Chapter 5
Bacterial and Archaeal Diversity in Permafrost

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5.1 Introduction

Microorganisms in permafrost survive in an extreme environment characterized by constant subzero temperatures, low water and nutrient availability, and prolonged exposure to background radiation. Despite the harsh conditions, considerable abundance and diversity of microorganisms inhabit permafrost. Pioneering studies focusing on permafrost microbiology simply attempted to determine if permafrost harbored viable microorganisms. For example, microorganisms cultured from Canadian (James and Sutherland 1942), Alaskan (Boyd and Boyd 1964) and Antarctic (Cameron and Morelli 1974) permafrost samples were generally poorly characterized, and the studies were hampered by an inability to demonstrate that drilling and sample handling were performed aseptically. Recent developments using fluid-less drilling (Gilichinsky et al. 1989; Khlebnikova et al. 1990; Juck et al. 2005), tracer microorganisms (Christner et al. 2005; Juck et al. 2005), nucleic acid stains (Christner et al. 2005) and fluorescent microspheres as microbial surrogates (Juck et al. 2005) have greatly improved our ability to recover intact permafrost samples and to monitor exogenous microbiological contamination of pristine permafrost samples.

Permafrost also contains various other geomorphological structures including massive ground ice, cryopegs, and ice wedges (Steven et al. 2006) that harbor microbial populations. The description of the abundance, diversity, activity and distribution of microorganisms in permafrost and associated environments will be fundamental to our understanding of how microorganisms survive in permafrost, and how they will respond to future climatic warming and permafrost thawing. Lastly, permafrost microorganisms and microbial ecosystems are considered significant terrestrial analogs for similar organisms that may inhabit permafrost environments that exist beyond the Earth, especially in light of the recent evidence of massive amounts of shallow ground ice near the surface of Mars (Gilichinsky 2002a; Gilichinsky et al. 2007).

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5.2 Viable *Bacteria* and *Archaea* in Permafrost

5.2.1 Microbial Abundance in Permafrost Environments

Substantial numbers (up to 10^9 cells g^{-1}) of microbial cells are detected in permafrost but vary over a large range among different permafrost environments (Table 5.1). In general, only a small proportion of the microbial community is represented by cultured isolates. In Arctic permafrost ca. 0.1–10% of the microbial community is recovered by standard culturing, while in Antarctic permafrost viable cell recovery is only 0.001–0.01% (Vorobyova et al. 1997). Microscopic investigations of permafrost microorganisms in situ have revealed the presence of partially degraded cells (i.e., ruptured cell walls and membranes) and empty "ghost cells" (Dmitriev et al. 2000; Soina et al. 2004); due to the constant subzero temperatures in permafrost, dead or compromised microbial cells may remain well preserved and contribute to total microbial counts. For example, Hansen et al. (2007) observed that 74% of the microbial community in Spitsbergen Island permafrost had compromised cell walls, based on differential staining and microscopy, and were considered non-viable. Intact microbial cells in permafrost are characterized by altered ultrastructures such as thickened cell walls and a non-homogenous cytoplasm that contains numerous aggregates (Soina et al. 1995, 2004). Perhaps most characteristically, Siberian permafrost appears to be dominated by populations of cells ≤1 μm in size (Dmitriev et al. 2000; Soina et al. 2004) with ultramicroforms of cells ≤0.4 μm in diameter making up as much as 80% of Siberian permafrost microbial populations (Vorobyova et al. 2001). Dwarfed cells are characteristic of the viable but non-culturable state (reviewed in Oliver 2005) and, therefore, many cells in permafrost may be in a physiological state that is recalcitrant to laboratory cultivation, partially explaining the low viable cell recovery.

The ability to recover viable cells from permafrost seems to be independent of permafrost temperature or depth, 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). Nevertheless, viable microbial cells were recovered from Siberian permafrost as old as 3 million years (Gilichinsky 2002a). The amount of ice in permafrost also has a large effect on cell recovery, as increasing ice content often greatly reduces viable cell counts. Viable bacteria are rarely recovered from nearly pure ice systems in permafrost such as ice wedges (Gilichinsky et al. 1995; Gilichinsky 2002b) or massive ground ice formations (Steven et al. 2008a), although viable bacterial numbers of up to 10^6 CFU ml^{-1} were recovered from an Alaskan ice wedge sample (Katayama et al. 2007). Therefore, the origin, age and physiochemical characteristics of the ice presumably determine the presence and abundance of a viable microbial community.

