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COMMENTARY

Antarctic subglacial lake exploration: a new frontier in microbial ecology

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To date, wherever life has been sought on Earth, it has almost always been found—from high in the stratosphere (Imshenetskii *et al.*, 1975, 1978, 1986; Wainwright *et al.*, 2003) to deep in the ocean trenches (Takamia *et al.*, 1997; D'Hondt *et al.*, 2004) and even within the Earth's crust itself (Pedersen, 2000). Microorganisms have also been found in some of the most extreme environments. They have been found to exist in ice, boiling water, acid, salt crystals, toxic waste and even in the water cores of nuclear reactors (Rothschild and Mancinelli, 2001).

Antarctic subglacial lake ecosystems have the potential to be one of the most extreme environments on Earth, with combined stresses of high pressure, low temperature, permanent darkness, low-nutrient availability and oxygen concentrations derived from the ice that provided the original meltwater (Siegert *et al.*, 2003), where the predominant mode of nutrition is likely to be chemoautotrophic. Yet, to date, the identification of significant subglacial bacterial activity in the Arctic, beneath glaciers (Skidmore *et al.*, 2000, 2005) and in subglacial lakes (Gaidos *et al.*, 2004), as well as extensive work on permafrost communities and work in the deep sea, suggests that life can survive and potentially thrive in these types of environment. Microbial life has been shown to function at gigapascal pressures (Sharma *et al.*, 2002) and bacteria recovered from the deep ocean at around 4000 m have been shown to retain both structural integrity and metabolic activity. They have shown activity in the Antarctic at −17 °C (Carpenter *et al.*, 2000) and to exist in the pore spaces between ice crystals (Thomas and Dieckmann, 2002).

It has been established for some time that viable microbial life is found in glacial ice, although estimates vary widely by study, geographical location and procedure—from less than one viable cell ml^{-1} in polar ice (Abyzov *et al.*, 1982) to 6×10^7 cells ml^{-1} in a Greenland ice core (Sheridan *et al.*, 2003). The identification of significant alpine subglacial bacterial activity has already been observed (Sharp *et al.*, 1999), and distinct bacterial communities have been characterized from beneath Arctic glaciers (Bhatia *et al.*, 2006). Elsewhere, viable microorganisms have been recovered from 1 million-year-old Antarctic permafrost (Kochkina *et al.*, 2001), which makes it likely that prolonged preservation of viable microorganisms may be prevalent in Antarctic ice-bound habitats. Thus, existing data strongly suggests that the Antarctic ice sheet may harbour a time-specific microbiological seed bank, which could provide a source of microorganisms to inoculate subglacial environments.

The Antarctic subglacial environment described so far consists of around 145 subglacial lakes and their interconnected watercourses (Siegert, 2005; Siegert *et al.*, 2005; Priscu *et al.*, 2008), although new lakes continue to be identified (Popov and Masolov, 2007; Peters *et al.*, 2008). In Antarctic subglacial systems, 100 cells ml^{-1} (glacial ice) and 400 cells ml^{-1} (accretion or glacial transition zone ice) have been estimated from the ice above Lake Vostok (Priscu *et al.*, 2008). Indeed, all samples in this accretion ice between 3541 and 3611 m depth were found to contain both prokaryotic and eukaryotic microorganisms (Priscu *et al.*, 1999; Price, 2000; Poglazova *et al.*, 2001; Christner *et al.*, 2001), and functional groupings have even been described, such as the thermophilic chemoautotrophic *Hydrogenophilus thermoluteolus* (Lavire *et al.*, 2006). More recently, microbes have been detected in sediments collected from beneath the West Antarctic Ice Sheet (Lanoil *et al.*, 2009) so the potential for microbial life in Antarctic subglacial lake systems is clear.

The estimated time of migration of microorganisms through the ice into Antarctic subglacial lakes, is of the order of 10 000–50 000 years—not long enough for the evolution of completely new species, but certainly long enough for novel biochemical, physiological and morphological diversity to potentially exist, or for the continued existence of relic populations that may have become extinct elsewhere. In such an extreme environment, the mere presence of life in itself would be a major scientific discovery, but there are reasons to expect that such microorganisms would possess special or unique adaptations to this unusual and potentially hostile environment. Analysis of the metabolic activity and capability or new physiologies (using a metagenomic or high-throughput sequencing approach)

and bioenergetics through the analysis of biochemical pathways of returned samples, will help to gain a better understanding of the potential role of such subglacial lake microorganisms in biogeochemical cycling and in their functioning and control of ecosystem processes, or indeed their biotechnological potential (Raymond *et al.*, 2008). In addition, the climate record locked in subglacial lake sediments has the potential to provide unique insights into past changes in ecosystem function and adaptation.

With the advent of molecular techniques, microbial ecology has entered a golden age of advancement and discovery. We have also reached the point at which technology can tackle one of the final frontiers of exploration in the search for life on Earth. It is now financially, logistically and practically possible to study Antarctic subglacial lake systems. Significant challenges still remain, however, particularly with respect to obtaining samples from such a remote and hostile environment, while preventing contamination (Vincent, 1999) of both the samples themselves and the subglacial environment (either microbiologically or chemically)—particularly as Antarctic subglacial lake systems are believed to be hydraulically interconnected (Price *et al.*, 2002), and in the unambiguous interpretation of microbiological material obtained. However, progress is being made on each of these fronts: resources have been made available for access at Lake Vostok and Lake Ellsworth www.nerc.ac.uk/press/releases/2009/03-ellsworth.asp (Figure 1), methods are already under development in analogous systems to effectively sample these environments (Doran *et al.*, 2008), particularly with respect to the potential for contamination (Alekhina *et al.*, 2006) and an initial assessment has already been made on what is needed to responsibly explore Antartic subglacial lake environments (National Research Council, 2007).

