8 Permafrost Model of Extraterrestrial Habitat

David A. Gilichinsky

Seven of nine planets of our Solar System (Fig. 8.1), as well as their satellites, comets, and asteroids are of a cryogenic nature, i.e. the permafrost is a common phenomenon in the cosmos. From an astrobiological point of view, the terrestrial permafrost, inhabited by viable cold adapted microorganisms may be considered as a model of extraterrestrial life. The microorganisms found in the Earth's permafrost provide a range of potential inhabitants on extinct space cryogenic bodies. Thus, in contrast to other disciplines, microbiology is looking not only for traces of extinct life on other planets, but, most intriguing for signs of existing life at the cellular level, especially within frozen materials.

8.1 The Importance of Permafrost

The ability of microorganisms, the most ancient life forms on the Earth, to live in a variety of natural environments continually forces us to redefine the limits of life in biosphere. Microorganisms not only have adapted to the cold and populate the main ecological niches, but also survive under conditions that seem absolutely unsuitable for life in large populations and a high diversity. More than 80% of the Earth's biosphere is permanently cold and cold adaptation of microorganisms would appear to be an important trait. Because of their ability to cope with low temperatures, cold adapted microbes, first isolated by Forster [1], can be regarded as Earth's most successful colonizers [2].

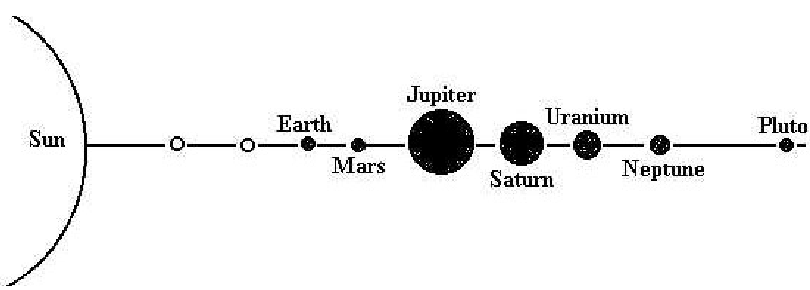

Fig. 8.1 The planets of cryogenic type.

A significant volume of the Earth's cold environments consists of permafrost (perennially frozen ground) - a naturally occurring material with a temperature below 0 °C, underling 20% of the land surface [3]: on the northern reaches of North America and Eurasia (Alaska, Siberia and Canada). In these regions, the permafrost reaches a thickness of more than 700-1000 m in the north, thinning toward the south. It also occurs in the ice-free regions of Antarctica and Greenland and surrounding Arctic and Antarctica as offshore permafrost. Alpine permafrost can be found in the high mountains of Europe, Asia, and America.

This considerable mass of frozen sediments, up to several hundreds of meters deep, harbors significant numbers of viable microorganisms. The first data on the existence of bacteria in permafrost appeared at the end of the 19th century, in relation to the discovery of mammoths and soil studies in Siberia [4, 5]. Later microbes were discovered in Holocene to late Pleistocene permafrost deposits in many Arctic regions, as well as viable cells in more ancient sediments were found in associated with Antarctic Dry Valley Drilling Project [6, 7]. In these studies the procedures and the application of drilling solutions did not guarantee the sterility of cores. Nevertheless, these authors have priority in raising the question of the possible preservation of living matter in permafrost.

In recent investigations, new aseptic methods of drilling, sampling, storage, transportation, and strict control, as well as new specialized tests, have shown that the microorganisms seen in permafrost samples could not have penetrated from the outside, but are present *in situ* [8-10]. They have been isolated (Fig. 8.2) from the cores up to 400-m deep in the Canadian Arctic and at the lowest ground temperatures (–27 °C) in Antarctica. The age of the microorganisms corresponds to the longevity of the permanently frozen state of the soils. The oldest viable cells date back to 2-3 million years (Ma) in northeastern Siberia, and probably older cells may be found in Antarctica. They are the only known forms of life that have retained viability over geological time periods and upon thawing renew physiological activity. Thus, the permafrost can be characterized as a unique physical-chemical complex, which can maintain life incomparably longer than any other known habitats. If we take into account the thickness of the deep permafrost layers, it is easy to conclude that they contain a total microbial biomass many times higher than that of the modern soil cover. This great mass of living matter, 10^2-10^7 cells per 1 g of soil (Table 8.1A.), is peculiar to permafrost strata only.

Therefore, permafrost is of great significance for research in cryobiology, biotechnology, ecology, molecular paleontology. Specially, the occurrence of a viable Cenozoic generation of microorganisms within the permafrost is intriguing for the newly emerging field of Astrobiology. If life should be found to be distributed beyond the planet Earth, the recovered organisms may possess unique mechanisms that probably might work for billions of years to allow them to maintain viability. Cameron and Morelli [11] first advanced the idea to look of Martian life by using terrestrial permafrost models.

8.2 Parameters of Permafrost Microbial Habitat

The continual subzero temperatures, as well as the main physical-chemical parameters, make permafrost one of the more stable and balanced of the natural environments. This determines the necessity to consider the traditional physical-chemical characteristics of permafrost as abiotic parameters of the habitat, what provides life support and ensures the formation of microbial communities that realize unknown possibilities of physiological and biochemical adaptation to prolonged cold and remain virtually invariable for millions of years. It may be suggested that the mechanisms of such adaptation are universal and operate within the broad limits of modification for natural ecosystems on and beyond the planet Earth.

8.2.1 Temperature

The temperature regime, the most fundamental aspect of any environment, acts as a regulator of all physicochemical reactions and biological processes [12]. In addition, the subzero permafrost temperatures are favorable to the preservation of biological systems, and is the main factor contributing to the long-term survival of cells. Evidently, the long-term impact of subzero temperatures should be regarded not as the extreme and limiting but rather as a stabilizing factor supporting the viability of microorganisms adapted to these conditions.

