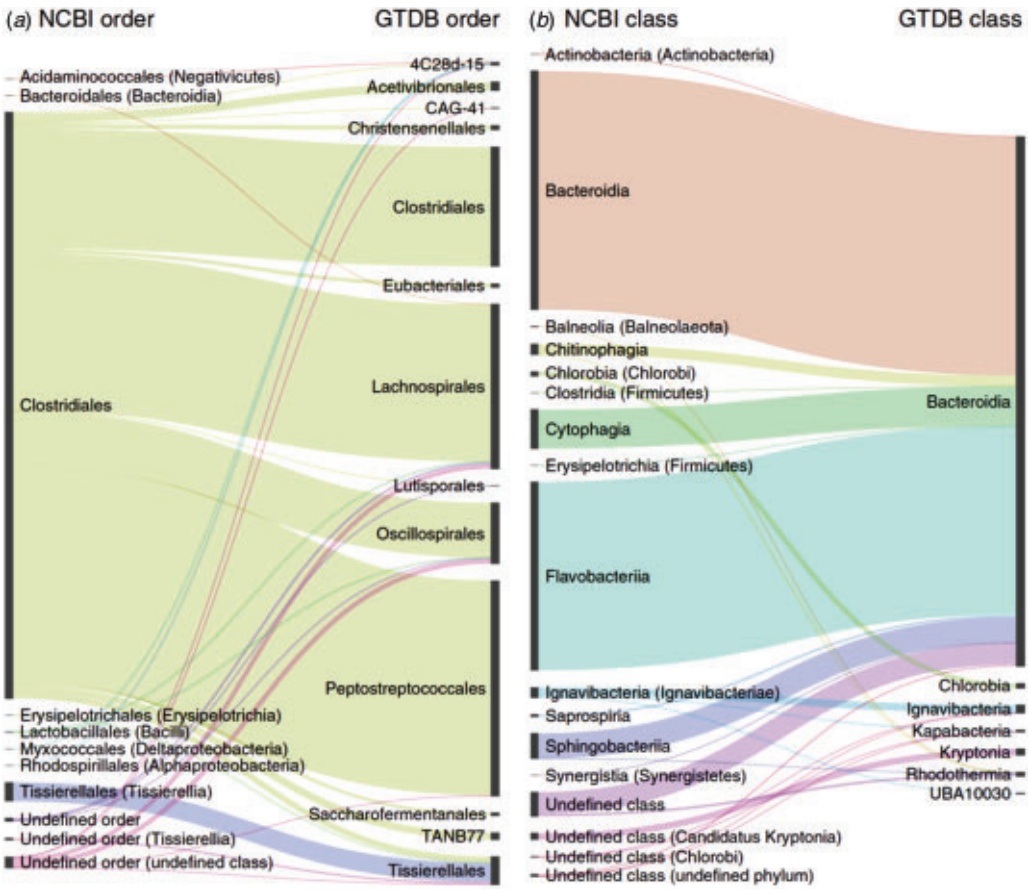

Figure 1. Comparisons of the NCBI classification based largely on 16S rRNA sequencing and the GTDB classification based upon 120 genes obtained from genome sequences. (*a*) Comparison of NCBI (left) and GTDB (right) order-level classifications of the 2368 bacterial genomes assigned to the class *Clostridia* in the GTDB taxonomy. Genomes classified in a class other than *Clostridia* by NCBI are indicated in parentheses. (*b*) Comparison of NCBI and GTDB class-level classifications of the 2058 bacterial genomes assigned to the phylum *Bacteroidetes* in the GTDB taxonomy. Genomes classified in a phylum other than the *Bacteroidetes* by NCBI are indicated in parentheses. Figure reproduced from Park *et al*.[12].

sequencing to initiate the next stage of utilisation of this important biological resource. Because their phenotypic and physiological properties are typically well characterised, type strains are well suited for studies of functional genomics, which unite genome content with the biological properties of bacteria and archaea. The proposed studies will systematically investigate genome function of type strains by a combination of (1) genome sequencing to determine gene content, (2) transcriptomics to elucidate gene expression, (3) secreted metabolites (or exometabolomics) to examine function, and (4) pangenomics to identify the core genome and evolutionary processes.

The rationale for these projects is as follows. While genome sequences provide valuable insights into phylogeny and systematics, they are only the first layer of information available from type strains. Organism function depends critically upon gene expression. Transcriptome studies will identify the highly expressed genes central to an organism's growth and metabolism. Many of these genes are expected to be 'character' genes, which play critical roles in an organism's specific adaptation to the environment[20]. Exometabolomics provides a different view of function, and spent culture media will be screened for nonpolar as well as polar metabolites. Nonpolar metabolites are expected to include many secondary metabolites, such as antibiotics, polyketides and phenolics. These compounds play fundamental roles in cell-cell signaling, competition with other microorganisms, metal uptake and other important biological functions. Polar metabolites are expected to include amino acids, sugars, small organic acids and other hydrophilic compounds. These analyses will identify the components of complex culture media that are consumed, providing direct evidence for the culture's metabolism. They will also identify compounds produced during growth by incomplete metabolism or fermentation.

Lastly, the genome content of any specific strain does not fully represent the gene content of the species[21,22]. Sequencing of closely related strains will identify the core and pangenome as well as the nature of evolutionary processes occurring within the taxon. The core genome is of special importance in the description of a species because it encodes those properties that are conserved across all members[20]. Thus, it captures the phenotypic basis for the species, including those factors which are responsible for

speciation and adaptation to its environment. The dispensable genome is also interesting and provides insight into strain specific differences. For instance, in many pathogenic species, the dispensable genome encodes processes associated with host interactions. Finally, the pangenome provides important insights into the frequency of horizontal gene transfer and sources of diversity within a species[23]. For instance, a pangenome can be either 'closed' or 'open'. The number of genes in a closed pangenome increases to a maximum value as the number of strains increases, suggesting that there is a finite limit to the genetic diversity within a species. The number of genes in an open pangenome is unbounded, and the genetic diversity is, in theory, unlimited.

To realise these goals, GEBA VI welcomes contributions of samples from individual investigators (Figure 2). To participate, submit a description of your project at our website (https://gold.jgi.doe.gov/gebaVI) or send an email to whitman@uga.edu (include 'GEBA VI' in the subject line). The types of projects being considered are genome sequencing of type strains, pangenome sequencing of species poorly represented in the databases, and functional genomics of type strains. The GEBA VI project will support two types of experimental designs, although other good ideas are encouraged. The first will be in-depth studies of single species, including characterisation of the pangenome or transcriptomes and exometabolomes under different growth conditions. In studies of this type, the genome of the type strain may have been sequenced in previous studies. We expect that individual investigators will be the primary contributors for these studies, and the goal is to provide sufficient data for a substantive publication. In this design, the project will support transcriptome studies of 3–4 replicates of up to four conditions. Exometabolome analyses will be dependent upon the suitability of the culture medium for analysis by mass spectroscopy. It may include analysis of the spent medium of up to four conditions for nonpolar metabolites and two conditions for polar metabolites. The pangenome is expected to comprise 5–10 reference strains. The second type of experimental design will comprise comparative studies of groups of related type strains and surveys of genome sequences, transcriptomes and exometabolomes. These designs will try to encompass collections of related species or genera. Type strains will be cultured under identical conditions for preparation of samples for the transcriptomes and exometabolomes.

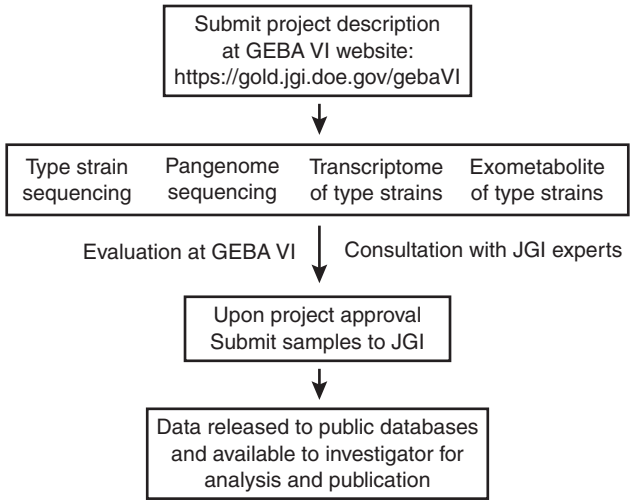

Figure 2. Workflow of investigator initiated GEBA VI projects.

Our primary intention is to survey a large amount of phylogenetic diversity, and, for that reason, these studies will not be comprehensive. For instance, complete sampling of a pangenome may require hundreds of sequences. The transcriptome can only be partially elucidated in the limited studies proposed here. Similarly, production of various classes of exometabolites often depends critically on the cultivation conditions (e.g. media, time, temperature, limiting nutrients, oxygenation, pH/buffering). Historically, screening programs use batteries of complex media designed to trigger secondary metabolite production based on nutrient limitations, and spent media is extracted using a battery of solvents ranging in polarity and protonation. The cell pellet is also often extracted to recover bound metabolites. Thus, the studies proposed here are unlikely to uncover the full range of exometabolites. Nevertheless, these studies will identify candidates for subsequent in-depth studies and provide valuable comparative information.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgement

The authors are grateful to Nikos Kyrpides, Tanja Woyke and Trent Northen for helpful discussions. This research did not receive any specific funding.

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Biographies

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Philip Hugenholtz is a microbiologist who has made contributions in the field of culture-independent analysis of microorganisms. He has contributed to the development and application of metagenomics, the genome-based characterisation of microbiomes, including their taxonomic classification.

Who said this?

The Panama Canal was built with a microscope

The Panama Canal is a 51 mile waterway that links the Atlantic and Pacific oceans. Over 26 000 workers lost their lives during its construction from work-related accidents and disease, mainly yellow fever and malaria.

The statement was made by Sir Ronald Ross (1857–1932), a British medical doctor who received the Nobel prize for Physiology or Medicine in 1902 for his work on the transmission of malaria, becoming the first British Nobel laureate. His discovery of the malarial parasite in the gastrointestinal tract of a mosquito in 1897 proved that malaria was transmitted by mosquitoes and laid the foundation for how to combat the disease.

The term malaria originates from Medieval Italian, mala aria or 'bad air'; the disease was formerly called ague or marsh fever due to its association with swamps and marshland.

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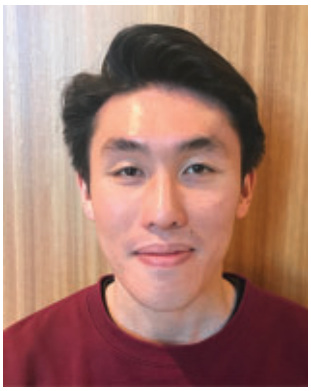
Australian medical mycology culture collections: fundamental resources for mycological diagnosis and research

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Currently in Australia, there are four major medical mycology culture collections that form a close collaborative network. They provide fundamental resources for diagnosis and research and are part of the World Federation of Culture Collections.

