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The resistance of viable permafrost algae to simulated environmental stresses: implications for astrobiology

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Abstract: 54 strains of viable green algae and 26 strains of viable cyanobacteria were recovered from 128 and 56 samples collected from Siberian and Antarctic permafrost, respectively, with ages from modern to a few million years old. Although species of unicellular green algae belonged to *Chlorococcales* were subdominant inside permafrost, green algae *Pedinomonas sp*. were observed in Antarctic permafrost. Filamentous cyanobacteria of *Oscillatoriales*, *Nostocales* were just found in Siberian permafrost. Algal biomass in the permanently frozen sediments, expressed as concentration of chlorophyll *a*, was 0.06–0.46 μg g^{-1}. The number of viable algal cells varied between $< 10^2$ and 9×10^3 cfu g^{-1}, but the number of viable bacterial cells was usually higher from 10^2 to 9.2×10^5 cfu g^{-1}. Frozen but viable permafrost algae have preserved their morphological characteristics and photosynthetic apparatus in the dark permafrost. In the laboratory, they restored their photosynthetic activity, growth and development in favourable conditions at positive temperatures and with the availability of water and light. The discovery of ancient viable algae within permafrost reflects their ability to tolerate long-term freezing. In this study, the tolerance of algae and cyanobacteria to freezing, thawing and freezing–drying stresses was evaluated by short-term (days to months) low-temperature experiments. Results indicate that viable permafrost microorganisms demonstrate resistance to such stresses. Apart from their ecological importance, the bacterial and algal species found in permafrost have become the focus for novel biotechnology, as well as being considered proxies for possible life forms on cryogenic extraterrestrial bodies.

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Key words: Antarctica, astrobiology, biotic survival, cyanobacteria, freezing, green algae, permafrost, Siberia, thawing.

Introduction

Unicellular microalgae, in particular cyanobacteria, are very resistant to extreme environmental factors. They inhabit various hyperthermic, saline, acidic, endolithic and low-temperature environments. Cyanobacteria and green algae are photoautotrophs and use sunlight as their source of energy and CO_2 as their source of carbon to build organic compounds during the process of photosynthesis. Obligate phototrophic organisms are unlikely to thrive in the complete darkness of subsurface environments. Nevertheless, photoautotrophs are known to survive in deep-sea water, subterranean rocks and sea ice (Malone *et al*. 1973; Sinclair & Ghiorse 1989; Palmisano & Garrison 1993). However, viable algae have not been isolated from ancient glacial ice. In fact, the presence of clones allocated to the green algae family Chlamydomonadaceae was shown in Greenland ice cores dated as 2000–4000 years old (Willerslev *et al*. 1999), and diatom cell wall fragments and remains of green algae were observed in Antarctic ice sheet samples from the Holocene and Pleistocene (Abyzov *et al*. 1998). But, viable green algae and cyanobacteria were detected inside buried permanently frozen sediments. It was established that algae viability and biodiversity depended on the duration of time spent at freezing temperature and decreased with increasing permafrost age (Vishnivetskaya *et al*. 2001).

From the present consideration, terrestrial permafrost and surface habitats in Antarctic deserts can serve as an analogue for Mars and other cryogenic planets. The cryptoendolithic, epilithic and permafrost communities exist near the limits of life but they could preserve their viability, therefore they are interesting objects for studying Arctic–Antarctic–Mars connections. The cryptoendolithic algae persist in Antarctic surface habitats, even after extinction by excessive low temperature, desiccation and ultraviolet-B (UV-B) stress within the Ozone Hole (Friedmann & Ocampo 1976; Friedmann

1982). There is still no direct evidence of photosynthetic life on early Mars, but cyanobacteria and unicellular green algae appear to be the most ancient of oxygen-releasing photosynthetic organisms on Earth. The stromatolite fossils and carbon isotope ratios confirm that autotrophs fixing carbon via the Calvin cycle must have existed 3.5 Ga (Schopf & Packer 1987). It is generally thought that green algae originated from an endosymbiotic event between a cyanobacterium-like ancestor and a non-photosynthetic eukaryotic phagotroph (Margulis 1970; Douglas 1998).

The fact of recovery of algae from permanently frozen sediments may suggest their resistance to both primary and long-term freezing. The most critical steps where cells may receive injuries are the processes of primary freezing and recovery from the frozen state. The aim of this study is to show that permafrost algae themselves could survive the stress associated with transition through the freezing point, as it can be hypothesized for near-surface Martian layers; and water loss during dehydration, as may happen under ultra-low temperatures and deep freezing there.