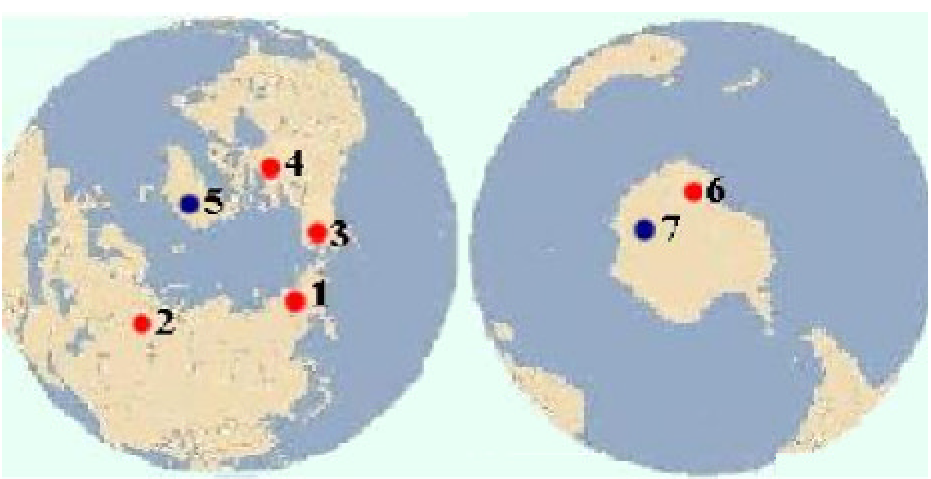

Fig. 8.2 Locations of microorganisms discovery in permafrost (red) and ice cores (blue). Arctic: 1 - Kolyma lowland; 2 - West Siberia; 3 - Alaska; 4 - Mackenzie Delta; 5 – Greenland Ice Sheet; Antarctica: 6 - McMurdo Dry Valleys; 7 - Vostok station on Ice Sheet [21].

Table 8.1. Viable microorganisms in permafrost

A. The number of viable aerobes in permafrost at different temperatures

Kolyma lowland, T= –10 to–12 °C		Mackenzie Delta, T= –5 to –6 °C		West Siberia, T= –1 to –2 °C		Dry Valleys, T= –18 to –25 °C	
depth, m	cells/g	depth, m	cells/g	depth, m	cells/g	depth, m	cells/g
21.0-62.4	2.2×10^5 - 1.4×10^7	9.5-326.4	1.4×10^3 - 1.6×10^5	2.0-18.5	1.4×10^2 - 2.7×10^3	1.0-19.3	5.2×10^2 - 6.2×10^4

B. The number of viable aerobic bacteria (cells/g) on the culturing temperatures

depth, m	temperature, °C			
	40	30	20	4
Antarctica, Tailor and Meyr Valleys, eolian, glacial and lake bottom sands; T= –20 °C				
0.5-16.3	0-<10^1	0-1.1×10^3	<10^2-5.9×10^4	0-9.1×10^3
Antarctica, Mt. Feather (formation Sirius) and Beacon Valley (sands); T= –23 to –27 °C				
1.0-3.0	0	0-1.5×10^2	7.6×10^1-6.3×10^4	0
Canadian Arctic, Mackenzie Delta, sands and loam				
9.5-41.2	0	6.4×10^1-1.9×10^3	1.4×10^3-1.6×10^5	0-6.5×10^3
Kolyma lowland, marine and alluvial sands and loam				
5.7-55.2	0	1.7×10^1-5.3×10^4	3.2×10^1-1.1×10^5	1.2×10^2-2.3×10^4

C. The number of viable permafrost anaerobes (cells/g) growing at 15 °C

period (age, years)	depth, m	metanogens (CO_2+H_2)	denitrifing (NO_3+citrate)	sulfate reducers (SO_4+ lactate)
QIV (5-10×10^3)	1.0	2.0×10^7	2.0×10^7	2.0×10^2
QIII (1-4×10^4)	4.4-17.0	2.5×10^7	2.5×10^5 -2.5×10^6	0
QII (1-6×10^5)	30.0-35.7	(2.0-2.3) $\times10^7$	2.3×10^3 -2.0×10^6	0-2.3×10^2
N_2-QI (0.6-1.8×10^6)	37.2-64.3	(2.0-2.5) $\times10^7$	2.5×10^3 -2.5×10^7	0-2.0×10^2

In Arctic the mean annual permafrost temperature, as registered at high altitudes, is –10 to –12 °C, rising toward the southern border of the permafrost to –1 to –2 °C. In Antarctic polar desert, with its background of below freezing air temperatures even in summer, the maximal (–18 °C) mean annual permafrost temperature in free ice areas, such as the Dry Valleys, is registered at low hypsometric levels near the coast. This temperature decreases when moving inland and toward higher altitudes, reaching the lowest value on the Earth, −24 to −27 °C, however, higher that of Mars. But at some depths below the surface, where the amplitude of temperature oscillations goes down, the mean temperatures of Martian permafrost could be comparable with those of the Earth. And what is more, icy caps on Martian poles isolate the underlying permafrost from the ultra low temperatures prevailing in the atmosphere as ice sheets or glaciers on the Earth. The Martian dust in equatorial or moderate zones apparently plays the same role as finely dispersed sediments in terrestrial conditions - reduce sharply the temperature oscillations and the depth of their penetration. As a result, at depths below the annual temperature oscillations, the temperature of Martian permafrost (both, on the poles and at the equator) should be approximately –30 °C, not much different from the extreme values found on Earth. At these temperatures permafrost could present an inhabited environment identical to that of the Earth.

