Extremophiles (2006) 10:259–267
DOI 10.1007/s00792-006-0506-3

REVIEW

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Microbial ecology and biodiversity in permafrost

Received: 5 July 2005 / Accepted: 18 November 2005 / Published online: 21 March 2006

Abstract Permafrost represents 26% of terrestrial soil ecosystems; yet its biology, essentially microbiology, remains relatively unexplored. The permafrost environment is considered extreme because indigenous microorganisms must survive prolonged exposure to subzero temperatures and background radiation for geological time scales in a habitat with low water activity and extremely low rates of nutrient and metabolite transfer. Yet considerable numbers and biodiversity of bacteria exist in permafrost, some of which may be among the most ancient viable life on Earth. This review describes the permafrost environment as a microbial habitat and reviews recent studies examining microbial biodiversity found in permafrost as well as microbial growth and activity at ambient in situ subzero temperatures. These investigations suggest that functional microbial ecosystems exist within the permafrost environment and may have important implications on global biogeochemical processes as well as the search for past or extant life in permafrost presumably present on Mars and other bodies in our solar system.

Keywords Permafrost · Cold-adaptation · Dormancy · Astrobiology

Communicated by D. A. Cowan

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Introduction

A recent and significant development in microbial ecology and evolution is the realization that microbial life, primarily prokaryotic, is extremely hardy and can survive and, indeed, thrive in cold environments previously thought uninhabitable on Earth. Cold-adapted microorganisms have been found in Antarctic sub-glacial and permanently ice-covered lakes, cloud droplets, ice cap cores from considerable depth, snow, and glaciers (see reviews of Deming 2002; Priscu and Christner 2004 and references therein). Permafrost represents 26% of terrestrial soil ecosystems and can extend hundreds of meters to >1,000 m into the subsurface (Williams and Smith 1989). Yet its biology, essentially microbiology, remains comparatively unexplored. It was assumed that microorganisms found in this very harsh environment are present in a dormant or dead state. However, a number of recent studies indicate that microorganisms are capable of both growth and metabolic activity at subzero temperatures characteristic of permafrost. Given the considerable mass of permafrost, this represents a potentially vast repository of viable microorganisms that could have important implications on global nutrient cycling and biogeochemical processes. The survival of these organisms at such low temperatures also raises the question of what constitutes the low temperature limit of microbial life (Gilichinsky et al. 2003; Rivkina et al. 2004). Understanding permafrost microbial communities will also lead to the exploitation of potential biotechnological applications (cold-adapted enzymes and compounds) of indigenous microorganisms (Cavicchioli et al. 2002). Recent evidence of massive amounts of shallow ground ice near the surface of Mars (Boynton et al. 2002) and the realization that the majority of cosmic bodies beyond Earth are cryogenic in nature have made terrestrial permafrost environments significant extraterrestrial analog sites (Rivkina et al. 2004; Jakosky et al. 2003; Gilichinsky 2002b; Gilichinsky et al. 2005). This review describes the permafrost environment and highlights

recent studies attempting to define and characterize permafrost microbial communities.

The permafrost system

Permafrost is "ground that remains at or below 0°C for at least two years" (Permafrost Subcommittee 1988, p. 63). It reflects a thermodynamic balance between ground surface temperature, which is controlled by air temperature, and the geothermal gradient. Locally, the nature of the ground surface including water, vegetation, and snow cover determines the degree to which air temperatures influence permafrost. Seasonal temperature variations, including warm summer temperatures, are rapidly attenuated with depth such that only a thin layer experiences thaw and the top 10 m experiences a temperature fluctuation of >1°C. The seasonal variation in temperature is limited to this upper 10 m of ground. During summer, air temperatures rise above 0°C producing thaw of a thin layer at the ground surface, called the active layer (Fig. 1). Active layer depths range from a few decimeters in the high Arctic to more than 2 m in more southern latitudes. Permafrost is strictly a thermal phenomenon and does not depend on the composition of the ground. The boundary between the active layer and permafrost is the permafrost table. The permafrost table acts as a physical and biogeochemical barrier that limits infiltration of both surface water and external environmental factors (Gilichinsky 2002a).

