

Antarctic metagenomics



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The DNA sequencing of whole environmental samples (referred to as metagenomics, environmental genomics, ecogenomics or community genomics) captures genome surveys of uncultured microorganisms. By moving away from a species-by-species investigation of the environment, metagenomics enables a greatly expanded view of the diversity and metabolic function of the resident microbial communities. The southern polar region plays a critical role in maintaining microbial processes that are essential for the health of the world's ecosystems, yet we know less about microorganisms on the vast Antarctic continent than we do about the mould in our refrigerators. Metagenomics offers an unprecedented means to reveal the secrets of Antarctic microorganisms and to learn about the unique, cold-adapted processes that they perform.

From genome to metagenome using random shotgun sequencing

The first complete genome sequence of an organism, 1.8 million base pairs for *Haemophilus influenzae*, was published in 1995¹. The approach used to sequence the genome was random shotgun sequencing. This approach relies on the generation of a redundant coverage of a genome (e.g. 5–10 times the size of the genome) to enable overlapping DNA sequences to extend around the entire genome. Despite being more than 1000 times larger, random shotgun sequencing was subsequently used to generate the 2.9 billion base pair human genome, and the paper describing this was published just six years after the *H. influenzae* genome in 2001².

MICRO-FACT

For a controversial read, see Pace NR. Time for a change. *Nature*, 2006; 441(7091):289.

The demonstration that shotgun sequencing could be applied to biological complexity as high as the human genome provided confidence that it could be used for sequencing whole environmental samples of microbial communities. In 2004 two papers described large scale metagenome studies: 76.2 million base pairs for a low complexity, acid mine drainage community from Iron Mountain³, and 1.04 billion base pairs for a moderate complexity Sargasso Sea community⁴. These large scale sequencing efforts have since been applied to a number of diverse environmental samples including, the human gut⁵, symbionts of a marine worm⁶, sludge⁷, soil⁸, water from an iron mine⁹, whale-fall⁸ and the ocean¹⁰⁻¹². In March 2007, a collection of papers in *PLOS Biology* (<http://collections.plos.org/plosbiology/gos-2007.php>) described the analysis of 6.3 billion base pairs of metagenome data collected from ~50 sites from the Atlantic and Pacific oceans. As a continuation of the Sargasso Sea study⁴, this Global Ocean Survey (GOS) uncovered nearly 6 million genes (more than the total number of genes known prior to the study). The study also demonstrated that new genes continued to be discovered at a rate that was directly proportional to the extent of sequence coverage. In other words, clearly showing that the combined efforts of all DNA sequencing performed to date has only just scratched the surface of life's collective genetic code.

The cold biosphere: an ecosystem particularly critical to the health of the planet

Psychrophilic microorganisms proliferate in cold environments including sub-zero temperatures^{13,14} and have recently been suggested to be able to metabolise, albeit at very low rates at temperatures down to -196°C¹⁵. Most (~75%) of the earth's biosphere is cold, and psychrophiles can be found in permanently cold (e.g. polar and deep ocean habitats), seasonally and artificially cold environments. Despite the fundamental role that the cold biosphere plays in maintaining the health of the planet, relatively little is known about the resident microorganisms and the biogeochemical processes they drive^{13,14}.

It has only recently been appreciated that archaea perform diverse functional roles in a wide range of cold environments¹⁴. The extent to which they transform the cold biosphere can be appreciated from their phylogenetic and functional diversity, abundance, and range of cold biotopes they inhabit. Psychrophilic archaea contribute to global energy cycles through the processing of organic and inorganic carbon and nitrogen compounds; this

occurs at all levels, from the cycling of simple carbon compounds to anaerobic ammonia oxidation to transformation of complex biomolecules¹⁴. Metagenomic based studies have proven crucial in establishing the role of specific microbial groups in driving biogeochemical processes (e.g. Schleper *et al*¹⁶). For example, the important role that Crenarchaeota play in the nitrification of soil and ocean waters through the oxidation of ammonia was greatly facilitated by metagenomic studies (reviewed in Cavicchioli *et al*)¹⁷.