8.2.2 Iciness and Unfrozen Water

The permafrost is characterized by a multiphase state of water, which plays a dual role from the biological point of view [13]. The solid phase (ice) makes up 92-97% of total water volume in permafrost and serve as a cryoconservant for biological objects. The iciness of sandy-loam-based frozen ground in Arctic varies from minimal values of 10-20% to a wide range of maximal values, 40-50%, or more in the presence of ice wedges. At the higher ice contents the pores are completely filled with ice, obviously excluding any migration inside the stratum. The unexpectedly high (25-40% and more) content of ice in the subsurface coarse-grained Dry Valleys sands firmly cemented into a massive cryogenic structure, has no explanation. These data disprove the concepts that ground ice is unstable because of the active processes of sublimation at ultra low air temperature in Antarctica: –60 to –70 °C, as well as the thesis of the “dry Antarctic permafrost” and makes to have a new look at the regularities of the formation of frozen strata. In contrast, McKay with co-workers [14] think that some mechanism is recharging the ground ice from atmosphere. This is why one cannot exclude the high ice presence in Martian permafrost, too.

In permafrost 3-8% of the water is in an unfrozen state ($W_{unf.}$), most commonly as thin films and, possibly, as brine pockets in saline soils. Among other parameters the unfrozen water play the leading role in the preservation of microorganisms [13]. These films, by coating the organo-mineral particles, protect the viable cells sorbed onto their surface from mechanical destruction by growing crystals of intrusive ice and make possible the mass transfer of microbial metabolic end-products within the permafrost, preventing the cell's biochemical death. Probably, they also may serve as a nutrient medium, because the unfrozen water contains high concentrations of various ions and molecules, as well as represent firmly bound, liquid, water with binding molecules; this is why permafrost may be considered as biologically dry environment.

The dependence of unfrozen water on the physical-chemical parameters of frozen strata has been studied by the many scientific schools [for review see 15]. The quantity of the unfrozen water and thickness of its films is independent of the total ground iciness, decreases with temperature [16, 17] and associates with texture composition: the more dispersed the sediments are, the larger is the $W_{unf.}$ in them and the thicker are its films, 5-75 Å (this is why the finely-dispersed Martian dust appears favorable for the presence of unfrozen water). At Arctic permafrost temperatures (–9 to −12 °C) the magnitude of $W_{unf.}$ in loam is 3-5%. The maximal values of (10%) are related to higher temperatures or saline marine facies. In sands, $W_{unf.}$ is minimal, tending to zero, although there a silt fraction in them that retains a measurable $W_{unf.}$. Only in Antarctic sands, because of the low temperatures, the $W_{unf.}$ values there are so small that the instrumental methods fail to record them.

8.2.3 Ice and Permafrost as a Habitat

The validity of unfrozen water as a main ecological niche, where the microorganisms might survive, can be demonstrated by a comparison between the numbers of viable cells recovered from permafrost and ice cores. In contrast to frozen soils with an abundance of microorganisms [18-20], the viable cells recovered from cores of the pure ice of the Antarctic Ice Sheet taken at the Vostok station [21] or Greenland Ice Sheets, is on the order of a few dozens per 1 ml of thawed water and increase [22] with increasing concentrations of dust particles in the core. It should be noted that reliable data are available only for young, not older than Holocene, ice layers. To date, no viable cells have been found in the fossil ices of Arctic - neither in intrusive ice nor in polygonal ice wedges [7, 23]. Also, it should be remembered that permafrost not only contains more information because of the high microbial numbers and diversity, it is also much older than ice sheets and glaciers. The oldest glacial ice, 400-700 thousand years old, is found at Vostok station in Antarctica and in Gyliya ice cap in the Tibetan mountains [24, 25], while the permafrost ages can reach millions of years.

The viable organisms in the Earth's Cryosphere - permafrost, ice sheets, and glaciers (Fig. 8.3) - represent a significant part of Biosphere thus, it might be called Cryobiosphere. Both pure ice and permafrost are at the same subzero temperatures. The only difference between the two is the unfrozen water associated with suspended solids. In nature, pure ice, lacking suspended solids and their associated $W_{unf.}$, has insufficient protection and transport abilities and cells are destroyed mechanically by ice crystals [26]. Permanently frozen fine-grained soils provides more favorable as microbial niche and represent the most inhabited part of the Earth Cryobiosphere [27, 20].

8.2.4 Gases and Supercooled Water

The composition of the gaseous phase of permafrost is different from that of atmospheric air. The pore space of frozen strata is occupied by oxygen, nitrogen, methane, carbon dioxide, etc. The concentration of O_2 and N_2 does not differ significantly from their values in the air, while the content of biogenic gases may be appreciably higher: CH_4 in Arctic permafrost varies 2 to 40 and CO_2 1-2 to 20 ml/kg. The facts suggest that these gases be held within the frozen layers in a clathrate form [28]. From a planetary science perspective it is important to note that CO_2 and CH_4 do not prohibit long-term cryopreservation of viable cells: CO_2 is expected to be a major constituent of the gas phase within Martian permafrost, and CH_4 in the permafrost of the satellites of the gas giants.

Any data about free water on Mars, because of the ultra low temperatures on the planet, means salt water. We have such model on the Earth. In Arctic, at some depths, super cooled (up to –10 °C) saline water lenses were found within permafrost. These lenses are formed after the retreat of the sea and the freezing of marine sediments. Because of the freezing out of salts from the deposits, the concentration of salts is up to 300 g NaCl per 1 liter. And even in these environments viable cells were found, both aerobic and anaerobic, and non-halophylic. It remains to be determined whether the salt tolerance of these cells may also be associated with cold tolerance.