Materials and methods

Permafrost sites location

Samples were collected in the tundra zone of the northeast sector of Siberia (67°–70° N, 152°–156° E) and the Dry Valley region of Antarctica (77°–78° S, 160°–163° E). Siberian permafrost deposits consist of sands, loamy sands and loams with an average annual temperature from −10 to −12 °C. Iciness varied from 18–25% in sands to a maximum of 45–70% in loamy sands and loams. Antarctic permafrost comprises fluvial and eolian ice cemented sediments that are coarse- to medium-grained sands and fine gravels. The ice content of the samples was from 25% up to over 40%. The mean annual temperature of the Antarctic permafrost varied from −18 °C (Taylor Valley) and −24 °C (Beacon Valley) to −27 °C (Mt Feather) (Wilson *et al.* 1996). Samples came from different layers, representing modern sediments to layers that correspond to sedimentation laid down 2–3 Ma. Samples from both syncryogenic layers (where sedimentation occurred concurrently with freezing from below) and epicryogenic layers (where freezing occurred from the top after sedimentation) were collected.

Algae isolation

Algae from tundra soil or permafrost were isolated during enrichment of thawed samples in simple liquid mineral medium under constant illumination from fluorescent white lamps at an intensity of 500 lux for 2 months on static at 20 or 4 °C. The medium composition was as follows ($g\,l^{-1}$): $NaNO_3$ 0.25, KH_2PO_4 0.25, $MgSO_4$ 0.15, $CaCl_2$ 0.05, NaCl 0.05, Fe_2Cl_6 trace (Gollerbakh & Shtina 1969). The algae growth rate and biomass was estimated, correspondingly, by dry weight determination at three time points (Kumar & Singh 1971) and by measurement of chlorophyll *a* (Steubing 1973). The chlorophyll *a* was extracted in 80% aqueous acetone at 4 °C in the dark for 1 h on a shaker. After filtration, the absorption and excitation spectra of the filtrate were determined using a spectrofluorometer (Hitachi 850, Japan). The chlorophyll *a* concentration was calculated using the following equation: Chl *a* ($\mu g\,ml^{-1}$) = $0.58 \times D_{652}$ (Hipkins & Baker 1986). The average amount of chlorophyll *a* per 1 g of dry weight was estimated.

Freeze–thaw cycles

The undisturbed samples from both syngenetic and epigenetic sediments with high content of algal biomass were subjected to repetitions of two, five and ten freeze–thaw cycles in complete darkness. Aluminium tins with 10 g of the sample each were placed in a calorimeter where the temperature ranged from −20 to 20 °C. The samples were exposed to this range of temperatures over 18 h. Temperature changing usually took 6 h with an average speed of about 6.8 °C h^{-1}. At each time point, 1 g of sample was taken in duplicate before freezing for microbiological analysis.

Freezing inside soil a matrix

Previously autoclaved coarse sands (1 g) with low organic content were inoculated in duplicate with 50 µl of liquid culture. Preliminary, cyanobacteria and green algae were grown in liquid Bold basal medium (Rippka 1988), yeasts in liquid peptone–glucose medium (GmbH, Germany) and bacteria in trypticase soy broth (Difco). Cell concentration was determined by counting colony-forming units (cfu) on appropriate nutrient media supplemented with 1.5% agar (Difco). Populations of *Chlorella vulgaris* and *Chodatia tetrallantoidea* were estimated at 6.2×10^7 and 9.6×10^6 cfu ml^{-1}, respectively. Populations of *Arthrobacter sp.*, *Flavobacterium sp.* and *Rhodotorula sp.*, also isolated from permafrost, were 3.9×10^8, 1.2×10^9 and 4.9×10^8 cfu ml^{-1}, respectively. The inoculums were maintained at −4.5 °C for 3 months. As incubation chambers simple aluminium boxes covered with Styrofoam insulation were designed. These boxes had no active coolers or heaters in or around them. The insulation provided a stable temperature of about −4.5 °C inside the boxes while the outside temperature was −10 °C. Each box had a thermocouple in it that was monitored. The temperature during long-term incubation was stable to within about 0.5 °C. At specific time points the number of viable cells was estimated by carrying out serial dilution and counting cfu on the appropriate solid nutrient media mentioned above.