Antarctic metagenomics

Antarctica is the fifth largest continent and the Australian Antarctic Territory represents around 42% of this large land and ice mass. While the integration of metagenomic data with ecological principles and physical, chemical and earth sciences represents one of the biggest challenges in modern ecology, it also provides the greatest opportunities for facilitating effective ecosystem management^{18,19}, including the Antarctic environment²⁰⁻²³. Metagenomics will play a major role in unravelling the secrets of Antarctic biology.

Permanent Antarctic stations with laboratory facilities exist at Casey and Davis to facilitate scientific research (<http://www.aad.gov.au/>). Both stations are situated within coastal areas of Antarctica within part of the small area (<0.4%) of Antarctica that is not permanently covered by ice, and offer unique opportunities for studying Antarctic biology.

Lakes in the Vestfold Hills

Davis is situated in the Vestfold Hills; a region that contains an immense variety of lake systems²⁴. More than 300 lakes and ponds from freshwater (<0.1%) to hypersaline (32%) are present, including ~20% of the world's meromictic lakes. Meromictic lakes contain water (normally anoxic bottom waters) that never mixes with the remaining lake water and thus provide stratified habitats. In the Vestfold Hills, meromictic lakes (e.g. Ace Lake and Organic Lake) and highly saline mixed lakes (e.g. Deep Lake) are marine derived, having been isolated from the ocean about 5000 years ago. As a result the lakes offer a variety of model systems for studying the evolution of marine microorganisms. Moreover, lake systems that impose harsh conditions for growth and border on being able to sustain life, provide opportunities to define biological properties required for surviving at the very extreme limits of life.

First visited by scientists in 1974, Ace Lake is the most intensively studied meromictic lake in Antarctica, and probably the world^{25,26}. The saline stratification maintains an upper zone that is rich in oxygen, through to permanently cold (1–2°C) anoxic bottom waters and sediment that support a methane cycle that has existed for 3,000 years. Viruses, protists, copepods, archaea and bacteria are present, and the biogeochemistry has been carefully examined²⁵. Lipid, carotenoid and 16S rRNA analyses have identified the presence of unusual biomarkers in the lake, in addition to the detection of archaea from 10,000-year-old sediment samples and a range of faunal microfossils^{27,28}.

Genome sequence data is available for two methanogenic archaea from Ace Lake, *Metanococcoides burtonii* and *Metanogenium frigidum*²⁹. Building on findings from genomic studies and a large number of laboratory-based studies (reviewed in¹⁴), a broad range of questions have been generated that can only be addressed by performing metagenomics and associated functional studies on samples from Ace Lake^{14,19}. For example, it was previously shown that transposons are expressed at 4°C under laboratory growth conditions³⁰, and metaproteomic and metagenomic studies will help to determine whether transposons are expressed in the environment and how they affect the overall genetic microheterogeneity of the natural population of *M. burtonii*. Previous studies of Ace Lake identified a large proportion of novel archaeal 16S rRNA sequences that were not related to known classes of methanogens^{26,27}. Metagenomics will enable the functional capacity of the novel archaea to be assessed, and to place this in context of the roles that *M. burtonii* and *M. frigidum* play in the ecology of the Antarctic lake.

To facilitate metagenomics, in December 2006 an expedition collected samples from Ace, Deep and Organic lakes (Figure 1). This research program is funded by the Australian Research Council, and the Australian Government Antarctic Division (AGAD) which provided logistical support for the expedition. DNA from the samples is currently being sequenced at the Department of Energy Joint Genome Institute (DOE-JGI) and at the J Craig Venter Institute (JCVI) in the USA, and metaproteomics is being performed at the Bioanalytical Mass Spectrometry Facility (<http://www.bmsf.unsw.edu.au/>) at UNSW. It is expected that these studies will provide a profound understanding of the ecology, adaptation biology and undiscovered properties of cold adapted microbes in the lakes, delivering a new understanding of the fundamental biology of psychrophiles and greatly improved knowledge of Antarctic microbial processes (e.g. methane and sulfur cycles).

