LIFE IN THE ICE. C. C. Allen[1], N. R. Wainwright[2], S. E. Grasby[3], R. P. Harvey[4]
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Introduction: The current Martian surface environment is extremely hostile to any known form of life. The combination of subfreezing temperature, low atmospheric pressure and high ultraviolet flux, combined with desiccated and possibly oxidizing soil, could destroy even the hardiest microorganisms. The Viking biology experiments are generally interpreted to indicate that the surface of Mars is currently devoid of life and organic molecules at the part-per-billion level [1].

Speculation on the possibility of extant or preserved microbial life on Mars thus centers on refuges in some manner protected from the current surface environment, either in space or time. Terrestrial analogs include hydrothermal systems, lakes, caves and subsurface aquifers as well as more clement conditions in the distant past [2].

We are examining the evidence for microbiology in Earth's glaciated polar regions as analogs to the polar caps of Mars. This research concerns the detection of microorganisms or their preserved remains at the surface and within polar glacial ice.

Surface Ice and Snow – Antarctica: Earth's polar deserts, particularly in Antarctica, are some of the least hospitable places on this planet for life's survival and growth. Air temperature rarely exceeds 0º C and the small amounts of liquid water are ephemeral. Dry, cold katabatic winds regularly sweep from the polar plateau to the surrounding ocean [3]. The ultraviolet flux, particularly in springtime, is among the highest on Earth. Yet small numbers of bacteria from many genera, some viable and some apparently metabolizing, have been reported.

We are extending these studies to previously unsampled areas in the Transantarctic Mountains. Our study employs a technique that is under development for bioassay on future planetary missions.

Field Setting: Ice and snow samples (Fig. 1) were collected for bioassay at six locations adjacent to the MacAlpine Hills in the Transantarctic Mountains (84º 13' S, 160º 30' E) during the 2002-2003 Antarctic Search for Meteorites [4]. The glacial ice in this region of the continent is essentially isothermal, with an average annual temperature near the surface in the range of -30º C to -40º C [5]. Average air temperatures measured in 1997 by an automated weather station in the Transantarctic Mountains ranged from -30º C in summer to -50º C in winter, with summer highs rarely exceeding the freezing point and winter lows approaching - 60º C (unpublished). In the perpetual sunlight of summer, moraines and rock surface temperatures in the MacAlpine Hills exceeded the ambient air temperature by around 10º C, but only for a few hours each day. Temperatures several centimeters deep in the snow can be 10º C colder than those in the air [6].

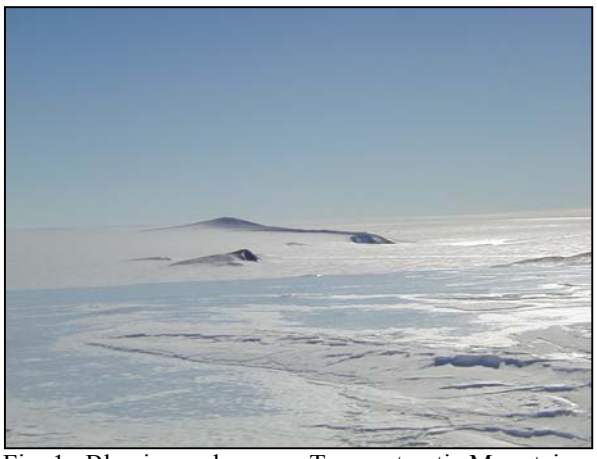

Fig. 1. Blue ice and snow – Transantarctic Mountains (photo by A. Caldwell)

Samples and Analyses: Samples of blue glacial ice, ablated by katabatic winds, were collected from freshly-chipped holes 10 to 20 cm deep at three sites. Samples of melted and subsequently refrozen ice and snow were collected from two locations on moraine gravel surfaces. A final sampling location, also in a moraine, was immediately below a rock face where snow had recently melted and the meltwater refrozen. Samples of immediately adjacent windblown snow were also collected at all six locations.

Quadruplicate ice and snow samples (48 total) were collected with autoclaved stainless steel spatulas. Several cm^3 of ice chips or snow were placed in each sterilized plastic vial, and the vial was immediately capped. The samples were kept frozen in the Antarctic environment during the remainder of the field season, approximately three weeks, except for one set that was inadvertently thawed and refrozen the same day. Following return from the field the samples, in their vials, were allowed to melt and remain at room temperature for approximately six weeks prior to analysis.

