SIGNIFICANCE TO ASTROBIOLOGY OF MICRO-ORGANISMS IN PERMAFROST AND ICE

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1. Introduction

Astrobiology is a newly emerging multidisciplinary field concerned with the limitations and distribution of life on Earth and in the Cosmos. The discovery of chemical and mineral biomarkers and possible microfossils in the Allen Hills meteorite (ALH84001) indicated that microbial life may have existed on Mars more than 3 billion years ago. Meteorites on Earth that have come from the moon and Mars (SNC meteorites) establish that impact ejection processes can result in the transplanetary transfer of astromaterials. It is now widely recognized that the transfer of cometary water, organics, and volatiles to early Earth and the impact synthesis of organics may have played a significant role in the Origin of Life on Earth. by Chyba and Sagan, in 1992 [12]; Mumma in 1996[41]; Delsemme in 1997 [13]; Oro *et al.* in 1980 [45]. New results by Mosjis and Arrhenius in 1996 [40] indicate that microbial life has existed on Earth for the past 3.5 billion years. Over the eons, deep impacts of asteroids, comets and meteorites could have ejected large quantities of debris into space from planets or frozen moons. It is now clear that ancient Earth (and possibly even ancient Mars) was teeming with microbial life. Ejecta from marine sediments, permafrost, deep crustal rocks or polar ice must have contained biominerals, organic chemicals, microfossils, and perhaps even intact cells and cryopreserved viable microorganisms. The possibility of biological cross contamination of other planets, moons, comets, and the parent bodies of meteorites can not be excluded. The long held paradigm that Earth represents a closed ecosystem must be re-examined.

Water, ice, and permafrost appear to be extremely common in the Cosmos. Most of the planets and their moons, comets, and asteroids of our Solar System are frozen worlds. Consequently, cryoenvironments, which comprise a major portion of the terrestrial biosphere, are of great significance to Astrobiology. These low temperature regimes have great thermal stability over long time periods. Cryoenvironments include the deep sea floor, sea-ice, permafrost, glaciers, the polar ice sheets. The cryosphere provides an best analog for the environmental conditions of the frozen worlds of the Cosmos. The investigation of microbiota from the permafrost and polar caps of Earth may help develop techniques and methods needed to explore the permafrost and polar ice caps of Mars and icy moons of Jupiter. The terrestrial cryophiles provide analogs

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for microbiota that might be able to inhabit the permafrost and ice caps of Mars or the oceans of Europa or Callisto.

On Earth, complex microbial ecosystems thrive in cold sea-floor sediments and at the water/ice boundaries of sea-ice and in permanently ice covered Antarctic lakes. Halophiles (Archaea and bacteria) have been found living at temperatures as low as –24C in the high salinity Don Juan Pond of Antarctica. Microbes inhabit brine channels in glaciers, under-cooled water in ice-bubbles, and the ice/rock interfaces in permafrost, glaciers, and ice-sheets. Hence, we can not rule out the possibility that comparable microbial ecosystems may also be found in the permafrost or ice caps of Mars, the ice of comets, and the ice crust, ocean/ice boundaries, water and sea-floor sediments of Europa and Callisto. Since permafrost and glaciers permits long-term preservation of intact and viable cells and microbial ecosystems the polar caps and permafrost of Mars afford an ideal opportunity for Astrobiology. Viking images (Fig. 1.a.) obtained on May 18, 1976 reveal the presence of snow on the surface at the Viking 2 Lander site (48N, 226W). Since the detector temperature was –18C this must have been due to water rather than CO_2. The presence of snow or frost at the Viking Lander Site and water ice in the Polar Caps greatly enhances the possibility of metabolically active microbial life on Mars today.

The Mars meteorite results and the ensuing debate demonstrated the importance of establishing conclusive biochemical, biomineral, and morphological biomarkers. It is important to develop morphological biomarkers including an image database of the microbial extremophiles of Earth. New instruments and spacecraft are being developed to search for evidence of microbial life elsewhere in the Solar System. It is important to know where and how to search for evidence of life and to establish valid chemical, mineral, and morphological biomarkers. This knowledge is required to detect and recognize evidence for extinct or extant microbial life and for the development of acceptable planetary protection protocols as we prepare to return samples from other Solar System bodies.

2. Biomarkers and Microfossils in Meteorites

McKay *et al.* in 1996 [39] reported the detection of indigenous chemical and mineral biomarkers and possible minute microfossils (nanofossils) in the Mars meteorite (ALH84001). These mineral and chemical biomarkers (carbonate globules, greigite, magnetites, and Polycyclic Aromatic Hydrocarbons) were found in close association with the possible nanofossils. This association of indigenous chemical, mineral and morphological biosignatures was interpreted as evidence of biogenicity. Minute nanobacteria (Fig. 1.a.) may have inhabited water filled cracks and been fossilized in the rock over 3 billion years ago—long before a large impact ejected the rock into a trajectory that would bring it from Mars to the blue ice of Antarctica.

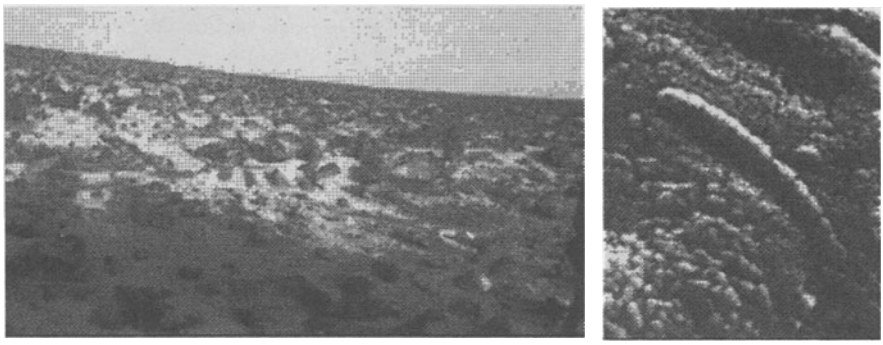


Figure 1.(a.) Snow and Frost on Mars at Viking 2 Lander Site (48N,226W) obtained on May 18, 1976 15:50:43Z (Frame #21I093; Detector Temperature –18.10C) (b.) Image of possible microfossil in Mars Meteorite ALH84001*(Photos Courtesy NASA & David McKay).*

The discovery of biomarkers and possible microfossils in ALH84001 stimulated research in meteorites and microbiology and may have triggered a paradigm shift concerning the limits of life on Earth and the possible distribution of microbial life in the Cosmos. This discovery also provided the initiative for the new field of Astrobiology. The ensuing scientific debate helped to delineate research areas crucial for the recognition of biomarkers and microfossils that may be encountered in ancient terrestrial rocks, meteorites and in Astromaterials returned by future space missions. Critics of ALH84001 forms argued that the minute nanofossils in ALH84001 were far to small to represent microbial cells. Subsequent studies reveal microbiota can exist in the 100-400 nanometer size range. Independent researchers have also detected the chemical and morphologcal biomarkers found by McKay *et al.* [39] in ALH84001. Benoit and Taunton in 1997 [7] found abundant clusters of small (30 nm x 200 nm) rod-shaped forms in ALH84001 carbonate nodules. Taunton [7] discounted contamination effects, since similar microstructures were not found during a careful examination of eight lunar meteorites and two other Mars meteorites (EET 79001 and ALH 77005), which had comparable terrestrial and laboratory histories

Extensive studies of terrestrial nanobacteria have also continued to refine the lower limit of the size of microbial life on Earth. The possible nanofossils in ALH84001 have been shown to be similar in size, morphology, and colonial appearance to terrestrial nanobacteria which had been found living deep within the Earth's crust in Columbia River Basalt as reported by Kieft in 1997 [37], Stevens and Mckinley in 1995 [44]. Several other groups have shown that minute culturable nanobacteria (as small as ~100 nm) live in mammalian blood as Kajander in 1997 [36], in soil as Vainshtein *et al.* in 1998 [59] and in deep rocks as Onstott *et al.*, in 1997 [44]. These microorganisms exhibit size, morphology, colonial associations, and biomineralization characteristics that are not significantly different from many of the possible nanofossils found in the Martian meteorite. Similar nanofossil-like forms have been found in terrestrial rocks and carbonaceous meteorites by Folk in 1993 [15] and in 1997 [16]. These independent studies show that the biogenicity of these microstructures in the Mars meteorite cannot

be dismissed on the basis of size alone. These results highlighted the need for further studies of ancient terrestrial rocks and meteorites to seek additional evidence of chemical and mineral biomarkers and microfossils;