Total cell counts were determined by counting cells stained with 5-(4,6-dichlorotriazin-2-yl)-aminofluorescein (DTAF) following the protocol of Bloem *et al.* (1994). Microscopy was performed using a Leitz Ortholux fluorescence microscope using the 63× objective and a filter with a band pass of 450–490 nm. The images were collected using a 10 s exposure on a digital camera and analysed using IPLab Spectrum computer software for Macintosh (Signal Analitics Corporation, USA).

Freezing–drying

Suspensions of cyanobacterial cells were rapidly frozen in a −80 °C bath (dry ice in alcohol) and dried completely under high vacuum. After 3 months, the dry material was

Table 1. *The observation frequency of viable bacteria, cyanobacteria and green algae within Siberian permafrost*

Age of the sediments (years)	Geneses and texture	Samples with viable microorganisms (%)		
		Bacteria	Cyanobacteria	Green algae
Holocene (1–10 kyr)	Lake-swamp loam	91	17	50
Late Pleistocene (20–30 kyr)	Alluvium sandy loam	80	9	18
Late Pleistocene (20–30 kyr)	Channel-fill sands	40	0	0
Middle Pleistocene (100–200 kyr)	Marine (littoral) sands	40	0	0
Middle Pleistocene (200–600 kyr)	Lake-alluvium loam and sandy loam	90	8	39
Late Pliocene–Early Pleistocene (0.6–1.8 Myr)	Lake-alluvium loam and sandy loam	38	6	15
Late Pliocene–Early Pleistocene (2–3 Myr)	Lake-alluvium loam and sandy loam	44	13	9

[a] Altogether 128 Siberian permafrost samples were studied. The observation frequency is expressed as a percentage of samples with viable microorganisms.

inoculated in fresh Bold basal medium (Rippka 1988) and cultivated at 20 °C, under constant illumination from fluorescent white lamps at an intensity of 1000 lux for 30 days. The formation of young cyanobacterial trichomes was examined under a phase-contrast microscope.

Results and discussion

Viable algae from the permafrost environments

Remarkably, viable cyanobacteria and green algae were detected inside permanently frozen sediments from both Polar regions. They were found in surface layers (as an isolation control) and permafrost sediments with a range of texture, chemical composition, organic matter and ice content, temperature and age, including the oldest strata, which had been frozen for several million years (Table 1). Viable algae were more abundant inside Siberian permafrost layers in comparison with those from the Antarctic. The survivability of algae was associated with the presence of fine loam and sandy-loam regardless of the origin of sediments. The ice content of the samples (25–70 %) did not prevent algae survival.

Pseudococcomyxa sp., *Gloeocapsae sp*. and *Chroococcidiopsis sp*. were identified in surface sandstone samples collected in the Beacon Valley sector of the McMurdo Dry Valleys in east Antarctica. *Chlorella sp*., *Chlorococcum sp*. and *Trebouxia sp*. were found in upper layers of tundra peaty-gley soil in northeastern Siberia (outcrope 305-94, Sukharnaya River).

The phototrophic organisms isolated from permafrost were referred to the order of *Chlorococcales*, *Pedinomonadales* (*Chlorophyta*) and *Oscilatoriales*, *Nostocales* (*Cyanophyta*). *Chlorella vulgaris* (Fig. 1) isolated more often from Arctic permafrost had non-motile globular small cells about 3–4 μm in diameter with bowl-shaped chloroplast and one pyrenoid. Reproduction was by means of four autospores, which formed inside larger 6 μm diameter generative cells. Unicellular green algae of the genera *Chlorella*, *Pseudococcomyxa* (Fig. 2) and *Mychonastes* (Fig. 3) were discovered inside Arctic horizons of different ages, but *Chlorococcum*, *Scotiellopsis* as well as rod-shaped algae of genus *Stichococcus* (Fig. 4) and *Chodatia* were isolated only from relatively young, late Pleistocene–Holocene layers. *Chodatia tetrallantoidea* had slightly curved cells with a single parietal chloroplast (Fig. 5). *Chlorococcum sp*. was represented by non-motile spherical cells, which vary much in size (Fig. 6a). The chloroplast was parietal with one pyrenoid. Two phases of the life cycle were typical for this alga: non-motile mature vegetative and motile generative cells. At favourable growth conditions the vegetative cells increased in size and the protoplast divided giving commonly 32–64 biflagellate zoospores, which were released from a single vesicle. Zoospores narrowed down toward the forward end and had two flagella. Flagella located along the cell body from each side were the same in length but twice as long as the cell itself (Fig. 6b). Unicellular green algae *Mychonastes sp*. (non-motile cells) and *Pedinomonas sp*. (motile uniflagellate with thin chloroplast lying along the cell wall without any pyrenoid) were discovered within Antarctic permafrost. Heterocystous (*Nostoc*, *Anabaena*) and non-heterocystous (*Oscillatoria*, *Phormidium*) filamentous cyanobacteria were isolated from Arctic permafrost only.