Mycological cultures have been deliberately cultured, stored and maintained for centuries, mainly as starter cultures in fermenting food and drinks, including in the beer and wine production process. At least since the discovery of penicillin, fungal cultures have been preserved as resources of biological, pharmacological and industrial substances. At the turn of the century living fungal cultures started to be stored as an important resource for research and teaching, with the first fungal cultures having been made commercially available from the German Technical University in Prague at the end of the nineteenth century by Professor Frantisek Karl[1]. Since then many countries have established government funded microbial/mycology culture collections, with the oldest being the Centraalbureau voor Schimmelcultures (now Westerdijk Institute for Fungal Biodiversity Institute) in Baarn in 2004 and now situated in Utrecht, The Netherlands[1].

In the absence of a national mycological culture collection a number of diagnostic and medical mycology research related collections have been established throughout Australia. There are currently four major medical mycology culture collections in Australia, holding together over 30 000 fungal cultures, which are briefly described below. They form a close collaborative network and are part of the World Federation of Culture Collections (WFCC; http://www.wfcc.info).

Medical Mycology Collection of the Australian National Reference Laboratory in Medical Mycology (AMMRL), NSW Health pathology

WFCC registration number: AMMRL-42; Curator: Ms Kerry Weeks; Contact: Tel: +61 2 9926 4319, Email: Kerry.Weeks@health.nsw.gov.au

The medical mycology collection of the Australian National Reference Laboratory in Medical Mycology (AMMRL) was established in 1949 at the Royal North Shore Hospital (RNSH) in Sydney, NSW, when a small number of live cultures from overseas were received, which were first stored in the Department of Bacteriology. In 1954 with the appointment of a full-time research employee, medical mycology became an officially acknowledged unit within the framework of the Department of Bacteriology. With assistance from the Institute of Medical Research, the mycology unit and its associated culture collection grew steadily. In 1964, Dr Dorothea Frey was appointed Principal Mycologist until she retired in 1982. In 1965 the Department of Medical Mycology was designated the National Reference Laboratory in Medical Mycology by the National Health and Medical Research Council (NHMRC). It was maintained by the Board of the Hospital and the Council of the Institute of Medical Research. From 1954 to 1965 it was supported by grants from the NHMRC. In 1983, the mycology laboratory was incorporated into the Microbiology Department at RNSH and is now under the mantle of NSW Health Pathology. From 1975 until 1999 Mr. David Muir was the principle Mycologist. On his retirement, Ms. Kerry Weeks was appointed as curator. The collection is part of the Atlas of Living Australia (ALA; http://www.ala.org.au).

The collection currently holds approximately 2200 clinical, veterinary and environmental strains, including 670 yeast strains, representing 84 species, 1372 filamentous fungal strains, representing 350 species, and 165 aerobic actinomycetes, which were obtained from routine clinical diagnostics, outside referred and published reference sources, both nationally and internationally. The cultures are mainly identified using classical morphological/phenotypic characteristics and some are further identified by ITS1/2 sequencing and MALDI-TOF. A genomics program is currently being developed. Isolates are stored in 5 mL sterile water vials at room temperature. Associated metadata are stored as a Microsoft Word document (currently being converted to MS Excel format) and in a Laboratory Information System (LIS). Cultures are open access and are available commercially on a fee basis and freely for collaborative teaching/research studies.

The collection has been involved in many publications involving first time reports of fungal pathogens. Recently it formed the basis for a study of the *Metarhizium anisopliae* species complex implemented in human disease[2].

National Mycology Reference Centre, SA Pathology, Mycology Culture Collection

WFCC registration number: ACH-47; Curator: Dr Sarah Kidd; Contact: Tel: +61 8 8222 3544, Email: Sarah Kidd@sa.gov.au

The Adelaide Children's Hospital Mycology Culture Collection (ACH) was established by Ms. Geraldine Kaminski (née Brown) in the early 1960s in Adelaide, SA, collecting fungal cultures stored under oil and/or in sterile water. A special teaching collection of 100 cultures was maintained via monthly sub-culturing. Professor David Ellis took over the collection in 1983 and after his retirement in 2011, Dr Sarah Kidd took over the curation.

While the Adelaide Children's Hospital became the Women's and Children's Hospital in 1989, and the Mycology Unit moved to the Institute of Medical and Veterinary Sciences (IMVS) in 2013 and became the National Mycology Reference Centre, under the banner of SA Pathology. The laboratory offers molecular identification and genotyping of human and animal pathogenic fungi as well as antifungal susceptibility testing. Cultures remain available to non-profit organisations for use in teaching and collaborative research. Many of the earlier isolates belong to the *Cryptococcus gattii* species complex and formed the basis of the fundamental studies by Professor Ellis on the environmental niche of *C. gattii*[3]. The collection has also been used to screen for multi-azole resistant *Aspergillus fumigatus* isolates, the first confirmed isolates in Australia[4].

The collection now houses ~22 700 fungal cultures comprising local and referred clinical, veterinary and environmental isolates of around 80 yeast species (~12 300 isolates) and 340 filamentous fungal species (~10 400 isolates). The isolates were initially stored at −80°C, but new isolates are now largely stored as water cultures. Early cultures were identified by morphological/phenotypic means but are now confirmed by MALDI-TOF and ITS1/2, D1/D2 rDNA gene cluster, and/or β-tubulin sequencing as required. Electronically stored metadata include antifungal minimum inhibitory

concentrations, which have been used in a number of international collaborative studies to assess susceptibility patterns and set interpretive criteria for yeast and fungal species[5].

The PathWest Laboratory Medicine WA, Queen Elizabeth II Medical Centre Medical Mycology Culture Collection

Curator: Mr Ian Arthur; Contact: Tel: +61 8 6383 4531, Email: Ian.Arthur@health.wa.gov.au

The medical mycology culture collection at the Department of Microbiology at Path West at the Queen Elizabeth II Medical Centre (DMFMC), Nedlands, WA was established in 1966 by Ms. Rose McAleer for reference and clinical fungal strains obtained by the Mycological Laboratory in Perth., at what was at that time 'The State Health Laboratory Services (SHLS), WA'. It has since been transferred across to institutions that have superseded SHLS, now being Path West. It continues to accumulate clinical and quality-controlled reference strains as deemed important to store by the curator, Ian Arthur. The collection is part of the Atlas of Living Australia (ALA; http://www.ala.org.au).

The PathWest QEII culture collection currently contains 3500 human and animal pathogenic fungal strains, predominantly fungi but also *Nocardia* spp., which have been obtained through affiliated institutions state-wide. Early cultures were identified solely by morphological criteria, but now strains have MALDI-TOF or relevant sequence data. The cultures are stored in 15 mL sterile water suspensions in glass bottles at room temperature and as 2 mL glycerol stocks in cryogenic bottles stored at –80°C. Associated metadata are stored in an access database. Cultures are available for collaborative studies. The collection offers molecular identification and genotyping of human and animal pathogenic fungi. The laboratory provides diagnostic and referred identification services including fungal pathogens and aerobic actinomycetes.

The collection has formed the basis for a number of national studies of mycoses including the investigation of Australian *Sporothrix* isolates by whole genome sequence analysis[6].

Westmead Medical Mycology Collection (WM), University of Sydney

WFCC registration number: WM-1205; Curator: Professor Wieland Meyer; Contact: Tel: +61 2 8627 3430, Email: wieland.meyer@sydney.edu.au

With the establishment of the Centre for Infectious Diseases and Microbiology (CIDM) at Westmead Hospital in 1980 the first collection of fungal cultures was established by Professor Tania Sorrell forming the basis of the WM culture collection, which was formally established by Professor Wieland Meyer at the Molecular Mycology Research Laboratory (MMRL) at CIDM at the Faculty of Medicine and Health of the University of Sydney in 1995 and is currently housed at the Westmead Institute for Medical Research. The collection is partially funded by grants from the NHMRC and the Western Sydney Local Health District. The collection is part of the Atlas of Living Australia (ALA; http://www.ala.org.au).

Currently the WM culture collection holds 11 137 strains, representing 580 human and animal pathogenic fungal species, isolated from clinical, veterinary and environmental sources from 63 countries. The majority of strains were collected as part of national and international clinical, molecular epidemiological and basic science projects. It maintains 174 type cultures and the reference strains of the major molecular types of the *Cryptococcus neoformans* (VNI, VNII, VNB, VNIII and VNIV) and *C. gattii* (VGI, VGII, VGIII and VGIV) species complexes[7]. Among the 11 137 fungal cultures 9242 are yeast strains and 1671 are filamentous fungal strains. Among the 9242 yeast isolates, 5135 are *C. neoformans* and *C. gattii* species complex isolates, representing 49 out of 623 *C. neoformans* and 88 out of 551 *C. gattii* globally identified MultiLocus Sequence (MLST) Types (ST). The second largest proportion of yeasts in the collection are from *Candida* spp. with 2798 isolates, representing 117 species.

Strains are initially identified phenotypically and biochemically followed by either sequencing of the ITS1/2 region (primary fungal DNA barcode[8]) or the *translocation elongation factor 1α (TEF1α)* (secondary fungal DNA barcode[9]), and LSU D1/D2 region of the rDNA gene cluster for yeasts, or MALDI-TOF analysis. After identification, strains are subcultured onto Sabouraud dextrose agar for 48h at 30°C for yeast and 20°C for filamentous fungi to prepare long-term storage cultures. The strains are stored as lyophilised and glycerol cultures at –80°C, with a small number being also stored as living cultures at 14°C. The strain associated metadata are stored electronically using the BioloMICS software package (http://www.bio-aware.com, Hannut, Belgium). Strains are freely available for collaborative research projects or as reference strains for diagnostics and molecular typing studies. The collection offers molecular identification and genotyping of human and animal pathogenic fungi in outbreak settings.

The collection formed the basis for global molecular typing studies, especially using Multilocus Sequence Typing (MLST) (e.g. Meyer *et al.*[7]) and whole genome sequencing based SNP analysis for the *C. neoformans* and *C. gattii* species complexes (e.g. Firacative *et al.*[10]), *Scedosporium* spp.[11] and *Pneumocystis jirovecii* at http://

mlst.mycologylab.org. It was also the primary resource for the development of molecular identification methods for human pathogenic fungi, especially in the development of the Dual DNA barcoding scheme, combining the ITS1/2 region of the rDNA gene cluster (primary fungal DNA barcode[8]) and the *TEF1α* (secondary fungal DNA barcode[9]), and it's associated quality controlled reference sequence database (http://its.mycologylab.org or http://www.isham.org) for which more than 1000 strains are reference strains[12,13].