8.2.5 Age and Radiation

The age of Mars is approximately the same as the age of the Earth. Therefore, the Earth and Mars may have experienced similar stages of development, including the emergence of early life forms. Indeed, the frozen subsurface environments of Earth and Mars seem to provide quite similar conditions for microbial habitation [27] (Fig. 8.4). From an exobiological point of view, the main difference between the terrestrial and outer planets permafrost, inhabited by microbes, is their age. The oldest continuously late Cenozoic permafrost, where the age of microorganisms corresponds to the longevity of sediments frozen state, found in the north hemisphere of the Earth dates back to about 3 Ma in northeastern Eurasia ([29], Fig. 8.5a, b), while on Mars, the age of permafrost is estimated at approximately 3-4 Ga. This difference in time scale could result in different patterns and have a significant impact on the possibility for life preservation. The Antarctic permafrost may be somewhat closer to Mars. The analysis of numerous publications, best reviewed by Wilson [30], indicates that permafrost has existed under Antarctic climatic conditions for last dozens of millions of years, greater than the duration of Arctic permafrost by a factor of ten [27]. To the moment, viable bacteria were found in bore holes in one prospective site, Mt. Feather [31]. The age of glacial deposits of the Sirius Group dates back at least 2 or, probably, 15 Ma [32, 33]. $^{40}Ar/^{39}Ar$ dates on ash layers suggest that the most ancient ground ice below these layers may be more than 4.5 Ma old in Arena Valley [34] and 8.1 Ma old in Beacon Valley [35]. Viable bacteria have been isolated in the frozen sediments and ice below this ash layer and if this age is correct, this is the oldest viable life on Earth (Fig. 8.5c, d).

Estimation of ground radiation has been made by McKay and Forman, using both elemental analysis of the radioactive elements in sandy and loam samples and direct *in situ* measurements in the bore holes on the Eurasian northeast. The dose received by the permafrost bacteria is about 2 mGy per year. Taking into account the age of bacteria, late Pliocene to late Pleistocene, the total dose received by cells would therefore range from 1 kGy in soils dozens of thousands years old to 6 kGy at over the 3 Ma age of microorganisms. Experiments of Vorobyova have shown that up to 30 kGy are required to sterilize soil at above-freezing temperatures and the results obtained by Japanese researchers, indicate that bacteria in soil have a much greater resistance to

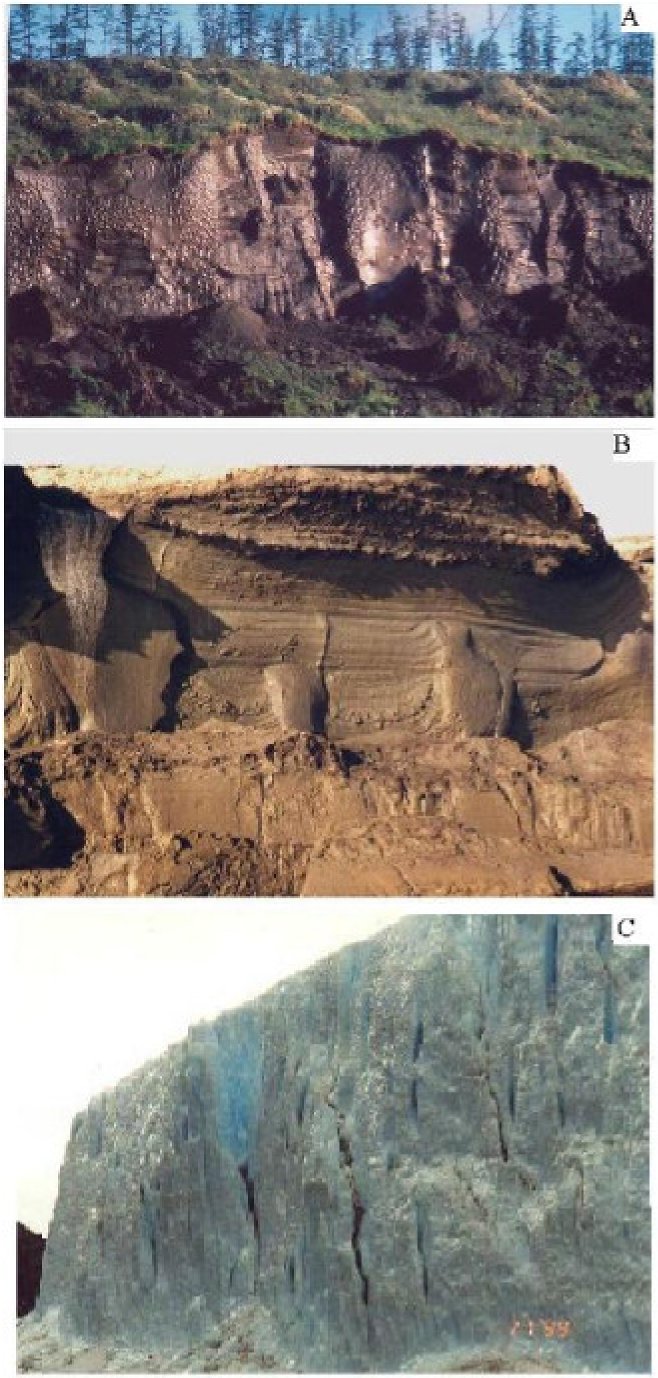

Fig. 8.3 Permafrost (A, B) and glaciers (C) exposures. A, B: frozen layers with ice veins in Arctic tundra (photo by M.Grigor'ev) and forest-tundra; C: Taylor Glacier in Antarctica