The permafrost algal isolates had a low growth rate in a liquid culture; they usually required 30–40 days to reach a stationary phase. The variation of the growth conditions, e.g. increasing nitrogen (up to 1.5 mM as KNO_3) or phosphorus (up to 0.54 mM as K_2HPO_4) concentration, media saturation with 2–4 % CO_2, did not affect the growth rate. The growth rate for a number of permafrost algae was equal to 0.07–0.1 division day^{-1}. The media composition indeed influenced the algal growth. Cyanobacterial growth was completely inhibited by 1.5 mM ammonium chloride or 0.05 mM ferric ammonium citrate. The sources of organic ammonium such as Na-glutamine, asparagine or glycine in concentrations of 1.5 mM led to the reduction of heterocysts and the development of akinetes in the genus *Nostoc*. When *Nostoc* was transferred into a fresh-made BG-11 medium (Rippka 1988), the germination of each akinete gave rise to a new uniseriate trichome (Figs 8b–e). The green algae strains grew well at 4, 20 and 27 °C, but cyanobacterial strains did not grow at 4 or 27 °C.

Our observations showed that the appearance, morphology, cell size and growth rate of the ancient algae isolated from permafrost did not differ significantly from the findings available in the literature on modern algae from cold regions.

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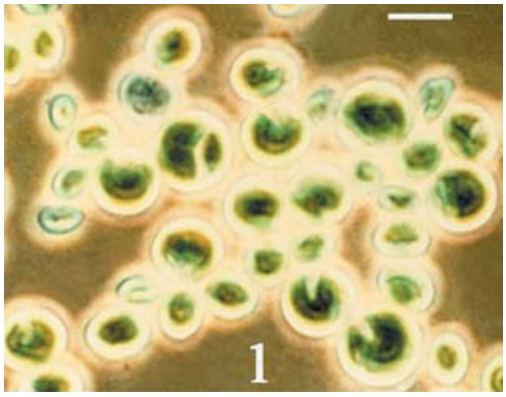

Fig. 1. Biodiversity of green algae isolated from different depth of Holocene lake-swamp sediments. The depth, which algae were discovered on, is given in parentheses. Bar markers represent 5 μm. *Chlorella vulgaris* (10.1 m).

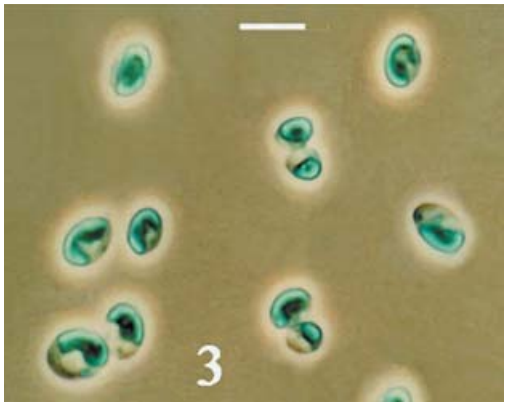

Fig. 3. As in Fig. 1 for *Mychonastes sp*. (3.6 m).

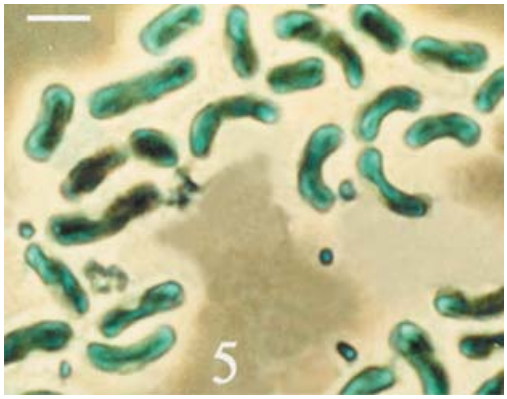

Fig. 5. As in Fig. 1 for *Chodatia tetrallontoidea* (9.35 m).