Mission statement

The main purpose of the four major Australian Medical Mycology Culture Collections is to preserve and provide the mycological research community with fungal strains and their associated metadata, representing Australian and global clinical biodiversity of human and animal pathogenic fungi and related environmental fungal isolates. In addition, these collections preserve quality controlled, well documented reference strains for validation and interlaboratory comparisons of diagnostics tests. They maintain a bank of cultures used in research studies, which provide the basis for reproducibility studies of the obtained research findings and for the inclusion of those isolates into future national and international research studies.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank all national and international clinicians and researchers to entrust their fungal isolates to the Australian Medical Mycology Culture Collections building a major resource of human and animal pathogenic and environmental fungal biodiversity. This research did not receive any specific funding.

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Biographies

Professor Wieland Meyer is a Molecular Medical Mycologist and academic at the Faculty of Medicine and Health, The University of Sydney and the Fundação Oswaldo Cruz (FIOCRUZ) in Rio de Janeiro, Brazil, heading the MMRL within the CIDM, Westmead Institute for Medical Research, with a PhD in fungal genetics from the Humboldt University of Berlin, Germany. His research focuses on phylogeny, molecular identification, population genetics, molecular epidemiology and virulence mechanisms of human and animal pathogenic fungi. He is the Convener of the Mycology Interest Group of ASM, and the President of the International Mycological Association (IMA).

Ian Arthur is the Senior Medical Scientist at PathWest Laboratory Medicine, QEII network with B. App Sci (Med Tech) and 26 years' experience in medical mycology. Ian provides various lectures and support for the University of WA.

David Ellis is an emeritus medical mycologist at SA Pathology and A/Professor at the School of Biological Sciences, University of Adelaide with a major interest in dermatophytes and the natural niche of *Cryptococcus gattii*.

Alex Kan is a research assistant at the MMRL within the CIDM, Westmead Institute for Medical Research, who graduated from the University of Sydney after completing an Honours project investigating *C. gattii* in free-ranging hosts and the environment. He conducts the molecular identification of *Cryptococcus* spp. using *URA*5-RFLP and MLST, undertakes fungal DNA barcoding studies and manages the WM culture collection database.

Dr Sarah Kidd is a medical scientist heading the National Mycology Reference Centre at SA Pathology. She graduated with a PhD from the University of Sydney (within the MMRL) in 2004 on the molecular epidemiology of *Cryptococcus neoformans* and *C. gattii* species complexes. She is an adjunct Senior Lecturer at the University of Adelaide, Secretary of the Australia and New Zealand Mycoses Interest Group (ANZMIG) and convenes the biennial Mycology Masterclass.

Krystyna Maszewska is a research assistant at the MMRL within the CIDM, Westmead Institute for Medical Research, who has graduated in Poland. She manages the WM culture collection and carries out molecular identification using ITS1/2, D1/D2 and EF1α sequencing and genotyping of pathogenic fungi using MLST.

Kerry Weeks is a Senior Hospital Scientist in charge of the Australian National Reference Laboratory in Medical Mycology, RNSH, NSW Health Pathology, with a B. App. Science (Biomedical Science) and M. App. Science (Occupational Hyg.). She performs routine identification and susceptibility testing of fungi and aerobic actinomycetes and provides a referral service for difficult fungal identifications. Kerry was the Mycology SIG convener for the NSW ASM branch. She is actively involved with Microbiology Registrar training and the RCPA Microbiology Program.

Biodiscovery and the Queensland Plant Pathology Herbarium





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The Queensland Plant Pathology Herbarium (BRIP) and its associated collection of fungal and bacterial cultures have obtained Australian and international recognition as critical resources for agricultural research and plant biosecurity. For decades, many key agricultural and mycological studies published in international journals have examined Australian reference specimens obtained from BRIP. The Queensland Plant Pathology Herbarium is now seeking to reposition itself as a significant provider of unique Australian cultures. This ambitious journey could unlock the potential of Australian specimens to provide novel bioactive natural products that may benefit society.

The Queensland Plant Pathology Herbarium was established in 1901. In 1966, the herbarium was registered under the acronym BRIP by its first curator, Dr John Alcorn (b. 1937), who held the position for almost 40 years (1956–1997). BRIP has a unique collection of microfungi that dates back to the 1850s. The earliest plant pathogens found in Queensland date back to the 1800s, when the botanist Frederick Manson Bailey (1827–1915) collected and sent specimens to European mycologists for study. Bailey conducted numerous expeditions across Queensland and collected more than 1000 specimens of fungi including plant pathogens. Bailey's collection was housed in the Queensland Herbarium, Mount Coot-tha, Brisbane until 1968 when it was transferred to BRIP.

John Alcorn was a taxonomic mycologist who worked in an era when fungal taxonomy was determined by morphological differences. John Alcorn discovered and described many new species and genera of plant pathogenic fungi in Queensland. Importantly, the type specimens were deposited in BRIP with ex-type cultures preserved in the associated culture collection. John's contribution to Australian taxonomic mycology has been recognised by the generic name *Johnalcornia*[1], as well as the species *Avettaea alcornii*[2], *Colletotrichum alcornii*[3], *Curvularia alcornii*[4], *Muyocopron alcornii*[5], *Teratosphaeria alcornii*[6] and *Ustilago alcornii*[7]. John Alcorn also introduced a computerised database that catalogued about 50 000 specimens at the time of his retirement in 1997.

Digitisation of plant disease records began in the early 1990s using the software Titan 3.2. The Plant Pathology Herbarium then migrated to KE Texpress in 1997 with an upgrade to KE EMu in 2002. In the late 1990s, John Alcorn and the curators of the plant pathology herbarium in New South Wales and Victoria formed the National Collection of Fungi (NCOF) and agreed to use the same database and standardised fields to enable data interchange[8].

Since John Alcorn's retirement, BRIP has grown to about 90 000 herbarium specimens and 23 000 living cultures preserved in a metabolically inactive state (Figure 1). Many of the herbarium specimens represent groups of obligate plant pathogens, e.g. rusts, smuts, downy mildews and powdery mildews, which cannot be cultured. Since the late 1990s the taxonomy of fungi and bacteria has been based on molecular phylogenetic analysis. Significantly, in 2012 the nuclear internal transcribed spacer (ITS) region, was recognised as the universal DNA barcode marker for most fungi[9]. Over the past two decades, staff at BRIP have taken a prominent role in using molecular methods to unravel cryptic diversity in plant pathogenic fungi. This work has led to revisions and taxonomic contributions to our knowledge of the diversity of Australian downy mildews[10–12], smuts[13–15], rusts[16–19] and some important plant pathogenic ascomycetes (*Bipolaris*[20], *Botryosphaeriaceae*[21,22], *Colletotrichum*[23,24], *Curvularia*[1,25], *Diaporthe*[26,27]).

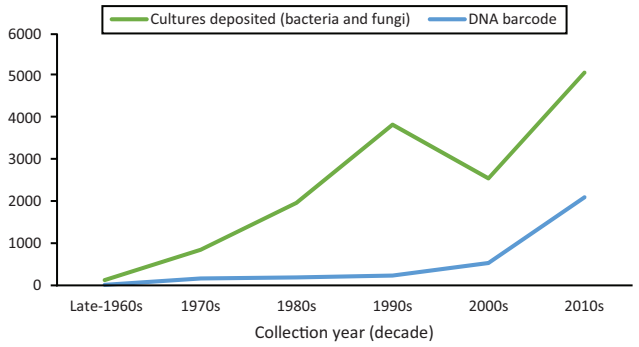

Figure 1. Growth of BRIP since its inception in 1966. The graph shows the number of bacterial and fungal cultures deposited in BRIP, as well as the number of cultures that have a DNA barcode.

The Queensland Plant Pathology Herbarium holds authoritatively identified specimens of most of the known plant pathogenic fungi in Queensland. The Office of the Chief Scientist has recognised BRIP as a State Significant Collection. The collection records for almost 60 000 specimens are available through the website *DAF Biological Collections* (https://collections.daf.qld.gov.au/).

Interest in bacteria and fungi as an untapped resource for novel bioactive natural products (enzymes, proteins, primary and secondary metabolites) has gained momentum in recent years. Over 50% of all pharmaceutical drugs currently on the market are directly derived from or inspired by natural products[28]. This resurgence was considered due to novel approaches towards bioprospecting, which included: (1) the targeting of species not known to produce bioactive natural products; (2) exploring non-traditional environmental niches and methods for the isolation of species; and (3) genome mining[29]. Based on these criteria, many specimens in BRIP are potential candidates for bioprospecting.

The culture collection associated with BRIP holds about 22 000 fungal cultures and 1500 bacterial cultures. Some of the significant taxonomic groups represented amongst BRIP cultures are (1) entomopathogenic fungi from insects and spiders found in tropical Australian rainforests; (2) diverse yeasts found on the leaves of Australian native plants, including the spectacular red and orange ballistosporic species that discharge their spores into the environment with an acceleration of 25 000 times the force of gravity; (3) ascomycetes (helminthosporioid and cercosporoid fungi) that cause specific diseases on native plants, and (4) endophytic fungi that live symbiotically inside the plant tissues of rainforest plants without causing disease.

The Queensland Plant Pathology Herbarium has many unique specimens. The collection houses 493 holotypes (the specimen on which the description and name of the species is based), of

which 129 are available as ex-holotype cultures. The genera with most ex-holotype cultures in BRIP are *Bipolaris* (16 species), *Curvularia* (24), *Diaporthe* (18) and *Pseudocercospora* (7). Each of these genera contain many well-known plant pathogenic ascomycetes. Recent taxonomic studies of the helminthosporioid genera (*Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum*)[1,20,25,30,31] that mostly cause leaf spots on grasses, were built upon the work started by Alcorn 60 years ago.

During 2018, as part of a Queensland Government initiative called the Technology Commercialisation Fund (TCF), the provision of cultures from BRIP to organisations or companies for commercial purposes rather than solely for traditional research purposes was investigated. The TCF aims to find opportunities and pathways for the commercialisation of research outputs from the Department of Agriculture and Fisheries (DAF). The aim of the culture collection project is to make BRIP less financially dependent on government funding and use funds generated to increase staff levels for the effective long-term maintenance of the collection. Through an open Expression of Interest (EOI) process in late 2018, three companies made submissions to express their interest in accessing the collection. DAF and representatives from these companies are currently in discussion regarding terms and access arrangements. All parties interested in accessing the cultures in BRIP have to comply with the Queensland Government legislation, the *Biodiscovery Act 2004*. A Biodiscovery Plan approved by the Department of the Environment and Science and a Benefit Sharing Agreement (or Commercialisation Agreement) approved by the Minister for Science are required.