MICRO-FACT

Carl Woese was the originator of the RNA world hypothesis in 1967.

MICRO-FACT

The Great Barrier Reef is the largest, and one of the most biological diverse reef systems of its kind worldwide.

Southern Ocean studies

As a direct consequence of climate change, the quantity of sea ice surrounding Antarctica has declined by ~20% since the 1950s resulting in major changes to the ecology of the region^{31,32}. Impacts on upper trophic levels (e.g. krill to whales) are evident; however, the impacts on the microbial populations are not well documented. A metagenomics based program (DNA sequencing at the DOE-JGI) will compare winter and summer archaea and bacteria to better understand the genomic and functional differences in the populations that occur during these seasons (<http://www.jgi.doe.gov/sequencing/why/CSP2006/>

[antarctic.html](http://www.jgi.doe.gov/sequencing/why/CSP2006/antarctic.html)). This will provide information on the diversity, energy generating processes and adaptive capabilities of the microorganisms and enable predictions to be made about the impact of continued global warming.

As part of the International Polar Year program (IPY 2007–08), the research forms an important component of the Census of Antarctic Marine Life (CAML; <http://www.caml.aq/>), an international cooperative project that will investigate the distribution and abundance of Antarctica's marine biodiversity and how it is affected by climate change. In other work planned during the IPY Southern Ocean voyages, opportunities to collect microbial associates with benthic species (e.g. sponges) and additional open ocean microbial samples, will create synergies of discovery between trophic levels that should greatly assist the CAML program.



Figure 1. Sampling at Ace Lake for metagenomics and metaproteomics. A) Voyage 2 south on the Aurora Australis. B) The field site on Ace Lake. C) Using equipment from the Sorcerer II GOS, microbial biomass was sampled from various depths throughout Ace Lake and collected sequentially onto 3, 0.8 and 0.1 μm filters. D) The 0.1 μm filtrate was concentrated using a 50kDa tangential flow filter to capture very small cells and viruses. E) Biomass from the bottom of the lake was sampled.

A diverse range of Antarctic metagenomic opportunities

The Vestfold Hills lakes and Southern Ocean metagenomics programs provide important precedents for the capacity to perform Antarctic metagenomics. In order to better define Antarctic microbial diversity and mechanisms of adaptation, an expansion of metagenomic analyses to other Antarctic regions and different types of ecosystems is needed. Potential metagenomic projects may be considered within the scope of priority programs of Australia's current Antarctic Science Strategy (<http://www.aad.gov.au/default.asp?casid=13949>). Following are a few examples that fall within two of the priority programs.

I) Adaptation to environmental change

- Examining communities indigenous to Casey and Davis will enable comparisons between ice-free regions that are separated by more than 1000 km. Addressing questions as fundamental as this will allow the first insight into the scope of diversity within similar but geographically separated areas.
- Unique communities are likely to exist within glacial ice and lake sediment dating back thousands of years. Using methods of DNA extraction specially designed for extracting ancient DNA, such as those devised at the Australian Centre for Ancient DNA (<http://www.adelaide.edu.au/acad/>), insight into the evolution of microorganisms on the Antarctic continent will be gained, and the changes in microbial populations will be able to be modelled with global paleontological changes.
- In addition to ancient communities, metagenomic analysis holds great potential for examining transient communities. A good example is the dense microbial communities that form in hypersaline pools on the shallow seabed below sea-ice (M. Riddle, personal communication). These microbial blooms appear to develop before the sea ice melts in summer while waters remain sufficiently calm to maintain stratifications of semi-anaerobic, pools of brine that have formed as a result of salt exclusion from the sea ice.
- Epiglacial lakes form on the down, ice flow side of mountains that penetrate the polar ice cap as a result of glacier ice melt. They appear to be a common lake type in Antarctica, but are surprisingly little studied³⁵. It is not known whether the organisms that inhabit the lake are recent (post-glacial) or ancient inhabitants of Antarctica. Epiglacial lakes in the Framnes Mountains (~30 km inland from Mawson Station) are permanently covered by up to 5 m of ice, cold (<0.2°C),

MICRO-FACT

Coral is a symbiotic relationship between an animal and its symbiotic partners (eukaryotic, bacterial, archaeal and viral).

slightly brackish, well mixed, oxygenated and have a high pH (~11). Preliminary studies have identified cyanobacterial mats and a variety of metazoans (e.g. nematodes, tardigrades and rotifers). Given the abundance of these types of lakes and the distinct lack of knowledge about the diversity and metabolic capacity of the resident organisms, metagenomic studies would greatly improve understanding of the microbial component.