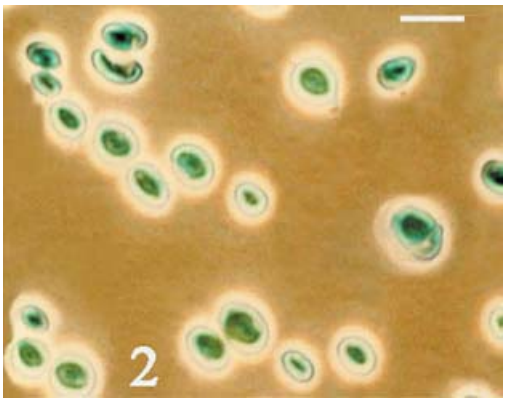

Fig. 2. As in Fig. 1 for *Pseudococcomyxa sp*. (2.9 m).

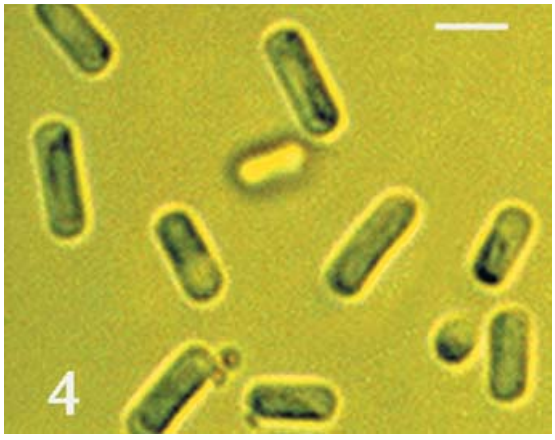

Fig. 4. As in Fig. 1 for *Stichococcus sp*. (4.7 m).

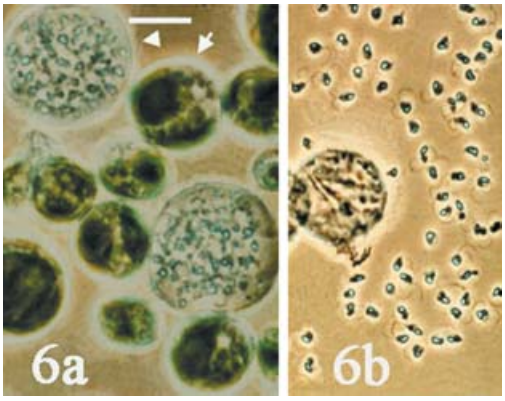

Fig. 6. As in Fig. 1. (a) *Chlorococcum sp*. (7.3 m) contains both vegetative (arrow) and generative (arrowhead) cells. (b) Liberation of zoospores.

Simulated freeze–thaw stresses

The surface and subsurface samples with relatively high algal biomass and numerous cultivatible green algae units were selected for freeze–thaw experiments. In nature, algae inhabiting tundra soils show high resistance to annual temperature fluctuations, which cause repetitive phase transitions of water through the freezing point. But how would permafrost microorganisms particularly algae conduct themselves in such a situation? Therefore, freeze–thaw of both pristine permafrost and tundra soil samples in complete darkness with only temperature fluctuations were run. Algal isolation from tundra soil was expected to be very fast and easy: visible green films appeared in less than 2 weeks during primary thawing. Algal isolation from permafrost during primary thawing was a time consuming process with visible algal

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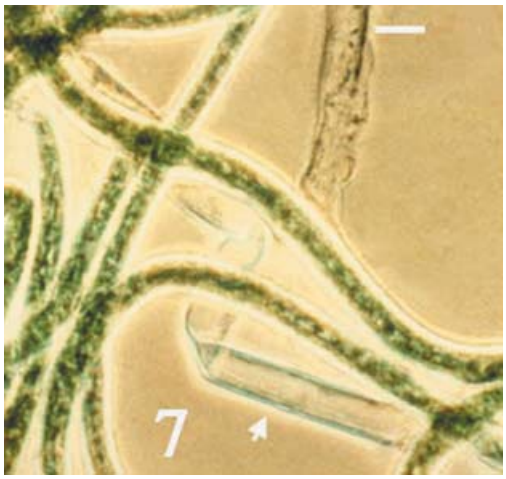

Fig. 7. The beginning of cyanobacterial growth after application of freezing–drying stresses. Arrows indicate the empty protective envelopes. *Oscilatoria sp*. (13.7 m). Trichomes composed of similar vegetative cells.

