Methanogenesis in subglacial sediments

Eric S. Boyd,1,* Mark Skidmore,2 Andrew C. Mitchell,3 Corien Bakermans2† and John W. Peters1
1Department of Chemistry and Biochemistry, The Astrobiology Biogeocatalysis Research Center, Montana State University, Bozeman, MT 59717, USA.
2Department of Earth Sciences and 3Center for Biofilm Engineering, Montana State University, Bozeman, MT 59717, USA.

Summary

Methanogenic archaea have a unique role in Earth’s global carbon cycle as producers of the greenhouse gas methane (CH₄). However, despite the fact that ice covers 11% of Earth’s continental landmass, evidence for methanogenic activity in subglacial environments has yet to be clearly demonstrated. Here we present genetic, biochemical and geochemical evidence indicative of an active population of methanogens associated with subglacial sediments from Robertson Glacier (RG), Canadian Rockies. Porewater CH₄ was quantified in two subglacial sediment cores at concentrations of 16 and 29 ppmv. Coenzyme M (CoM), a metabolic biomarker for methanogens, was detected at a concentration of 1.3 nmol g sediment⁻¹ corresponding to ~3 × 10³ active cells g sediment⁻¹. Genetic characterization of communities associated with subglacial sediments indicated the presence of several archaeal 16S rRNA and methyl CoM reductase subunit A (mcrA) gene phylotypes, all of which were affiliated with the euryarchaeal order Methanosarcinales. Further, CH₄ was produced at 9–51 fmol g dry weight sediment⁻¹ h⁻¹ in enrichment cultures of RG sediments incubated at 4°C. Collectively, these findings have important implications for the global carbon cycle in light of recent estimates indicating that the Earth’s subglacial biome ranges from 10⁴ to 10⁶ km³ sediment.

Introduction

Approximately 90% of atmospheric CH₄ on Earth is produced by methanogenic archaea (Conrad, 1996; Thauer et al., 2008). Recent modelling studies indicate that subglacial methane production may be important in the global carbon cycle and may have had a significant role during the Laurentide glaciation (Weitemeyer and Buffett, 2006; Wadham et al., 2008). However, empirical data on methanogens and methane cycling in cold environments are currently limited to permafrost, cold marine sediments, freshwater sediments and basal ice with nothing known on subglacial aqueous environments (Cavicchioli, 2006; Wadham et al., 2008). A better understanding of methanogenic activity at low temperatures in subglacial settings is pertinent given the scale of the present day ice covered sediment biome, which has been estimated to range from 10⁴ to 10⁵ km³ beneath Antarctica alone (Priscu et al., 2008). The abundant water-saturated sediments recently discovered at the base of the Antarctic ice sheet have been suggested as the Earth’s largest wetland (Priscu et al. 2008), representing a significant and largely unexplored environment for subglacial methane cycling.