Other thermal structures can exist within permafrost. For example, unfrozen taliks occur under deep-water bodies or in areas of discontinuous permafrost. Many processes and landforms unique to permafrost regions are directly related to the aggradation and degradation of ground ice. The term ground ice refers to "all types of ice formed in freezing and frozen ground" (Permafrost Subcommittee 1988, p. 46). It ranges from disseminated ice crystals in a soil matrix (pore ice) to thick 10–20 m horizontally layered bodies termed massive ground ice, of nearly pure ice that extend for several kilometers. Although many types of ground ice are recognized, pore ice, wedge ice, segregated ice and buried ice are most significant in terms of volume and frequency of occurrence (Mackay 1972; Harry 1989). Ice wedges are linear v-shaped bodies of nearly pure ice that form through the infiltration of surface water into thermal contraction cracks. Repeated cracking over long periods of time can produce wedges several meters wide and 5–10 m deep (Mackay 1972). Cryopegs (aka: brine or salt lenses) are saline groundwater lenses formed during the regression of the polar ocean ~100–120,000 years ago when sediments surrounding the remaining pockets of water froze (Gilichinsky 2002b). Due to salt fractionation during freezing, residual water within the cryopegs is enriched with salt (140–300 g/l) and forms supercooled brines that remain liquid at well below 0°C (Gilichinsky et al. 2003; Bakermans, et al. 2003).

Various physiochemical parameters have been measured in permafrost samples from both polar regions (Table 1), although current data sets are incomplete and remain limited. Permafrost temperature ranges from 0 to −17°C in the Arctic while Antarctic permafrost temperatures were measured from −18 to −27°C (Vorobyova et al. 1997). Information on other physiochemical parameters of permafrost is reviewed by Gilichinsky (2002a) but is derived from a limited number of sites. In Arctic permafrost samples, organic carbon ranged from 0.35 to 10%, pH was neutral, E_h ranged from +40 to −256 mV (indicative of a reducing environment), and the sediments were low in salinity (5–7%). In comparison, measured Antarctic permafrost contains much less organic matter, higher E_h, and more alkaline pH values (Gilichinsky 2002a; Pollard 2000; Vorobyova et al. 1997; Gilichinsky et al. 1992). A variety of gasses (oxygen, carbon dioxide, nitrogen, and methane) have been detected within the pore spaces present in permafrost. Oxygen and nitrogen concentrations are similar to those

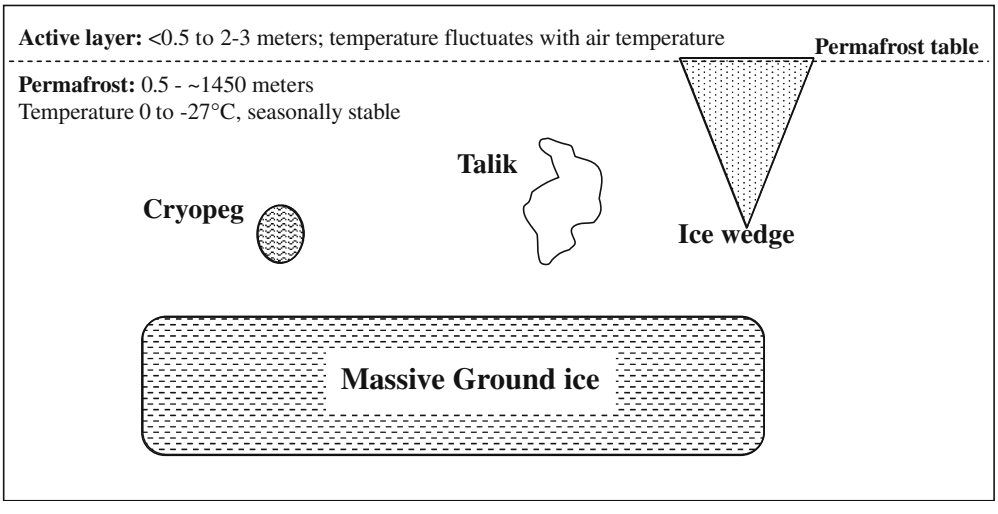

Fig. 1 Schematic diagram of the permafrost zone. Positions are generalized and do not reflect the actual depth or size

Table 1 Physical/chemical properties of permafrost soils

Location	Depth (m)	Age[a]	Temperature (°C)	pH	Organic carbon[b]	Ice[b] (%)	E_h (mV)	References
Arctic Permafrost								
Kolyma lowlands, Siberia	0–100	Present to 5 Ma	−7 to−11	5.6–7.8	0.35–10	17–164	40 to −250	Vorobyova et al. (1997), Gilichinsky (2003), Shi et al., (2004), Vishnivetskaya et al. (2000)
Eureka, Ellesmere Island, Canada	0–15	Present to 20,000 years old	−10 to −17	6.5	2.19	30–100	NA[c]	Pollard, personal communication or our data, unpublished
Antarctic Permafrost								
Dry Valley, Taylor Valley, Miers Valley, Mt. Feather	0–17	150,000 to 2 Ma	-18 to −27	7.8–9.8	0–0.43	NA	260–480	Vorobyova et al. (1997)

