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Life in Solid Ice on Earth and Other Planetary Bodies

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Abstract. Theory and direct observation indicate that micro-organisms exist in liquid veins in ice and permafrost, provided the temperature is above the eutectic for H_2O and soluble impurities present. Microbes can exist and metabolize in glacial ice and permafrost on Earth, Mars, and Europa. One can search directly (with fluorescence microscopy at liquid veins in Vostok ice core samples) or with a biologging instrument (for microbial fluorescence in a borehole in terrestrial or martian permafrost or ice). The viability lifetime against DNA destruction of bacterial spores can be measured with analytical techniques that identify calcium dipicolinate, which is unique to spores.

1. Liquid veins in polycrystalline ice as a microbial habitat

In phase equilibrium at temperatures below the liquidus and above the eutectic, polycrystalline ice comprises a two-phase system consisting of pure ice crystals bounded by liquid veins where all the soluble impurities are concentrated. To maintain thermodynamic equilibrium, the size of the veins changes with temperature, adjusting the ionic concentration in order for the liquid to remain at the freezing point. Eutectic temperatures range from a few degrees below 0°C to ≈ -100°C, depending on impurity composition. In Antarctic ice the major impurity is H_2SO_4, with a eutectic temperature low enough (-73°C) to guarantee that a three-dimensional network of liquid veins will be present. For the highly pure Antarctic ice, at T as low as -55°C, the vein diameter must be only a few μm in order for some liquid phase to be present. I recently proposed liquid veins in ice as a habitat for bacteria and archaea (Price 2000) and showed that atmospheric drops and particles deposited in snow provide nutrients (through redox reactions catalyzed by microbial enzymes) and bioelements such as C, H, O, N, S, and P.

Acidophilic psychrophiles capable of surviving the harsh conditions in liquid acidic veins in glacial ice exist. Thiobacillus spp. live in a Greenland mine (83°N) at $T \approx -30$°C in winter (Schleper et al. 1995, Johnson 1998), and they generate H_2SO_4 at pH ≈ 0.

The idea of a microbial habitat in solid ice has a number of interesting consequences:

- Karen Junge has detected bacteria living in liquid veins in both sea ice and Arctic lake ice. See Junge et al. (2001) for the technique and observations of bacteria in sea ice.

- Sharma et al. (2002) studied physiological and metabolic activity of E. coli and Shewanella oneidensis (a piezophile) in a diamond anvil cell. At pressures 1.2 to 1.6 Gpa the bacteria resided in liquid veins in ice-VI crystals and were able to oxidize formate and move in the veins.

- I have initiated a search for microbes in Vostok ice core samples by scanning with fluorescence microscopy at $-20°$C. I estimate that a few tens to hundreds of cells per cm^3 can survive in veins in the oldest Vostok ice, $\approx 420,000$ years at a depth of ≈ 3500 m.

- In their study of gases CO_2, N_2O, and CH_4 trapped in Bolivian glacial ice, Campen et al. (2002) found gas concentrations orders of magnitude larger than those measured in polar ice cores of the same age. They concluded that the gases were the metabolic products of microorganisms living in veins within the ice at $-10°$C. I estimate that for a microbial concentration of $\approx 10^3$ to $\approx 10^5$ g^{-1}, a metabolic rate of $\approx 10^{-4}$ to $\approx 10^{-6}$ g carbon per g carbon per year during the 14,000 yr age of the ice would account for the excess gases.

- Veins in ice on a young planet may facilitate a cold origin of life by concentrating prebiotic solutes and increasing rates of encounter and reaction. Levy et al. (2000) froze ammonia and cyanide into ice at $-78°$C. The ice turned brownish, and 25 years later they analyzed it and found adenine, guanine, and simple amino acids dominated by glycine. My interpretation is that the reaction rate is higher in liquid veins into which the ammonia and cyanide were concentrated at $-78°$C than if the experiment had been carried out at a much higher temperature in a liquid solution. Here is a case in which high encounter rates in narrow channels result in faster reactions than in bulk solution, due to the concentrating effect of the solutes.

2. Biologging for microbes in ice, permafrost, subglacial lakes and the Martian subsurface

My colleagues and I have developed a biologger (Bay et al. 2002) that builds upon the success of our dust logger (Miocinovic et al. 2001, Bay et al. 2001). As the dust logger is lowered into a borehole, it records light that is emitted in a horizontal plane from the instrument, that then escapes into the surrounding ice, permafrost, or water, and that finally is either absorbed in the medium or is scattered back into the borehole and detected. Its success in studies of volcanic ash layers and dust concentrations as an indicator of climate changes led us to devise a 7-channel biologger that uses an ultraviolet source to excite biomolecules and microbes. Phototubes record the fluorescence spectrum in 7 different wavelength bands. The biologger could have excitation sources at several wavelengths : at $\approx$ 270 nm, in order to detect tryptophan, which is present in all proteins; at $\approx$ 370 nm, in order to detect NADH; and at $\approx$ 420 nm, to detect the coenzyme F420, a marker for methogenic archaea. A live-dead test for microbes in acidic veins would be to measure the ratio of signals from tryptophan and adenosine. Adenosine fluoresces at $\approx$ 390 nm when excited at $\approx$ 270 nm, but only if the protoplasm of the bacterial cell is exposed to acid at