We are now, therefore, in a position to ask some very interesting questions of these systems, such as: do the Antarctic subglacial lake environments contain life, and if so, what, where and how? What can subglacial lake microorganisms tell us about the distribution and evolution of microbial life in on Earth? What are the biogeochemical resources of this unique gene pool? What unique historical climate change record is locked within subglacial lake sediments, and how do Antarctic subglacial lakes interact with and influence the overlying ice sheet? To address these questions, developments and improvements in key techniques can now be

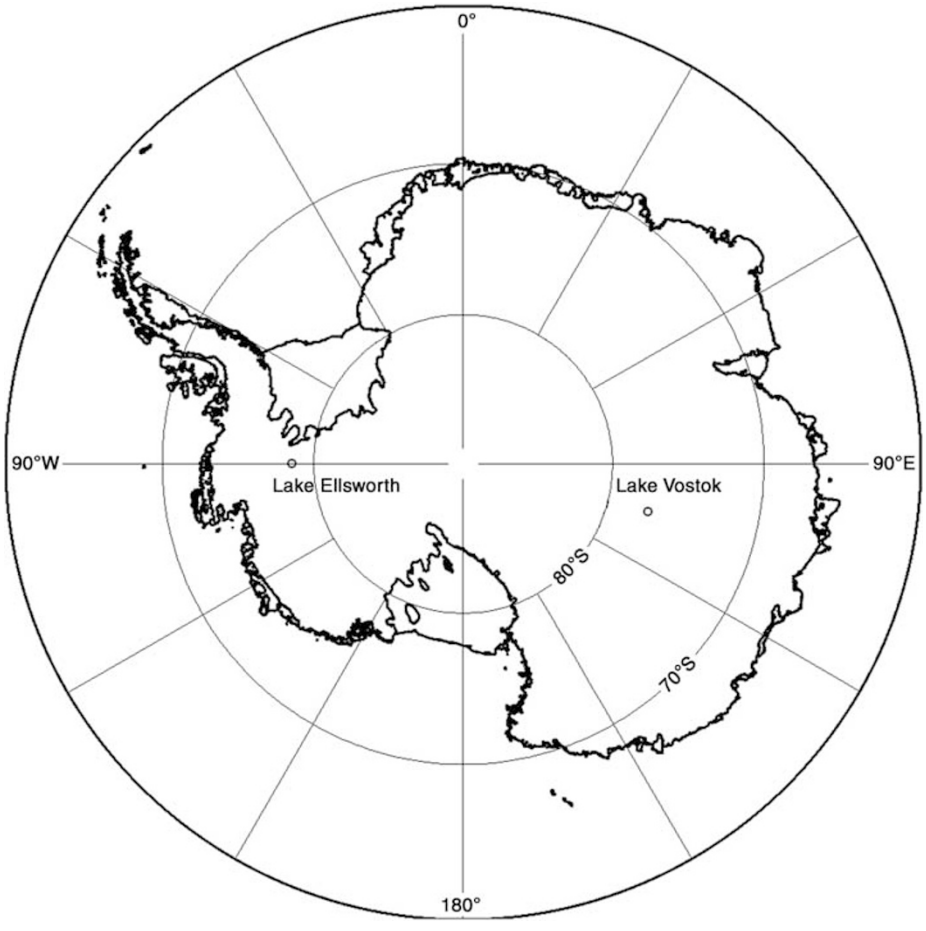

Figure 1 The location of Lake Ellsworth and Lake Vostok in West and East Antarctica, respectively.

applied to subglacial lake samples. These include: microscopy; fluorescent and electron microscopy (linked to specific gene probes), molecular biology; genomic DNA extracted from material obtained and used to construct metagenomic libraries (to screen for new physiologies), physiology and biochemistry (to investigate biogeochemical cycling), direct culture and biomarkers or tracers (Wackett, 2007).

Advances in molecular technology have vastly improved life detection limits, such that microscopy and PCR are now capable of detecting individual cells per ml, or the DNA itself at 0.1–0.2 μl^{-1}. To date, 16S rDNA-based community reconstruction has shown sequences between 6–93 from Lake Vostok accretion ice (though this figure is known to include contaminants). Adopting a culture-based approach from Antarctic ice cores, 0, 2 and 10 cfu ml^{-1} have been isolated from Dyer Plateau, Siple Station and Taylor Dome respectively (Christner *et al.*, 2000), and 1–16 cfu ml^{-1} from a Dronning Maud Land ice core (Pearce, unpublished data). Radiolabelled substrates can yield uptake rates at the level of several hundred cells (Karl *et al.*, 1999). However, not one approach is likely to provide a complete unbiased picture of the microorganisms residing in a sample or their relative numbers, and the design of specific, clean sampling strategies is extremely important.

Although Antarctic subglacial lakes were identified almost 40 years ago (Robin *et al.*, 1970), we are only now at a stage where the exploration of Antarctic subglacial ecosystems is a reality, and this will open a new frontier in microbial ecology. Initial results from Lake Vostok accretion ice, access into Arctic subglacial lakes and preliminary work with shallow Antarctic subglacial systems, suggests we are about to enter an exciting phase in Antarctic subglacial lake research. Perhaps most significantly, if Antarctic subglacial lake ecosystems are found to be sterile, it would be a major discovery in itself.

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