High concentrations of CH₄ have been measured in the basal ice of the GRIP (Greenland Ice Core Project) and GISP2 (Greenland Ice Sheet Project 2) cores from the Greenland Ice Sheet (Souchez et al., 1995; Price and Sowers, 2004). The δ¹³C of the CH₄ recovered from the GRIP and GISP2 basal ice layers ranged from −81‰ to −84‰ and −74‰ to −79‰ respectively (Souchez et al., 2006; Miteva et al., 2009), indicating that the CH₄ is of biogenic origin. However, it remains unknown whether the CH₄ was produced in situ in the ice over the last glacial cycle or incorporated into the basal ice during inception and development of the Greenland ice sheet, although Souchez and colleagues (2006) argue for the latter explanation. An independent examination of the GISP2 ice core revealed the presence of cells exhibiting F₄₂₀ fluorescence which were interpreted as methanogens (Tung et al., 2005). However, F₄₂₀ is present in a diversity of non-methanogenic archaea and a number of bacteria including the Actinobacteria (Lin and White, 1986; Purwantini et al., 1997; Purwantini and Daniels, 1998) and is not strongly correlated to the production of CH₄ in representatives of several methanogen orders, including Methanobacterium bryantii (Euryarchaeota: Methanobacterales) and Methanosarcinaarkeri (Euryarchaeota: Methanosaicina) (Heine-Dobbernack et al., 1988). Importantly, 16S rRNA genes recovered from DNA extracted from the GISP2 ice core revealed the presence of a single phylotype affiliated with the methanogen Methanococccoides...
burtonii (Euryarchaeota: Methanomicrobiales) and a diversity of Actinobacteria phylotypes (Miteva et al., 2009), suggesting that the F<sub>420</sub> fluorescence signal previously identified in the GISP2 ice core might not be derived solely from methanogens. Perhaps the most straightforward evidence for the presence of viable methanogens in glacial environments comes from John Evans Glacier (JEG), Canada where enrichments containing melted basal ice produced significant CH<sub>4</sub> following 12 months’ incubation at 4°C (Skidmore et al., 2000). However, it is unclear whether the CH<sub>4</sub> producing methanogen population(s) was remnant or active in the basal ice (Skidmore et al., 2000). Here, we provide the first genetic, biochemical and geochemical evidence of an active assemblage of methanogens associated with subglacial sediments sampled from Robertson Glacier (RG), Alberta, Canada. In addition to methanogens, the data suggest that methanotrophic archaea might also be important constituents of the RG subglacial biome.

Results and discussion

Subglacial sediments from RG, Canadian Rockies (Fig. S1), contain significant organic carbon [25 ± 14 mg particulate organic carbon (POC) g sediment<sup>−1</sup>] and organic nitrogen [213 ± 54 μg Particulate organic nitrogen (PON) g sediment<sup>−1</sup>]. Such conditions are favourable for heterotrophic microbes, which through their activity create localized anoxic zones where organisms often couple organic carbon oxidation with methanogenesis or with the reduction of sulfate [sulfate reduction (SR)] (Conrad, 1996). Sulfate reduction accounts for the majority of the anaerobic degradation of organic carbon in environments with sulfate at concentrations > 60 μM (Winfrey and Zeikus, 1977; Lovley and Klug, 1983). The concentration of sulfate in RG porewater sediments in July 2007 was 0.3 mM and sulfate in late season (November) concentrated meltwaters at RG can be as high as 2.2 mM (Sharp et al., 2002). Despite the high concentrations of sulfate, PCR using degenerate primers specific for the alpha and beta subunits of the bisulfate reductase gene (dsrAB) and genomic DNA from four separate RG subglacial sediments sampled approximately 25 m apart failed to yield PCR products, even when 40 cycles of PCR was employed. This observation, although not definitive, suggests that SR may not be an important process in this environment despite the presence of abundant sulfate; a finding which would be consistent with geochemical and microbial data derived from a number of geochemically disparate subglacial environments (Tranter et al., 2002; Foght et al., 2004; Skidmore et al., 2005). In the absence of significant SR activity, organic carbon utilization in anoxic environments often proceeds via methanogenesis (Lovley and Klug, 1983; Conrad, 1996).

Two separate sediment cores sampled from RG contained 16 and 29 ppmv CH<sub>4</sub>, approximately 9–17 times greater than the current atmospheric concentration of 1.7 ppmv (Khalil and Rasmussen, 1990), indicating CH<sub>4</sub> production. Therefore, we screened the sediments for biomarkers indicative of methanogens to determine the potential for biological methane production, including coenzyme 2-mercaptoethane sulfonate [coenzyme M (CoM)], isoprenoid glycerol dialkyl glycerol tetraether (GDGT) lipids, methyl CoM reductase subunit A (mcrA) genes, and 16S rRNA genes. While none of these biomarkers are necessarily unique to methanogens (Allen et al., 1999; Hallam et al., 2003; Schouten et al., 2007), the identification of multiple biomarkers in sub-ice environments coupled with observations of elevated CH<sub>4</sub> concentrations would strongly suggest the presence of an active assemblage of methanogenic archaea.