Thermophilic chemolithotrophic microbes are very abundant in deep aquifers as shown by Olsen *et al.*, 1981 [42]; Pedersen *et al.*, 1990 [47] and in deep crustal rocks by Frederickson *et al.*, 1989 [17]; Pedersen, 1993 [47]; Pedersen and Ekendahl, 1990 [48]; Stevens and McKinley, 1995 [55]; Frederickson and Onstott, 1996 [18]; Onstott *et al.*, 1997 [44]. The subsurface lithotrophic microbial ecosystems may well comprise the dominant component of the biomass of planet Earth as was suggested by Gold in 1992 [27]. Chemolithotrophic microorganisms could possibly inhabit the deep crustal rocks of Mars, Venus, Io or other planetary bodies of the Cosmos as reported by Hoffman and Farmer, 1997 [29]. Consequently thus the study of the microbiota and microfossils found in deep rocks, hydrothermal vents, ancient rocks, meteorites and other astromaterials is of great importance to Astrobiology.

3. ESEM and FESEM Study of Ancient Rocks, Meteorites and Ice at MSFC

The ALH84001 announcement led us to initiate electron microscopy studies of meteorites and ancient rocks at the NASA Marshall Space Flight Center (MSFC) to explore the morphology, texture, and chemical composition of *in-situ* minerals, biomorphic microstructures, and possible microfossils at the micron and sub-micron level. The chemical composition, size, and morphology of biomorphic microstructures and possible microfossils found *in-situ* in Proterozoic and Phanerozoic rocks and carbonaceous meteorites were compared with known living microorganisms grown in pure culture. The study used advanced imaging and analysis instrumentation, tools, methods and aseptic collection and handling protocols. We had previously applied similar techniques to the study of living thermophiles from geysers, deep-sea hydrothermal vents (Rainbow and Snake Pit) and deep crustal rocks (Driefontein gold mines of South Africa). We have also used these methods, in combination with a cold stage, to explore living and cryopreserved microbiota from Glaciers (Matanuska, Cascade, and Holbrook), deep-ice cores (Vostok), permafrost, ice-wedges and frozen thermokarst ponds in Beringia (Alaska and Siberia) and Antarctica (Beacon Valley).

3.1. INSTRUMENTATION AND METHODOLOGY

The MSFC study was carried out using the Environmental Scanning Electron Microscope (ESEM) made by the ElectroScan Corporation and the Hitachi Field Emission Scanning Electron Microscope (FESEM). Both of these instruments can be used with uncoated samples and non-conductive materials and have sensitive Energy Dispersive X-Ray Spectroscopy detectors and software for elemental analysis (Boron and above) and can generate 2D x-ray maps of elemental distributions.

The Environmental Scanning Electron Microscope (ESEM) uses a partial pressure of water vapor (~10 Torr vacuum) to image uncoated, non-conductive samples (e.g. plastics, ceramics, and living biological materials). Uncoated non-metallic samples

build up a negative charge in a conventional SEM which degrades the imaging properties of the instrument. To overcome this problem, non-conductive samples are usually coated with a thin conductive film for conventional Scanning Electron Microscope (SEM) study. However, this coating process can produce artifacts and alter the surface features and chemistry. Furthermore, it is almost always lethal to living cells. Bacteria and microbial cells must also be fixed to minimize the distortion effects that occur as a result of exposure to the high vacuum. The SEM usually requires a high vacuum (at least 0.0001 Torr or lower) in the sample chamber.

The ESEM overcomes the vacuum and grounding limitations of most SEMs by utilizing an environmental electron detector and a differentially pumped sample chamber that is backfilled with a small amount of water vapor. The water vapor is ionized by the electron signal generated on the sample and the environmental detector then detects the ionized gas. The gas in the chamber also serves as a ground path so excess electrons (charging) does not collect on the sample. This removes the need for any conductive surface coatings. Hence the ESEM is ideal for the study of the morphology of living microorganisms. The cells do not suffer significant structural alterations as are often encountered under the ultrahigh vacuum of conventional electron microscopes. The ESEM operates at a voltage of 10-30 kV and affords magnifications up to 100,000X and is equipped with a 3-axis cold stage and micromanipulator. The images are recorded with digital image capture (4Pi Analysis) with a 4096K by 4096K pixel array with 8 bit (256 grays) or 16 bit (65K grays) digital image depth. Chemical analysis is accomplished by Energy Dispersive Spectroscopy (EDS) by Noran Instruments with a detector sensitive to light elements (Boron and above). Higher spatial resolution images at magnification up to 250,000X can be obtained with the Hitachi Field Emission Scanning Electron Microscope (FESEM). The FESEM uses a field emission electron source allowing operation at reduced accelerating voltage (0.5 to 30 kV). The FESEM also permits imaging of uncoated non-conductive materials but requires harder vacuum than the ESEM and is not as well suited for the study of living biological materials.

3.2. CONTAMINATION CONTROL AND SAMPLE HANDLING PROTOCOLS

At MSFC, aseptic methods have been used for storage, preparation, and preservation of all research samples. Samples of meteorites, rocks, and deep-ice for Astrobiology research are typically stored in a freezer at -80C in sterile vials purged with filtered dry Nitrogen. (Permafrost, ice and glacier samples are typically stored in at –20C.) All chisels, electron microscopy tweezers, tools, and mount stages are sterilized before handling, fracturing and mounting the samples. For EDS analysis of the samples, polished Boron nitride which have been cleaned with alcohol and flame sterilized are typically employed. Immediately after mounting, the sample is inserted into the ESEM sample chamber and pumped down. For studies of Vostok ice or permafrost, the temperature controlled cold stage can keep the specimen frozen and permit controlled melting while digital or video images are recorded. To avoid recent microbial contamination, we usually study only freshly fractured interior surfaces. Gerasimenko, Hoover and many others [59], have observed in 1999 significant contamination of

recent actinomycetes growing on the fusion crust and within small cracks in a sample of the Murchison carbonaceous meteorite provided by the Field Museum. Of course, exterior surfaces must be examined when it is desirable to examine meteoritic fusion crust. However, any microbial material encountered on the fusion crust or in existent cracks in meteorites or other Astromaterials are highly suspect and must be discounted as probable recent terrestrial contaminants. In order to minimize contamination effects, the samples studied with the ESEM are usually not acid cleaned, coated, or permitted to come into contact with any fluids or stains. Since acids destroy many microfossils, we rejected the methods commonly used by previous meteorite researchers for the extraction of acid resistant microfossils were rejected.

4. Microfossils in Ancient Rocks and Carbonaceous Meteorites

Investigations were carried out at MSFC on meteorites, graphites, shungites, phosphorites, and living microorganisms by Hoover, 1997 [32], again by Hoover and Russian colleagues.,1998 [33], and by Zhegallo, Rozanov, Yu, Ushatinskaya, Hoover, Gerasimenko and Ragozina.1999 [68]. Similar studies were conducted independently in Russia at the Institute of Microbiology and the Institute of Paleontology of the Russian Academy of Sciences. Zhmur, Rozanov and Gorlenko in 1997 [69] as well as Zhegallo Rozanov, Yu and Ushatinskaya in.1998 [67] and McKay and his co-workers also in 1998 [39]. Subsequently, we jointly studied several carbonaceous chondrites (e.g. Alais, Allende, Murchison, Efremovka, Orgueil, Murray, Mighei, and Nogoya). Many of these carbonaceous chondrites were found to contain large numbers of biomorphic microstructures and possible microfossils. Many of the microstructures were lithified and embedded in the matrix and interpreted as indigenous bodies. Several forms found in these meteorites resemble microfossils of Cyanobacteria that we have found in Cambrian phosphorites and Devonian graphites as published by Rozanov *et al.,* 1998 [49] (Figure 2a, 2b). Energy Dispersive Spectroscopy (EDS) X-ray analysis indicates that these meteoritic forms are lithified and/or carbonized (diagenetically converted to kerogen or graphite). Although some microorganisms fossilize rapidly, lithification into a rock matrix and diagenesis to kerogens typically require geological time periods. These phenomena could have occurred within the past few decades since the chondrites fell to Earth.