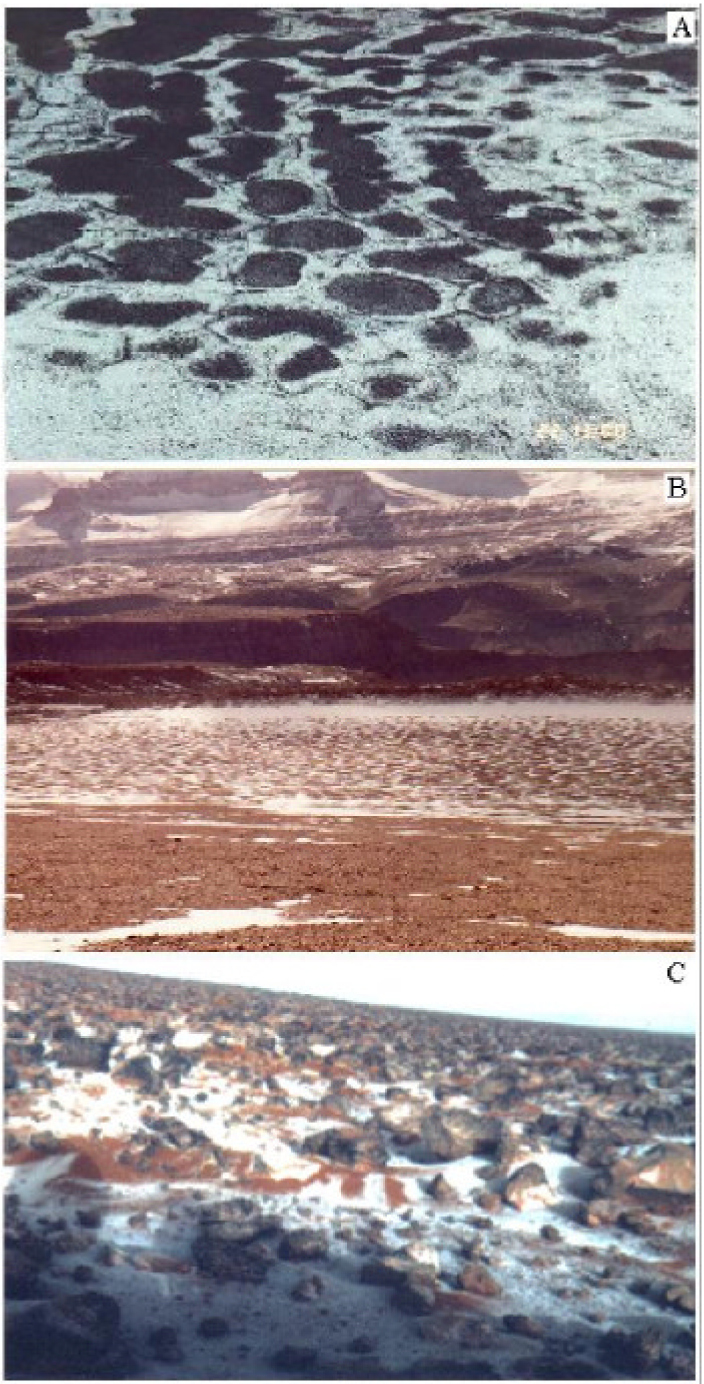

Fig. 8.4 Permafrost landscapes. A: Arctic in October; B: Antarctica in January; C: Mars in spring (NASA):

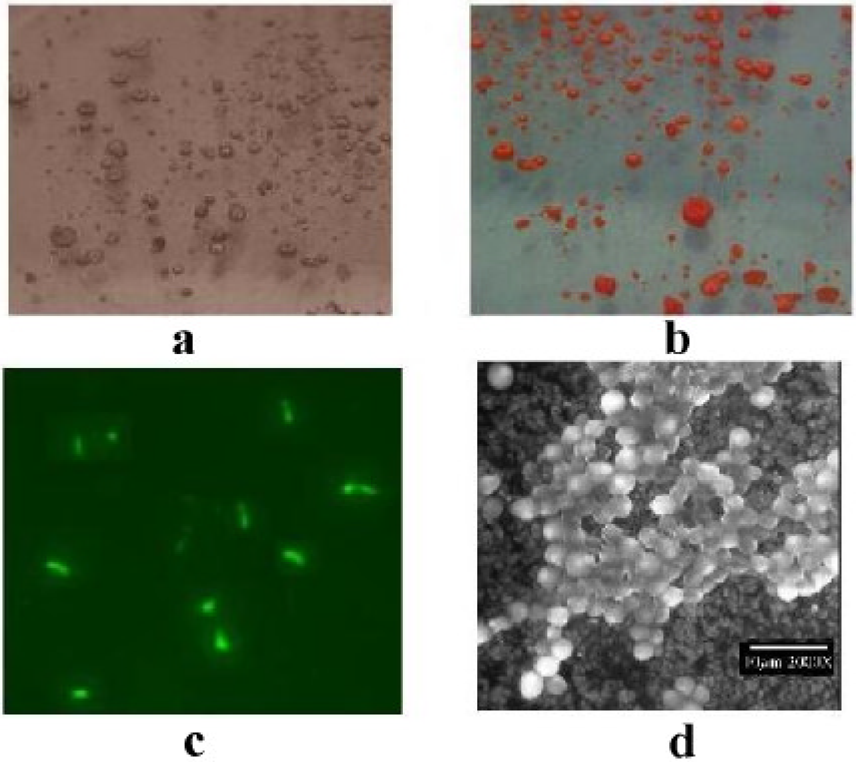

Fig. 8.5 The oldest viable microorganisms. The morphology of bacteria (a, b) from the late Pliocene Arctic permafrost; bacteria (c) and green algae Mychonastes (d) isolated from frozen sediments in Antarctica (DTAF, photo by E. Spirina and A. Shatilovich).