The melted ice and snow samples were analyzed using the limulus amebocyte lysate (LAL) assay [7]. This assay detects and quantifies trace levels of lipopolysaccharides (LPS), which are major components in the cell walls of all Gram-negative bacteria, one of the two dominant bacterial groups. As such, LAL has been developed as a broad-based yet highly sensitive assay for bacteria, without reference to particular species. This assay does not distinguish among active, dormant or dead cells.

The LAL assay is being tested as a possible supplement to NASA's standard microbial assay for spacecraft and laboratory planetary protection [8,9]. LAL is also among a suite of life detection sensors being developed for future planetary landers [10].

Results: The concentration of LPS in each of the 48 ice and snow samples was below the detection limit of the LAL assay. The practical limit of detection for this assay was 0.01 Endotoxin Units (EU), equivalent to 1×10^{-12} g of LPS, per ml of melt water.

This value can be converted to an approximate detection limit for Gram-negative bacterial cells. The mass of carbon in a typical bacterium is approximately 10^{-14} g, and the ratio of carbon to LPS in typical bacterial cells is 11.7 ± 5 [11]. Given these assumptions and uncertainties, the calculated LAL detection limit is approximately 10^3 cells / ml. Culture experiments conducted at the Marine Biological Laboratory [10] suggest that this value is a conservative maximum. Ongoing experiments at the same laboratory demonstrate that storage of the ice, meltwater and snow samples in plastic vials for up to nine weeks before analysis apparently did not alter the LPS concentrations.

Discussion: The 48 samples tested in the present study, while taken from the same overall environment, actually address three distinct environmental niches: blue ice, refrozen liquid water in contact with rock and gravel, and snow. As discussed below, microbial life has been detected in each of these niches. The low levels, less than 10^3 cells / ml, implied by the present results illustrate just how rare life is in Earth's extreme polar deserts.

Blue ice. These samples represent snow that fell on the Antarctic plateau millenia ago and was compressed to glacial ice. This ice flowed downslope and locally stagnated upon encountering the Transantarctic Mountains. In these areas sublimation caused by the dry katabatic winds sublimated significant volumes of the ice, leaving behind blue ice from deep within the glaciers [12].

This blue ice is equivalent to material sampled for microbiological assay in a number of drill holes across the continent. Christner et al. [13] recovered bacteria and fungi from ice cores collected at Taylor Dome (77º 40´ S, 157º 40´ E), Siple Station (75º 55' S, 84º 15' W) and Dyer Plateau (70º 40' S, 64º 52'W). The ice from Taylor Dome and Dyer Plateau is approximately 1,800 years old, while ice from Siple Station is 150 years old. 16S rDNA sequencing identified members of the bacterial genera *Acinetobacter*, *Bosea*, *Bradyrhizobium*, *Methylobacterium* and *Sphingomonas* (Taylor Dome) and *Bacillus*, *Norcardioides* and *Sphingomonas* (Siple Station). Culture experiments yielded approximately 10 bacterial colony-forming units (cfu) / ml of snowmelt from the Taylor Dome sample, 2 cfu / ml from Siple Station and no culturable organisms from Dyer Plateau. These values may represent only a fraction of the cells present in a given sample. Comparison of culture experiments against DNA analyses in samples from a non-Antarctic glacier indicated that only 1 % of the cells in that ice core formed colonies [13].

Higher microbial counts have been reported from studies of much deeper and older ice. Abyzov et al. [14] extracted a diverse mixture of prokaryotes and eukaryotes from 110,000 to 240,000 year old glacial ice beneath Vostok station (78º 28' S, 106º 48' E). Using these core samples from depths of 1500 to 2750 m, still well above the frozen ice of subglacial Lake Vostok, they derived total cell counts ranging from 10^3 to 10^4 cells / ml. The biomass values for these samples were positively correlated with the presence of dust in the samples. The presence of viable microorganisms was demonstrated by the consumption of ^{14}C-labeled organic material.

Refrozen Water. Liquid water does occasionally form at the MacAlpine Hills site, due to melting of snow and ice. The water exists as ephemeral surface rock coatings and ice-covered pools a few centimeters deep. The rock coatings and pools generally refreeze within hours, as changing insolation and wind lower the surface temperature below 0º C. The meltwater pools can reform several times in the same place over the course of an Antarctic summer, but remain permanently frozen for most of the year.