A single GDGT lipid containing no cyclopentyl or cyclohexyl rings (GDGT-0) was detected in the RG subglacial sediments (Table 1), suggesting the presence of archaea. Aside from the hyperthermophile Methanopyrus kandleri which synthesizes GDGTs containing 0–4 cyclopentyl rings, GDGT-0 is the only known GDGT synthesized by pure culture methanogens screened to date (Schouten et al., 2007). To further characterize the archaeal community, we PCR-amplified archaeal 16S rRNA and mcrA genes (Appendix S1) from all four subglacial sediment samples, and sequenced 37 and 35 clones of each from a single representative sample from the East stream respectively. Six 16S rRNA gene phylotypes were recov-

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<th>Biomarker</th>
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<td>CH&lt;sub&gt;4&lt;/sub&gt; in sediment porewater at 16 to 29 ppmv</td>
<td>Conrad (1996)</td>
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<tr>
<td>Glycerol dialkyl glycerol tetraether lipids with no cyclopentyl or cyclohexyl rings (GDGT-0)</td>
<td>Schouten et al. (2007)</td>
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<td>Methanogen 16S rRNA genes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Simankova et al. (2003)</td>
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<tr>
<td>Methyl coenzyme M reductase genes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Simankova et al. (2003)</td>
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<td>Coenzyme M 1.3 ± 0.1 pmol g sediment&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>Elias et al. (1999)</td>
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<td>Viable methanogens in subglacial sediments</td>
<td>Skidmore et al. (2000)</td>
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<sup>a</sup> See Fig. 1.
<sup>b</sup> See Appendix S1.

References for the physiology and or ecology of each biomarker are also presented.
cloned from the RG East subglacial sediments, all of which clustered in two distinct lineages within the euryarchaeal order *Methanosarcinales* (Fig. 1). One of the phylotypes (RG76), representing 5.5% of the sequenced clones, was 99% identical to environmental clones recovered from sediment-associated communities from Lake Huron and Lake Michigan; both of which are glacial in origin. Importantly, we also PCR-amplified and sequenced 12 16S rRNA archaeal gene clones from a long-term CH4-producing enrichment culture from JEG (discussed above) and the only phylotype recovered (clone 2) also clustered with the RG76 sequence. Together, this clade of environmental sequences are > 98% identical to *Methanosarcina lacustris*, a psychrotolerant methanogen that utilizes H2/CO2, methanol, methylamines, or acetate for growth (Simankova *et al*., 2003).

The other five 16S rRNA gene phylotypes, which together represented 94.5% of sequenced clones, clustered within an undefined clade of sequences (defined as Cluster 1 here for simplicity) that branch within the *Methanosarcinales*. Among the environmental clone sequences present in cluster 1 are those recovered from sediments from Lake Coeur d’Alene, also a former glacial lake, and a permafrost soil as well as a sequence obtained from an anaerobic denitrifying and methane-utilizing enrichment culture obtained from anoxic freshwater sediment. The anaerobic oxidation of methane (AOM) is typically catalyzed by a consortium of methanotrophic archaea and SR bacteria (Zehnder and Brock, 1980; Iversen and Jorgensen, 1985) although recent studies suggest that the SR population can be replaced by a denitrifying population (Raghoebarsing *et al*., 2006). Representatives of cluster 1 are yet to be characterized in detail, precluding a straightforward inference of the physiology of the environmental clones comprising this cluster. However, activity studies performed on subglacial sediments sampled from RG (E.S. Boyd, M. Skidmore, A.C. Mitchell and J.W. Peters, in prep.) indicate the presence of an active denitrifying community. Thus, the recovery of 16S rRNA gene phylotypes from RG basal sediments that exhibit affiliation with a methanotrophic Archaean grown in coculture with a denitrifying bacterium would be consistent with the presence of denitrifying populations and the apparent absence of SR bacteria in RG sediments. Further investigation is warranted to determine the physiology of the organisms comprising this cluster of sequences.