- Linking microbial populations to higher trophic levels will facilitate a greatly improved understanding of the food chain and biological web of life. Metagenomics has proven valuable for studying the microbial populations of the human gut⁵ and symbionts of a marine worm⁶; therefore, the approach holds great potential for probing the biotransformation processes driven by microorganisms resident in krill, fish, penguins, seals and whales.
- Metagenomics has been applied to studying the unculturable microbial residents (Crenarchaeota) of marine sponges³⁴. In view of the discovery of similar archaea in Antarctic sponges³⁵, a good opportunity exists to use comparative metagenomics to obtain insight into cold adaptation. Under sea ice near Casey (<http://www.aad.gov.au/default.asp?casid=4353>) and below the Amery ice shelf (<http://www.aad.gov.au/default.asp?casid=29216>), the sea floor is teeming with sponges. Metagenomics holds great potential for studying microbial communities that are locked away within these sponges.

II) Impacts of human activities in Antarctica

- Metagenomics has a real capacity to facilitate bioremediation of contaminated sites. Studies are already underway to compare marine benthic communities of plants and animals at sites close to old refuse tips with sites nearby that are outside the influence of contamination (<http://www.aad.gov.au/default.asp?casid=6754>). Research is also addressing microbial processes in remediation of both contaminated terrestrial soils and marine sediments (<http://www.aad.gov.au/default.asp?casid=22397>), and toxicity tests are being performed to determine acceptable levels of soil contamination in Antarctic environments (<http://www.aad.gov.au/default.asp?casid=3113>). Integrating this information with metagenomics of microbial communities will not only identify how communities change as a result

