

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



Wieland Meyer^{A,E}, Ian Arthur^B, David Ellis^C, Alex Kan^A, Sarab Kidd^C, Krystina Maszewska^A and Kerry Weeks^D

^AMolecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Faculty of Medicine and Health, Sydney Medical School, Westmead Clinical School, Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Westmead Hospital (Research and Education Network), Westmead Institute for Medical Research, Westmead, NSW 2145, Australia

^BMycology Laboratory, Department of Microbiology and Infectious Diseases, PathWest Laboratory Medicine WA, QEII Medical Centre, Nedlands, WA 6009, Australia

^CNational Mycology Reference Centre, Microbiology and Infectious Diseases, SA Pathology, Adelaide, SA 5000, Australia

^DAustralian National Reference Laboratory in Medical Mycology, Royal North Shore Hospital, NSW Health Pathology, St Leonards, NSW 2065, Australia

^EEmail: wieland.meyer@sydney.edu.au

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 Karl¹. 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 Netherlands¹.

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 *Metarbizium anisopliae* species complex implemented in human disease².

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 Australia⁴.

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 species⁵.

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 analysis⁶.

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 complexes⁷. 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 barcode⁸) or the *translocation elongation factor 1 α* (*TEF1 α*) (secondary fungal DNA barcode⁹), 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 barcode⁸) and the *TEF1α* (secondary fungal DNA barcode⁹), and its 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 meta-data, 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.

References

1. von Arx, J.A. *et al.* (1978) The CBS fungus collection. *Adv. Appl. Microbiol.* **24**, 215–236. doi:10.1016/S0065-2164(08)70641-5
2. Nourrisson, C. *et al.* (2017) Species of *Metarbizium anisopliae* complex implicated in human infections; retrospective sequencing study. *Clin. Microbiol. Infect.* **23**, 994–999. doi:10.1016/j.cmi.2017.05.001
3. Ellis, D. (1987) *Cryptococcus neoformans* var. *gattii* in Australia. *J. Clin. Microbiol.* **25**, 430–431. doi:0095-1137/87/020430-02
4. Kidd, S.E. *et al.* (2015) Multi-triazole-resistant *Aspergillus fumigatus* infections in Australia. *Mycoses* **58**, 350–355. doi:10.1111/myc.12324
5. Espinel-Ingroff, A. *et al.* (2015) Multicenter study of epidemiological cutoff values and detection of resistance in *Candida* spp. to anidulafungin, caspofungin, and micafungin using the Sensititre YeastOne colorimetric method. *Antimicrob. Agents Chemother.* **59**, 6725–6732. doi:10.1128/AAC.01250-15
6. New, D. *et al.* (2018) Identification of multiple species and subpopulations among Australian clinical *Sporobrix* isolates using whole genome sequencing. *Med. Mycol.* **2018**, 30. doi:10.1093/mmy/myy126
7. Meyer, W. *et al.* (2009) Consensus multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococcus gattii*. *Med. Mycol.* **47**, 561–570. doi:10.1080/13693780902953886
8. Schoch, C.L. *et al.* (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci. USA* **109**, 6241–6246. doi:10.1073/pnas.1117018109
9. Stielow, J.B. *et al.* (2015) One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia* **35**, 242–263. doi:10.3767/003158515X689135
10. Firacative, C. *et al.* (2016) MLST and whole-genome-based population analysis of *Cryptococcus gattii* VGIII links clinical, veterinary and environmental strains, and reveals divergent serotype specific sub-populations and distant ancestors. *PLoS Negl. Trop. Dis.* **10**, e0004861. doi:10.1371/journal.pntd.0004861
11. Bernhardt, A. *et al.* (2013) Multilocus sequence typing of *Scedosporium apiospermum* and *Pseudallescheria boydii* isolates from cystic fibrosis patients. *J. Cyst. Fibros.* **12**, 592–598. doi:10.1016/j.jcf.2013.05.007
12. Irinyi, L. *et al.* (2015) International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database - the quality controlled standard tool for routine identification of human and animal pathogenic fungi. *Med. Mycol.* **53**, 313–337. doi:10.1093/mmy/myv008
13. Meyer, W. *et al.* (2019) Database establishment for the secondary fungal DNA barcode *translational elongation factor 1α* (*TEF1α*). *Genome* **62**, 160–169. doi:10.1139/gen-2018-0083

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 *URA5-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 EF1 α 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



Roger G Shivas^{A,C}, Dean R Beasley^A, Kaylene Bransgrove^A, Yu Pei Tan^A and Geoff Bulow^B

^AQueensland Plant Pathology Herbarium (BRIP), Department of Agriculture and Fisheries, Ecosciences Precinct, Dutton Park, Qld 4102, Australia

^BTechnology Commercialisation Fund Project (TCF), Department of Agriculture and Fisheries, 41 George Street, Brisbane, Qld 4000, Australia

^CEmail: roger.shivas@daf.qld.gov.au

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.