The abundance of methanogens in RG subglacial sediments was investigated by quantifying CoM. Coenzyme M is present in all methanogens and is involved in the final steps of methane formation, accepting methyl groups...
from methylcobalamin to form methylcoenzyme M, which is subsequently reductively demethylated, yielding methane (Taylor and Wolfe, 1974). The concentration of CoM in pure cultures of taxonomically and metabolically diverse methanogens is proportional to the growth status of the cells, the concentration of viable cells, and to cellular protein (Elias et al., 1999). Coenzyme M is labile and not likely to be preserved in environmental samples, as indicated by an approximate 46–83% decrease in detectable CoM in a variety of environmental samples following a single freeze-thaw cycle when compared with unfrozen samples (Elias et al., 1999). Thus, CoM recovered from environmental samples is likely to be associated with not only viable, but active populations of methanogens. Robertson Glacier sediments contained CoM at a concentration of 1.3 ± 0.1 pmol g dry weight sediment⁻¹ (Table 1), indicating the presence of an active population of methanogens in the subglacial ecosystem. The abundance of CoM in RG east subglacial sediments was approximately an order of magnitude lower in comparison with other anaerobic sediments such as landfill sediment (10 pmol CoM g⁻¹ sediment) and pond sediments (20 pmol CoM g⁻¹ sediment) (Elias et al., 1999). The quantity of CoM determined in RG east sediments corresponds to ~3 x 10⁸ methanogen cells g⁻¹ dry weight sediment, when converted using the CoM cell⁻¹ values previously determined for Methanosarcina barkeri str. Fusaro when grown on acetate (Elias et al., 1999). It is important to note that it has been suggested that CoM could be involved in the AOM (Hallam et al., 2004); however, this hypothesis remains untested. If true, it implies that a fraction of the CoM detected in the subglacial sediments could be attributed to this class of organism especially considering a large fraction (~95%) of the 16S rRNA gene clones from RG subglacial sediments were affiliated with a clone from an archaeon that may be involved in the AOM.

The rate of CH₄ production in cultures incubated at both 4°C and 15°C, appeared to be independent of the composition of the medium (mineral or enriched) and only differed slightly depending on the source of the subglacial sediments (east or west stream) (Fig. 2), suggesting the presence of a similar number of viable methanogens with similar activities at each sampling location. The CH₄ production rate in cultures in mineral medium incubated at 4°C averaged over the 374 day duration of the experiment was 9 and 51 fmol CH₄ g dry weight sediment⁻¹ h⁻¹ for subglacial sediments sampled from the eastern and western stream respectively. In contrast, the CH₄ production rate in cultures grown in mineral medium and incubated at 15°C, when averaged over the 150 day experiment, was 126 and 319 fmol g dry weight sediment⁻¹ h⁻¹ for subglacial sediments sampled from the eastern and western stream respectively. The CH₄ production rates in RG sediment enrichments in mineral medium when incubated at 4°C was approximately one to two orders of magnitude greater than that (~0.2 fmol CH₄ g dry weight sediment⁻¹ h⁻¹) previously determined for deep marine sediments sampled from Hydrate Ridge, Cascadia Margin incubated at 4°C (Colwell et al., 2008) and was approximately four to five orders of magnitude lower than that (~234 pmol CH₄ g sediment⁻¹ h⁻¹) previously determined for peat bog samples when incubated at 4°C (Kotsyurbenko et al., 2004). We estimate the CH₄ production potential beneath the Laurentide and Fennoscandian Ice sheets during the last glacial cycle using the rates of CH₄ production measured in enrichment cultures from RG sediments incubated at 4°C and the assumptions of Wadham and colleagues (2008) (see Appendix S2). Based on these assumptions and using the CH₄ production rates of 0.1–0.6 pg C g dry weight sediment⁻¹ h⁻¹ determined for RG sediments, we estimate that a total of 0.5–3.1 Pg C could be converted to CH₄ during the ~85,000 years ice cover. Our values are similar to one of the lowest previously reported estimates of 2 Pg C converted to CH₄ for the glacial cycle (table 6, Case C, Wadham et al., 2008) which was based on CH₄/CO₂ ratios determined in basal ice from the GISP2 ice core (Souchez et al., 2006). This is interesting as the ‘Lower estimate Case C’ value of 2 Pg is the only calculation in the various modelling scenarios of Wadham and colleagues (2008) that uses field measurements from the base of an ice sheet, rather than theoretical or laboratory-based values as was used in their Case A & B calculations. Therefore, it may be that ~2 Pg C converted to CH₄ over the last glacial cycle is a more realistic estimate than the most probable value of 63 Pg C converted to CH₄ that Wadham and colleagues (2008) ultimately advocate for.