irradiation when frozen than when thawed. Our own results demonstrate that, at similar levels of ionizing radiation, a known quantity of viable cells and a total radiation dose of 5 to 50 kGy, most of the cells in frozen samples survived, while most of the cells in unfrozen samples died. This facts shows that freezing increased the cell's resistance to radiation and uniqueness of permafrost as an environment, where microorganisms display a high resistance to radiation. From these data the dose from radionuclides diffused through the permafrost is far from being sufficient for complete sterilization, i.e. is not fatal for viable cells, but should be large enough to cause some selection effect and to destroy the DNA of ancient cells. Their viability and growth on media implies the capacity for DNA repair. Probably DNA repair is occurring in the frozen environment, i.e. at the stable rate of damage accumulation, a comparable rate of repair also exists. From the biological point of view it is important that the permafrost preserves the cells from diffuse ground irradiation for thousands and millions of years. And what is more, the oldest, Pliocene cells, which had received an *in situ* maximal dose during the time of cryopreservation, were found to be more resistant to radiation than microorganisms from modern tundra soils.

8.3 Biodiversity in Permafrost

The permafrost microbial community has been described as "a community of survivors" [36], which have overcome the combined action of extremely cold temperature, desiccation and starvation. Under these conditions, the starvation-survival lifestyle is the normal physiological state [37]. According to their growth temperatures [38], the microbial community, even after its long-term existence within the permafrost, is not composed of psychrophilic bacteria (Table 8.1B.) [39]. However, it is resistant to sharp temperature transits through the freezing point and to freezing/thawing stress. Most of the isolated cells did not grow at temperatures higher than +30 °C, but were often capable of growth on Petri dishes at subzero temperatures as low –10 °C in the presence of cryoprotectants such as glycerol [7]. According to Russell [2], this correlates with the lower growth temperature limit of psychrophilic microorganisms. Because this term is not clearly delimited, one can define the isolated microbes as psychrotolerant organisms.

It is interesting to note that Arctic and Antarctic sediments contain about the same number of microorganisms (estimated from direct counts using epifluorescence microscopy [20]), although the Arctic sediments are rich in organic carbon, whereas in the Antarctic ones the content of carbon and unfrozen water is close to zero. This total number of microbial cells is relative constant from modern up to the oldest frozen layers. In other words, there is the minimal constant level of microbial colonization that forms in natural systems in the presence of only mineral soil particles and short term existence of free water. Possibly, this unexpected result shows that these minimal conditions are enough even for origin of life.

Overall, a number of different morphological and physiological groups of microorganisms (spore forming and spore-less, aerobic and anaerobic, prokaryotic and eukaryotic) have been found (Fig. 8.6). Morphologically, they are coccoid, coryneforming, nocardia- and rod-like Gram-positive or Gram-negative bacteria. The ancient permafrost microbial community is predominantly bacterial, as is the community in the depths of Antarctic Ice Sheet [21]. This is in contrast with modern soils, where the fungal mass is much greater than the bacterial mass. Prokaryotes with thick well preserved cell walls and capsules, surrounded by additional surface layers of low electron density are observed rather frequently [40]. Evidently, good preservation of cell structures is determined by the equilibrium-state of the unfrozen water inside and outside the cells. Violation of the equilibrium results in cell death. As a consequence, ice formation in the experiments on Petri dishes results in cell death [26]. Eukaryotic cells, while present, seem less able to survive long-term cryopreservation [40]. In Arctic permafrost, non-spore-formers predominate while in Antarctic permafrost spore-formers dominate. Recently, phototrophs, organisms that had preserved their photosynthetic apparatus in the full permafrost darkness were isolated [41]. These included cyanobacteria, perspective the most ancient organisms (the fossil cyanobacteria often found in meteorites and basalt deposits by ages 3.5 billion years) that use light of almost all wave lengths to synthesize organic material. Green algae and mosses represent the lower plants, and now from the buried Arctic soils were isolated even Protozoa. Viable seeds of high plants were found in the late Pleistocene thickness, and these seeds from Canadian Arctic still are able to grow [42].