[a] *Ma* millions of years
[b] Percent of dry weight soil
[c] Data not available

present in the atmosphere while methane and carbon dioxide can be higher. Carbon isotopic analyses suggest that some permafrost methane may be biologically produced (Vorobyova et al. 1997). The presence of sulfides and viable sulfate-reducing bacteria in permafrost possibly suggests a biological origin of these compounds (Rivkina et al. 1998). The low reducing potential measured in permafrost indicates a primarily anaerobic environment although microaerophilic or aerobic microzones are presumably present.

Even at subzero ambient temperatures, it is generally accepted that liquid water is present within permafrost, existing as a very thin film surrounding soil and ice particles; adsorption forces due to the proximity of the particles stabilize the liquid phase (Lock 1990). In Siberian permafrost, water films were predicted to be ~15 nm thick at −1.5°C and shrank to ~5 nm when the sample was cooled to −10°C (Rivkina et al. 2000). At temperatures below −10°C, the effect of temperature is offset by the role of particle size of the soil (Ostroumov 1995). Smaller particles have more surface area interacting with the water and, therefore, increase soil–water interactions. For example, Arctic loamy soils contain a maximum of 10% unfrozen water at in situ temperatures (−9 to −12°C) whereas in sandy soils containing larger particles, the amount of unfrozen water approaches zero (Gilichinsky 2002a; Gilichinsky et al. 1995). In clay soils, a liquid water film was preserved to temperatures as low as −60°C although this layer was theoretically only a few molecules thick (Ostroumov and Siegert 1996).

In situ microscopic analysis of Arctic sea ice indicates that bacteria are predominantly located in brine veins surrounding ice particles (Junge et al. 2001, 2004). Permafrost liquid water films are too small to harbor microorganisms or allow for the migration of microbial cells. However, they are considered to be a crucial component of the permafrost habitat for microbial survival and, possibly, in situ activity as these films protect viable bacteria from destruction by ice crystal formation and serve as a "nutrient medium" for microbial growth (Gilichinsky 2002a). Similar to brine vein formation in sea ice (Deming et al. 2002), nutrients may concentrate in the thin films as the surrounding water freezes. These films also allow for the mass transfer of nutrients and metabolic end products; if mass transfer did not occur in permafrost, cells would eventually die from a combination of starvation and accumulation of toxins. Mass transfer of potassium and chlorine ions was demonstrated in microzones of permafrost located near ice-rich inclusions, although pure ice was impermeable to similar ion diffusion (Ostroumov and Siegert 1996). Solutions with dissolved NaCl, $CaCl_2$, or $MgCl_2$ can significantly depress the freezing point of water and thus increase their mobility within permafrost. Elevated levels of salinity have been documented in permafrost and ground ice in the high Arctic (Pollard 2000) and recharge through brine films has recently been described as a ground ice formation mechanism in Antarctic permafrost (Dickinson and Rosen 2003). Viable microorganisms that inhabit or colonize the ice–water interfaces (eutectic interfaces) of frozen environments have been termed eutectophiles (Deming 2002).

Microbial biodiversity of permafrost

The initial objectives of early studies in permafrost microbiology were to determine if viable microorganisms could be isolated (James and Sutherland 1942; Boyd and Boyd 1964; Horowitz et al. 1972; Cameron and Morelli 1974). These pioneering investigations revealed that both significant numbers and varieties of viable microorganisms were present in permafrost from several Arctic and Antarctic sites. Isolated organisms included aerobic heterotrophs, anaerobic bacteria, nitrogen-fixing bacteria, sulfur-oxidizing and sulfur-reducing bacteria. Most of these isolates were psychrotrophic rather than psychrophilic, although mesophiles

and even limited numbers of thermophiles were detected. Early Russian studies reported aerobic heterotrophic plate counts in 192 of 220 (87%) samples from nine Siberian permafrost bore holes (Gilichinsky et al. 1989).