4.1.EVIDENCE OF MICROFOSSILS IN THE MURCHISON METEORITE

Some of the biomorphic microstructures interpreted as microfossils in the Murchison meteorite are more than 100 microns in size and exhibit extremely complex morphology. That is orders of magnitude larger than the forms found in ALH84001 that were interpreted as nanofossils. The large size of the Murchison forms makes it possible to easily conduct valid x-ray spectroscopic analysis without interference from the matrix or substrate and to generate 2D X-ray maps revealing chemical differences between the form and the rock matrix. Many of these highly complex embedded microstructures are found in close proximity to other bodies that are consistent in the context of microbiology or microbial ecology. Several are found close association with

associated reproductive bodies, providing additional evidence for biogenicity. Analysis of the chemical composition of the forms detected and their response to the electron beam makes it possible to differentiate indigenous lithified remains (microfossils) from recent contaminants such as fungal hyphae, actinomycetes, pollen, and museum dust.

The primary condition for indigenicity is considered to be satisfied, since the lithified and/or carbonized forms do not exhibit the characteristics observed in recent or living microbiota and were found *in-situ* in freshly fractured surfaces of the meteorite rock matrix.

Many of the forms encountered in the Murchison meteorite appear to represent embedded and lithified microbial remains, and can not be dismissed as recent terrestrial contaminants.

They are interpreted as the remains of microbiota (microfossils) that were present in the matrix when the meteorite fell in Murchison, Australia at 11:00 A.M on September 18, 1969.

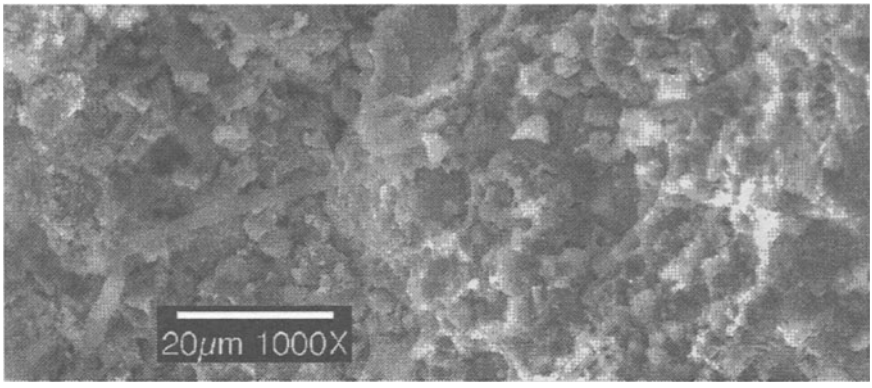
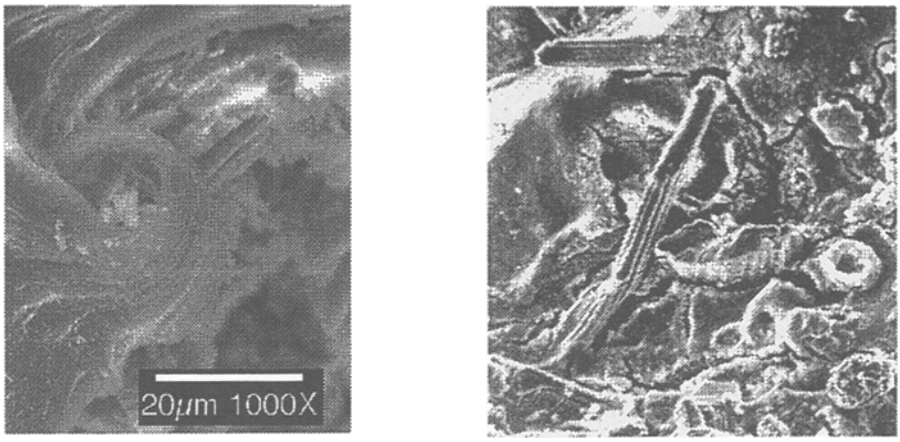


Figure 2. Microfossil of *Nostoc*-type cyanbacteria embedded in Murchison mineral matrix. Trichome, hollow filament, and segmented flattened sheath found near multiple remnants of biologically consistent reproductive structures (hormogonia). *(Photo: Greg Jerman and Richard B. Hoover, Courtesy NASA).*

Figure 2 is an ESEM image of a freshly fractured surface of the Murchison meteorite. The embedded microfossils seen are recognizable with size, morphology, and life cycle aspects consistent with known cyanobacteria. The cyanobacteria trichome has emerged from the hollow sheath at the far left. The filament is broken near the center and it is clearly seen that is a hollow tube or sheath. Further to the right, the sheath becomes flattened and embedded in the matrix. Segment can be seen in the flattened sheath. In this same image it is possible to see the lithified remains of a number of toroidal forms. These toroidal structures (many of which have a small trichome-like body protruding from the center) are interpreted as the reproductive hormogonia of cyanobacteria. The hormogonia of cyanobacteria are multicellular reproductive structures in which trichome fragments are released from the sheath. In *Nostocacean* species they have been observed to form a hormogonial swarm in which they coil into tightly wound toroidal spirals, with the tip of the trichome protruding out of the center of the toroidal coil. The presence of multiple lithified remains of structures with these characteristics embedded in the matrix near other bodies consistent with known terrestrial cyanobacteria provides strong evidence of biogenicity. Consequently, this complex assemblage of embedded microstructures is considered to provide strong evidence of microfossils of cyanobacteria indigenous to Murchison meteorite.

During the course of this research, several hundred hours of electron microscopy studies have been exclusively devoted to electron microscopy studies of freshly fractured samples of the Murchison meteorite from several sources (V. I. Vernadsky Institute, Field Museum, etc.) After this extensive study, we observe that these toroidal (hormogonia-like) structures are rarely found in Murchison. However, in figure 2 we see a number of these hormogonia-like forms in very close proximity to the structures interpreted as a lithified cyanobacterial filament and trichome. In addition to the toroidal forms that we interpret as the remains of coiled hormogonia from *Nostocacean*–type cyanobacteria, rounded forms can also be seen in his image that resemble cyanobacteria resting cells (akinetes).

We have also found in the Murchison meteorite other complex assemblages of microfossils that can be compared with living cyanobacteria and Cambrian microfossils (Fig. 3). The large form (Fig. 3.a.) resembles living *Microcoleus chthonoplastes* with multiple trichomes in a common laminated sheath overlain by a glycocalyx. A bundle or cluster of more or less parallel filaments *(fascicle)* is also seen in this image. The individual filaments in this fascicle are ~2 micron diameter. These characteristics are similar to cyanobacteria of the genus *Phormidium* as defined by Kuetzing ex Gomont in 1892. Nearby there is a single larger tapered filament (~6 micron diameter) in slightly coiled hyaline sheath with a calyptra or capitate apical cell.



(a) **(b)**

Figure 3. (a.) Murchison meteorite with microfossils resembling cyanobacteria comunity *(cf. Microcoleus., Nostoc & Phormidium)*; (b.) Microfossils of cyanobacteria filaments, fascicles and coiled hormogonia (~ *Microcoleus.)* in Cambrian phosphorite of Khubsugul, Mongolia. *(Photos:3.a. NASA;3.b. Institute of Paleontology, Russian Academy of Sciences)*

Figure 3b shows a similar assemblage of the lithified remains of microfossils of cyanobacteria in Cambrian phosphorite from Khubsugul, Mongolia. In this image it is possible to recognize the Cambrian cyanobacteria *Siphonophycus* (analogous to the modern *Microcoleus or Phormidium*) exhibiting fascicles (multiple filaments generally parallel in a common sheath).

Also found in this beautifully preserved microbial community in Cambrian phosphorite are toroidal forms, some with small trichome-like protrusions.