Table 5.1 Microbial abundance in various permafrost environments

Location	Cell type	Viable cell counts[a]	Direct microscopic counts[b]	References
Antarctic Dry Valley	Aerobic heterotrophs	$0–10^5$	$10^5–10^{6c}$	Horowitz et al. (1972)
	Methanogens	$0–10^3$		Cowan et al. (2002)
	Sulfate reducers	$0–10^3$		Gilichinsky et al. (2007)
	Denitrifying bacteria	$0–10^1$		
Siberian permafrost	Aerobic heterotrophs	$0–10^8$	$10^3–10^8$	Rivkina et al. (1998)
	Methanogens	$0–10^7$		Gilichinsky (2002a)
	Sulfate reducers	$0–10^3$		
Canadian high Arctic permafrost	Aerobic heterotrophs	$10^1–10^4$	$10^7–10^8$	Steven et al. (2007a)
				Steven et al. (2007c)
Spitsbergen Island	Aerobic heterotrophs	10^5	10^9	Hansen et al. (2007)
	Anaerobic heterotrophs	10^5		
Tianshan Mountains, China (alpine permafrost)	Aerobic heterotrophs	10^5	NA[d]	Bai et al. (2006)
Qinghai-Tibet Plateau (high altitude permafrost)	Alkaliphilic and psychrotolerant bacteria	$10^2–10^5$	NA	Zhang et al. (2007)
Siberian Cryopeg	Aerobic heterotrophs	$10^2–10^5$	10^7	Bakermans et al. (2003)
	Anaerobic heterotrophs	$10^1–10^2$		Gilichinsky et al. (2003)
	Sulfate reducers	10^6		Gilichinsky et al. (2005)
	methanogens	10^2		
Alaskan ice wedge	Aerobic heterotrophs	$10^5–10^6$	NA	Katayama et al. (2007)
Canadian high Arctic ground ice	Aerobic heterotrophs	0	10^4	Steven et al. (2007c)
Greenland glacier ice/permafrost	Aerobic heterotrophs	10^2	10^7	Miteva et al. (2004)

[a]CFU g^{-1} (only the order of magnitude of the counts are presented)
[b]Cells g^{-1} (only the order of magnitude of the counts are presented)
[c]Estimated from ATP content/cell
[d]Data not available

5.2.2 Diversity of Viable Bacteria and Archaea

The catalog of viable *Bacteria* recovered from permafrost and associated environments, currently includes at least 70 genera (Table 5.2). Cultured isolates recovered from permafrost are capable of a wide range of metabolic processes including aerobic and anaerobic heterotrophy, chemolithoautotrophy, sulfate-reduction, methanotrophy, methanogenesis (Gilichinsky et al. 1995; Steven et al. 2006) and even phototrophy (Chap. 6). Both Gram-positive and Gram-negative cells are represented, and spore-forming *Bacteria* are also commonly isolated, although the abundance of spore-forming *Bacteria* varies widely between geographically separated permafrost samples. For example, spore-forming genera dominated the culturable community from 2 to 9 m (69% and 100% of isolates, respectively) Canadian high Arctic permafrost samples (Steven et al. 2007a, 2008a), whereas spore-forming genera only composed 30, 5 and 1% of Siberian (Shi et al. 1997), Spitsbergen Island (Hansen et al. 2007) and Chinese alpine (Bai et al. 2006) permafrost isolates, respectively. *Firmicutes* and *Actinobacteria* generally represent a high proportion of the permafrost microbial community, accounting for up to 100% of Canadian high Arctic isolates (Steven et al. 2008a), 60% of Chinese alpine permafrost isolates (Bai et al. 2006) and 45% of Siberian permafrost isolates (Shi et al. 1997). To date, the phylogenetic groups that account for the anaerobic *Bacteria* community in permafrost remain poorly characterized.

Cryopegs are lenses of supercooled, saline liquid water within the permafrost (Bakermans et al. 2003) that can harbor substantial numbers of viable microbial cells (Table 5.1). These include a variety of anaerobic and aerobic, spore-less and spore-forming bacteria (Table 5.2), with a *Psychrobacter*-related isolate accounting for 53% of all isolates, suggesting this organism was a dominant community member (Bakermans et al. 2003).