These early studies were hampered by the inability to demonstrate or experimentally test sampling procedures. In the 1980s, Russian scientists began developing sampling methods using fluid-less drilling and bacterial tracers to ensure that samples were pristine (Khlebnikova et al. 1990; Shi et al. 1997). To demonstrate non-contamination in permafrost drill samples, we recently developed a two-step methodology using fluorescent microspheres during permafrost drilling and a green-fluorescent protein-marked *Pseudomonas* strain for downstream culture-dependent and culture-independent microbial analyses (Juck et al. 2005). This methodology does not require the release of microorganisms into the environment, and the ability to detect contaminating nucleic acids in drill core samples is important given the widespread utilization of culture-independent molecular techniques for characterizing microbial biodiversity.

In many cases, bacterial counts in permafrost were equal if not greater than in other cryoenvironments, even when compared to cold aquatic environments where there is no osmotic stress due to the presence of ice (Table 2). A wide diversity of bacteria including aerobic heterotrophs, anaerobic heterotrophs, methanogens, iron reducers, sulfate reducers, nitrifying and nitrogen fixing bacteria representing over 30 bacterial genera including *Bacillus*, *Arthrobacter*, *Micrococcus, Cellulomonas*, *Rhodococcus*, *Flavobacterium*, *Pseudomonas*, *Aeromonas*, *Myxococcus*, *Exiguobacterium*, *Nitrobacter*, *Nitrosomonas*, *Nitrosospira*, and *Streptomyces* have been isolated from Arctic permafrost (Gilichinsky 1995; Shi et al. 1997; Vorobyova et al. 1997). In Antarctic permafrost the genera *Arthrobacter*, *Bacillus, Streptomyces* were predominant (Vorobyova et al. 1997).

Although viable eukaryotes are rarely recovered from permafrost, direct microscopy of permafrost has indicated that the total biomass of eukaryotes can be up to ten times higher than the biomass of prokaryotes (Vorobyova et al. 2001). Fungi are present in permafrost solely as spores (Vorobyova et al. 2001) while well-preserved green algae and plant seeds have been found in permafrost samples more than 40,000 years old; seeds recovered from permafrost generally cannot sprout under laboratory conditions, although the germination of plant seeds from ~10,000-year-old Alaskan permafrost has been reported (Gilichinsky et al. 1992). The most frequently isolated eukaryotic organisms were yeasts, primarily belonging to the genera *Cryotococcus*, *Rhodotorula*, and *Saccharomyces* (Vorobyova et al. 1997). In some samples, yeasts account for 20–25% of total aerobic heterotrophs, although they were generally associated with younger permafrost samples, less than 20,000 years old (Vorobyova 2001).

Several recent reports have begun to describe the microbial communities present in Siberian cryopegs ~100,000–120,000 years old, but have been hampered by the difficulties associated with obtaining pristine samples (Gilichinsky et al. 2005). A variety of anaerobic and aerobic, spore-less and spore-forming, halotolerant or halophilic, psychrophilic and psychrotrophic bacteria as well as mycelial fungi and yeast have been isolated and characterized from these cryopegs. Direct microbial counts revealed $\sim 10^7$ cells/ml while culturable aerobic heterotrophs ranged from 7×10^1 to 1×10^6 cfu/ml. Bakermans et al. (2003) recovered 17 bacterial isolates from cryopeg water belonging to nine genera: *Psychrobacter*, *Arthrobacter*, *Frigoribacterium*, *Bacillus*, *Paenibacillus*, *Subtercola*, *Rhodococcus*, *Microbacterium*, and *Erwinia*. The non-spore-forming isolates were more tolerant to salt

Table 2 Enumeration of microbial populations from permafrost and other cryoenvironments