These forms are considered to represent multiple coiled hormogonia in association with a community of Cambrian cyanobacteria (*cf. Phormidium sp., Nostoc sp. & Microcoleus sp.).*

The Cambrian microfossils from the phosphorites of Khubsugul, Mongolia are virtually identical in size, morphology, and complex microstructure to the forms found in the Murchison meteorite *(see also Figure 2).*

Many of the possible microfossils and communities found lithified, carbonized or converted to kerogen in the Murchison meteorite exhibit complex microstructures that resemble fossil and living cyanobacteria belonging to the genera *Nostoc, Microcoleus,* and *Phormidium.* These particular genera of cyanobacteria are known to inhabit glaciers, and are associated with snow, ice and Antarctic soils. They have been reported as growing together in a community in meltwater under dark rocks on glaciers or ice-sheets (a cryoconite microbial ecosystem). These genera of cyanobacteria are also known as mat builders also comprise a major component of thick benthic

cyanobacterial and algal mats found at the bottom of perennially ice covered lakes in Antarctica as reported from Warwick in 1997 [62].

Although the parent body of the Murchison meteorite is unknown, it is thought that carbonaceous meteorites may be of cometary origin. Comets are conventionally considered "dirty snowballs" in which rocks, dust, and debris is embedded in ice. Glacial ice with entrained rocks and dust (cryoconite) and ice-bubbles may comprise the best terrestrial analogue for comets and perhaps even the parent body of Murchison.

5. Microorganisms from Glaciers

Hoover has collected cores and samples of snow, firn, ice, rocks, lichens, and mosses for microbiological research from the Matanuska piedmont glacier of Alaska. Cores were also taken from chunks of floating ice (many with entrained rocks) that had recently calved from tidewater glaciers in Prince William Sound (Cascade, Baker) and the Gulf of Alaska (Holgate). Aseptic methods were employed in the collection and handling of the samples. Interior ice materials were obtained with (35cm long X 1cm diameter) stainless steel tubes. All sampling tubes were cleaned and then sterilized by autoclave and individually wrapped in sterile aluminum foil. In the field, and after the samples were collected, all sampling materials were kept frozen in a cooler with blue ice at –10C. At the Matanuska glacier, core samples were obtained by driving the .3 M tubes into the snow or blue ice. After extraction, the ends of the tubes were sealed with sterile caps, wrapped in autoclaved aluminum foil, and placed in the cooler for transport to MSFC while still frozen.

The glacier samples are being studied at MSFC. We are also exploring the microbial ecosystems found in deep-ice, snow, firn, ice-bubbles, and cryoconite assemblages. The deep-ice cores from the Central Antarctic ice-sheet above Lake Vostok are being studied in collaboration with Academician Michael Ivanov and Dr. Sabit Abyzov of the Institute of Microbiology of the Russian Academy of Sciences. The chemical composition, morphology, ultramicrostructure, motility and viability of living and dead microorganisms and microbial remnants are being evaluated in Russia and at MSFC using optical, epifluorescence, and Scanning Electron Microscopy methods. In some cases the cells can be grown in pure culture. The microbiota that can be isolated from pure culture will be studied more thoroughly. The enzymes, proteins, and lipids of psychrophiles, psychrotrophs and psychrotolerant microorganisms can be explored and PCR amplification and gene sequencing (16s or 18s rRNA) may permit precise identification.

The optical microscopy and ESEM investigations have already shown that a very complex microbial ecosystem (bacteria, cyanobacteria, actinomycetes, green algae, diatoms, flagellates, etc.) exists in deep ice cores, ice-bubble cavities, and rocks entrained in glacial ice and cryoconite holes. These ice communities may be even more complex than those reported associated with snow ecosystems.

5.1.1. *Snow Microbiota.* Fogg [14] investigated the algal components of the red, green, and yellow colored snow of the South Orkney Islands in late summer. During thaws, he observed rapid development of chrysophytes *(Ochromonas spp)* and green flagellates (e.g. *Chlamydomonas nivalis* and *Chlorosphaera Antarctica).* These green flagellates have spores that are intensely colored. Large numbers of these red spores frequently result in red and pinkish colored snow. The maximum concentration of the green colored motile cells of these species is usually found at the top of the firn layer just beneath the fresh snow. A filamentous green alga *(Hormidium subtile)* is primarily responsible for green snow. Diatoms *(Nitzschia spp., Navicula spp.)* and cyanobacteria *(Phormidium spp.)* may also produce snow with green and brownish coloration. Yellow snow may be the result of concentrations of chlorotic cells of species of *Raphidomena.* Ochre, brown and black snow or ice can result from diatom or cyanobacterial assemblages, but could also be produced by inclusions of rocks and fine rock dust. The snow ice algae have been relatively well studied. However, comparatively little is known about the associated bacteria and heterotrophic communities that may be present in snow and on rocks entrained within the glacial ice.

5.1.2. *Rock Crystal, Cryoconite and Ice-Bubble Habitats.* Very diverse microbial assemblages have been found in the cryoconite habitats. The Arctic explorer Nordenskjold used the term "cryoconite" to describe the "cold rock dust" sediments he found at the bottom of holes in the Greenland Ice Cap. Rock dust can be blown onto the ice sheet and absorb solar energy to initiate local melting. Much rock debris is produced by the glacial destruction of mountains and nunataks. This debris is often brought to the surface by wind transport or by the ablation of ice exposing rock debris entrained in the glacial ice. The dark rocks absorb solar energy and become sufficiently heated that they melt the ice. As they sink into the glacial snow and ice they produce micro-Edens; cryoconite holes that contain liquid water, rock dust, organics, and mineral nutrients.

The rock crystal habitats and ice-bubble microbial ecosystems are also very interesting. Friedmann and Ocampo Friedmann (1976) first described the cyanobacteria that live under the surface of sandstones in the Antarctic Dry Valleys. The cryptoendolithic microbiota are extremely important to Astrobiology as they provide a model for microbial life capable of surviving in very dry cold environments such as present day Mars.

Ice-bubbles and entrained rocks are common in glacial ice and act as solar energy traps developing their own microenvironments of gas, water and nutrients. Ice bubble communities from ice-covered lakes in Antarctica contain benthic diatoms, cyanobacteria, or purple sulfur bacteria mat material as indicated by Wharton *et al.* in 1981 [63] and in 1985 [64]. Oxygen, methane, and nitrogen gasses produced by metabolic processes buoy the algal or bacterial mats. When bits of mat material break free, they are buoyed by trapped gasses and float upward until they strike the bottom of the overlying ice layer and freeze. As more ice and frozen mat forms at the bottom of the ice sheet and the top surface is ablated away by katabatic winds and sublimation, the entrained mat material migrates upward through the ice column. Wilson in 1965 [66] and Parker and others in 1982 [46] have shown that this may constitute a

significant mechanism for escape of nutrients and organic matter from ice covered lakes.

6. Astrobiological Significance of the Polar Ice Caps

Due to specific conditions that are characteristic of Antarctic glacier (low temperatures, dry climate, enhanced radiation level), the polar ice cap on the Antarctic continent provides an ideal regime for Astrobiology research. The Antarctic ice sheet comprises the best terrestrial analog for the polar ice cap of Mars. The ice fields of Antarctica (and Greenland) serve as a natural trap for precipitation and airborne particles. The sediments that are trapped in the glacier represent a unique historical record of both cosmic and terrestrial events. Inclusions of terrestrial and extraterrestrial origin (ancient air bubbles, volcanic ash, interstellar particles and dust, micrometeorites, micro-algae, pollen of ancient plants, etc.) have accumulated in the ice over the course of many millennia. Quantitative analysis of these materials can provide valuable information for solving many important problems of modern science.