Priscu et al. [15] reported a viable microbial community located in meltwater pockets within the ice cover above Lake Bonney in the Antarctic Dry Valleys. The community included filamentous cyanobacteria of the genera *Phormidium*, *Chamaesiphon* and *Leptolyngbya*. Bacteria in numbers equivalent to 10^5 cells / ml were found concentrated in a layer approximately 20 cm thick, located near the center of the 4 m thick ice layer. The bacteria concentration correlated with a layer of windblown sediments enriched in organic and inorganic nutrients. The biomass in ice even a few cm away from this layer rarely exceeded 10^3 cells / ml. The bacterial community within the ice

cover is apparently distinct from the abundant bacterial mats on the floor and within the water of Lake Bonney.

Snow. Limited fresh snowfall and considerable blowing snow were encountered throughout the field season at MacAlpine Hills. Thus, the samples constitute a mixture of freshly fallen snow and snow that had fallen days to weeks earlier. The source of any bacteria and their organic nutrients are likely to have been the ocean surrounding Antarctica. For much of each year katabatic winds blow from the polar plateau and limit transport of organic material from the sea [3]. However, limited wind transport poleward does occur, sometime on time scales as short at two days [16].

Carpenter et al. [17] counted bacteria in snow collected during two summers at the South Pole. 16S rDNA analyses identified members of the bacterial genera *Deinococcus*, *Cytophaga*, *Alcaligenes* and *Bacteroides*. Biomass values ranged from 10^2 to 10^3 cells / ml of snowmelt. Similar concentrations of bacteria were reported in surface snows from the Ross Ice Shelf [18]. Low levels of metabolism were demonstrated for the South Pole snow samples, as indicated by DNA and protein synthesis [17].

Glacial Springs – Arctic Canada: Ice sheets and glaciers in the arctic also represent extremely challenging environments for life's survival and growth. Christner et al. [13] found < 1 cfu / ml in ice from two cores in Greenland. Dancer et al. [19] reported 1 to 5 cfu / ml of culturable bacteria in glacial ice from the Canadian high arctic. Skidmore et al. [20] cultured bacteria in surface meltwater on the John Evans Glacier of eastern Ellesmere Island and derived a concentration of 10^3 cfu / ml. However, they could measure no microbial activity in samples of the ice itself.

We have recently completed the study of a unique set of englacial springs in arctic Canada [21]. These springs contain evidence for a complex community of subsurface bacteria existing within or beneath a thick sheet of glacial ice.

Field Setting: The springs are located at 81º 01'N, 81º 35' W on northern Ellesmere Island. Extensive glaciers occur in the high mountain ranges and flow down and coalesce within a valley. Temperature logging of an exploration well drilled 43 km SW of the springs site indicates 540 m of permafrost and a 22º C / km geothermal gradient. The mean annual air temperature is -20º C.

Ten springs and seeps discharge from the surface of an approximately 200 m thick glacier. The springs were sampled during the summers of 1999, 2000 and 2001, and sulfur deposits on the ice were reported as early as 1988. Native sulfur is typically thinly dispersed over several square meters of the ice surface around the discharge sites (Fig. 2). Active discharges of approximately 1 liter / min were observed at some locations, whereas diffuse seeps were more common at others.

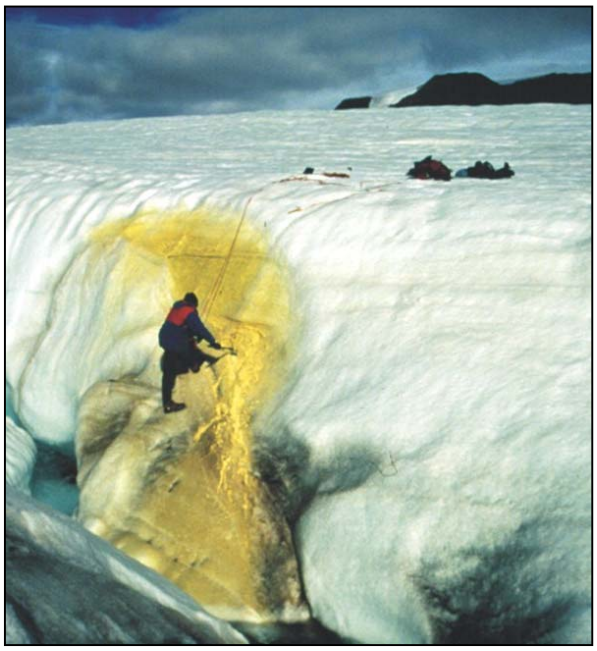

Fig. 2. Sulfur spring in glacial ice – Ellesmere Island (photo by S. Grasby)

Samples and Analyses: Water samples and precipitates were collected from five actively flowing spring sites and samples of glacial meltwater were collected from nearby streams. Temperature, pH, conductivity and deuterium excess were measured for each water sample. Total direct counts of bacteria were performed using fluorescence microscopy. Total community DNA was extracted from spring precipitates and assessed by agarose gel electrophoresis. Polymerase chain reaction (PCR) was performed on each extract using universal primer sequences. DNA extracts were also PCR amplified and analyzed using denaturing gradient gel electrophoresis (DGGE).