A single report of the microbial community in an Alaskan permafrost ice wedge indicated relatively high numbers of viable microbial cells (Table 5.1), although the diversity of the recovered isolates was low (Katayama et al. 2007). The phylogenetic groups of the isolates were similar to those identified in permafrost soils (Table 5.2).

The description of viable *Archaea* in permafrost remains limited. Methanogenic *Archaea,* generally occur in low numbers (10^2–10^3 g^{-1}) and not in all samples (Rivkina et al. 1998, 2002). Recovered isolates related to the genera *Methanosarcina* and *Methanobacterium* (Rivkina et al. 2007) and methanogenic activity detected in Siberian permafrost samples suggests that methanogenesis occurs at in situ permafrost temperatures (Rivkina et al. 2000, 2002). We recently detected halophilic *Archaea* in saline enrichment cultures from Canadian high Arctic permafrost, indicating that these organisms are members of a viable permafrost microbial community (unpublished data).

5.2.3 Increasing Representation of Cultured Isolates

Methods to increase the representation of cultured microbial isolates from permafrost have recently been applied. For example, Vishnivetskaya et al. (2000) used natural permafrost sediment (NPS) enrichment to recover microbial isolates. NPS, consisting

Table 5.2 Phylogenetic groups of *Bacteria* cultured from permafrost[a]

Phylogenetic group	Canadian high Arctic permafrost[b]	Siberian permafrost[c]	Siberian cryopeg[d]	Spitsbergen Island permafrost[e]	Antarctic permafrost[f]	Chinese alpine permafrost[g]	Alaskan ice wedge[h]
Actinobacteria							
Arthrobacter	+	+	+	+	+	+	+
Brachybacterium	+			+			+
Cellulomonas		+		+	+		
Cryobacterium				+			+
Frigoribacterium			+			+	
Kocuria	+			+			
Leifsonia				+		+	
Microbacterium		+	+			+	+
Micrococcus	+	+		+	+		
Nocardia				+		+	
Promicromonospora		+			+		
Rhodococcus	+	+	+	+	+	+	+
Streptomyces		+		+	+		
unique genera	–	1	1	10	–	5	–
CFB							
Flavobacterium	+	+				+	
Pedobacter	+			+		+	
unique genera	–	2	1	1	–	1	–
Firmicutes							
Bacillus	+	+	+	+	+	+	
Exiguobacterium		+				+	
Paenibacillus	+	+	+	+			
Planococcus	+	+				+	+
Planomicrobium		+				+	
Sporosarcina	+	+				+	
unique genera	3	–	–	–	–	2	1
Proteobacteria							
Aeromonas		+			+		
Myxococcus		+			+		
Psychrobacter		+	+			+	
Pseudomonas	+	+		+	+	+	+
unique genera	1	9	1	5	1	7	1

[a]Genera represented in at least two permafrost environments are indicated (+). The number of genera that were unique to the distinct permafrost environments are also indicated. *Bacteria* phyla are shown in **bold**

[b]Steven et al. (2007a, 2007b);

[c]Shi et al. (1997), Vorobyova et al. (1997) and Vishnivetskaya et al. (2006)

[d]Bakermans et al. (2003) and Gilichinsky et al. (2005)

[e]Hansen et al. (2007)

[f]Vorobyova et al. (1997) and Gilichinsky et al. (2007)

[g]Bai et al. (2006) and Zhang et al. (2007)

[h]Katayama et al. (2007)

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. Similarly, preliminary incubation in anaerobic and aerobic liquid media prior to plating greatly increased the recovery and diversity of recovered organisms from deep Greenland ice core samples and a Spitsbergen Island permafrost sample (Miteva et al. 2004; Hansen et al. 2007). Preliminary incubations may permit damaged, stressed, or dormant cells to repair damage induced by long-term exposure to thermal, osmotic, and nutritional stresses imposed by permafrost environments. Ideally, osmoprotectants such as salts, alcohols, and/or sugars could be incorporated in culture media, not only to enhance cellular survival and recovery, but to lower the freezing point of culture media to ambient permafrost temperatures. The ability to isolate and culture permafrost microorganisms at in situ temperatures will be crucial in determining the cellular mechanisms and physiological adaptations required for indigenous microbes to survive in permafrost.