Environment	Cell type	Viable cell counts[a]	Total counts[a]	References
Permafrost environments				
Antarctic permafrost	Aerobic heterotrophs	$0–10^5$	$10^5–10^{6b}$	Horowitz et al. (1972), Cowan et al. (2002)
Siberian permafrost	Aerobic heterotrophs	$0–10^8$	$10^3–10^8$	Rivkina et al. (1998), Gilichinsky (2002)
	Methanogens	$0–10^7$	–	Rivkina et al. (1998)
	Sulfate reducers	$0–10^3$	–	Rivkina et al. (1998)
High Canadian Arctic permafrost	Aerobic heterotrophs	$10^1–10^3$	10^7	Steven et al. (2004)
Cryopeg water	Aerobic heterotrophs	$0–10^6$	10^7	Gilichinsky et al. (2003)
	Sulfate reducers	$0–10^6$	–	
	Methanogens	$0–10^2$	–	
Greenland Glacier ice/permafrost	Aerobic heterotrophs	10^2	10^7	Miteva et al. (2004)
Other cryoenvironments				
Arctic Sea ice	Aerobic heterotrophs	$10^8–10^{10}$	$10^5–10^{12}$	Monfort et al. (2000)
Arctic marine sediments	Sulfate reducers	10^5	NA[c]	Knoblauch et al. (1999a, b)
Antarctic lakes	Aerobic Heterotrophs	$10^5–10^6$	NA	Cowan and Ah Tow (2004)
High Arctic Glacial ice	Aerobic heterotrophs	$10^2–10^3$		Skidmore et al. (2000)
Lake Vostok accretion ice	Aerobic heterotrophs	NA	10^2	Karl et al. (1999);Christner et al. (2001)
Glacier cryoconite ice	Aerobic heterotrophs	10^4	NA	Christner et al. (2003)

[a]Cells per gram soil/ice or ml of water
[b]Determined by ATP content/cell
[c]Data not available

and showed higher metabolic activity at −10°C compared to the spore-forming bacteria (Bakermans et al. 2003). Anaerobic populations varied from 10^1 to 10^6 cells/ml and anaerobic heterotrophs, sulfate-reducing bacteria, and methanogens have been detected (Gilichinsky et al. 2005). A novel psychrophilic *Clostridium* strain adapted to low nutrient concentrations characteristic of the cryopeg environment was shown to produce lactate and butyrate, substrates utilized by heterotrophic *Psychrobacter* isolates, indicating the possibility of a trophic chain within the cryopeg microbial community (Gilichinsky et al. 2005; Shcherbakova et al. 2005).

The ability to recover viable cells from permafrost seems to be independent of permafrost temperature, but depends on the age of the permafrost. With increasing age, both the number and diversity of bacterial isolates decrease with an increase in the number of sterile samples (Gilichinsky et al. 1989, 1992; Khlebnikova et al. 1990), suggesting there may be an upper time limit that microorganisms can maintain viability in a frozen state. The amount of ice in permafrost also has a large effect on cell recovery, as increasing amounts of ice often reduces viable cell counts. Viable bacteria are rarely recovered from pure ice systems such as ice wedges (Gilichinsky et al. 1995; Gilichinsky 2002b) or massive ground ice formations (our data, unpublished). In the absence of liquid water, bacterial cells may be damaged by ice crystal formation, which causes a mechanical disruption of the cell ultrastructure. In addition, mass transfer is greatly reduced in pure ice, stopping the inflow of fresh nutrient and outflow of toxic metabolic waste products leading to the biochemical death of the cell.

Isolation of organisms from cryoenvironments has proven technically challenging. Direct plating methods require incubating samples at relatively high temperatures (~4°C) and the media used plays a strong role in the selection of organisms. A low-temperature enrichment technique, natural permafrost enrichment (NPS), consisting of thawing permafrost at 4°C and incubating the permafrost samples for up to 12 weeks before direct plating, increased the recovery of both the numbers and diversity of viable cells from most permafrost samples (Vishnivetskaya et al. 2000). In some samples, the NPS treatment increased direct bacterial counts, suggesting increased cell recovery was due to bacterial reproduction within the sample, while in other samples there was no increase in bacterial counts indicating that the NPS treatment facilitated a recovery of cells from a viable but non-culturable state. Similarly, preliminary incubation in anaerobic and aerobic liquid for up to one year prior to plating greatly increased the recovery and diversity of recovered organisms from deep Greenland ice core samples (Miteva et al. 2004). Preliminary incubations may permit damaged, stressed, or dormant cells to repair damage induced from long-term exposure to thermal, osmotic, and nutritional stresses imposed by the permafrost environment. Ideally, osmoprotectants such as salts, alcohols, and/or sugars could be incorporated in culture media to enhance cellular survival and recovery; osmoprotectants could also be used to lower the freezing point of culture media to ambient permafrost temperatures. The ability to isolate and culture permafrost bacteria at in situ temperatures will be crucial to determine the cellular mechanisms and physiological adaptations required for indigenous bacteria to survive in permafrost.