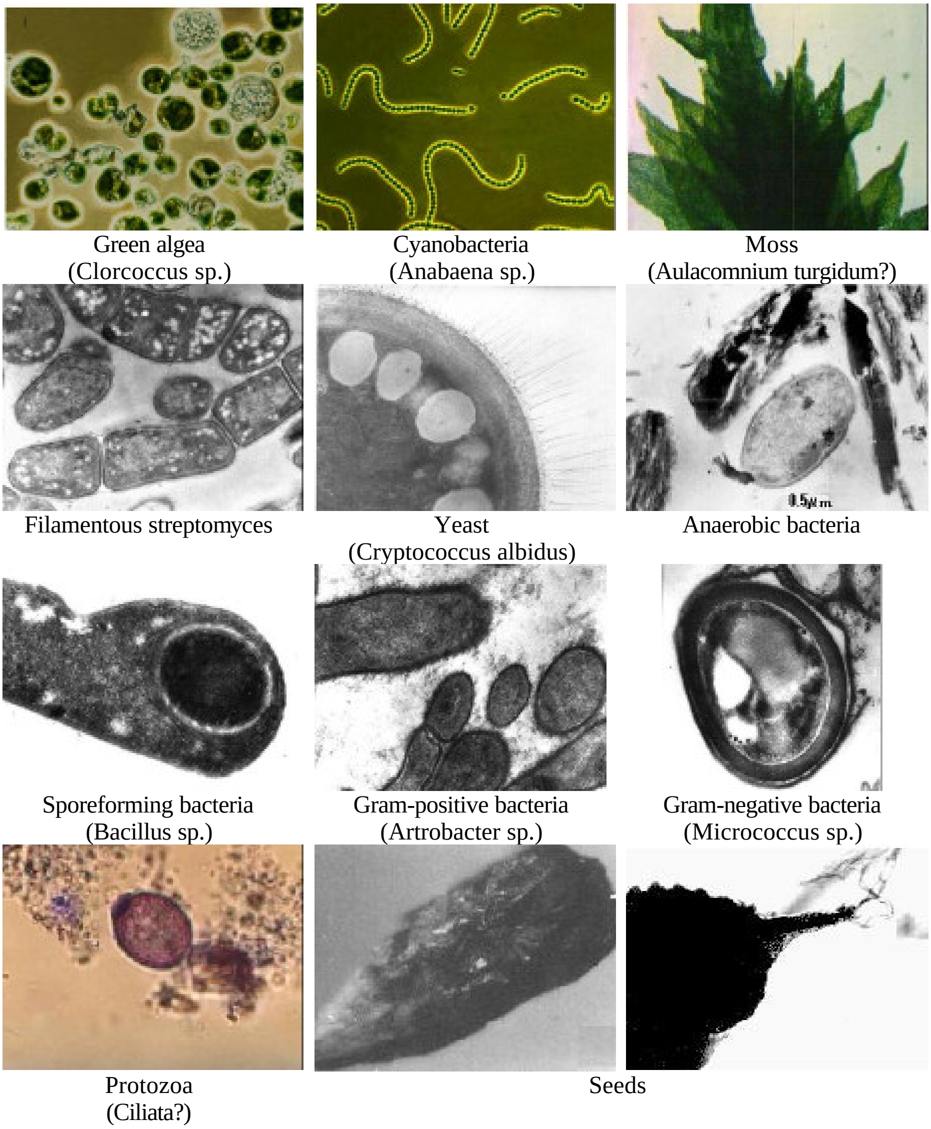

Fig. 8.6 Permafrost biodiversity (photo by V. Soina, T. Vishnivetskaya, N. Suzina, E. Spirina and S. Gubin).

The presence of methane, as well as the value of the redox potential (+40 to –250), indicate that conditions in Arctic permafrost are mostly anaerobic; these permafrost thicknesses are primarily lacustrine-alluvial and marine deposits. The *Eh* of studied Antarctic sands (mostly, eolian and glacial origin) vary from +260 to +480, indicating that the conditions here are not as anaerobic as in Arctic; this is confirmed by the absence of biogenic methane. In any case, it is the specific character of permafrost soils, that, until the moment of transition to the permanently frozen state, they contain both aerobic and anaerobic microzones. This is why the ancient microbial communities consist of comparable numbers of anaerobic and aerobic species. The ratio of anaerobes to aerobes is different in different geological facies, i.e. the part of the community that dominates depends on the origin of sediments. In one study, based on alluvial deposits, aerobic groups dominated [18], while in another study, the number of anaerobes (methanogens, denitrifyiers, sulfate-reducers and iron-reducers) in swamp and lagoon soils was several orders of magnitude higher than that of aerobes [28]. The reduced state and viable anaerobic bacteria provides a model for possible anaerobic life forms on cryogenic planets without free oxygen and even in the absence of organic matter. These chemolithotrophic, psychrotolerant bacteria, with their ability to assimilate CO_2 in an environment similar to that of Mars, have been suggested as prospective analogues to the living forms that might be found in subsurface Martian layers [43]. This thesis supported by distribution of anaerobic bacteria in permafrost layers - it is determined by the anaerobic origin of the frozen strata, and if the community exists, their number is independent of permafrost age [28] (Table 8.1-C). For aerobes, after the prolonged exposure of the microorganisms to the permafrost environment, the ratio of readily culturable (hypometabolic) bacteria to those that may represent viable but nonculturable forms (deep resting cells) is determined by the extent and duration of exposure to subzero temperatures [20]. The greater the permafrost (not sediments) age, the lower the number of viable cells, the lower their qualitative and morphological diversity.

Some cells may have died, when subjected to the stresses of thawing and exposure to oxygen as the samples were suddenly melted in the laboratory for microbial study at relatively high temperatures. While these stresses are known to inhibit the recovery of a fraction of the community, strategies and techniques for the low-temperature recovery of bacteria from permafrost environments are only just beginning to be developed [44]. In isolations aerobic bacteria from Arctic permafrost at +20 °C, a significant fraction was found to be spore-formers [10]. This contrasts sharply with isolations carried out at +4 °C, where spore-formers were only rarely obtained. Perhaps this reflects an intriguing paucity of spore-forming bacteria in frozen environments [44]. The permafrost microbes have not received as much study and the need development of improved protocols for their recovery from ancient frozen environment, as preparation for the analysis of life in extraterrestrial materials.

For this reason we need to keep in mind that all above mentioned discussions are based on the culturable cells (analysis of Arctic sediments showed that only 0.1-5% of the cells counted by fluorescence microscopy grew on nutrient media and in Antarctica, the percentage is reduced to 0.001-0.01%) and, as is in other environments, a large portion of the permafrost microbial community remains uncultured. The phylogenetic diversity of the permafrost community has only recently begun to be addressed and the data based on DNA extracted from modern tundra soils suggest that the clones are phylogenetically diverse, and that many probably reflect new genera or families. Hence, most of this bacterial community has never been isolated and the physiology and function of its dominant members is unknown [45]. The first molecular data have recently been obtained from ancient permafrost bacterial communities, using 16S rDNA sequencing and the phylogenetic trees derived from this data indicate that the Arctic isolates fall into four major groups, partly determined by the age of the permafrost. Most are high-GC Gram-positive bacteria and β-proteobacteria. All γ-proteobacteria came from the samples, Ma old. Most of the low-GC Gram-positive bacteria came from the age, 3000-8000 years [10]. The communities also include members of Archaea, which have recently been detected in permafrost [46]. The results demonstrated good preservation of ancient genomic DNA in both, permafrost, and Greenland ice cores [47].