5.3 Phenotypic Characteristics of Permafrost Isolates

The recovery of viable cells from Arctic and Antarctic permafrost samples is generally facilitated by using nutrient-poor media (Gilichinsky et al. 1989; Bai et al. 2006; Steven et al. 2007a), suggesting that permafrost communities are primarily oligotrophic; although organic carbon is more abundant in Arctic permafrost (Vishnivetskaya et al. 2000; Gilichinsky 2002a; Steven et al. 2006). Microbial abundance and activity in subsurface soils is affected by soil porosity, as subsurface pores are required for the movement of liquid water, with larger pore sizes associated with an increased availability of organic compounds (Kaiser and Bollag 1990). The sequestering of liquid water as ice in permafrost reduces porosity and may therefore act to limit the availability of organic carbon, selecting for oligotrophic microbial populations.

Permafrost microorganisms also tend to be more halotolerant than organisms from the overlying active layer soil (Gilichinsky 2002a; Steven et al. 2008a). Microbial survival in extremely cold environments is under the influence of ice formation and, consequently, little biologically available liquid water is present. Therefore, water activity is probably an important factor influencing microbial survival in permafrost (Gunde-Cimerman et al. 2003). In addition, during freezing and the binding of water in ice crystals, ions are expelled and concentrate in the remaining liquid phase (Price 2007). Thus, there may be a connection between halotolerance and microbial survival at extremely low temperatures.

Permafrost microorganisms are primarily cold-adapted, with very few mesophilic or thermophilic isolates identified (Gilichinsky 2002a; Steven et al. 2006) Most isolates described are psychrotolerant (growth optimum ≥20°C) rather than psychrophilic, although both psychrotolerant and psychrophilic microorganisms capable of growth at subzero temperatures are isolated from permafrost (Ponder et al. 2005; Bai et al. 2006; Steven et al. 2007a, 2008a), suggesting the potential for growth and metabolism at the ambient subzero temperatures in permafrost.

Many of the microorganisms isolated from permafrost represent potentially novel microbial species or genera (Bakermans et al. 2003; Bai et al. 2006; Ponder et al. 2005; Rivkina et al. 2007; Steven et al. 2007a, 2008a, b). Recent genomic (see Chap. 11) and proteomic (Qiu et al. 2006; Bakermans et al. 2007; see Chap. 12) investigations of species from the genera *Exiguobacterium* and *Psychrobacter* will help define the physiological and genetic adaptations that have allowed these organisms to survive in permafrost. Presumably, these and future studies will lead to a better understanding of long-term survival at subzero temperatures and the low temperature limits for microbial growth and metabolism.

5.4 Culture-Independent Bacterial and Archaeal Diversity in Permafrost

Culture-independent methodologies have recently been applied to the study of microbial diversity in permafrost. These studies, which use molecular-based tools to analyze DNA extracted directly from permafrost (Spiegelman et al. 2005 and references therein), bypass the need for culturing and have increased the number of phylogenetic groups of *Bacteria* and *Archaea* associated with permafrost (Table 5.3). For example, the culturable microbial community in a Canadian high Arctic permafrost sample was dominated by *Firmicutes*-related isolates, whereas *Actinobacteria*- and *Proteobacteria*-related sequences were predominant in a culture-independent analysis, with the phyla *Gemmatimonadetes,* CFB and *Planctomyces* identified in the culture-independent survey but not among the isolates (Steven et al. 2007a). A diverse *Bacteria* community, comprised of 13 *Bacteria* phyla (Table 5.3), including three candidate phyla (phyla that have no cultured representatives), was detected in Spitsbergen Island permafrost 16S rRNA gene clone libraries (Hansen et al. 2007), while only four phyla (*Actinobacteria,* CFB, *Firmicutes* and *Proteobacteria*) were represented by cultured isolates (Hansen et al. 2007). 16S rRNA gene clone libraries constructed from Siberian permafrost DNA (Table 5.3) were dominated by sequences related to the *Proteobacteria*, *Actinobacteria* and *Firmicutes,* with *Arthrobacter* being abundant in both the culture-dependent and culture-independent surveys of microbial diversity (Vishnivetskaya et al. 2006). The proportion of 16S rRNA sequences related to the high G + C Gram-positive *Bacteria* was also found to increase with increasing age of Siberian permafrost (Willerslev et al. 2004a). Antarctic Dry Valley permafrost 16S rRNA gene clone libraries were composed of the phylogenetic groups *Proteobacteria* and *Actinobacteria*, with *Arthrobacter, Bacillus,* and *Pseudomonas* detected in all of the Antarctic permafrost clone libraries (Gilichinsky et al. 2007).