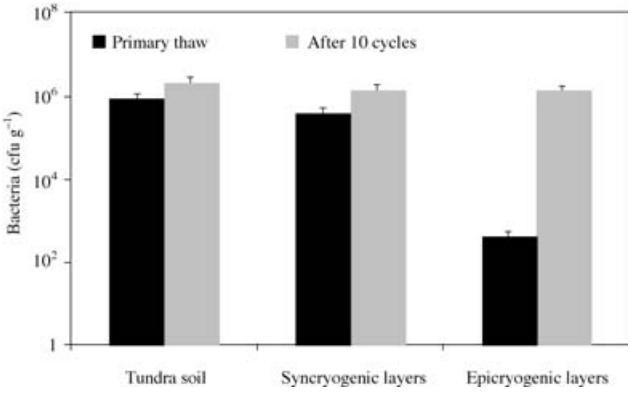

Fig. 9. Effect of freeze–thaw cycles on the recovery of bacteria from permafrost versus surface tundra soil. Data are the mean and the range of three replicate plate counts on trypticase soy broth medium supplemented with 1.5% agar. Black bars denote the number of bacterial cells at primary thaw and grey bars denote the number of bacterial cells after 10 freeze–thaw cycles.

growth observed over the 30 day course of the natural sediment enrichment (Table 2). Algae revived from permafrost are likely to endure freezing, a long period of frozen dormancy, desiccation and thawing. Even though the surviving cells may have resistance and protective mechanisms against stresses mentioned above, they would still have incurred some reversible metabolic damage due to natural radiation or chemicals. The observation that long incubation periods were often necessary to obtain primary visible algal growth are consistent with the idea that such aged cells need time, before beginning growth, to repair the accumulated damage. Algae isolation after repetitive cycles of freeze–thaw took shorter periods. These algae were readily reversible towards proliferation. About 70% of the permafrost samples did not contain any viable algae after primary thawing. No algae were isolated from these samples either after different enrichment procedures or after repetitive freeze–thaw cycles.

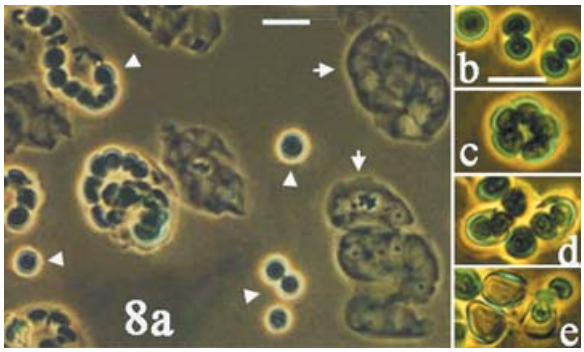

Fig. 8. As in Fig. 7. (a) *Nostoc sp*. (14.8 m). The trichomes consist presumably of akinetes (arrowheads). (b)–(e) Stages of akinete germination. The mature akinete swells, and the first divisions often occur inside the akinete envelope (b, c). After subsequent cell division the envelope ruptures but may remain attached to the developing filament (d); empty envelopes retain their shape (e). Young filaments subsequently give rise to mature filaments.

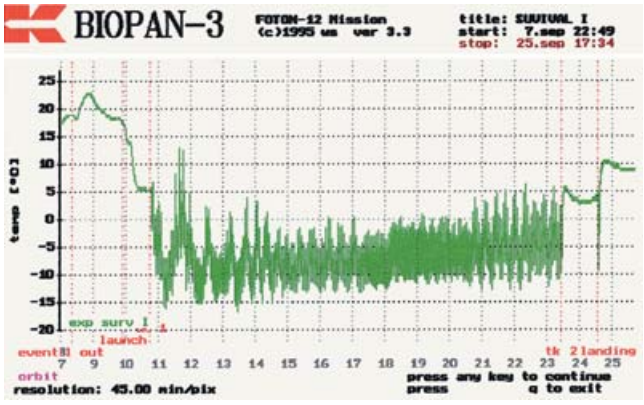

Fig. 10. The spectrum of temperature fluctuations in space near the orbit of the Earth as determined by the ESA Foton-12 mission. The *x*-axis gives us the date on the orbit.

As a control, the amount of bacterial isolates was studied. After 10 freeze–thaw cycles, the bacterial plate counts from modern tundra soil and syncryogenic layers did not change significantly. But similar freeze–thaw cycles led to increasing plate counts from 4.5×10^2 to 1.5×10^6 cfu g^{-1} within epicryogenic permafrost (Fig. 9). These results may be explained as follows. The temperature changes did not affect the microbial communities of the modern tundra soils and syncryogenic layers, which formed and functioned under similar conditions. Microorganisms isolated from epicryogenic permafrost sediments did not experience the action of temperature fluctuation in their natural habitat. In the first stage, the frequent transitions through the freezing point may lead to a mass of cell death (Gilichinsky *et al*. 1993). In the following stage, the remaining cells stop dying and start to adapt to the new conditions.