Organisms isolated from permafrost tend to be psychrotrophic rather than psychrophilic (Table 3). The paradox of finding primarily psychrotrophic bacteria in permanently frozen soils led to the description of permafrost microbes as a "community of survivors" (Friedmann 1994). Bacteria isolated from permafrost tend to be more halotolerant than isolates from the

Table 3 Growth characteristics of permafrost isolates

Isolate	Description	Minimum growth temperature[a] (°C)	Optimal growth temperature (°C)	Generation time (days)[b]	References
Psychrobacter cryopegella	Psychrotrophic, halotolerant aerobic Gram-negative heterotroph	−10	22	62.5[c]	Bakermans et al. (2003)
Carnobacterium pleistocenium	Psychrotrophic Gram-positive facultative anaerobe	0	24	NA	Pikuta et al. (2005)
Clostridium algoriphilum	Psychrophilic anaerobic spore-former	−5	5	2.1	Shcherbakova et al. (2005)
Psychrobacter sp. 273-4	Aerobic Gram-negative heterotrophs	−2.5	26	3.5	Ponder et al. (2005)
Psychrobacter sp. 215-51	Aerobic Gram-negative heterotroph	−2.5	26	3.5	Ponder et al. (2005)
Exiguobacterium sp. 255-15	Aerobic Gram-positive heterotroph	−2.5	42	5.5	Ponder et al. (2005)
Virgibacillus sp. nov	Psychrotrophic spore-former, aerobic heterotroph. Capable of growth in 20% NaCl	0	30	2.4	Our data, unpublished
Sulfobacillus sp. nov	Psychrotrophic facultative autotroph. Sulfur oxidizer	−4	25	3.5	Our data, unpublished

[a]Minimum temperature at which growth was recorded
[b]Generation time at minimal growth temperature
[c]Calculated generation time from specific growth rate of 0.016 day^{-1} at −10°C (Bakermans et al. 2003)

overlying active layer soil, suggesting a possible link between halotolerance and psychrotolerance. Permafrost can be considered an environment of low water availability, as below 0°C temperature primarily exerts its effect on microbial communities by removing available liquid water in the form of ice (Franks 2003). Permafrost bacteria are extremely resistant to cold temperature assaults. For example, the number of viable bacteria recovered from permafrost samples did not change significantly after 30 freeze–thaw cycles (Soina et al. 2004) and permafrost isolates have been observed as intact cells when stored in distilled water at temperatures as low as −90°C (Vorobyova et al. 2001).

Bacterial direct counts as determined by epifluorescence microscopy tend to be similar between most permafrost samples, ranging from 10^7 to 10^9 cells/g, even in samples from which no viable cells were recovered. In Arctic samples, viable cell recovery was on average 0.1–10% of total cell counts, whereas in Antarctic permafrost only 0.001–0.01% of total cells was represented by cultured isolates (Vorobyova et al. 2001). The lack of cultured organisms suggests that many bacteria in permafrost are unculturable, or the proper culturing conditions have not been attempted. There are presently very few reports using culture-independent molecular techniques to describe microbial diversity in permafrost. Phylogenetic analysis of bacterial 16S rDNA sequences isolated from 10 to 20,000-year-old permafrost from the Canadian high Arctic revealed that sequences clustered into six phyla: *Proteobacteria*, *Actinomycetes*, *Firmicutes*, *Planctomyces*, *Cryptophaga-Flavobacteria-Bacteroides* (CFB), and Gemmatimonadetes (Steven et al. 2004). Archaeal 16S rDNA genes related to both the major Archaeal domains (*Crenarchaeota* and *Euryarchaeota*) were also detected, suggesting the presence of a diverse Archaeal population. Previously the presence of Archaea in permafrost was limited to the detection of methanogens (Rivkina et al. 1998, 2001, 2004) and a short report of the detection of 16S rRNA genes related to the *Crenarchaeota* (Ochsenreiter et al. 2003). It remains unclear if the detection of these organisms is due to their presence in a viable form or due to the long-term preservation of DNA in permafrost. As naked DNA is degraded rapidly in most environments, the persistence of bacterial DNA in permafrost suggests that these organisms have in the past or currently maintain a slow metabolic activity to repair DNA damage (Willerslev et al. 2004). The emerging view of microbial biodiversity in permafrost is one in which the species composition is determined primarily by the selection of organisms able to survive prolonged exposure to subzero temperatures, desiccation starvation, and background radiation.