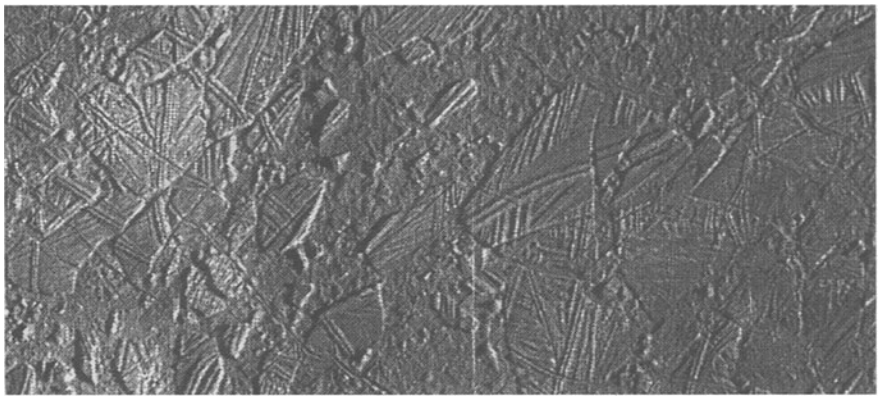


Figure 4. Ice Rafts of Europa indicate underlying Ocean. The blue and white in this image resemble glacial blue ice and snow. The other hues may be due to minerals, but are also consistent with pigments of diatoms and cyanobacteria. *(Photo NASA)*

These investigations become especially important in connection with the discovery of an under-glacier lake at Vostok station, Antarctica. This lake is considered by the worldwide scientific community to be an important object in the search for relict forms of life on the Earth. The large enclosed body of water beneath 3.7 km of ice at Vostok Station (Lake Vostok) provides the best terrestrial analog for ice moons of Jupiter (e.g. Europa, Callisto and Ganymede), which appear to have a liquid water ocean beneath a thick ice crust. Europa and Mars are prime candidates for the existence of microbial life elsewhere in the solar system. Figure 4 is a color enhanced Galileo image of

Europa, showing surface ice rafts that clearly indicate the existence of a liquid water ocean underlying a thick ice crust. The liquid water lake (Lake Vostok) underneath the thick Central Antarctic ice sheet is very significant as a model for solving methodological problems in Astrobiology. It is vital to the development of instruments and methods needed to penetrate into oceans that lie beneath the ice crust of Jupiter's moons Europa and Callisto

6.1. MICROORGANISMS OF LAKE VOSTOK

Although it has not yet been possible to penetrate into the waters of Lake Vostok, we may have glimpses the lake microbiota by the study of the microorganisms entrapped in the deepest ice layers from just above the lake. Some of the material found in these deep ice layers may represent benthic microbiota from mats and sediments at the bottom of Lake Vostok. It is well known from studies of perennially covered lakes in Antarctica that pieces of mats of cyanobacteria, bacteria and algae can become buoyant from metabolically produced gasses. They break free from the lake bottom and float upwards until they strike the ice cover and become frozen into the overlying ice sheet. As more mat material and ice collects at the bottom and is removed from the top surface by ablation, the organic mat material migrates upward through the ice.

The microbiota of Lake Vostok and the deep ice layers above it provide a model for the types of life forms that might be capable of living in the Europa ocean or be found in the overlaying ice sheet. Hence, the deep ice at Vostok is of great importance in the quest for evidence of extraterrestrial life forms and the development of planetary protection and quarantine protocols. The existence of viable microorganisms from deep ice layers at Vostok may constitute Nature's successful attempt to solve the problem of long-term anabiosis. Of course we will only be certain about the origin of microbial materials when robotic samplers venture into the lake. This must wait until samplers are developed that can enter the lake without introducing chemical or microbiological contaminants into this pristine, ancient terrestrial ecosystem.

6.2. MICROBIOTA FROM DEEP ICE LAYERS ABOVE LAKE VOSTOK

A 3670-m-deep drill hole has been bored at Vostok, and samples for microbiological and other investigations have been taken. The water-ice interface of Lake Vostok lies at 3700 M and the lake is thought to be 500 M deep. Preliminary data taken by the radioisotopic analysis provide evidence for the cryopreservation of some viable microorganisms in the layers deeper than 3000 meters (~ 300,000 years old). Extensive microbiological investigations have been carried out at Vostok Station, Central Antarctica Abyzov and his co-workers in 1983 [2], 1993 [3] and in 1998 [5]. The development of special methods for boring, collection and preservation of ice cores, and aseptic sampling has made it possible to reveal viable microorganisms belonging to different taxonomic groups in the layers of the glacier. The frozen microbiota encountered include diatoms and other algae, bacteria and bacterial spores, cyanobacteria, fungi, yeasts, actinomycetes, and plant pollen.

Microflora of the ancient horizons of ice sheet consisted of approximately the same groups of microorganisms as the contemporaneous forms. The ancient microorganisms found belonged to various taxonomic groups and were characterized by a significant morphological diversity. Prokaryotic microorganisms were encountered and were represented by bacteria (cocci, diplococci, and bacilli as straight and curved rods). Acinomycetes were also encountered, including a new species that was named *Nocardiopsis antarcticus* by Abyzov in 1983 [2].

Diatoms and various species of yeasts, both budding and multiplying by binary fission represented the eukaryotic microorganisms. Several samples contained remains of fungal mycelia, partially or completely lysed. In contrast to mycelia, the fungal conidia appeared to be rather well preserved. In some horizons, the pollen of higher plants was found in abundance and detected by its luminescence microscopically by Abyzov and co-workers in 1998 [6]. A great diversity of microscopic algae was revealed using the luminescent and scanning electron microscopes by Abyzov and co-workers in 1996 [5]. Detailed studies of microbial cultures and investigations of microbiota collected by unique methods on membrane filters were carried out in Russia using fluorescence and scanning electron microscopy.

We have recently conducted investigations at MSFC in collaboration with Dr. Sabit Abyzov and Academician Mikhial Ivanov using the Environmental and Field Emission Scanning Electron Microscope. During these *in-situ* studies of ice and membrane filters, we have found abundant microorganisms at all layers of the ice sheet examined in selected depths between 386 M to 3611 M. Interesting, and sometimes bizarre, microbial forms were encountered at all layers examined. Great variations, in composition and density of dust and microorganisms occur from one layer to another.

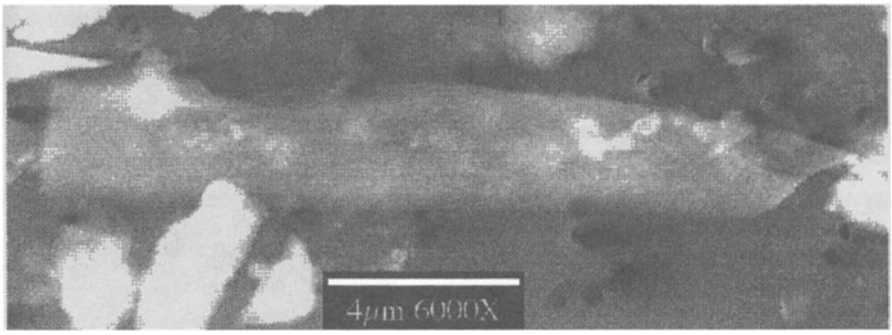


Figure 5. Diatom *Rhizosolenia alata* var. *gracillima* from Vostok ice layer at 1002 M.

Figure 5 is an ESEM image of a beautifully preserved diatom found on a filter from 1002 meters deep in the Vostok ice. This diatom is identified as *Rhizosolenia alata* var. *gracillima* (Cleve) Gran. That is one of the smaller representatives of the family *Rhizosoleniaceae.* (Some of the *Rhizosolenia* are gigantic; with *R.. styliformis* may have valve diameter up to 100 micron and length exceeding 1.5 mm.) The detection of

this *R. alata* in the Vostok ice is interesting, since the family *Rhizosoleniaceae* is truly planktonic and wholly marine with no freshwater forms known. *Rhizosolenia* are found in enormous numbers in temperate seas, but are rarely encountered in waters around the polar caps according to Hendey in 1964 [28]. This diatom most probably arrived in the Antarctic ice layers above Lake Vostok by wind transport from a very great distance.

Figure 6a is an example of cyanobacteria found on the filter from the 1249M depth of the core. Fig. 6b is an image of two complete valves (in girdle view) of the diatom *Fragilariopsis sp.* from the ice layer at 2827 M. This is approximately 100 M above the ice-water interface. *Fragilariopsis antarctica* (Castracane) Hustedt is abundant in Antarctic waters and the bacillar cells are usually united into ribbon-like colonies. These perfectly preserved frustules are seen in girdle view and the diatoms are still encased in polysaccharide.

This makes it difficult it very difficult to identify the form to species. *Fragilariopsis* is a small pennate diatom that is very abundant in sea ice. *Fragilariopsis nana,* sometimes dominates the Southern Ocean biomass in late summer. Communities of *Fragilariopsis* have been found growing in brine pockets and on the bottom layers of hard pack ice and at the base of the Antarctic ice sheet near Syowa Station (69S, 39E) as reported by Hoshiai in 1977 [34].