![Fig. 2. Methane production from enrichment cultures from Robertson Glacier incubated in mineral (m) or enriched (e) medium at 4°C, over 374 days and 15°C, over 150 days. No methane increase was observed in parallel killed controls.](image-url)
In summary, we describe genetic, biochemical and geochemical evidence indicating the presence of an active assemblage of methanogens, and perhaps methanotrophic archaea, in a subglacial environment. These results highlight the importance of anaerobic microbial processes in subglacial environments and suggest that they may have an important and previously overlooked role in global biogeochemical cycles both at present, and at times in the past, especially when ice cover on the planet was greater than at present such as during the Quaternary glaciations and the long periods of pervasive low latitude glaciation ('Snowball Earth') in the Late Proterozoic (Kirschvink, 1992).

Experimental procedures

Field site description and sample collection

A detailed description of the hydrology and geology has been previously reported for RG (McMechan, 1988). Robertson Glacier (115°20′W, 50°4′N) drains the northern flank of the Haig Icefield in Peter Lougheed Provincial Park, Kananaskis Country, Alberta, Canada. The glacier is approximately 3 km long, spans an elevation range from 2900 to 2370 m, and currently terminates on a flat till plain, although glacially smoothed bedrock surfaces are exposed along the glacier margins (Fig. S1). Two principal subglacial meltwater streams, referred to here as RE (eastern) and RW (western), drain from beneath the ice front.

Fine-grained basal sediments were collected from two locations at both RE and RW in July 2007 and July 2008 as previously described (Bhatia, 2004). Sediments were collected at the terminus of the glacier where RE and RW emanate, such that the sediments could be collected from beneath the ice. (Supporting information). Briefly, sediments were collected aseptically using a flame-sterilized spatula and aliquots were transferred to sterile polypropylene bottles. Bottles and their contents were flash frozen on site using a dry ice/ethanol slurry. Cores of basal sediment were also taken at the RE ice margin and kept at 0–1°C during 4 h of transit out of the field site and then frozen at −20°C. Samples were maintained at −20°C during transit to Montana State University where they were stored at −80°C until further processed. Corers consisted of pre-cleaned threaded aluminium tubes (7.5 cm diameter × 30 cm) that, following core collection, were sealed with polypropylene screw caps and gas impermeable thread tape.

Methane gas concentrations

Sub samples (‘pucks’—7.5 cm diameter × 7 cm) representing ~300 cm³ of volume were cut from the sediment core using an angle grinder in a −20°C cold room. Subsamples were inserted into 1 l Tedlar bags and the bags were evacuated to 1 atm and were sealed using a vacuum sealer. Tedlar bags were equipped with sampling port consisting of a polypropylene stopcock with an attached 3 cm piece of Noprprene tubing closed with two tube clamps. HgCl₂ (100 ml volume) was injected through the sample port tubing to achieve a final concentration of 500 μM. Ten millilitres of oxygen-free N₂ gas was added to the bags such that a negative pressure did not develop during gas-phase sampling. Subsamples were allowed to thaw and equilibrate at 4°C for 18 h. Negative controls were prepared as described above; however, the bags did not contain glacial sediment.