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low pH due to loss of membrane integrity. The biologger can also detect PAHs, flavins, and other biomolecules. A search for microbes with the instrument in a 1000 m borehole at Siple Dome, Antarctica, showed that the technique is promising but that the paths of the fluorescent light must be well collimated so that the light must pass through the notch filters at nearly normal incidence.

With the modified instrument, we plan to measure fluorescence from microbial life as a function of depth in Lake Tahoe, in conjunction with other methods that will record microbial concentrations for calibration. In his studies of microbes in Vostok ice cores, John Priscu (unpublished) melts ice samples and examines the filtrate with SEM and epifluorescence microscopy. Concentrations are typically $\leq 10^2$ /ml (or $\approx 10^3$ /ml in ice containing rocky debris).

The biologger should become a rapid survey tool in searches for microbial life in various locations in addition to terrestrial glacial ice:

- Once the international community has agreed upon a sterile drilling procedure, we can use the instrument to search for life in subglacial lakes such as Lake Vostok, which has been isolated from the Earth's surface for as long as 25 Myr.

- Airborne radar surveys have led to detection of ≈ 100 subglacial Antarctic lakes as inferred from flat radar profiles between ice and bedrock. Lake Vostok is by far the largest. Not all the flat regions are guaranteed to consist of liquid water. Price (2002) showed that the base of the glacial ice at South Pole is at $-9°C$ and that the flat region at the base of the ice 10 km from South Pole is almost certainly frozen. They proposed that this "fossil lake" be used as a test bed for sterile drilling. The advantage of drilling into a frozen lake is that a failure would not contaminate other subglacial lakes, whereas drilling with an unsterile drill into a liquid lake would contaminate many others, due to their subglacial interconnectivity. Attractive features of the South Pole lake are its proximity to logistical support and the likelihood that its permafrost, with its likely soil or rock content, may contain a high concentration of microbes.

- Mars permafrost and European ice diapirs are the extraterrestrial environments most likely to contain primitive life. Chyba and Phillips (2001) have estimated that tidal heating of Europa's interior and charged particle irradiation of its surface will maintain the disequilibrium and local warm regions necessary for life to have arisen. They estimated that the microbial concentration might be ≈ 0.1 to 1 cell cm^{-3} if distributed uniformly throughout its ocean, or as high as $\approx 10^2$ to 10^3 cm^{-3} if concentrated in the upper 100 m of its ocean. Neutron and gamma ray spectrometer results on Mars Odyssey (Feldman et al. 2002, Mitrofanov et al. 2002, Boynton et al. 2002) provide strong evidence for H_2O within a meter of the Martian surface, especially at high latitudes. Carlton Allen is principal investigator on a proposal to develop an automated drill that would produce a 10 m deep borehole in Martian permafrost. As a co-investigator, I would construct a lightweight version of the biologger to search for life in the top 10 m.

3. Viability lifetime of bacterial endospores in ice and sea sediments

There is strong motivation to determine the viability lifetime of bacterial spores. One could test the concept of panspermia: can spores survive interplanetary or interstellar transport in the vacuum, low temperature, and radiation background of space? One could assess claims that Bacillus cells or spores trapped for 25 to 100 Myr in amber (Greenblatt et al. 1999) and trapped for $\approx$ 250 Myr in halite (Vreeland et al. 2000) can repair DNA damage and germinate. Adrian Ponce (JPL) and I propose to perform a live-dead test by exploiting the unique signature of a bacterial spore - the presence of up to $\approx$ 1 molar calcium dipicolinate (CaDPA) in the spore but not in the cell. Step 1, applied to one aliquot of spores, would use L-alanine in solution to force those spores to germinate and thus to release CaDPA, which is detected in the solution with a sensitive analytical technique. Step 2, applied to a second aliquot, would be to destroy the spores with microwaves, thus forcing the release of the CaDPA into the second solution, which is then analyzed with the same technique. Successful execution of steps 1 and 2 for spores as a function of age and ambient temperature would lead to determination of viability lifetime, which might be less than a millennium if the amber and halite results are wrong, or possibly a billion years if they are right. Spores would be sought in Vostok ice, with residence times up to 420 kyr, in million-year-old ice from Beacon Valley, Antarctica, and in sea sediments, with residence times up to millions of years.

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