To date, very few studies have described the *Archaea* communities in permafrost using culture-independent methodologies. Other than a report of the detection of 16S rRNA genes related to the *Crenarchaeota* (affiliated to environmental group 1.1.b) in Chinese alpine permafrost (Ochsenreiter et al. 2003), all of the culture-independent characterizations of *Archaea* diversity in permafrost are from the Canadian high

Table 5.3 Phylogenetic groups of *Bacteria* and *Archaea* detected by culture-independent methods in various permafrost environments

Phylogenetic group	Canadian high Arctic permafrost[a,b]	Kolyma lowlands Siberia[c]	Spitsbergen Island[d]	Dry Valleys Antarctica[e]	Alaskan ice wedge[f]	Canadian high Arctic massive ground ice[b]
Bacteria						
Acidobacteria	+		+	+		
Actinobacteria	+	+	+	+	+	+
CFB	+		+	+		+
Firmicutes	+	+	+	+	+	+
Gemmatimonadetes	+					
Planctomyces	+		+			
Proteobacteria	+	+	+	+	+	+
Spirochaetes			+			
Thermomicrobia			+			
Verrucomicrobiae			+			
OD1[g]			+			
OP10[g]			+			
TM7[g]			+			
Unclassified	+		+			
Archaea						
Environmental *Crenarchaeota*	+					+
Environmental *Euryarchaeota*	+					+
Halophilic *Archaea*	+					+
Methanogenic *Archaea*						+

[a]Steven et al. (2007a)
[b]Steven et al. (2007b)
[c]Vishnivetskaya et al. (2006)
[d]Hansen et al. (2007)
[e]Gilichinsky et al. (2007)
[f]Katayama et al. (2007)
[g]Candidate divisions for which there are no cultured representatives

Arctic. Our studies have revealed that both of the major *Archaea* phyla (*Euryarchaeota* and *Crenarchaeota*) are present in Canadian permafrost, with sequences belonging to the *Euryarchaeota* being numerically dominant (Steven et al. 2007a, 2008a). Although methanogens have been isolated from Antarctic and Siberian permafrost (Rivkina et al. 1998; Gilichinsky et al. 2007), 16S rRNA gene sequences related to methanogenic *Archaea* were not detected in Canadian high Arctic permafrost, with the exception of a single sequence detected in a massive ground ice deposit (Steven et al. 2008a). An interesting result of the culture-independent characterization of *Archaea* communities in Canadian high Arctic permafrost was the detection of a significant number of sequences related to the halophilic *Archaea*, although the salinity

in the permafrost was only moderate (Steven et al. 2007a, 2008a). The detection of halophilic organisms in only moderately saline permafrost provides circumstantial evidence that the primary microbial habitat in permafrost exists as thin saline liquid water veins surrounding soil particles (Price 2007).

It should be noted that the detection of a DNA sequence is not conclusive evidence that the phylogenetically related organism is active or even viable in permafrost, as the constant subzero temperatures are ideal for DNA preservation (Willerslev et al. 2003, 2004a; see Chap. 4). Thus, developing novel methods will be essential to determine if microorganisms identified in culture-independent surveys exist as viable cells or are the microbial equivalent of mammoths, frozen in time in the permafrost environment.

5.5 Biogeography of Permafrost Microorganisms

One of the longstanding theories of microbial biogeography is the paradigm that "everything is everywhere, but the environment selects" (Baas-Becking 1934, cited in O'Malley 2007). However, various studies have started to challenge this traditional theory with research showing divergence of microbial types due to geographical constraints on microbial migration, and environmental factors driving spatial and temporal distributions (Hughes Martiny et al. 2006). Comprehensive descriptions of permafrost environments encompassing both molecular and culture-based approaches are only starting to emerge in the literature (Vishnivetskaya et al. 2006; Gilichinsky et al. 2007; Hansen et al. 2007; Steven et al. 2007a, 2008a); therefore, it may be premature to put these into a biogeography context. Nevertheless, trends are beginning to appear including the dominance of high G + C Gram-positive organisms within permafrost as revealed by culture-dependent and culture-independent methods (Tables 5.2 and 5.3). The high similarity between 16S rRNA gene sequences and isolates recovered from permafrost samples (Gilichinsky et al. 2007; Hansen et al. 2007; Steven et al. 2007a, 2008a) and those from other similar cryoenvironments (e.g., glacial ice, sea ice, and Lake Vostok accretion ice) also suggests that cosmopolitan groups of microorganisms adapted to life at subzero temperatures exist. Conversely, several *Bacteria* genera detected in each of the above mentioned studies also seem to be unique to the specific location under investigation (Tables 5.2 and 5.3). Taken together, these results indicate both cosmopolitan and endemic populations of microbes residing in geographically separated permafrost. However, one cannot conclusively prove an organism is not present in any given environment, due to the limitations of current technologies used in microbial ecology (Ramette and Tiedje 2007). It is also important to note that studies of the microbiology in permafrost are from a relatively small number of sites, and do not reflect a comprehensive survey of permafrost environments.