BRIP is currently reviewing the collection and testing of specimens to estimate their viability and biological diversity. Cultures in BRIP have been stored by several methods, including in freeze-dried ampoules; under water in vials; and under glycerol at –80°C (Figure 2). Initial results show a high level of viability and diversity, although there are some notable failures, such as the long-term storage of culturable of the oomycetes *Phytophthora* and *Pythium*.

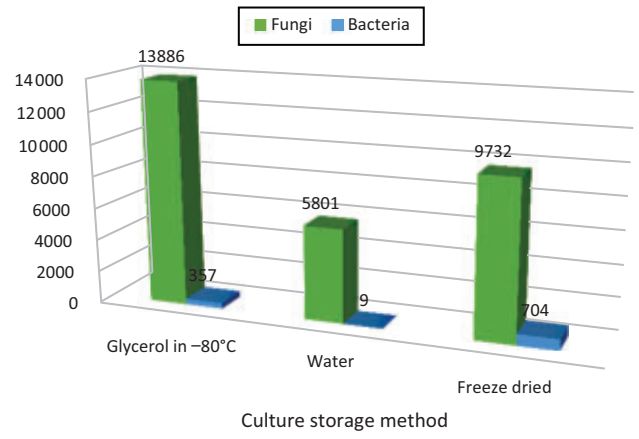

Figure 2. The number of bacterial and fungal cultures stored under various methods in BRIP.

Conclusion

BRIP is undergoing a transformation to make the collection more open and accessible for companies and researchers to undertake biodiscovery research. If successful this may lead to the development of commercial products that benefit society in diverse ways, including crop protection, drug development, and the production of fine chemicals. With the collection being primarily sourced from subtropical and tropical environments it offers a range of potentially unique cultures for research and development. The Department is willing to enter in to more arrangements should other companies want to access the collection.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank Dr Sharon Bishop-Hurley for her contribution to the TCF project.

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Biographies

Professor Roger Shivas is a plant pathologist and co-curator of the Queensland Plant Pathology Herbarium, as well as a mycologist in the Centre for Crop Health, University of Southern Queensland. His research interests are the systematics of fungi, especially those that cause diseases of plants and insects. He has described and classified over 500 new species of Australian fungi.

Dr Dean Beasley is a plant pathologist who has developed and maintains the Queensland Plant Pathology Herbarium and Insect Collection database. He has developed computer based diagnostic tools for plant pathogenic fungi. He has extensive experience in south-east Asian countries, training plant pathologists in biosecurity and diagnostics.

Kaylene Bransgrove is a plant pathologist, mycologist, co-curator of the Queensland Plant Pathology Herbarium and a research scientist at the University of Queensland. She has worked in Australian and international herbaria in mycological and botanical taxonomic roles and has a keen interest in both micro- and macrofungi. Current taxonomic groups of interest include the ascomycete plant pathogens *Phyllosticta* and the powdery mildews.

Dr Yu Pei Tan is a molecular biologist and mycologist who uses phylogenetic analyses to identify and classify fungal plant pathogens. She has made significant taxonomic contributions for a diverse range of plant pathogenic fungi, including *Botryosphaeriaceae*, *Colletotrichum*, *Elsinoë*, *Fusarium*, helminthosporioid fungi and downy mildews (*Oomycetes*).

Geoff Bulow is responsible for the commercialisation of technologies, and as a planner and organiser provides leadership in managing the requirements and processes for technology commercialisation within a government framework, overall project management and resource allocation.

Biobanks – serum and cells – human and animals

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The Australian Animal Health Laboratory (AAHL), CSIRO is a high-containment facility and a vital part of Australia's national biosecurity infrastructure. AAHL closely collaborates with veterinary and human health agencies globally, as approximately 70 per cent of emerging infectious diseases in people originate in animals. The facility is designed to allow scientific research into the most dangerous infectious agents in the world and contains a large collection of serum and cell lines.

The National Animal Serum Bank (NASB) is in the care of the Diagnostic, Surveillance and Response Group (DSR) at the CSIRO Australian Animal Health Laboratory, Geelong, Victoria (Figure 1) and an important component of Australia's biosecurity arrangements. It is a catalogued collection of valuable animal sera stored for the primary purpose of retrospective investigations into exotic or newly discovered agents. Availability of these types of sera is important as an integral component of Australia's animal health biosecurity arrangements even though accessions are rare. There is greater than 30 000 different sera in the collection and CSIRO maintains careful control over the serum repository, utilising a scientific review board to determine which requests for serum will be granted.

The NASB was initiated in 1979 to supply a planned and properly catalogued collection of sera, frozen in liquid nitrogen to preserve their biochemical and immunological characteristics. The earliest sera housed in the NASB was collected from a representative sample of animals located throughout Australia, and from animals imported through quarantine stations. Originally each of the Australian states and the Northern Territory collected serum from a minimum of 100 animals annually. In 1994, national annual sampling of cattle was discontinued and since this time the submissions to the NASB consist of quarantine samples from imported animals[1]. The aim is to continue to accumulate these samples indefinitely.

At AAHL, all physical containment systems are duplicated, and all essential systems, such as electricity generators, steam and compressed air plants, are triplicated. Containment and sample integrity of the NASB and cell culture collection would therefore not be at risk from a computer or power failure.

In establishing the serum bank, the most relevant question was to develop effective methods for submission, processing and storage of samples and establishing a database, which would be fit and flexible for such a long-term collection. AAHL staff developed a simple and practical method to effectively store a serum submission in 4–8 semen straws of 0.5 mL to remove the need for freeze/thawing when accessing and risk compromising the samples integrity. Since 2010, each serum submission has been stored in duplicate cryogenic vials.

Originally, sera were stored in liquid nitrogen at –196°C but this was changed to – 80°C in the 1990s with the move to mechanical freezers and resulted in simpler management and larger capacities. There have been little changes to the way sera are submitted to DSR since the NASB's inception but accessions are now computer based in a Laboratory Information Management System, which facilitates logistics and retrieval of stored samples.

The public perception about the value of stored sera is often low, but this is a problem of lack of information rather than a reality. The stored sera are largely there as an insurance against an emergency need. Lack of access for this purpose can therefore be interpreted as a positive rather than a negative aspect of the NASB – it indicates that other components of Australia's livestock biosecurity are working effectively.

Some sera has been used on rare occasions, in 1993/94 AAHL scientists used bovine sera from the NASB to validate an ELISA test, samples were retrieved and tested during the Equine influenza outbreak in horses in 2007 to assist with the initial diagnosis at Eastern Creek and some quarantine samples have also been accessed by AAHL scientists to verify earlier findings.

In some instances access may allow a research group to add value to a set of recently collected sera by obtaining extra information from

Figure 1. CSIRO Australian Animal Health Laboratory.

the source properties during epidemiological studies. This may also be a valuable use of sera whose value to biosecurity is waning. Such access to the NASB would be a matter of judgement on a case by case basis.

AAHL also holds a large collection of sera from designed surveys mostly from the northern regions of Australia and bordering countries.

The AAHL Cell Culture Unit has over 350 cell lines stored in liquid nitrogen –196°C cabinets in the same storage area as the National Serum Bank. The Cell Culture Unit is a service provider for the facility and supplies appropriate cell culture to order.

AAHL has both primary and continuous cell lines from a variety of species including, humans, bovine, equine, piscine, mosquito, amphibian, reptile, murine, caprine, feline, canine, porcine, avian, ovine and other species. The collection was started at the CSIRO Division of Animal Health at Parkville and was moved to AAHL in 1984 when the Laboratory opened with some of those cell lines dating back to 1970s. Many of the cell lines have been obtained from commercial organisations or other research Institutions, although a certain proportion have been developed by the staff at the Laboratory.

Production of primary cell cultures requires the use of animals, and to reduce the usage of animals, cells are produced in large quantities and then stored long term in liquid nitrogen. Primary animal cell cultures are used to diagnose many diseases. For example, porcine bone marrow and porcine alveolar macrophages (PAMs) are used for the highly contagious African Swine Fever virus (ASFV) that causes high mortality rates in pigs and is currently causing huge problems in several countries.

Cell lines are required in most instances for the isolation of virus from field samples, for example in Australia they are used for Hendra virus, Australia Bat Lyssa virus and a range of aquatic viruses. Both of these collections have existed at AAHL since its opening in 1984 and have been carefully managed and expanded, and provide a valuable resource to Australian veterinary research and diagnosis into animal diseases in Australia. They will continue to be preserved to play a vital role in maintaining the health of Australia's animals, the international competitiveness of Australian agriculture and trade, the well-being of Australians and the quality of our environment.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This research did not receive any specific funding.

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Biography

Lynda Wright is an Operations Manager CSIRO Australian Animal Health Laboratory primarily responsible for the Biorisk Management Group and data platform systems. She has previously worked in the mammalian Virology Laboratory, working with viruses exotic to Australia in PC3 and PC4 containment laboratories.

Historical perspectives and new opportunities for Australian collections of microorganisms in the microbiome era

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A new microbiology support program for Australian microbial resources centres is essential to take full advantage of the exciting information and biological materials emerging from molecular studies of microbiomes. At a time when taxonomic capacity is in decline, culture collections, with the appropriate level of infrastructure support and funding, are well positioned to enhance the outcomes of microbiome research. The importance of microbial biodiversity and its contribution to life on earth have never been more appreciated in the history of science than now. This appreciation came initially through the systematic study of microbial cultures, their ecological interactions, evolution and genetics. But now in the genomics era, uncultured microorganisms and whole microbial biomes are increasingly being studied using advanced DNA sequencing and bioinformatic techniques bringing greater insight into complex microbial communities, revealing interactions between microbes and the host affecting health and wellbeing. However, it should be remembered that the inference of identity and interpretation of functions of members of these uncultured communities relies heavily on knowledge gained from the study of cultured microorganisms. Advances will be greatly enhanced by bringing novel, and other significant, species in these environments into culture for laboratory study and accession into collections for future biodiscovery.

While Australian biodiversity programs are world leading with respect to eukaryotic flora, fauna and fungi, they have not been sufficiently inclusive of prokaryotic microorganisms (bacteria and archaea), and also viruses. It is essential that Australian microbial biodiversity is more extensively studied, described, and protected securely in microbial collections for immediate and future research and biotechnological applications. Changes are needed so that microbial biodiversity studies and culture collections are integrated equally into Australian biodiversity studies and collections infrastructure. Several proposals have been made over a long period of time to achieve these goals[1–4] and to transition culture collections into Biological Resource Centres in line with OECD recommendations and guidelines[3–7].

Microbial biodiversity and culture collections in Australia

I have written previously in more detail on the importance of microbial diversity and the history and role of culture collections in Australia[1,3,4]. Microorganisms were the first forms of life on earth and have evolved into the most ecologically, genetically and metabolically diverse species known. Microorganisms belong to all three Domains of life: The Bacteria, Archaea and Eukarya (algae, fungi, yeasts, 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 the sustainability of the planet and society depends[4]. Culture-independent molecular studies of environmental samples using rRNA sequence information and metagenomics continue to confirm that the vast majority of microbial species remain so far uncultured thus limiting our knowledge of microbial functions and ecology[8]. A recent molecular estimate of the Earth's bacterial and archaeal diversity has been determined as 2.2–4.3 million species[9].