8.4 How Long the Life Might Be Preserved

To answer the question how long life might be preserved, we have to establish the principal mechanism of cell's behavior in permafrost conditions. Probably this mechanism, for example for spore-formers, might work for billions years. A key question regarding viable paleobacteria in permafrost is whether they are active at permafrost environment or whether are in a suspended "dormant" state. This is why the mechanisms for microbial survival during the both long-term anabiosis or low metabolic state must be studied. It is necessary to consider 2 ways of surviving viable cells. First, in Arctic, at temperatures around –10 °C, where cells are only in a cooled state, and the second, in Antarctic, at temperatures below –20 °C, where cells are preserved in a frozen state.

There are only a few data about metabolic activity of microorganisms below the freezing point, and only for recent microbial communities. Measurements show that Antarctic lichens may be active at temperature –17 °C [48]. Recently it was also obtained evidence of low rates of bacterial DNA and protein synthesis in Antarctic snow, which indicates that the organisms were metabolizing at ambient temperatures –12 to –17 °C [49]. The following facts provide indirect evidences that paleomicroorganisms can be active in the permafrost nutritional-temperature regime and allow to speculate on the possibility of biogeochemical processes *in situ*: the ability of immobilized enzymes in permafrost to become instantly activated [50]; the presence of usually metastable nitrites and ferrous sulfides [51, 52]; the simultaneous presence of methanogenic archaebacteria and methane [28, 46]. Experiments using ^{14}C acetate show that microbial ancient communities from 3 Ma old permafrost at temperatures down to –20 °C are able to form bacterial lipids [53]. However, the question about the metabolic state of microorganisms, microbiological and biogeochemical processes and life forms within permafrost still remains open.

8.5 The Perspectives for Future Studies

Permafrost is a depository of ancient biomarkers (Fig. 8.7), including biogenic gases, biominerals, biological pigments, lipids, enzymes, proteins, nucleotides, RNA and DNA fragments and molecules, microfossils, died and viable cells. They provide a range of analogues that could be used in the search for possible ecosystems and potential inhabitants on other planets. They might have been preserved and their biosignatures could be found at depths within permafrost on other planets, if life existed during the early stages of their development.

The existing data indicate the possibility to transport ingmicroorganisms embedded within the sryogenic Space bodies (meteorites) to the Earth. The longevity of life forms and their preservation obtained in the Arctic permafrost can be used as base to assess the survival of microorganisms during a hundreds of thousand of years transfer from Mars to Earth (see Chap. 4, Horneck et al.). These very microorganisms can be the Space Frontiers of Life confirming the possibility of panspermia. This is, why it is necessary to search for the presence of viable cells in the oldest Antarctic permafrost layers. The recent data from the Cape Rogers drilling project, show that early Oligocene sediments (38 Ma) old contain pollen spectra, indicating very cold conditions, and similar observations were made in studies in Australia. Such expedition to

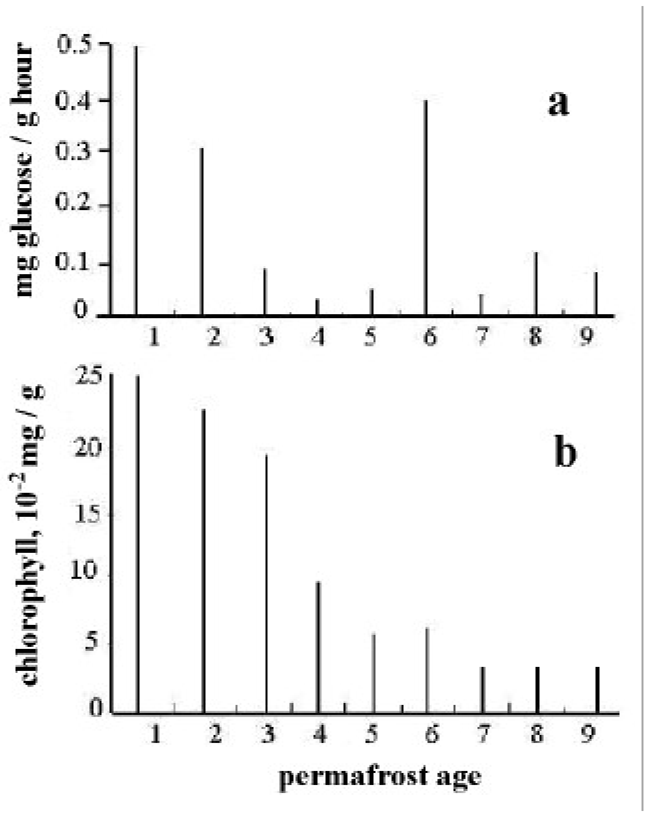

Fig. 8.7 Examples of biosignatures in Arctic permafrost. a: free extracellular enzymes: invertase (54); b: pigments: chlorophyll (41). Permafrost age: 1- modern tundra soil; 2- Holocene; 3, 4- late Pleistocene; 5, 6- middle Pleistocene; 7- early Pleistocene; 8- late Pliocene-early Pleistocene; 9- late Pliocene.

Antarctica is expensive, but thousand times cheaper than a mission to Mars. The limiting age - if one exists - within the Antarctic cores, where the viable microorganisms were no longer present, could be established as the age limit for life preservation within permafrost. Any positive results obtained from the Antarctic microbial data will extend the geological scale and increase the known duration of life preservation.

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