Work undertaken by Steven et al. (2008a) has also demonstrated the importance that comparisons between microbial communities in geographically separated permafrost should be made from similar horizons, as the composition of microbial

communities varies with permafrost depth. For example, 55% of the *Bacteria* 16S rRNA gene sequences from a 1-m depth permafrost sample (Steven et al. 2008a) were most closely related to 16S rRNA gene sequences recovered from a ca. 1-m deep Spitsbergen Island permafrost sample (Hansen et al. 2007), compared to 15% of clones from a 2-m permafrost sample, while none of the clone sequences from a 9-m sample (Steven et al. 2007a) had closest relatives identified in the Spitsbergen Island permafrost sample.

The application of new techniques in biogeography theory, taxonomic level resolution and exhaustive sampling methods, and novel molecular approaches such as microarray and metagenomic technologies (Ramette and Tiedje 2007; Xu 2006) will lead to a greater understanding of microbial biogeography and the environmental factors in permafrost that control the abundance, distribution and diversity of the microbial populations.

5.6 Permafrost Microorganisms: Ancient Survivors or an Active Ecosystem?

Without a fossil record or detectable events of when a group of specific microorganisms appeared for the first time, we have little knowledge concerning the timeline or age of microbial species, or how to calibrate their evolutionary divergence (Vreeland and Rosenzweig 2002). Therefore, the age of supposed ancient organisms, including permafrost isolates (Willerslev et al. 2004b), is assumed from the age of their surrounding environment (Drancourt and Raoult 2005). A molecular clock has been postulated estimating that there is a characteristic rate of evolution in small subunit rRNA genes (Ochmann et al. 1999; Vreeland and Rosenzweig 2002). However, there is doubt regarding the validity of assuming a universal molecular clock of sequence evolution, as rates differ between bacterial taxa, and it may be unrealistic in regard to native species of environments such as permafrost that are subjected to low nutrient levels, extremely low temperatures, and long microbial doubling times (Vreeland and Rosenzweig 2002). In addition, recent studies demonstrating microbial activity in permafrost samples at ambient subzero temperatures (Steven et al. 2006; see Chap. 9) further complicate the determination of the age of microorganisms isolated from permafrost. These findings suggest that at least a subpopulation of the permafrost microbial community may constitute an active modern microbial ecosystem rather than “ancient” frozen microbial survivors.

5.7 Conclusion

Both culture-dependent and culture-independent methods have revealed that permafrost harbors diverse and novel microbial communities. The future challenge for the study of permafrost microbiology is to begin to address the ecology of these

unique microbial ecosystems. The knowledge gained from culture-independent surveys of microbial diversity can be used to design targeted culturing strategies in order to determine if phylogenetic groups detected by molecular strategies are part of the viable microbial community. Moreover, the characterization of the microbial component of permafrost will provide important insights into how these environments will respond to climate change in regard to the increased metabolic rates associated with higher temperatures and nutrient availability due to the melting of permafrost. The application of technologies such as stable isotope probing (Dumont et al. 2006) and FISH-microautoradiography (Lee et al. 1999) could identify active microorganisms, and better define the functioning and maintenance of permafrost microbial ecosystems at ambient subzero temperatures. As microbial activities in situ are expected to be extremely slow and minute, new methods and technologies specific to the permafrost environment will be required. For example, we have recently described a method to measure microbial respiration at subzero temperatures that was effective at detecting low amounts of microbial respiration occurring at temperatures as low as −15°C from a variety of Arctic environments (Steven et al. 2007b). Developing methods to detect and characterize the active *Bacteria* and *Archaea* in permafrost will allow for the differentiation of the active microbial populations presumed to exist in permafrost from cryopreserved microbial fossils that may have remained frozen for geological time scales.

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