Currently there are 34 collections with 82 946 cultures listed for Australia in the WFCC World Directory of Culture Collections of Microorganisms (http://www.wfcc.info/ccinfo/index.php/home/content), down from 50 in 1998. These collections have mainly institutional roles and the host institutions are usually universities, CSIRO, hospitals, government laboratories, and industry. Most cultures within these collections are bacteria, fungi, yeasts, and microalgae with minor holdings of protozoa and viruses. The collections are engaged in medical, veterinary and plant pathology, agriculture, marine science, forest microbiology, Antarctic

microbiology, food science, wine research, ecology, taxonomy and education.

Due to the distributed nature of microbial culture collections in broadly different disciplines, the Heads and Curators of Australian microbial collections rarely had an opportunity to meet to discuss common objectives for the development of culture collection resources in Australia. These issues were addressed independently in various forums and special interest groups within separate scientific societies covering microbiology, medical sciences and plant pathology but a mechanism for all to meet together was missing.

Initially set up as an ARC Seed Funding Project for Research Networks in 2004, the Australian Microbial Resources Research Network (AMRRN)[2,3] was the first attempt to bring microbial collections and biodiversity researchers together and was involved as a partner with the Council of Heads of Australasian Herbaria (CHAH), Council of Heads of Australian Faunal Collections (CHAFC) and other stakeholders in the proposal to NCRIS (National Collaborative Research Infrastructure Strategy) which led to the Atlas of Living Australia (ALA) (https://www.ala.org.au/). The vision of AMRRN was to develop a world class research network to discover and exploit Australian microbial resources and to make these resources and associated information available for applications in science, research, industry and education. The AMRRN would link and support researchers working in a range of disciplines, including microbial diversity, taxonomy, evolution and genomics, ecology, identification, culture collections, bioinformatics, biodiscovery and biotechnology[2–4]. The AMRRN proposed three mechanisms to deliver this vision:

- ACM: an integrated network of Australian Collections of Microorganisms to conserve and supply cultures;
- AMRIN: the Australian Microbial Resources Information Network to facilitate access to information on Australian microbial resources; and
- AMRS: Australian Microbial Resources Study to undertake taxonomic research on Australian microbial diversity.

Unfortunately, the proposal was not funded as it did not meet the criteria for ARC Research Networks. However, the exercise allowed some progress to be made. The later development of the ALA has meant that the data aggregation and search functions proposed for AMRIN can be delivered through the ALA Natural Collections Hub (https://collections.ala.org.au/) and now also through the Global Catalogue of Microorganisms (http://gcm.wfcc.info/) at the WFCC World Data Centre for Microorganisms. Although by no means complete there is an open-ended opportunity for collections to expand the information available on their holdings and for new collaborating collections to join these initiatives. Australian collections are encouraged to register with the World Data Centre for Microorganisms and the Atlas of Living Australia and to connect their entries.

In 2009, the AMRRN held a meeting in Brisbane with representatives of the ALA to establish the Council of Heads of Collections of Microorganisms (CHACM). This marked the first comprehensive meeting of Heads and Curators of Australia's microbial collections. The meeting established the minimum standards for data in Australian microbial collections compliant with international standards to facilitate sharing of data through the ALA. A few collections had suitable database software, but the meeting identified that the lack of modern database software was a major impediment for many collections to digitise their collection records which would allow sharing of information and the ALA is commended for providing BioloMICS software to those collections in need. The WDCM Global Catalogue of Microorganisms now provides a complementary means to assist with the generation of digital catalogues.

There are 31 collections of microorganisms listed with the ALA (https://collections.ala.org.au/) but not all have committed to provide strain data at this stage of its development usually due to lack of staff within the collection to carry out the work and sometimes due to patient privacy concerns in some medical collections, biosecurity issues in some plant pathology collections and commercial sensitivity in others. Information on Australian collections is available through the ALA (https://collections.ala.org.au/) and the WFCC World Directory (http://www.wfcc.info/ccinfo/index.php/home/content).

A new era for Australian collections

The microbiome era opens up new opportunities and challenges for microbial collections, not only for the conservation of complex genetic material but also for collaborative research on the microbial taxonomy and ecology of the expanding number of microbiomes across a wide range of environments. The completion of genomic analysis of microbiomes is not the end of the story for scientific discovery. Rather, it is the beginning, an insight into the microbial complexity of different environments revealing novel microbial diversity and significant microbial functions and interactions. Current statistics indicate that 15% of 154 904 microbial genomes belong to uncultured microorganisms (https://gtdb.ecogenomic.org/stats) compared with 85% based on 16S rRNA surveys[8,10]. This apparent discrepancy is simply a reflection of the limited number of genome sequences available (P. Hugenholtz, pers. comm.).

Clearly, a more complete knowledge of Australian microbial diversity and microbial taxonomy will be achieved by encouraging the systematic microbial study of Australian microbiomes. To achieve

this, previous recognition by government biodiversity policy[11] and reviews[12] to accelerate Australian microbial diversity studies and recommending the need to strengthen and support collections of microorganisms will need to be urgently achieved.[4] Many government ministries and agencies support programs in agriculture, trade, food, health, quarantine, industry, science and education which depend on accurate taxonomic decisions and access to standard cultures for quality assurance and regulatory compliance. New long-term infrastructure funding mechanisms are needed to support microbial collections to improve their security, meet OECD guidelines[6,7], and help reverse the loss of collections and microbial biodiversity when researchers retire, or host institutes change direction and priorities.

As a matter of principle, representative Australian microbial diversity obtained in publicly funded research must be accessioned into permanent national Australian collections and protected as part of our natural scientific heritage as occurs with the native flora and fauna. As well, it would not be unreasonable to expect that cultures described in publications from publicly funded research be accessioned in the same way as a condition of funding. The OECD is strongly promoting that biological resource centres are essential to underpin advances in biotechnology, the life sciences and the bioeconomy[6,7]. Microbial resource centres are more than collections. 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. They work within the framework of the Convention on Biological Diversity (CBD) (https://www.cbd.int/convention/) implemented to support the conservation and utilisation of biodiversity and recognising the principles of fair and equitable benefit sharing. With the coming into force of the Nagoya Protocol on Access and Benefit-Sharing (https://www.cbd.int/abs/), culture collections and microbiologists generally are addressing best practices to adhere to the Protocol for the receipt, supply and management of biodiversity material and associated information and records[13,14].

There is an urgent need to train and mentor the next generation of taxonomists and curators in collections[4]. Many curators are approaching retirement and many who have already retired are not being replaced. The Taxonomy Australia (https://www.taxonomyaustralia.org.au/) initiative is calling for accelerated research on describing Australian biological diversity over the next decade and highlights the slow progress being made with microorganisms, particularly bacteria, archaea and viruses. This is an excellent initiative but brings no additional funding, an issue which must be addressed by funding agencies if realistic progress is to be made.

There is also a need to reverse the decline in teaching and postgraduate research training in microbial taxonomy and ecology in universities. One model[4] for consideration is the establishment of research centres of excellence in microbial diversity, taxonomy and ecology either within or in collaboration with microbial resource centre collections to investigate microbial diversity in Australian microbiomes. This would accelerate discovery and assist in training the next generation(s) of research scientists and academics in microbial biodiversity and taxonomy and will be important for providing high-level research training and careers in taxonomy and identification for PhD and postdoctoral scientists.

Historically, Australian Collections of Microorganisms have made a significant contribution to microbiology. They have supported research and essential functions in their host institutions, but also provided essential service across the broader scientific community. Many microbiologists associated with these collections have been proactive advocates and responsible for some key advances. Notably, the establishment of the WFCC World Data Centre for Microorganisms by Professor Vic Skerman at the University of Queensland in 1966 and now hosted by the Institute of Microbiology at the Chinese Academy of Sciences in Beijing, has matured into a vital well respected global repository of digital information and support for collections.

Improved infrastructure for collections and microbial taxonomy has been a fundamental driver of the Australian Microbial Resources Research Network. Culture collections have always needed to adapt to advances in microbiology and changes in regulatory compliance and scientific priorities to remain relevant to current and future needs. This will be even more important in the future adapting to new technologies which are rapidly expanding information on the vast scale of microbial diversity in microbiome genomic research. This provides significant opportunities for collections to engage and collaborate in this research bringing microorganisms into culture for taxonomic study and biotechnology.

Culture collections will always remain the conservators of our natural microbial heritage. With proper funding arrangements to transition collections into OECD compliant microbial resource centres and centres of excellence for microbial taxonomy, collections will have important roles in adding significant value to Australian biodiversity knowledge and the outcomes of microbiome research. Collections and the microbiology community are encouraged to engage through CHACM to maximise the future success of Australian Collections of Microorganisms and help make the vision of AMRRN a reality.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This research did not receive any specific funding.

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Biography

Emeritis 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 Microorganisms 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 phylogenetic relationships amongst species in diverse bacterial and archaeal divisions, and to the development of molecular tools for the identification of bacteria and archaea. He was awarded Fellow of the Australian Society for Microbiology (FASM) in 1989, and Fellow of the Australian Institute of Biology (FAIBiol) in 1992. In 2001 his outstanding contributions to systematic bacteriology were recognised with the prestigious international Bergey Award and in 2010 he received the WFCC Medal for outstanding contributions to the World Federation for Culture Collections. He was president of the World Federation for Culture Collections from 1996 to 2000 and Foundation Chair of the Council of Heads of Australian Collections of Microorganisms in 2009.

ASM2019 report

Stephen Kidd

The local organising committee and the ASM Executive were very pleased to be able to hold the Australian Society for Microbiology (ASM) Annual: National Scientific Meeting and trade exhibition (ASM2019), in Adelaide and held at the Adelaide Convention Centre (ACC).

There was a diverse and stimulating scientific program with leading microbiologists from around Australia and the rest of the world presenting an exciting array of science, research and the advances in the field of microbiology. There was an overarching theme of Humanitarian Development and Solutions and a wonderful social program to encourage discussion and interaction.