These diatoms may represent a component of the microbiota that lived at the ice/water interface of Lake Vostok. It should be pointed out that although diatoms are photosynthetic algae, they sometimes are heterotrophic and can live in total darkness in deep-sea sediments and caves.

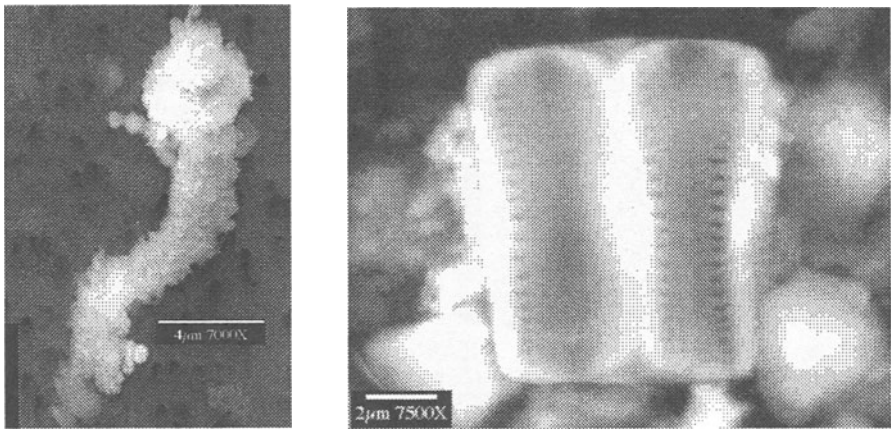


Figure 6. (a) Cyanobacteria from Vostok ice layer at 1249M (b) Complete frustules of the diatom *Fragilariopsis sp.* from Vostok Ice at 2827 M.

7. Polar Ice Caps as Microbial Environments

The polar ice caps of Earth and Mars represent a profoundly important resource for Astrobiology. Earth's south Polar cap overlying the Antarctic continent is the best analog the polar caps of Mars. The Earth's North Polar cap provides a better analog for the ice crust overlying the oceans of Europa. Impact events and the interaction of glacier with bedrock, mountains and nunataks entraps dark rocks in the ice that trigger localized melting create microenvironments (liquid water, salts, organic chemicals, minerals and nutrients). The cryoconite microenvironments, brine channels, and ice-bubbles could support microbial life in glaciers and polar caps, even at temperatures well below 0C). We cannot rule out the possibility that analogous microbial ecosystems might have lived on ancient Mars and may remain today.

7.1. THE POLAR CAPS OF MARS AS PALEOMICROBIOLOGICAL TRAPS

Elevation measurements collected by the Mars Orbiter Laser Altimeter aboard Global Surveyor during the spring and summer of 1998. The volume of the Mars north polar ice cap is 1.2 million cubic kilometers, which is about half the size of the Greenland ice cap. The spatial resolution of the instrument was 1 km with vertical accuracy of 5-30 M. These observations revealed the summer Mars North Polar Cap to be 1,200 km across by Zuber in 1998 [70] with a thickness of 3.8 kilometers. Figure 7(a) is a three dimensional image from MOLA data of the Mars North Polar Ice Cap. Large areas of the ice sheet are very smooth—varying only a few feet over many miles. Other areas of the glacier exhibit pinnacles and are cut by deep crevasses and moulins that plunge to 1 km depth. Figure 7(b) is a photo of the tongue of the Matanuska glacier in Alaska exhibiting smaller but analogous pinnacles, crevasses and moulins. The blue ice of the Matanuska glacier is similar to the blue features of Europa (Fig. 4). The shape of the Mars polar ice cap indicates that it is primarily composed of water ice. The North Polar Cap of Mars is almost identical to the thickness of the Central Antarctic Ice Sheet at Vostok.

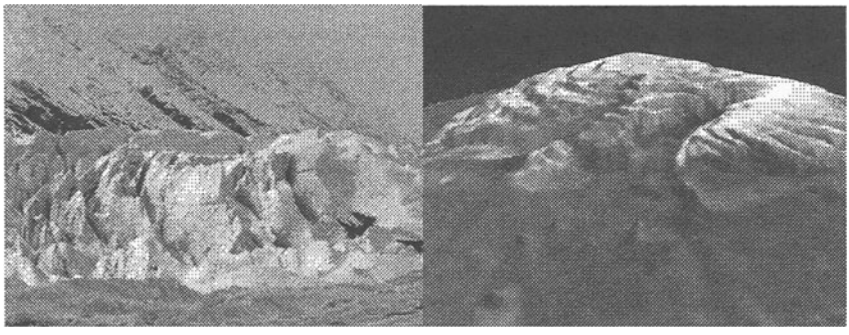


Figure 7. (a) Matanuska Glacier of Alaska (b) North Polar Ice Cap of Mars.
(Photos Courtesy: (a.) Richard B. Hoover; (b) MOLA/NASA)

The polar ice caps of Earth serve as paleomicrobiological traps --- cryopreserving ancient microfossils, organic remains and intact microbial cells in dead and viable states. The microbial remains can represent native forms or microbial remains that have arrived from great distances by wind transport or other mechanisms such as meteorite ejecta. The cratered surfaces of planets and moons clearly establish that large impacts happen relatively frequently. Meteorite, comet, and asteroid impact may have played a crucial role in the origin and distribution of life in the Cosmos. The Mars meteorites establish that large impact events are capable of ejecting material into space that can then arrive at another planet. Microbial laden debris ejected from ancient Earth (or ancient Mars) might have been able to contaminate other solar system bodies with organic chemicals, biominerals, biochemicals, microfossils, cellular components, nucleic acids, microbial remains, and perhaps even viable microbiota. Small impact events are very common on Earth, and occur even more frequently on planetary bodies that do not possess such a thick atmosphere for protection. The regoliths of the Moon and Mars are continually gardened by meteorite impacts. The present atmosphere of Mars is thin and provides little protection from meteorites, but is thick enough for clouds and large, wind driven dust storms. Meteorite impacts would eject into the atmosphere crustal rocks, ice, dust and debris from glaciers, permafrost, river, lake, or ocean sediments. Consequently, if microbial life has ever existed on Mars, the remains and biosignatures of microbiota would have been widely distributed across the planet by meteoritic events and volcanic activity combined with winds. If ancient microbial life was ever present in the permafrost, surface rocks as cryptoendoliths, or even as chemolithotrophs in deep subsurface aquifers or crustal rock of Mars, microbes could have been released into the atmosphere by meteorite impacts and volcanic events.

On Earth, the polar ice caps, permafrost and glaciers constitute time capsules for microbial life. They are capable of perfectly preserving ancient microorganisms that may have lived in a wide variety of ecosystems and been transported from distant and inaccessible localities. Organic chemicals and molecular biomarkers, intact microbial cells, and even viable ancient microbiota remain cryopreserved on Earth for eons in permafrost, ice wedges, glaciers, and the polar ice sheets. If similar phenomena operate on Mars, and rock fragments or dust with associated microbiota and microfossils that fell on glaciers or polar ice sheets may have remain frozen and preserved for geological time periods.

7.2. POLAR ICE CAPS AND SEARCH FOR EVIDENCE OF LIFE ON PRESENT MARS

The Arctic and Antarctic glaciers and permafrost and the deep-ice of the polar caps contain a magnificently preserved record for paleoclimatological, paleoenviromental, and microbial paleoecology research. If microbial life exists on Mars today, or has ever existed since these ancient glaciers formed, the evidence of this ancient life could be preserved in ancient dust laden glaciers and icecaps of Mars. These frozen time capsules might preserve a record of the paleoclimate, paleoatmosphere,

paleoenvironment, and paleoecology of Mars. Ivanov and Lein in 1995 [35] have discussed biogeochemical evidence of microbial activity on Mars.

The cryosphere of Mars may contain remains of ancient microorganisms, some of which may yet be viable. Consequently, the polar caps, permafrost and glaciers of Mars constitute an ideal locality to search for evidence of microbial life in the form of metabolically active microorganisms and microbial ecosystems. On the glaciers and polar ice caps of Earth, sunlight absorbed by dark rocks on the surface of the ice sheet triggers localized melting, even when the air temperature is far below zero. The microenvironments containing liquid water and rich mineral nutrients support abundant microbial life (cryoconite communities). Hence the polar caps and permafrost of Mars may presently harbor metabolically active microbial communities.