Gas-phase CH₄ concentrations were determined using a Varian CP-4900 Dual Channel Micro-Gas Chromatograph, equipped with two thermal conductivity detectors (one per channel), a 10 m 5 Å molecular sieve column equipped with backflush and heated injector (to eliminate condensation) and a 10 m PPQ (PoraPlotQ) column equipped with a heated injector. Sub-samples (2.5 ml) of gas were sampled through the tubing using a gas-tight syringe. Concentrations of CH₄ were determined by comparison with a standard curve generated using certified gas standards (Matheson). The values and standard deviations reported reflect three replicate measurements for each sediment ‘puck’. Reported gas concentrations reflect the difference between the sediments and negative controls. The quantity of CH₄ recovered was likely too low to perform carbon isotopic analysis (K. Muehlenbachs, pers. comm.).

Sulfate concentrations

Porewaters from the subglacial sediments were filtered through a 0.45 μm filter and major anion concentrations including sulfate were measured on a Metrohm Peak ion chromatograph using a Metrosep A-2-250 (4 × 250 mm) analytical column, and chemical suppression with 100 mM H₂SO₄. The eluent was a 3.2 mmol l⁻¹ sodium carbonate/1.0 mmol l⁻¹ sodium hydrogen carbonate solution.

Particulate organic carbon and nitrogen determinations

Sediments were dried (80°C, 24 h) and were ground to a fine powder using a porcelain mortar and pestle. Powdered sediments were analysed for POC and PON as described previously (Harris et al., 2001) using an elemental analyser model (CE Flash series 112) (Thermo Fisher Scientific, Waltham, MA). Briefly, 300 mg of each sample was placed into a glass scintillation vial, were fumed with 12 N HCl for 48 h in a glass desiccation chamber, and were subsequently dried at 60°C for 2 h. Samples were weighed on a Cahn model C 31 microbalance (Thermo Fisher Scientific) (measurement error ≤ 1 μg) and placed into tin disks for combustion GC analyses. Standard method parameters were used with the instrument.

The GDGT lipid analysis

A 10 g (wet weight) subsample of subglacial sediment was subjected to lipid analysis as previously described (Pearson et al., 2004; Zhang et al., 2006), using a cyanoto column and ion-specific quantification methods (Hopmans et al., 2004; Huguet et al., 2006). Each pooled sample was analysed once. The GDGTs identified by liquid chromatography-mass spectrometry are reported according to the nomenclature of Pearson and colleagues (2008).
DNA extraction and 16S rRNA and mcrA gene amplification and sequencing

Genomic DNA was extracted from approximately 250 mg of sediment in duplicate as previously described (Boyd et al., 2007). Negative extraction controls contained no added template DNA. The concentration of extracted genomic DNA was estimated using the High DNA Mass Ladder (Invitrogen, Carlsbad, CA) and approximately 15 ng of DNA was used as template for PCR amplification of mcrA using primers McrF (5′-TAYGAYCARATHGTY-3′) and McrR (5′-ACRTTCTATNGCRRARTT-3′) and an annealing temperature of 46°C (Springer et al., 1995). Similarly, 15 ng of DNA was used as template in the PCR amplification of dsrAB using primers 1F (5′-ACSCACTGGAAGCAGC-GTACCGCA-3′) and 4R (5′-GTGAGTACCGCA-3′) and an annealing temperature of 54°C (Wagner et al., 1998) using DNA from Desulfovibrio desulfuricans str. G20 as a positive control. Archaeal 16S rRNA genes were amplified using primers 21F (5′-GATCCYGCCGGA-3′) and 958R (5′-YCCGGCGTTGAMT-3′) and an annealing temperature of 55°C (DeLong, 1992). Thirty-five cycles of PCR were performed in triplicate for mcrA, dsrAB and 16S rRNA gene amplifications using conditions as previously described (Boyd et al., 2007). dsrAB amplicons were not generated using 35 cycles of PCR. Thus, a second set of PCRs employed 40 cycles were used for dsrAB genes. For mcrA and 16S rRNA gene amplifications, equal 40 μl volumes of three replicate PCR products were pooled, purified using the Wizard PCR Prep DNA Purification System (Promega, Madison, WI), quantified using the Low Mass DNA Ladder (Invitrogen, Carlsbad, CA), cloned using the pGEM Easy Vector System (Promega), and sequenced using the M13F and R primer pair as previously described (Boyd et al., 2007). Archaeal 16S rRNA and mcrA gene sequences have been deposited in the GenBank, DDBJ and EMBL databases under the accession numbers GU122859–GU122861 and GU122856–GU122858 respectively.