We kicked-off the conference on Sunday with a public lecture by Wendy Jackson and Hilda Zoleveke from the Pacific Region Infectious Disease Association PRIDA – empower, grow, sustain). This was a great talk outlining the history and work of PRIDA and the important work they do in the neglected areas of infectious disease including bacteriology, sepsis, other life-threatening infections and then hospital infection prevention. The importance and impact of seemingly simple but vital work and education in these remote areas was obvious and enlightening. The work they are doing goes into many areas and remote sites across the Pacific Region including Papua New Guinea, the Solomon Islands, Timor Leste and the Marshall Islands. Professor Tilman Ruff gave an inspiring Rubbo Oration (on Tuesday). He has a broad portfolio of amazing professional and personal activities; and his work has had an enormous impact in humanitarian development. He is an infectious disease and public health physician and has major roles in international organisations functioning for immunisation, public health, nuclear disarmament and peace. He helped establish the International Campaign to Abolish Nuclear Weapons (ICAN) and was its founding chair. ICAN was the recipient of the 2017 Nobel Prize for Peace.

Likewise, Dr Alan Landay, from Rush University Medical Center in Chicago (USA) talked about his research into HIV/AIDS and his part in the AIDS Clinical Trials Group and as an advisor to UN (United Nations) on AIDS and HIV and Aging. He presented research he has been at the forefront of in developing and providing an understanding of the role of immune activation in diseases related to aging in the HIV population. Intriguingly, there was some new research that was presented showing the links of the microbiota on HIV/AIDS disease progression. Indeed, there was a large number of talks presented at ASM2019 that showed important data reflecting the developments in our understanding of the role of the microbiome and pathology, and indeed, the role of the microbiome and antimicrobial resistance and tolerance. Commonwealth Serum Laboratories (CSL) annually provide direct support for the Bazeley Oration at the ASM 2019: we were pleased to have the Bazeley Oration given by Professor Luigina Romani. She is internationally recognised in the area of antifungal immunity. She presented some wonderful results on anti-fungal therapies but further to this, the role of the bacterial microbiota in mucosal homeostatsis and the function of metabolites in maintaining protection against pathogenic fungi. We were lucky to hear further on fungal infections (in plants) from Professor Sarah Gurr. Her interests in crop diseases, with particular emphasis on fungal infestations and in their global movement and control, highlighted the impact in modelling the rise and re-emergence of diseases in the global food supply.

Further to our understanding of infectious diseases, how they spread and how we treat these diseases, a global concern is the increasing number of infections that are resistant to antimicrobials and antibiotic resistance in particular. The Snowdon lecture was given by Dr Marnie L. Peterson (USA), an expert in antibiotic resistance, antimicrobial stewardship, experimental therapeutics, and microbial pathogenesis. Marnie provided a great platform for understanding the increase in antibiotic resistance, the global importance of monitoring the rise of resistance in infectious diseases and gave some case studies that showed the impact of research and development into new antimicrobial drugs.

Tuberculosis (TB) remains a major, global public health and infectious disease problem, particularly in developing countries, where in many parts it is endemic. Globally, almost 1 million people die from TB every year. Fittingly the conference was concluded by Professor Miguel Viveiros (from the Instituto de Higiene e Medicina Tropical da Universidade Nova de Lisboa, Portugal) with a wonderful talk on TB and antimicrobial resistance mechanisms.

Throughout the conference we had great environments to interact with each and with further science (in the poster sessions), and during the social events. We are proud to have hosted such a wonderful conference – the scientific program and the exciting social events. We especially thank all our sponsors of ASM 2019: they are providing essential and generous support.

Finally, the ongoing quality of ASM National Conferences is the end result of the effort of the ASM Executive (in particular, we acknowledge Dena Lyras, Cheryl Power and Kate Seib) as well as the Local Organising Committee members: Stephen Kidd (Chair), Chris Ossowicz (Deputy Chair), Peter Zilm (Scientific Program Chair), Paul Sideris, Stephanie Lamont-Fredrich (Social), Laura Weyrich, Tania Veltman, Haig Henry, Gianny Scoleri, Alexandra Tikhomirova, Katarina Richter, Nicky Thomas, Darren Trott, Mohammed Alsharifi, Gupta Vadakattu and Tania Veltman. We would also like to thank ASN Events (especially Kara Taglieri, the ASM National Office Manager) for their work in organising this conference.

EduCon 2019: event report

Karena Waller

ASM Ed SIG Chair
Email: klwaller@unimelb.edu.au

This year's ASM EduCon was held 3–4 July in the University of South Australia's new Health Innovation Building in Adelaide. It was a fabulous meeting, attended by 33 registrants from Australia, and from international locations. Registrants enjoyed a diverse program of engaging oral and poster presentations focussing on microbiology and broader biomedical education, teaching and learning in the higher education landscape and professional development opportunities for teaching-focussed academic staff. Throughout the program, attendees enjoyed plentiful and tasty catering supplied by *Food Lore* (caterers based within the Health Innovation Building).

The meeting commenced with Associate Professor Prue Bramwell (RMIT University) delivering an engaging presentation titled *Group work – perspectives on the student experience and assessment*. Prue, recipient of the 2018 ASM David White Excellence in Teaching Award, presented her perspectives and findings on the complexities of group work and its assessment. Prue's presentation included examples of the successful use of Spark^{PLUS}, a licensed software for peer assessment and feedback on group work.

Mr Adam Montagu, from Adelaide Health Simulation (University of Adelaide), then delivered an engaging presentation titled *Simulation under the microscope*, which included a live demonstration of the capabilities of the simulation unit by Adam and his colleagues (including physicians, technicians and actors). Following this, we were then treated to a guided tour of, and further demonstrations in, the Health Simulation unit, which is responsible for teaching active, clinical practice skills to medical, nursing, physiotherapy and health science students.

Wednesday's activities were rounded out by the EduCon Conference Dinner, held at the Union Hotel, a local gastronomic pub within walking distance of the Health Innovation Building. Once there, we enjoyed a delicious three-course meal accompanied by regional wines within an opulent, yet relaxed setting. A wonderful evening of networking and great conversation was had by all!

Thursday's program kicked-off with Dr Helena Ward and Professor Ray Peterson (both from the University of Adelaide) delivering an accessible presentation on *Scholarship of Teaching and Learning: Why it matters and how you can engage in it.* Given the increasing importance of scholarship of teaching and learning (SoTL) activities to the careers of many University academic staff, this presentation was highly informative, relevant, practical and timely.

Dr Nicolene Lottering, currently of Swinburne University of Technology but formerly with the University of Adelaide, delivered an engaging presentation titled *Re-inventing learning for the digital generation: A students-as-partners approach to maximise student engagement*, which exemplified the use of social media in increasing student engagement in her Anatomy classes. Nicolene outlined useful ways for using Instagram to boost engagement (not only with her students, but also with us via a live demonstration), and highlighted the many positive outcomes this had on student engagement, performance and the subject's reviews.

Associate Professor Wilhelmina (Willa) Huston (University of Technology Sydney) then presented *Student-Professional-Academic*

Co-creation of collaborative and active learning approaches in the new UTS PC2 Superlab. Willa's presentation articulated the consultation, design and development process that staff (academic and professional) and students at UTS underwent that lead to the new superlab laboratory spaces, which will be ready for teaching in 2020. During this process, utmost care was taken to ensure active learning approaches were incorporated into the curricula to be delivered in these engaging, multidisciplinary biomedical laboratory 'pods'.

Dr Rebecca LeBard (University of New South Wales) presented *How do we measure good teaching?*, which articulated the University's recent implementation of teaching-focussed academic roles, and the community of practice that Rebecca and colleagues established and developed to support these roles, and advance the careers of teaching-focussed academics at the University.

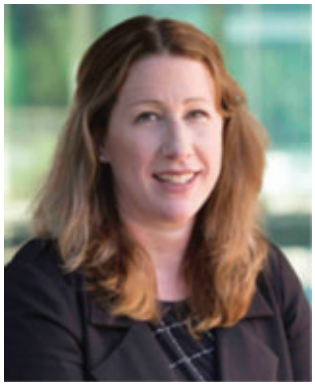
The conference program concluded with Dr Karina Riggs, from the University of Adelaide, delivering a presentation titled '*Flipping the Microbiology Lab' – A case study into Flipped Classroom design for improving student engagement.* Karina presented the strategies and pre-laboratory class activities that she has successfully used to increase student engagement, performance and outcomes in her microbiology labs.

This year our meeting was very proudly sponsored by Monash University and The University of Melbourne. We are extremely grateful for their very generous and continued support.

Given the huge success this year's EduCon, in addition to the many wonderful conversations and networking opportunities it provided, I am already looking forward to next year's ASM EduCon, which will be held in Melbourne in 2020. See you all then!

The conclusion of ASM EduCon 2019 also marks the end of my tenure as Chair of the ASM's Education Special Interest Group (Ed SIG). I wish to take this opportunity to thank all members of the SIG, and in particular the attendees at EduCon over the last two years for their participation, attendance and engagement. It really has been a pleasure working with you all. I now hand over the reins to Dr Megan Lloyd (Edith Cowan University, WA) who, I am sure, will continue leading the ED SIG, and EduCon, with passion and vigour!

ASM Summer Student Research Awards: 2019



Priscilla Johanesen

ASM Student and Early Career Microbiologist Engagement Coordinator

This year the society awarded nine ASM Summer Student Research Awards. These awards are run as a collaboration between national office and state branches and are a fantastic opportunity for students to complete a research project in a laboratory over the summer vacation period. This activity allows students to gain valuable experience in a microbiology research laboratory. This year the successful awardees were: Joshua Dubowsky from South Australia; Amy Griffith and Mikaela G Bell from Queensland; Cassandra Stanton, Joshua J Vido and Yinglei Hua from Victoria; and Wenna Lee, Andrew Vaitekenas, and Alicia Tan Yi Jia from Western Australia. As you will see from the student's abstracts, awardees performed research on wide-ranging areas of microbiology from microbial communities in a tropical rainforest ecosystem through to human and veterinary microbial pathogens. The society congratulates all of the winners of the 2019 awards.

South Australia

Investigating transcriptional disparity in components of the complement alternative pathway between different cell types infected with dengue virus

Joshua Dubowsky

Supervised by: Associate Professor Jill Carr

Microbiology and Infectious Diseases, College of Medicine and Public Health, Flinders University, Adelaide, SA, Australia

Dengue virus (DENV) is the most significant arbovirus affecting human health worldwide yet there is currently no approved treatment to combat the disease it causes. The severe symptoms that can result from DENV infection have clinical features similar to those caused by complement alternative pathway (AP) overactivity and our laboratory has previously shown that DENV infection induces complement factor H (FH) mRNA. This mRNA could encode for full length FH, or a shorter FH-like protein (FHL-1), which has different properties in regulating the AP. The aim of this project was to quantify the levels of these two alternative transcripts, in DENV infected cells. DENV infection in both HeLa and MDM significantly increased mRNA transcripts from the 5' end of FH however, significant increases in further 3' and full-length FH transcripts were only observed in DENV-MDM. Furthermore, DENV-MDM but not HeLa demonstrated a significant increase in FHL-1 mRNA. MDM represent the natural *in vivo* target for DENV infection and demonstrates that the full transcriptional response of FH to DENV-infection is not mimicked by routine laboratory cell lines such as HeLa. However, HeLa remain suitable for investigating mechanisms of the FH mRNA induction at the level of the promoter. Establishing this methodology will benefit further characterisation of DENV induction of FH and FHL-1 mRNA in other target cells for DENV replication, such as endothelial cells.