Ablation of the glaciers and ice sheets occurs by melting, evaporation and sublimation at the surface and glacial margin would result in the continual release of ancient rock debris, dust and entrained particulate matter. This may also include organic chemicals and cryopreserved microbial cells from different geological epochs. Consequently, space probes and instruments should be developed to explore the polar ice to search for evidence of life on Mars.

In-situ investigations at the glacial surface and cores from permafrost and ice at the tongue, crevasses and moulins exposed at the edge of the Martian ice sheet could provide ready access to deep crustal rock material without the difficulties associated with deep drilling.

The Martian Polar Caps afford an opportunity to search for evidence of active microbial life as well as ancient life on the planet. It is also very important to recognize that any microbiota found entrained in ancient ice from the polar caps could not be the result of recent contamination.

The detection of living or dead microorganisms frozen in the permafrost or polar ice of Mars would constitute definitive and unambiguous proof of the existence of microbial life elsewhere in the Cosmos. Such as discovery would answer the most fundamental question of Astrobiology – *Is Life a strictly terrestrial phenomenon, or is Life a Cosmic Imperative?*

8. Microbiota of Permafrost

The active microbial ecosystems as well as the cryopreserved anabiotic viable microorganisms and dead microbial remains and biomarkers frozen within permafrost, ice wedges, and pingos are of great significance to Astrobiology. The perennially frozen soils of Antarctica and Beringia (East Siberia and Alaska) provide some of the best terrestrial analogues to the permafrost of Mars. The investigation of microorganisms in permafrost, ice wedges, and glaciers should provide a better understanding the evolution of life on Earth and the spatial and temporal limitation of the microbiota of the deep cold biosphere. This data is important for understanding biostratigraphy, microbial evolution, global changes, geocryology, geomicrobiology, the physico-chemical aspects of abiotic and biological systems, conservation of the modern gene pool, and the long-term viability of microorganisms in deep anabiosis.

In order to be prepared to recognize evidence of life that may be encountered in returned samples of rock, permafrost, or ice from Mars, comets, Europa or other Astromaterials, it is important to understand the appearance, size distribution, morphology, ultra-microstructure, chemical, biochemical, and genetic characteristics of terrestrial cryophiles. This investigation should provide new information concerning the *in situ* morphological diversity of prokaryotic and eukaryotic cells in long-frozen permafrost for comparison with the morphological diversity of cultured viable microorganisms.

A joint project to investigate permafrost as *in-situ* microbial habitat is currently underway and is supported by the NASA Joint U.S./Russian Research in Space Science (JURRISS) Program. The morphology, ultramicrostructure and elemental distributions of microbiota in ice and permafrost are being explored in frozen state *in-situ* using the ESEM cold stage. The influence of the low-temperature is manifested primarily in the content of water accessible to cells that unavoidably has its effect on their activity and viability. At low temperatures (at least up to −20°C), part of moisture (which is in thermodynamic equilibrium) remains in the liquid phase in dispersed deposits and has different localization in them depending on their temperature, granulometric, mineralogical and chemical composition as clearly demonstrated by Vorobyova in 1996 [60], by Soina and Vorobyova in 1995 [52] and by Soina and Vorobyova in 1996 [53]. In frozen deposits from the Siberian Arctic and Antarctic, the number of cultured microbial cells correlates with the content of unfrozen water, whereas the total number of cells estimated by epifluorescence microscopy remains virtually invariable as attested by Vorobyova in 1998 [61]. This is indicative of a substantial influence of the content of unfrozen water in deposits and the character of its interaction with mineral particles and the physiological state of cells. Direct microscopic examination of such interactions is extremely difficult. (It may be feasible with advanced instruments which afford high spatial resolution but do not require hard vacuum.)

Some of these ancient microorganisms and plants can be grown in culture and isolated for PCR and DNA analysis and gene sequencing research. Other cryopreserved microorganisms are viable but non-culturable. It is well known that microbes can be metabolically active even though they are not culturable.

The existence of viable but non-culturable microbes indicates that culture methods alone are not adequate to determine whether the microorganisms found in ice and permafrost are still alive.

Many bacteria from extreme environments are very slow growing as is indicative of microbes capable of starvation-survival. Starvation-survival characteristics include nearly total metabolic arrest in some cell types, changes in macromolecular quantities and cellular density and resistance to starvation.

Among techniques used to study starved cells are visualization of viable cells with fluorescent dyes, respiratory activity, molecular probes and flow cytometry. In some cases, direct observation of cellular or flagellar motility or the assimilation of radiolabeled nutrients may provide evidence of viability. Viability and apoptosis may also be established in some cases by the use of live/dead stains and molecular probes in conjunction with epifluorescence microscopy.

8.1. PERMAFROST AS A PALEOMICROBIOLOGICAL TRAP

Permafrost also provides a paleomicrobiological trap in which viable microbiota and microbial ecosystem are cryopreserved for geological time periods. Gilichinsky has shown in 1992 [23] and in 1997 [25] that extremely ancient viable microorganisms may be recovered from the permafrost. The Beringian and Antarctic permafrost is both a microbial habitat for metabolically active microorganisms and a repository for ancient DNA, fossil bacteria and cryopreserved microorganisms and mosses.

Much less is known about the microbiota of the permafrost and ice than is known about Pleistocene mammals. In June 1977 a nearly complete frozen Woolly Mammoth (Dima) was extracted from the Kolyma lowlands of Siberia. In 1979, a magnificently preserved Pleistocene Steppe Bison (Blue Babe) was recovered from the vicinity of Fox, Alaska. Tissues and genetic materials from frozen life make possible entirely new areas of scientific research --- genetic and molecular paleontology. (Some researchers are extracting cryopreserved DNA from animals frozen within the permafrost of Beringia in an effort to clone these long extinct animals.) Very extensive paleontological studies have been carried out on the fossil mammals; but comparatively little research has been done on the fossil microbiota and microbial ecosystems. (For a review of permafrost studies in Siberia and Alaska see: *Terrestrial Paleonvironmental Studies of Beringia,* Proceedings of a Joint Russian-American Workshop, Fairbanks, Alaska, June, 1991.)

8.2. PERMAFROST TUNNELS -- PALEOMICROBIAL TIME WINDOWS

The U. S. Army Cold Regions Research and Engineering Laboratory maintains the CRREL Permafrost Tunnel in Eastern Beringia at Fox, Alaska (~10 miles north of Fairbanks.) The active microbial ecosystems, cryopreserved (anabiotic) viable microorganisms and dead microbial remains and biomarkers frozen within the permafrost and ice of the CRREL Permafrost Tunnel are of direct relevance to Astrobiology.

The CRREL Permafrost Tunnel was constructed at Fox, Alaska in the 1960's to develop permafrost mining and tunneling methods, to investigate Arctic geomorphology and to explore the properties of permanently frozen ground. This knowledge was crucial for construction projects such as the Alaska Pipeline. Radiocarbon dating studies (carried out since the 1960's) have provided accurate information about the geologic age of the stratified permafrost layers. The tunnel permafrost strata span the range of 30,000 to 40,000 BP and contain rich organic remains and a perfectly preserved diverse assemblage of fossil mammals, plants, and microbiota.

A similar research tunnel into ancient permafrost is located at the Melnikov Permafrost Institute in Yakutsk, Siberia (Western Beringia). Studies carried out at these facilities have yielded important information on the nature and characteristics of permanently frozen soils, paleoenvironments, paleoclimatology and global warming, and paleoecology. The Beringian permafrost has yielded a magnificent record of Pliocene, Pleistocene and Holocene life on Earth spanning more than 2.5 million years.

This record includes frozen fossil bacteria, archaea, algae, mosses, higher plants, insects and mammals. Consequently, these Permafrost Tunnels provide a window to the life on Earth during the last great Ice-Age.

They allow us to study living psychrotolerant microorganisms and provide direct access to the ancient microbial ecosystems that thrived when Earth was a frozen world. The Permafrost Tunnels represent a unique and precious planetary resource for Astrobiology research.

8.3. MICROBIOTA OF THE CRREL PERMAFROST TUNNEL AT FOX, ALASKA

Working in collaboration with Dr. Daniel E. Lawson, a CRREL Research Scientist, I used autoclaved stainless steel sampling tubes and aseptic methods to collect samples of microorganisms and living and cryopreserved microbial ecosystems from the CRREL Permafrost Tunnel.