Microscopic observations of permafrost microorganisms

Various light and electron microscopic investigations of permafrost samples from both the Arctic and Antarctica have revealed the presence of intact cells, partially degraded cells (i.e., with ruptured cell walls and membranes, with bleached cytoplasm, lysed cells), and empty "ghost cells" (i.e., containing only cell walls) with a wide range of morphologies, including cocci and rods (Dmitriev et al. 2000; Soina et al. 2004). Intact cells show characteristics of both Gram-positive and Gram-negative cell walls, and are typically coated with capsular material, have thickened cell walls and a non-homogenous cytoplasm that contains numerous aggregates (Dmitriev et al. 2000; Soina et al. 2004). Ribosomes of permafrost bacteria can be closely packaged and the nucleoid area can consist of fibrillar material with small vesicles (Soina et al. 1995). Transmission electron microscopy of permafrost samples showed no signs of ice crystals in bacterial cells whereas yeast cells showed large cavities presumably due to ice crystal formation (Soina et al. 2004). Investigations of permafrost samples in situ reveals a paucity of dividing cells and bacterial spores (Dmitriev et al. 2000; Soina et al. 2004). Instead, cyst-like forms appear to be more abundant and non-spore-forming bacteria, perhaps in a dormant state, are common (Soina et al. 2004 and references therein). Perhaps most characteristically, permafrost appears to be dominated by populations of cells that are less than 1 μm in size (Dmitriev et al. 2000; Soina et al. 2004). For example, ultramicroforms of bacteria less than 0.4 μm in diameter make up as much as 80% of permafrost populations (Vorobyova et al. 2001). Soina et al. (2004) reported observing curved forms as small as 0.2 μm in diameter. Microscopic observations of melted Greenland ice cores also revealed the predominance of very small (< 1 μm), but viable "dwarf" cells suggesting small cell size may be a physiological response to environmental stress (Sheridan et al. 2003; Miteva et al. 2004). It is thus likely that small cell sizes are predominant in such cryoenvironments, including permafrost.

Microbial activity in the permafrost environment

Are permafrost microbial communities biologically active in situ? What is the cold temperature limit for microbial life? These questions are at the foundations of studies in cryoenvironments such as permafrost. Although most bacteria isolated from cryoenvironments grow optimally at moderate temperatures, recent investigations have begun to explore their growth capabilities at temperatures below 0°C. However, the ability to detect and measure growth at subzero temperatures is presently constrained by the lack of reliable culturing techniques. To date, the lowest temperature at which microbial reproduction has been clearly demonstrated is −12°C in the Arctic sea ice bacterium *Psychromonas ingrahamii* (Breezee et al. 2004). In theory, permafrost should be an ideal environment to search for bacteria capable of subzero temperature growth because it is characterized by permanently subzero temperatures for geological time scales. In our characterization of

Canadian High Arctic permafrost aerobic heterotropic isolates, 20% of isolates are capable of growth at temperatures of at least −5°C (Steven and Whyte, unpublished). In the most thorough examination of bacterial growth at subzero temperatures to date, *P. cryopegella* maximized growth yield at low temperatures (−10 to 4°C) by streamlining growth processes at a critical temperature (4°C), below which the respiration requirements for *P. cryopegella* reproduction increased dramatically (Bakermans and Nealson 2004).

Several recent studies have detected microbial activities at temperatures substantially below the freezing point of water (Table 4). Using resazurin reduction as an indicator of metabolic activity, several cryopeg isolates could reduce resazurin at −10°C, while showing no cell division (Bakermans et al. 2003). The Arctic sea ice bacterium, *Colwellia psychrerythraea* strain 34H, possesses a minimum growth temperature of −5°C but is capable of motility at −10°C (Junge et al. 2003). The best evidence presently indicating permafrost microbial communities are biologically active in situ has been obtained from microcosm radiotracer analyses and novel fluorescent microscopy techniques. Indigenous microorganisms in Siberian permafrost microcosms were able to incorporate ^{14}C-labeled acetate into lipids at temperatures from 5 to −20°C (Rivkina et al. 2000). The rate of incorporation dropped significantly at −1.5°C, the temperature at which ice began to form in the microcosms. The rate and amount of acetate incorporation was also highly correlated with the estimated thickness of liquid water films within permafrost (Rivkina et al. 2000), suggesting that below 0°C temperature primarily exerts its effect on microbial communities by decreasing the available liquid water as it forms ice. In a comprehensive review of microbial growth and activities at subzero temperatures from a variety of cryoenvironments, Price and Sowers (2004) proposed that microbial metabolic rates of cold-adapted communities fall into three groupings: (1) a rate sufficient for bacterial *growth*; (2) a rate for *maintenance* of metabolism but too low for growth; (3) a rate for *survival* solely to repair macromolecular damage accumulated during prolonged storage over geological time scales in bacteria characterized as ''largely dormant''. In support of the latter case, aspartic acid racemization rates extrapolated to in situ permafrost temperatures by Brinton et al. (2002) demonstrated that the 1:1 ratio of D to L chiral forms, due to spontaneous racemization, had not yet been established, suggesting that Siberian permafrost communities can maintain D-amino acid recycling for at least 30,000 years. Similarly, the presence of ''ancient'' viable microorganisms in permafrost millions of years old implies the presence of active DNA repair systems which at least neutralize the cumulative effects of background terrestrial gamma radiation (Price and Sowers 2004; Rivkina et al. 2004; Ponder et al. 2005), which can result in doses as high as 2 mGy per year (Gilichinsky 2003).