Phylogenetic analysis

The phylogenetic position of archaeal 16S rRNA genes was assessed using PhyML (ver. 3.0) (Guindon and Gascuel, 2003) using the GTR substitution model with gamma-shaped rate variation and a proportion of invariable sites as recommended by ModelTest server (ver. 3.8) (Posada, 2006). The 16S rRNA gene phylogenetic tree was projected using FigTree (version 1.2.2).

Enrichment medium preparation and incubation conditions

The subglacial sediments contained approximately 0.75 g dry solids g⁻¹ wet weight. Four bottles were prepared initially using aseptic techniques with ~60 g (wet weight) of RG subglacial sediment per bottle, two of which contained sediments from the east stream and two of which contained sediments from the west stream. The bottles were immediately flushed with N₂ gas and 20 ml of anaerobic and sterile MS Mineral (MSM) and MS Enriched (MSE) (see below) was added to a bottle for each stream sediment sample to create a slurry. The four different sediment slurries were then each dispensed evenly among 70 ml serum bottles (~20 ml of slurry per bottle). An additional 45 ml of either MSM or MSE medium was added to each bottle and the bottles were immediately sealed. The MSM and MSE medium was based on Boone and colleagues (1989). Media were prepared by adding 4.0 g l⁻¹ NaOH and 1.0 mg l⁻¹ resazurin to 1.0 L distilled water, boiling for approximately 30 min, and chilling under a stream of 100% CO₂ for 30–45 min. At this point 8.4 g l⁻¹ NaHCO₃ was added to establish the carbonate buffer system. Subsequently, 10 ml l⁻¹ Solution A (100.0 g l⁻¹ NH₄Cl, 100.0 g l⁻¹ MgCl₂·6H₂O, 40.0 g l⁻¹ CaCl₂·2H₂O, pH 4.0), 1.0 ml l⁻¹ Trace Metal Solution (Boone et al., 1989), and 10.0 ml l⁻¹ 0.5 M sodium acetate was added to the carbonate-buffered solution. At this point, 0.5 g l⁻¹ yeast extract (Difco) and 0.5 g l⁻¹ peptone (Difco) were added to MSE media only. After sparging with N₂:CO₂ (80:20) for 30–45 min, the pH was adjusted to 7.0 with HCl and autoclaved. Sterilized media was supplemented with 2.0 ml l⁻¹ Solution B (200 g l⁻¹ K₂HPO₄, 3H₂O), 10 ml l⁻¹ Vitamin B solution (Fedorak and Hruday, 1984), and 20 ml l⁻¹ freshly prepared 2.5% Na₂S.

Methane concentration in the headspace of enrichments was monitored by gas chromatography using a Varian CP-4900 Micro Gas Chromatograph using freshly prepared standards. The CH₄ concentrations reported represent the difference between CH₄ measured in the biological controls and in the killed controls. Cell counts in the enrichment cultures were determined via fluorescent microscopy as described previously (Boyd et al., 2007).

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References


Bhatia, M. (2004) Molecular characterization of bacterial communities associated with a high Arctic polythermal...


Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Robertson Glacier (RG), Kananaskis Country, Alberta, Canada looking south (July 2008). Inset, east stream sampling site in September 2009 (photo credit: Everett Shock). Arrows indicate sub-ice sampling locations.

Appendix S1. Results and discussion.


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