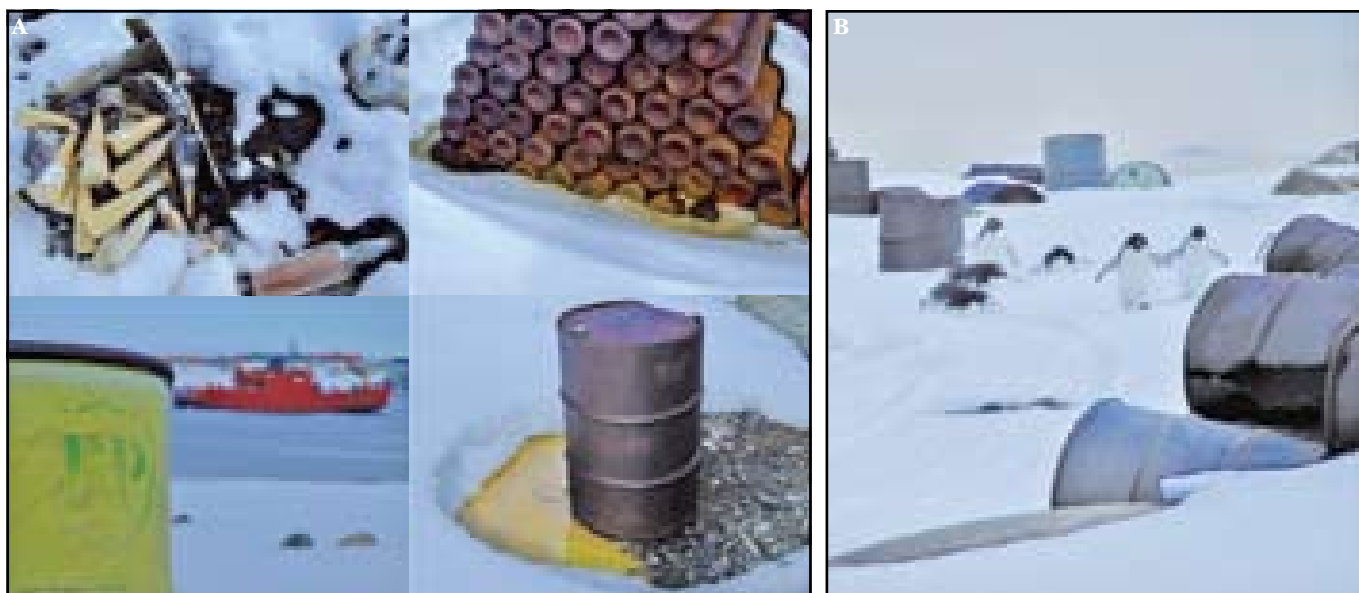


Figure 2. Sites at Wilkes Station where metagenomics may facilitate bioremediation efforts. A) Rubbish includes domestic waste, gas cylinders and petroleum drums. The BP drum is shown at the contaminated site relative to the Aurora Australis and Casey Research Station. B) Adelie penguins traversing through a waste site at Wilkes.

of contamination, but provide further avenues for the development of bioremediation regimes that function effectively in the Antarctic environment. While remediation of contaminated sites is underway (<http://www.aad.gov.au/default.asp?casid=3115>), there is a great need for this to be advanced. A good example of this need is the contamination that occurs at Wilkes Station, near Casey Research Station (Figure 2). Wilkes was first settled by the US in 1957 and transferred to Australia in 1959 (<http://www.aad.gov.au/default.asp?casid=1727>). Rubbish, including more than 3000 petroleum drums, was accumulated over 12 years of occupation and remains as a stark reminder of human activities.

In addition to research within the immediate scope of the AGAD priority programs, Antarctic metagenomics provides opportunities for bioprospecting. This may include the identification of novel, cold-active biological components, such as enzymes. The important commercial value of cold-active enzymes derives from their high activity at low temperature, which provides reduced energy costs for processes that would otherwise require heating (e.g. cleaning processes), or facilitates processes that must be performed at low temperature (e.g. food) or for practical reasons cannot be heated (e.g. biofilm remediation of water pipes)^{13,36,37}. The use of metagenomics for identifying novel

biocatalysts testifies to the strength of this approach³⁸⁻⁴⁴. The large sequencing houses (e.g. DOE-JGI, JCVI) that generate metagenomic data have strict policies ensuring the data is publicly released (www.sorcerer2expedition.org; <http://www.jgi.doe.gov/sequencing/collaborators/datarelease.html>), and much discussion has arisen surrounding intellectual property⁴⁴. As the majority of international chemical companies are heavily investing in white biotechnology that will slowly replace harsh chemical processes with softer enzyme-based methods^{41,45}, it is important to carefully consider how best to foster the biotechnological development of Antarctica's biological resource⁴⁶ (<http://www.aad.gov.au/default.asp?casid=14689>).

Conclusion

Metagenomics holds great potential for expanding our knowledge of Antarctic microorganisms. Linked to functional studies (e.g. metaproteomics), physical and geochemical data, an integrated view of microbial ecology will be able to be derived that will provide a greatly improved understanding of how the microorganisms have evolved, transformed and presently interact with the Antarctic environment. Facilitating this science in Australia will require significant investment from the Australian Government. To promote this, the Australian Genome Alliance has been established to lobby the Federal Government to establish a new genomics research fund to ensure that Australia's unique genetic heritage is harvested by Australia and not by other nations (<http://www.genomealliance.org.au/>).

MICRO-FACT

Scientists have predicted that over 50% of the coral reefs in the world may be destroyed by the year 2030.

MICRO-FACT

Over 700 bacterial taxa inhabit the oral cavity making it one of the most bacterially biodiverse regions of the human body.

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MICRO-FACT

Biofilms are essential for the wellbeing of the planet by driving biogeochemical cycles and other biotransformation processes.