Queensland

Characterising outer membrane vesicles (OMVs) of *Moraxella catarrhalis*

Amy Griffith

Supervised by: Dr Aimee Tan and Associate Professor Kate Seib

Institute for Glycomics, Griffith University, Qld, Australia

Moraxella catarrhalis is an important human restricted pathogen associated with otitis media in children and chronic obstructive pulmonary disease (COPD) exacerbations in adults. Outer membrane vesicles (OMVs) are naturally produced from the outer membrane of Gram-negative organisms including *M. catarrhalis* and have roles in virulence. This study characterised genes involved in OMV biogenesis, protein composition of OMVs and the effects of OMV formation on virulence mechanisms including biofilm formation and serum survival. We identified one gene involved in OMV formation, with ~2–4-fold increase in OMV production in the *M. catarrhalis* 195ME and 25239 mutant strains lacking this gene. This study also characterised OMV composition

of the wild-type *M. catarrhalis* 195ME and 25239 strains, identifying strain dependent differences in composition. In addition, data from phenotypic assays suggested that serum survival is altered by OMV concentration; *M. catarrhalis* 25239 has decreased serum survival when cells are washed prior to the assay representing a hypovesiculating phenotype and have increased serum survival when exogenous OMVs are added. This information helps to understand OMV biogenesis and pathogenesis of *M. catarrhalis*, while also aiding in the characterisation of *M. catarrhalis* OMV protein composition.

Development of a novel *Stenotrophomonas maltophilia* real-time PCR assay

Mikaela G Bell[A,B]

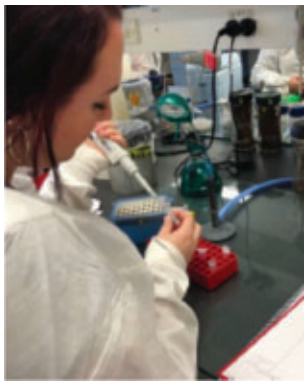
Supervisors and co-authors: Dr Tamieka A Fraser[A,B], Dr Patrick Harris[C,D], Professor Scott C Bell[E], Mr Haakon Bergh[C], Professor Graeme Nimmo[C], Dr Derek S Sarovich[A,B] and Dr Erin P Price[A,B]

[A]Sunshine Coast Health Institute, Birtinya, Qld, Australia; [B]GeneCology Research Centre, University of the Sunshine Coast, Sippy Downs, Qld, Australia; [C]Microbiology Department, Central Laboratory, Pathology Queensland, Herston, Qld, Australia; [D]University of Queensland Centre for Clinical Research, Herston, Qld, Australia; [E]QIMR Berghofer Medical Research Institute, Herston, Qld, Australia

Stenotrophomonas maltophilia is a Gram-negative, multi-drug-resistant environmental bacterium that is emerging as an important respiratory pathogen in those afflicted with the multiorgan disease, cystic fibrosis (CF). *S. maltophilia* is also recognised as a threatening nosocomial pathogen, especially among the paediatric population, and its presence in those afflicted with CF is associated with more advanced diseases. With a history of being overlooked as a pathogen in its own right, it is currently unclear how widespread *S. maltophilia* is amongst CF cases, so its role in disease pathogenesis remains enigmatic. Currently, there are no rapid, highly-accurate methods for the detection of *S. maltophilia*, meaning that its true prevalence is likely being underestimated. The goal for this study was to use large-scale comparative genomics of *S. maltophilia* and near-neighbour species to identify a highly-specific genetic target for this emerging pathogen, and to subsequently develop and test a newly designed PCR assay for its specific detection. The assay designed was successfully able to detect all 89 clinical *S. maltophilia* samples with 100% accuracy. Future work will entail screening of non-*S. maltophilia* strains, and determine the limits of detection and quantitation for this new assay.

Victoria

The genetic manipulation of bacteriophage, CSP3, for the detection of *Burkholderia cepacia*



Cassandra Stanton

Supervisors: Dr Steven Batinovic and Dr Steve Petrovski

Department of Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, Vic., Australia

A phage infective for two clinical strains of *B. cepacia*, CSP3, was previously isolated and characterised. In this study, CSP3 was used for the development of a biosensor through the addition of a fluorophore (GFP) onto the N-terminus of Orf25, the predicted major capsid protein. Two homology arms were PCR amplified from the CSP3 genome and cloned in either side of *gfp* with no stop codon to create the vector pBBR1-NW. pBBR1-NW was then conjugated into *B. cepacia* and grown with wildtype CSP3. It was hypothesised that as CSP3 infected *B. cepacia* containing pBBR1-NW, the regions of homology between the phage and vector would undergo a double crossover homologous recombination event, with *gfp* being inserted into CSP3. GFP would form a fusion protein at the N-terminus of Orf25, resulting in translation and expression of GFP during phage replication. Plaques were screened by PCR assays to detect recombinant phages. This study presents a method for producing phage biosensors able to detect *B. cepacia* or to be further applied to other phages and pathogenic bacteria for their sensitive detection.

Exploring the influence of soil abiotic components on microbial community structure within a tropical rainforest ecosystem

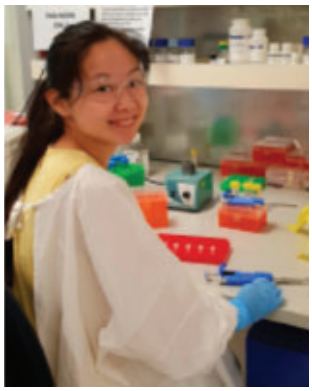
Joshua J Vido

Supervisors: Dr Jennifer Wood and Associate Professor Ashley Franks

Department of Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, Vic., Australia

Tropical rainforests harbour the greatest amount of plant diversity of all naturally occurring ecosystems on earth. However, the rapidly changing climate is placing a significant threat on the biodiversity within these ecosystems. Observations into the microbial community dynamics within soil may provide an understanding of how tropical rainforests may be altered from climate change. This is due to microbial communities responding rapidly to environmental change, while the response may be delayed in above-ground organisms such as mature plants. This study aimed to analyse abiotic factors (moisture, pH and electrical conductivity) of soil samples collected from a tropical rainforest in far North Queensland, Australia, in order to investigate the relationship between these factors as well as their influence on bacterial, fungal and oomycete communities. The elevation within the study site where each soil sample was taken was observed to influence microbial community structure, resulting in high beta diversity along the elevation gradient, it was proposed that pH was a major contributor as a strong negative correlation between pH and elevation was observed. This provides an understanding of the soil dynamics underpinning microbial community structure which can be furthered explored through more comprehensive analysis techniques such as next-generation sequencing.

Investigation of pneumococcal gene expression associated with pneumonia pathogenesis



Yinglei Hua[A,B]

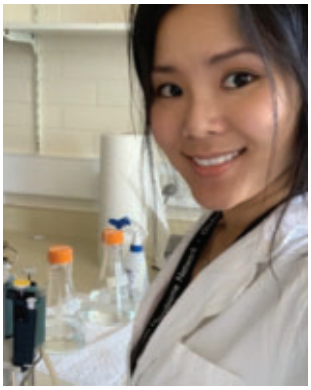
Supervisors: Dr Eileen Dunne[A,C] and Associate Professor Catherine Satzke[A,B,C]

[A]Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Vic., Australia; [B]Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, Vic., Australia; [C]Department of Paediatrics, The University of Melbourne, Parkville, Vic., Australia

Streptococcus pneumoniae (the pneumococcus) is the most common cause of community acquired pneumonia. However, it is also commonly found as an asymptomatic coloniser of the upper respiratory tract. It is not clear how this pathogen may change from the colonisation state to causing disease. This project investigated pneumococcal gene expression in the nasopharynx and the lung using a unique collection of paired nasopharyngeal swab and lung aspirate samples. It aimed to elucidate the molecular processes by which the pneumococcus can transition from the carriage to infection state, and identify potential genes involved in pathogenesis of pneumococcal pneumonia. RNA was extracted directly from clinical samples and transcriptomic changes were examined by reverse transcription quantitative PCR (RT-qPCR). Previously, expression of pneumococcal virulence genes has been examined using RNA from five pairs of culture-positive nasopharyngeal swabs and lung aspirates. For the remaining culture-negative clinical samples, sufficient pneumococcal RNA was obtained from nasopharyngeal swabs, but not from culture-negative lung aspirates, for RT-qPCR analysis. In the future, gene expression in the nasopharyngeal swabs from pneumonia patients will be compared with the swabs collected from healthy controls, to investigate whether the pneumococcus changes its behaviour dramatically in the nasopharynx of pneumonia patients compared with healthy controls.

Western Australia

Molecular characterisation of canine haemotropic *Mycoplasma* in Australia



Wenna Lee

Supervisors: Dr Charlotte Oskam, Dr Amanda Barbosa and Professor Peter Irwin

Murdoch University, WA, Australia

Emerging infectious diseases (EIDs) are a serious threat to public health and a quarter of EIDs are zoonotic, directly caused by arthropod vectors. However, efforts to control outbreaks of EIDs are sometimes limited by a lack of information, such as identification of aetiological agents, genetic diversity and transmission dynamics. Ticks are important arthropod vectors that transmit causative agents associated with tick-borne diseases (TBDs), such as *Babesia, Anaplasma* and *Mycoplasma* spp. This study aimed to molecularly characterise haemotropic Mycoplasma spp. in a group of dogs (blood samples $n = 10$) and haematophagous ticks (*Rhipicephalus sanguineus*; $n = 20$). While all ticks tested were negative for *Mycoplasma*, six out of 10 (60%) dogs screened were positive. Sanger sequencing revealed two dogs were infected with *Mycoplasma haemocanis* and four dogs were infected with *Mycoplasma haematoparvum*; both species were genetically identical (100%) to GenBank references. While *R. sanguineus* is a known vector for mycoplasmas overseas, *the R. sanguineus* analysed in this study were all negative. However, this is the first study to report 60% prevalence of mycoplasmas in dogs from an anonymous kennel.

Further research should focus on determining if the prevalence observed in this study is representative of other kennel dwelling dogs across Australia.