Permafrost is not dry. Water formed during thaw contains sufficient nutritive materials, many of which were frozen and trapped completely within the ice. These nutritive solutes are expected to be enough to support heterotrophic growth and prolongation of microbial communities. Finally, certain

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Table 2. *The temporal dependence of algal recovery on the application of freeze–thaw stresses*

Depth (m)	Sediment characteristics	Algal biomass,* (μg Chl *a* g dw^{-1})	Algae,** (cfu g dw^{-1})	Appearance of visible algal growth, days	
				Primary thawing	After 10 cycles of freeze–thaw
0.04	Arctic tundra peaty-gley soil	0.55	11×10^3	9	7
10.2	Arctic Holocene lake-swamp loams	0.46	9×10^3	36	28
56.3	Arctic late Pliocene sandy loams	0.06	NG	105	39
1.6	Antarctic Late Pleistocene lacustrine sediments	0.18	NG	NG	NG

* Algal biomass estimated by measuring of chlorophyll *a* during primary thawing.
** Algal cfu estimated during primary thawing by plating of soil suspension on Bold basal medium supplemented with 1.5% agar.
NG, no growth.

Table 3. *Viability of permafrost microorganisms after 3-months of exposure at −4.5 °C inside autoclave sands*

Strain	Description	Cell counts			
		Inoculums[a] (log cfu g^{-1})	Total[b] (log cell g^{-1})	Viable[c] (log cfu g^{-1})	Viability[d] as ratio [Viable]/[Inoculums]
Chlorella vulgaris	Green algae	6.5	5.7	5	0.08
Chodatia tetrallantoidea	Green algae	5.7	4.7	3.6	0.01
Arthrobacter sp.	Gram-positive	8.6	8.2	7.8	0.3
Flavobacterium sp.	Gram-negative	9.1	8	5.2	0.01
Rhodotorula sp.	Yeast	8.7	7.7	7	0.02

[a] Counted in the beginning of the experiment. Number of cells per 1 g of autoclave sands were determined by counting cfu on nutrient media.
[b] Counted after 3-months of exposure at −4.5 °C. Total cell counts were determined by counting cells stained with 5-(4,6-dichlorotriazin-2-yl)-aminofluorescein (DTAF) following the protocol of Bloem (1994).
[c] Counted after 3-months of exposure at −4.5 °C. Viable cell counts were determined by counting cfu on nutrient media.
[d] Ratio were calculated from viable cfu g^{-1} divided by inoculums cfu g^{-1}.

group(s) of microorganisms (monocultures in most cases) become adapted to water phase transitions between the melted and frozen state, occupying the unique microhabitats created by the thin films of unfrozen water in the permafrost (Gilichinsky 2002).

In these experiments we attempted to simulate the freeze–thaw processes that may take place in near-surface (0–100 cm) Martian layers that have now been shown to contain ice or water (Boynton *et al.* 2002; Feldman *et al.* 2002; Mitrofanov *et al.* 2002). In these three studies using remote sensing data from the Mars Odyssey, researchers suggest that large deposits of ice or water (35±15% of the layer by weight) are buried 1–2 ft below the surface of the planet. The results with modern tundra soils and permafrost sediments demonstrate the resistance of their microbial communities to prolonged freezing and freeze–thaw stresses. The same experiments also simulate the situation in near orbit of the Earth (as received from the ESA Foton-12 mission, Fig. 10), where comets and other icy planetesimals may reside for some time before impacting on the Earth. Fig. 10 comes directly from Survival BIOPAN's experiment and shows a temperature flux through the time from launch to landing. The Survival experiment was aimed at measuring the survival rate of microorganisms exposed to the harsh space environment of vacuum, extreme temperatures, radiation and solar UV. The temperature hovered between −5 and −10 °C most of the time, but there were transitions through 0 °C approximately six times per 24 h to a maximum of 5 °C. The launch and landing in turn were accompanied by temperatures of 5 °C and higher.

The survivability of ancient microorganisms in terrestrial permafrost over a hundred thousand to a few million years and the data illustrated the resistance of permafrost microorganisms to rapid temperature transitions through the freezing point add credibility to the theory of panspermia: the possibility that simple life forms may travel from one planet to another on meteorites.

