
The AWRI Wine Microorganism Culture Collection

What is the AWMCC?

The AWRI Wine Microorganism Culture Collection (AWMCC) is the largest repository of wine-associated yeast and bacteria in the southern hemisphere. It provides the Australian wine industry with novel, non-commercially available yeast and bacterial winemaking strains for efficient and reliable fermentations and to help shape and diversify wine style. The AWMCC is also essential for capturing the value of Australia's investment in microbial strain isolation and development, especially given the current focus on bioprospecting and generating Australian microbial isolates. It is fundamental to the success of wine-related biological research projects, which depend on ready access to correctly identified strains.

The AWMCC:

- is a secure state-of-art collection of Australian wine-associated yeast and bacterial strains that have been identified to genus and species levels using molecular and/or biochemical techniques
- provides yeast and bacterial strains for winemaking, research and quality assurance
- provides quality assurance, to internationally recognised standards, of strains deposited in the collection
- includes a secure off-site duplicate collection
- provides assistance with identifying yeast and bacterial strains.

What types of organisms are stored in the AWMCC?

Microbial strains from industry and research projects are continually being identified and added to the AWMCC, together with relevant supporting information. The AWMCC now contains more than 10,000 wine-related microorganisms with some Australian isolates dating back almost 80 years. One of the most commonly requested strains (AWRI 81) is a flor yeast that was isolated in 1940. From 2013 to 2017, 1,301 new strains sourced from researchers and industry were added to the collection and 2,423 strains were supplied from the collection to industry and researchers.

The collection contains:

- more than 3,000 natural yeast isolates for winemaking and laboratory-modified yeast strains for research
- more than 1,100 bacterial strains, the majority of which are malolactic bacteria
- yeast genome deletion libraries of more than 6,000 strains for research.

How does the AWMCC operate?

The technology

Microorganisms deposited in the collection are stored at -80°C in specialised tubes containing small ceramic beads immersed in a protective solution. The microorganisms coat the beads during the freezing process and can remain viable and stable for decades. When a microbe is requested by winemakers or researchers, a single bead is recovered and a new culture started. By returning to the original tube each time, the chance of contamination is reduced, and the drift in behaviour seen when active cultures are constantly regenerated is minimised. Each tube can be used for up to 20 subcultures before a fresh tube needs to be prepared. The AWRI's -80°C freezer is backed up by a secure off-site freezer which contains a duplicate version of the collection for long-term safekeeping.

Receival of microbes at the AWMCC

Strains are received throughout the year from researchers and from winemakers during the vintage period. When samples are received, whether from a ferment, cultured on media or freeze-dried, the microbes of interest are isolated on selected agar and purified. Each pure culture is given a unique AWRI number and physically placed into the collection at -80°C . The unique number ensures that each strain can be accurately traced in the future, even if the species name or other associated information changes or is updated.

Identification of microbes

Microbes submitted to the collection undergo an identification process; however, the AWMCC service does not deal routinely with problem-solving issues. Winemakers who are experiencing issues with microbial contamination should contact the AWRI helpdesk on helpdesk@awri.com.au or 08 8313 6600 for assistance with identifying the organisms responsible for the contamination.

Yeasts submitted to the collection are identified using microscopy, morphology and DNA sequencing, with sequences compared to international databases and the closest match used as the species name. Bacteria are identified via microscopy, morphology and biochemical testing. It is hoped that molecular identification of bacterial species through DNA sequencing will soon be used in addition to the current methods. Microorganism species identification is a dynamic area and names are constantly being updated, so names of cultures in the collection are periodically updated with new species designations. Sometimes requests are made for a species of yeast that is no longer valid (for example *Saccharomyces bayanus* has been reclassified as *Saccharomyces uvarum*), so using the AWRI number is the best way to identify required microbial strains.