Bacteriophage to treat *Pseudomonas aeruginosa* infection in cystic fibrosis' patient lungs

Andrew Vaitekenas

Supervisors: Associate Professor Anthony Kicic and Dr Stephanie Trend

Telethon Kids Institute, WA, Australia

Cystic fibrosis is characterised by recurrent bacterial infections principally with *Pseudomonas aeruginosa*. Treatment of infections is currently possible, but antibiotic-resistant bacteria, of which *P. aeruginosa* is a high priority, threaten to make this impossible. Bacteriophages are candidates for the treatment of antibiotic-resistant infections. Propagation is one of the first steps in preparing bacteriophages for therapeutic purposes and screening. Commercial and academic sources of *P. aeruginosa* bacteriophages and propagation conditions were identified for later use. A stock of bacteriophage E79 had a titre of 7.2×10^6, ~2 logs less than when it was prepared in 2016, indicating that it was relatively stable at 4°C over time. High multiplicity of infections (the ratio of bacteriophage to bacteria; MOI) was tested in a pilot experiment, which suggested that smaller MOIs (e.g. 0.001) were optimum and that initially high bacteriophage density impeded production of progeny. Changes to the protocol that included MOIs from 0.1–0.001 and infection of log-phase bacteria, did not have a marked effect on E79 titre but could be implemented to decrease the time propagation takes.

Using bioinformatics to identify novel virulence factors responsible for the persistence and threat of penicillin-resistant serogroup W *Neisseria meningitidis* in Western Australia

Alicia Tan Yi Jia

Supervisors: Dr Shakeel Mowlaboccus and Associate Professor Charlene Kahler

School of Biomedical Sciences, The University of Western Australia, WA, Australia

Neisseria meningitidis causes invasive meningococcal disease (IMD), which presents as septicaemia and/or meningitis. During asymptomatic carriage of *N. meningitidis* in the nasopharynx, acquisition of DNA via homologous recombination from closely related bacteria is frequent, resulting in a highly dynamic population structure. In Western Australia, IMD is predominantly caused by MenW:cc11 meningococci that belongs to two phylogenetically distinct clusters, A and B, which contain penicillin-susceptible and penicillin-resistant isolates, respectively. Interestingly, Cluster B has expanded more rapidly than Cluster A. In this comparative genomics project, we identified horizontal gene transfer events responsible for the emergence and evolution of the penicillin-resistant meningococci. Potential novel virulence factors were described and the prevalence of novel virulence factors in other genetic lineages of *N. meningitidis* was also studied. Various virulence factors such as genes involved in metabolism, iron uptake and transport, amino acid biosynthesis, lipooligosaccharide assembly and modification were identified. Homology to proteins in commensals and pathogens colonising the nasopharynx was observed, with possible acquisition of virulence factors *via* homologous recombination. Possible factors responsible for the global increase in reduced penicillin susceptibility have also been defined. These factors should be studied in mutagenic assays and targeted to address the fitness and persistence of penicillin-resistant meningococci.

Who said this?

But when it has been shown by the researches of Pasteur that the septic property of the atmosphere depended not on the oxygen, or any gaseous constituent, but on minute organisms suspended in it, which owed their energy to their vitality , it occurred to me that decomposition in the injured part might be avoided without excluding the air, by applying as a dressing some material capable of destroying the life of the floating particles. Upon this principle I have based a practice.

Who else but Sir Joseph Lister (1827–1912), a British surgeon and a pioneer of antiseptic surgery. While working at Glasgow Infirmary, Lister introduced the practice of sterilising surgical instruments and cleaning wounds with carbolic acid, now known as phenol. Lister had observed that the stench of fields irrigated with sewage was reduced by the use of carbolic acid. Cows subsequently grazing on the grass did not appear to suffer any side-effects so he concluded it would be a safe effective disinfectant. No TGA in those days!

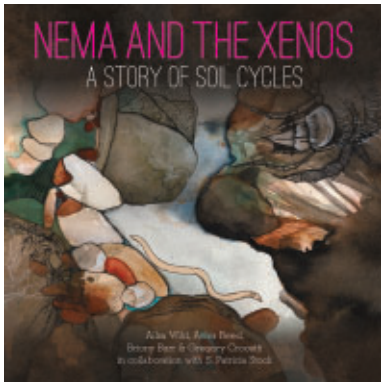
His patients suffered fewer post-operative infections and he became Britain's leading surgical authority, so much so that even in his late years, after suffering a stroke, he was consulted by surgeons called on to carry out an emergency appendectomy on King Edward VII, two days before his scheduled coronation. Lister advised them in the latest antiseptic surgical methods, which they followed, and the King survived.

Brought to you by the History SIG.

Book review

Nema and the Xenos. A Story of Soil Cycles

Ailsa Wild, Aviva Reed, Briony Barr and Gregory Crocetti in collaboration with S Patricia Stock
CSIRO Publishing, 2019



What goes on in the below-ground ecosystem of a tree? Many of the answers are revealed in this stimulating book, targeted to audiences that include the very young. My review included a 'field test' on my 4-year-old grandson, Isaiah. He was highly attracted to the story and wants me to read it each time I see him now.

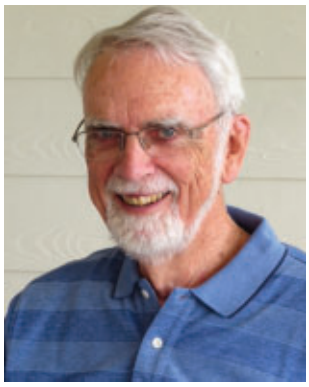
The story commences with the attack on a tree's roots by a grub. Chemical 'pain' signals released by the tree attract nematodes who make a perilous underground journey, with one nematode being lassoed by lasso fungi. So impressed by this, Isaiah now involves the lasso fungi in games with his kinder playmates. The main nematode, Nema, survives carrying its cargo of Xenos (xeno bacteria) on the journey to the grub. Along the way there are many other lifeforms in the soil including a worm, algae (surviving close to the soil surface), tardigrade (water bear), white fungus and soil bacteria. The stockpile of chemical weapons is shown, including their structures. Reaching the grub, the xenos are released to feast on the grub, which then become the nematode feast. The life cycle continues.

This 33-page story could be appreciated by anyone with a fascination for living systems, and from Isaiah's response is an excellent introduction to microbial ecology.

Following the story is a 16-page resource section that shows pictures of the actual story characters, as well as others that live in soil. The size differences are highlighted with examples including larger soil animals (moles, ants, earthworms etc.) to smaller soil animals (e.g. nematodes, mites, tardigrades etc.) to the microscopic life (e.g. protozoa, fungi, algae, bacteria etc.). This book follows two others in the series: *Zobi and the Zoox; A Story of Coral Bleaching* and *The Squid, The Vibrio & The Moon*. The book is well illustrated and would be very useful in the classroom, including in early primary education, stimulating early awareness of the interactions and diversity of life. I think it has given my 4-year-old grandson an unusual (for his age) introduction and awareness of microbial ecology. Thanks to all those involved in creating this book, including ASM. It is a great resource to our kids and may encourage them to pursue microbiology, or at least to think more about how microbes contribute to life's intricate ecosystems.

Professor Ian Macreadie
School of Science
RMIT University
Melbourne, Australia

Vale Dr Brian Mee



Brian was a generous mentor, a teacher with an amazing depth of microbiological knowledge, and a good friend to his academic and technical colleagues in the Department of Microbiology at UWA.

Brian received his PhD from Melbourne University in 1968 on his thesis entitled 'Biosynthesis of histidine in *Pseudomonas aeruginosa*'. He followed this with a post-doctoral position at Columbia University, College of Physicians and Surgeons, Department of Biochemistry where he continued to work on bacterial genetics.

In 1971, he started as a lecturer at the University of WA Microbiology Department, situated at Royal Perth Hospital, which then moved to its current location at the Queen Elizabeth II Medical Centre in Nedlands in 1973.

At the time, there was almost no bacteriology in 3rd year microbiology and Brian was instrumental in setting up these elements of the major. As a teacher Brian had a huge influence on the structure and content of the courses in microbiology, especially in bacteriology and genetics, and more recently in molecular biology. Many of the experiments he introduced into undergraduate laboratory classes are still in use today. He had a deep knowledge and understanding of bacterial genetics, physiology and biochemistry, and was the person everyone went to for advice and explanations of tricky points. You'd know that if you went to Brian asking for help, he would provide his time and expertise with great generosity, and that you could confidently rely on him knowing the answer!

In research, Brian supervised and mentored many Honours and PhD students. He inspired generations of students to pursue research, and many of these have gone on to great careers in Perth and elsewhere in the world. He published widely in aspects of bacteriology ranging from R plasmids to environmental biofilms, bacterial virulence factors to transcriptional response regulators, and melioidosis to *Helicobacter*.

Brian contributed much to the development of microbiology in Perth, being active in the Australian Society for Microbiology, and President of the WA branch for two years in the mid-1980s. He had numerous sabbaticals around the world at Bristol University, Nottingham University, Southampton University and McGill University.

He later retired to Denmark in WA in 2006. Brian will be very much missed by his family and by former colleagues in microbiology.

UNIVERSIDAD DE LA FRONTERA

BIOREN - UFRO
Scientific and Technological Bioresource Nucleus

CCCT
Chilean Culture Collection of Type Strains - CCCT

WFCC
World Federation for Culture Collections

15th International Conference on Culture Collections - ICCC15

Universidad de La Frontera, Campus Pucón, CHILE
25th – 29th November 2019

The Chilean Culture Collection of Type Strains - CCCT, hosted at the Scientific and Technological Bioresource Nucleus BIOREN-UFRO, of the Universidad de La Frontera (Chile) has the honour of announce the ***15th International Conference on Culture Collections (ICCC-15)***, which will be held in the ***Campus of Pucón, Chile from 25th to 29th November 2019***.

It is the third time that an ICCC Conference is organised in Latin-America. Both previous Conferences were organised in Brazil: ICCC2 in 1973 and ICCC13 in 2010. Organising the ICCC15 in Chile means an important achievement for the whole Spanish speaking Latin-American Countries.

The scientific programme has been organised in order to provide the latest developments on the different domains of Culture Collections, as well as in the related scientific fields of the Microbiology and Biotechnology, taking into consideration the motto of the ICCC15 Conference.

Over 4.5 working days of the ICCC15 Conference, the event will be aligned in balance with scientific sessions including keynotes, round tables, training courses and social events.

Pucón is an Andean Mountain Chilean city. It is the main door to the **Chilean Patagonia**. Pucón is located on the eastern shore of Lake Villarrica, and Villarrica Volcano. Pucón's location by a lake and a volcano, along with its relatively stable climate, especially in summer, make it a popular destination for tourists. It offers a variety of sports and adventure/ recreational activities for tourists, including water skiing, snow skiing, backpacking, white water rafting and kayaking, horseback riding, natural hot springs, zip line rides, skydiving and guided ascents of Villarrica volcano.

To travel to Chile, participants should take flights to La Araucanía Airport (ZCO). Then, participants can travel by taxi or transfer from the La Araucanía Airport to Pucón. The taxi fare is c.a. 25 Euros and takes 40 min. Additional information and Scientific Programme of the ICCC15 Conference is available at: www.iccc15.ufro.cl

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