Survival during freezing and desiccation stress

The permafrost microorganisms in their exponential growth phase were frozen inside coarse sands for the purpose of simulating permafrost formation and the transition of microorganisms from an active to a frozen state. The members of the permafrost community such as green algae, bacteria and yeasts were exposed for 3 months at −4.5 °C to complete darkness. Green algae *Chlorella vulgaris* and *Chodatia tetralontoidea* survived but the number of viable cells was lowered by a factor of 10 and more. The cell viability was 10 times higher for Gram-positive bacteria *Arthrobacter sp.* in comparison to Gram-negative bacteria and eukaryotic microorganisms (Table 3). It was shown earlier that because of the cryoprotective importance of unfrozen water, the number of viable cells under similar conditions depends on the textural composition of the permafrost, and increases with increasing

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soil dispersion. The finer the texture of sediments, the larger the unfrozen water content in them, the thicker the water films and the greater the number of viable cells (Gilichinsky 2002). The frozen coarse sands represent the environment with minimal content of unfrozen water, which cannot serve as a cryoprotective substance and cannot play a leading role in the preservation of microorganisms (Gilichinsky *et al*. 1993). Thus, in sands, the amount of unfrozen water is minimal, tending toward zero. Therefore, the cells (organelles and membranes) are destroyed mechanically by ice crystals, leading to death through cell lysis. It may be suggested that dead and lysed cells may play the role of cryoprotectors and nutrient supplies for surviving cells.

During the experiments, the microorganisms were subjected to freezing, frozen inactivity and thawing. These experiments demonstrated that both prokaryotic and eukaryotic microorganisms (bacteria, yeasts, algae) were able to survive at sub-freezing temperatures (−4.5 °C) over prolonged time (3 months) and simulated natural processes attesting to their resistance to extreme low temperatures and freeze–thaw fluctuation. It is possible that, even in low numbers, viable cells present in coarse sands after exposure to subzero temperatures bind with silt particles in permafrost, which retain unfrozen water that the microbes are able to assimilate.

Also in this study permafrost cyanobacteria belonging to five different genera namely *Nostoc*, *Anabaena*, *Phormidium* and two genera of *Oscillatoria*, were exposed to freezing–drying stresses. The inoculation of dried biomass into a fresh medium led to the release of cyanobacterial trichomes from sheaths (Figs 7 and 8a, arrows). In the case of *Nostoc* the trichomes were composed predominantly of akinetes (Fig. 8a, arrowhead). During the next few days, the formation of young trichomes from akinetes was observed (Figs 8b–e). It appears that freezing induces the formation of protective envelopes and resting cells and as a result, the permafrost cyanobacteria withstood dehydration and long-term inactivity.

Conclusions

Viable permafrost algae demonstrated resistance to freezing, thawing and freezing–drying stresses. They can survive below freezing point in complete darkness and show adaptation to living within permafrost. During the last several decades, permafrost microbiology studies have taken a growing role in astrobiology. Sediments frozen for thousands to millions of years and inhibited by microorganisms serve as an analogue for microbial life on Mars and other cryogenic bodies. The discovery of ancient viable cyanobacteria may have significant meaning for astro/exobiology. Cyanobacteria are the oldest photosynthetic microorganisms on the Earth and predate the evolutionary divergence of green plants. Cyanobacteria possess phycobilicomes, the structure of which can be adjusted depending on the availability of wavelengths for better light harvesting – a possible mechanism for survival under varying light intensity. Thus, to grow and develop cyanobacteria need water, carbon dioxide (CO_2), nitrogen as N_2 and light, so they might inhabit other planets. Since prokaryotic cyanobacteria and eukaryotic unicellular green algae survive in terrestrial permafrost for several million years and are known to be resistant to freezing temperatures and solar radiation, similar organisms, or their fossils, might be expected to occur in frozen Martian subsurface horizons. Live cyanobacteria and green algae contain chlorophyll and cyanobacteria have also phycocyanin and phycoerythrin. These photosynthetic pigments possess their own fluorescence, which can be detected by FT-Raman spectroscopy with 1064 nm excitation to avoid autofluorescence from the pigments (Wynn-Williams & Edwards 2000). So, photosynthetic pigments could be good biomarkers to show not only the presence of algae but also their micro-spatial distribution *in situ*. In fact, viable permafrost algae could make an important contribution to probing for buried biomolecules before future Mars missions.

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