Results: The spring waters had greater deuterium excesses than the local precipitation, but the spring water value was consistent with the range measured for glacial meltwater in the area. However, spring water temperatures (1 to 2º C) were higher than those of surface meltwater (~ 0.2º C). The spring waters had pH values of 9.0 to 9.5, distinctly different from glacial meltwaters that had pH values of 4 to 6. Conductivity values ranged from 115 to 230 mS, as compared to < 2 mS for meltwater elsewhere on the ice.

Total bacteria counts for the five water samples were consistent at approximately 10^4 cells / ml. Direct

cloning of PCR amplicons resulted in the identification of a single bacterium of the psychrophylic genus *Marinobacter*. The DGGE analysis produced 18 distinct bands. Sequencing of the three most dominant bands, based on band intensity, identified bacteria of the genera *Polaromonas*, *Pseudomonas* and *Burkholderia*.

Discussion: Stable isotope values for spring waters are indistinguishable from those of glacial melt waters, suggesting that the spring waters originate from meltwater rather than from precipitation or deep sources. The recharge zones for the spring system are likely to be in the nearby glaciated mountains.

Sulfur springs are often associated with an active volcanic source, but no evidence of recent volcanism has been reported in the region. An alternative source of sulfur is weathering of sulfur-rich geologic horizons. Pyrite veins associated with a nearby fault are chemically stable and are unlikely to form significant quantities of HS^-. The only other abundant sulfur-rich horizon in the area is a set of extensive anhydrite beds at an estimated depth of 1.5 km below the valley floor. These observations imply that the springs express a topography driven flow system along bedding and / or fracture planes, with circulation to a depth of at least 1.5 km.

Bacteria are ubiquitous in all spring samples, at consistent concentrations of 10^4 cells / ml. These concentrations are significantly higher that those reported for ice on the surface of other Canadian or Greenland glaciers. The spring waters apparently carry samples of a complex microbial community that exists within or beneath this thick sheet of glacial ice.

Implications: Microorganisms (bacteria and fungi) exist in Antarctic and Arctic ice, meltwater and snow. Bacteria from subsurface communities can also be brought to the surface in the mineralized waters of englacial springs.

These bacteria are rare compared to the biomass in more temperate environments. Bacterial counts in polar ice and snow consistently range from 10^2 to 10^4 cells / ml. Counts in the englacial spring samples cluster at 10^4 cells / ml. For comparison, bacterial population of surface soils can range from 10^8 to 10^9 / g [22].

Many different bacterial genera have been identified in polar ice and snow samples. Some of these baceria are closely aligned with known psychrophiles, while other genera are common in many environments. No consistent pattern obviously links the types of bacteria to their locations. These observations suggest that the bacteria represent a broad mixed population, probably carried to the glaciers from many locations by the winds.

The highest concentrations of bacteria in Antarctic ice are found in direct association with windblown dust and organic nutrients. Bacteria numbers in glacial ice and refrozen lake ice are strongly correlated with concentrations of windblown material.

Bacteria can be preserved in ice for millenia. Processes in the cryogenic water cycle – nucleation of snowflakes, transformation of snow to glacial ice and ice melting – preserve rather than destroy bacteria. Recognizable forms remain for hundreds of thousands of years and identifiable DNA is preserved for at least 1,800 years. A portion of these bacteria can be cultured after more than 1,000 years in the ice. In some cases a subset of the bacteria continues to metabolize within the snow, and some bacteria in ice may remain viable for hundreds of thousands of years.

Mars: The polar icecaps may be among the sites most likely to preserve evidence of life on Mars [13]. Windblown dust, carrying material from locations across the entire planet, forms recognizable layers in the ice [23]. Microorganisms carried to the poles on this dust could be trapped and protected from the hostile surface environment. Cellular morphology, organic molecules and even viability might be preserved in the ice through significant periods of Martian history.

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