These included mosses, tree fragments, sedges, grasses and insects. As well asmicroorganisms actively growing on the surface of ice wedges and frozen Pleistocene and Holocene microorganisms and microbial communities.

Modern (metabolically active) microbial communities were found to be growing at –4C on the exposed surfaces of ice wedges and frozen thermokarst ponds in the tunnel. These cryophilic microbial communities were fond *in-situ* as large (1-10 cm diameter) white patches growing on exposed surfaces of ice wedges and ice lenses.

These white patches clearly represented recent growth, as they quickly became tan when coated with Pleistocene loess stirred up by activity within the tunnel. These communities of non-pigmented organotrophic microorganisms (primarily bacteria, fungi and actinomycetes) were found growing on ice wedges in total darkness (beyond a region of roof collapse) at the far end of the Fox Tunnel.

Samples of brown ice were collected from the lower layers of a frozen Pleistocene thermokarst pond in the CRREL Permafrost Tunnel. ESEM studies of the brown ice sample indicated the presence of a complex ecosystem comprised of primarily of bacteria, fungi, actinomycetes, and yeast.

This sample also contains some very exciting and exotic flagellates (Fig. 8) and diatoms. Some of the microbial flagellates found in the Fox Tunnel ice wedges have unusual enlargements near the center of the flagella.

These forms are important as visual detection of flagellar motility would be conclusively indicate viability. Investigations are also underway to use fluorescence microscopy and molecular probes live/dead bacteria and yeast discrimination.

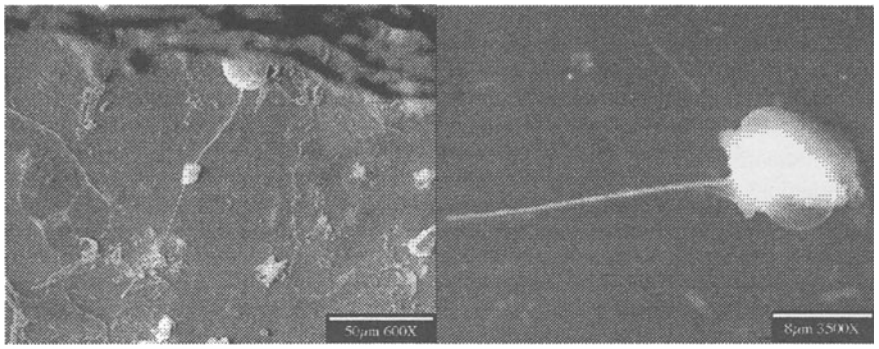


Figure 8. ESEM images of microbial flagellates found frozen in brown ice from lower portion of Pleistocene thermokarst pond of CRREL Permafrost Tunnel.

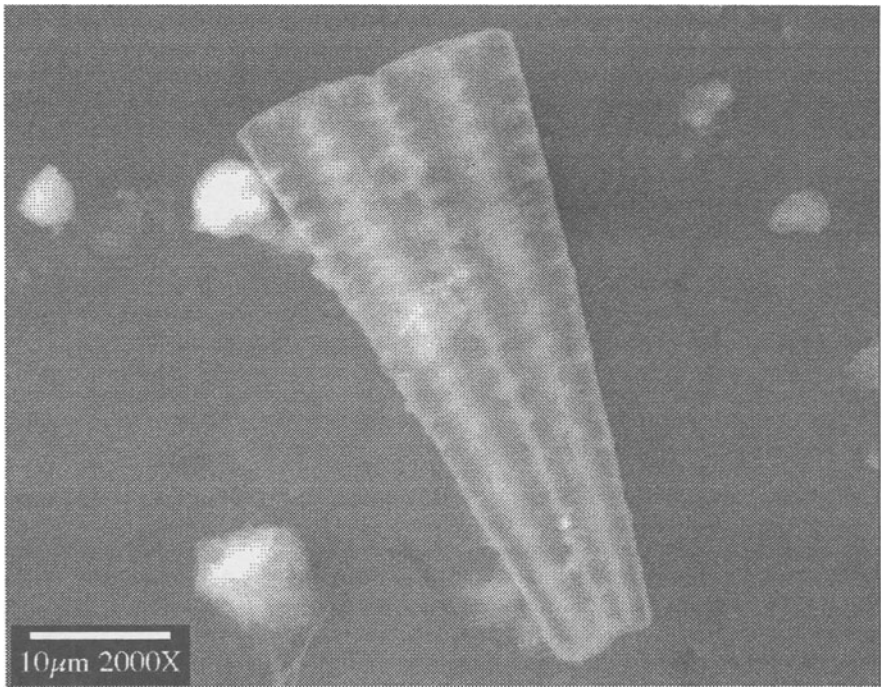


Figure 9. ESEM image of diatoms of the genus *Meridion (cf. Meridion circulare)*. These Pleistocene diatoms cells were frozen some 35,000 years ago in the lower layer of a thermokarst pond (brown ice layer) in the CREEL Permafrost Tunnel.

Figure 9 is an ESEM image of cryopreserved diatoms found in the brown ice from the Pleistocene thermokarst pond from the CRREL Permafrost Tunnel at Fox, Alaska.

Other interesting forms encountered include yeasts, actinomycetes, mosses, and cyanobacteria

8.4. DIATOMS, CYANOBACTERIA, LICHENS AND MOSSES ON GLACIERS

Orange lichens and green, orange, and black mosses were found growing on the surface of rocks and in finely broken rock debris on the ice at the tongue of the Matanuska glacier. The lichen and moss samples were collected and placed into sterile bags and frozen for subsequent identification and analysis for associated epiphytic diatoms, cyanobacteria and associated microbial communities. Some of the mosses collected from the Matanuska glacier exhibited black regions, which were possibly due to crusts of cyanobacteria. Broady in 1982 [11] reports that the black crusts he found coating mosses collected in the Antarctic were comprised of cyanobacteria (*Nostoc spp.)* and oscillatoriaceans. He also encountered abundant coccoidal cyanobacteria (*Synechococcus major)* and diatoms *(Pinnularia borealis)* in the depressions of moss cushions found at Mawson Rock, Antarctica.

On Signy Island, Broady in 1979 [10] observed a fascinating life cycle of the filamentous cyanobacteria *Nostoc muscorum* that grows epiphytically on moss shoots. Soon after the seasonal thaw the *N. muscorum* trichomes fragment and release motile hormogonia that propagate up the growing moss shoots. When they reach the site of the terminal bud, they produce heterocystous thalli and the lower *N. muscorum* communities become moribund and die. Consequently, the motile hormogonial stage in the *Nostoc muscorum* life cycle allows this cyanobacteria to maintain a population in the upper regimes of growing moss shoots, where there is more light available for photosynthesis.

9. Conclusions

We have investigated cryopreserved microbiota (viable cells or microbial remains and biomarkers) and metabolically active microorganisms in permafrost, sea-ice, snow, and glaciers. Environmental Scanning Electron Microscope (ESEM) images of some of the more interesting forms have been provided. We argue that the microorganisms of ice and permafrost are relevant to Astrobiology. These cryophiles provide the best terrestrial analogs for microbial life that might be capable of surviving (or cryopreserved) in the oceans or ice of Europa, the rocks and ice of comets, or in the permafrost and polar caps of Mars. In these regimes liquid water may exist as films or inclusions and therefore may afford the best opportunity to find bacterial fossils or metabolically active life. Cryopreserved bacterial fossils contain much more information than carbonized microfossils such as may be found in ancient terrestrial rocks and meteorites. Cryopreserved microbial cells retain information concerning the biochemistry, molecular biology, microbiology and the microbial ecology of these ancient life forms. The cryosphere of Earth provides a paleomicrobiological trap, perfectly preserving a record of ancient microbial life. Abundant microorganisms and intact microbial ecosystems have been found in deep ice cores from Vostok, and in the

glaciers and permafrost of Beringia and Antarctica. Analogous phenomena should function elsewhere in the Cosmos. Consequently, if microbial life has existed during the geological history of these icy bodies, microbial remains and possibly viable ancient microorganisms may be cryopreserved in the permafrost or polar ice caps of Mars, the ice and oceans of Europa, or on comets or other frozen worlds of the Solar System

10. Acknowledgements

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