Providing cultures to industry and researchers

When a request for a culture is received, a sample is retrieved from the -80°C freezer and plated onto nutrient agar to grow the microbe. After it has been confirmed that there is no contamination, the microbe is transferred to a nutrient agar slope for further growth. After it has grown the slope is sent out via Express Post or courier. Attempts are made to schedule the delivery to fall on a week day to avoid the culture sitting at the post office over the weekend. Given that live cultures may experience some deterioration during transit, a back-up culture is prepared ready to send again quickly if anything goes wrong. When the slope is received, it should be used as soon as possible. Storage in the fridge may be ok for up to two weeks depending on the type of culture, although this is not recommended for bacteria.

Turnaround times for the provision of cultures are nominally two weeks; however, some microbes (particularly yeast) may be able to be delivered more quickly. If a request is urgent, attempts will be made to send out the culture as soon as possible. The cost to retrieve a culture is up to \$50 for levy payers or Australian research institutions and \$90 for all other recipients. If the strain is being requested by the person who originally deposited it into the collection, the requester may be eligible for two free retrievals per year.

How does the AWMCC assist wine research in Australia?

The AWMCC has been crucial for several successful research projects by providing access to the genetic diversity of microorganisms collected over many regions and many decades. When a new research project requires a large number of strains it is rarely feasible to collect and identify sufficient numbers of strains on demand, or even within the life of the project. The number of suitable isolates that becomes available from industry during a single vintage

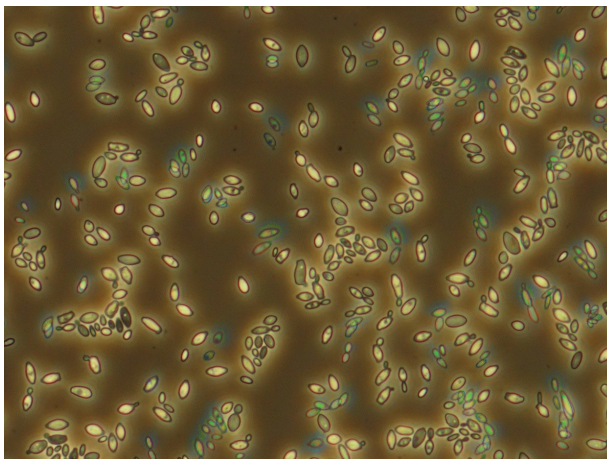


Figure 1. *Brettanomyces bruxellensis* under a microscope at 400x magnification.

can vary widely. Having ‘ready-to-go’ candidate strains previously deposited over many years can be critical in determining whether a research project is viable. Many strains in the collection pre-date the currently available commercial strains and thus provide a depth and complexity for genetic comparisons that would otherwise not be available.

What benefits are there for the winemaker in depositing strains?

There are several advantages in depositing and storing yeast and bacterial strains in the AWMCC rather than storing them in-house. These include: reduced expenses, identification of the microorganisms and long-term maintenance of deposited strains by expert microbiology staff in appropriate storage conditions.

If a winemaker identifies a wine that has interesting microbiological properties (e.g. favourable alcoholic or malolactic fermentation behaviour, or perhaps spoilage occurring pre-bottling) they are encouraged to contact Simon Dillon or Angus Forgan on culture@awri.com.au or 08 8313 6600 to provide some details about the situation. One of the team members can discuss whether storage in the AWMCC is a good option and assist with submitting a sample in the best possible condition. Typically, strains deposited in the AWMCC are done so under a Material Transfer Agreement that grants the AWRI permission to use the strain for research and extension activities.

Conclusion

Backed by the Australian wine industry, the AWMCC has grown into a large, genetically diverse and operationally secure microorganism collection. Future research projects, particularly those involving bioprospecting and metagenomics approaches for the study of microbial diversity, are expected to deliver several thousand more isolates to the collection in the coming years. To ensure efficient operation and enable support for complex experimental designs involving hundreds of strains, new high-throughput storage, handling and culturing tools, combined with latest generation genetic sequencing and data mining technologies, will be incorporated. This will improve handling of large numbers of isolates and help capture the full value of this resource for the benefit of the Australian wine community.

More information about the services available from the AWMCC can be found on the AWRI website: www.awri.com.au/research_and_development/wine-microorganism/winemaking-yeast-and-bacterial-strains/

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