In summary, these findings provide both direct and indirect evidence that permafrost environments may harbor active microbial ecosystems rather than frozen dormant microbial survivors, albeit acting at very low rates. Given the considerable microbial biomass believed to inhabit the significant areas of terrestrial permafrost, this may have important implications on global nutrient cycling and biogeochemical processes , such as the C, N, and S cycles that have not yet been accounted for (Rivkina et al. 2004).

Permafrost microbiology: future opportunities and challenges

In the last 5–10 years, an increasing number of reports from a limited number of permafrost environments have begun to characterize microbial biodiversity and activity in these unique ecosystems. Yet many fundamental questions remain unanswered. Detection and quantification of permafrost in situ activity will be technically challenging and require the development of novel methodologies specific for permafrost environments; as in situ rates, such as biogenic gas formation or uptake, are expected to be extremely slow and minute, ultrasensitive methodologies, able to differentiate background from biogenic activity, will be required. Similarly, expected advances in subzero culturing techniques will prove crucial to isolating and characterizing novel bacteria from permafrost; permafrost and cryopegs within probably harbor novel halophilic psychrophiles that will define the lower temperature limits of

Table 4 Microbial activities detected at subzero temperatures

Activity	Temperature (°C)[a]	Environment	References
Incorporation of ^{14}C-labeled acetate into lipids	−10	Arctic permafrost	Rivkina et al. (2000)
Incorporation of ^{14}C-labeled glucose	−15	Arctic permafrost	Gilichinsky et al. (2003)
Methanogenesis	−16.5	Arctic permafrost	Rivkina et al. (2004)
Sulfate reduction	−1.7	Arctic marine sediments	Knoblauch et al. (1999a, b)
DNA/protein synthesis	−17	South Pole snow	Carpenter et al. (2000)
DNA/protein synthesis	0	Cloud droplets	Sattler et al. (2001)
DNA synthesis	−1.5	Arctic Sea ice	Smith and Clement (1990)
CTC[b] uptake and rRNA targeted FISH[c]	−20	Wintertime sea ice	Junge et al. (2004)

[a]Reported as the minimum temperature at which activity was detected
[b]5-Cyano-2,3-ditoyl tetrazolium chloride
[c]Fluorescence in situ hybridization

terrestrial microbial life. Culture-dependent studies have quantified viable bacteria and begun to describe microbial biodiversity of selected permafrost communities but only describe 0.1–1% of the indigenous population. Permafrost microbial biodiversity has yet to be reported with state-of-the-art culture-independent molecular techniques that will certainly identify and characterize novel phylotypes. For example, recent advances in stable isotope probing could be used to identify actively metabolizing members of microbial communities at ambient subzero temperatures (Dumont and Murrell 2005). More detailed physical/chemical analyses of the permafrost environment could prove the existence of microenvironments such as large brine veins observed in sea ice and to identify the range of electron donors and acceptors and carbon sources required to support permafrost microbial communities.

Much of the current interest in permafrost microbiology is being driven by exobiology implications, as permafrost present on Mars is considered a primary target for the search for past or extant life which are primary targets for future robotic missions to Mars (Rivkina et al. 2004; Gilichinsky et al. 2003, 2005). Because of their prolonged age in a stable and permanently frozen state, terrestrial permafrost is currently being utilized as an analog for similar sites that could exist on Mars. In this respect, understanding the microbiology of terrestrial permafrost is paramount in determining how to best search for and detect the presence and/or activities of similar microbial communities that could possibly exist or existed on other bodies in our solar system rich in permafrost deposits.

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