Mean annual precipitation around 640 mm.

**Figure 1.** Permafrost cores of 1-m long were collected in July 2010 on Axel Heiberg Island in the Canadian High Arctic.

**Table 1.** Protein yield from different cryospheric samples.

<table>
<thead>
<tr>
<th>Cryospheric sample</th>
<th>Collection method</th>
<th>Protein yield (mg/kg of sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active layer</td>
<td>SDS-TCA</td>
<td>5.5</td>
</tr>
<tr>
<td>AL</td>
<td>SDSA-TCA</td>
<td>10.5</td>
</tr>
<tr>
<td>Full-thawed core</td>
<td>Phenol-Chloroform</td>
<td>25</td>
</tr>
<tr>
<td>Enrichment cultures</td>
<td>SDSA-TCA</td>
<td>50-400</td>
</tr>
</tbody>
</table>

DNA-derived microbial communities

**Figure 3.** Bacterial composition of total community DNA in native AL cryosol using 16S rRNA phylogenetic affiliation. 3655 16S rRNA gene sequences (515 sequences) identified using high-throughput sequencing with a 80 bp classification scheme, based on confidence threshold of 80%. Underlined phyla contain known methanotrophs, with detailed breakdown in Table 2.

**Table 2.** Number of proteins identified by different databases

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amplitcon_DB</th>
<th>AlaskanPDB</th>
<th>AMO_DB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>5 cm</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>Sample</td>
<td>35 cm</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Sample</td>
<td>65 cm</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Sample</td>
<td>80 cm</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

Results from Amplitcon_DB:

- DNA polymerase and b-lactamase were found at all three depths.
- Many enzymes, including Puccinellia reductase, HSP70, and Glucosidase, were identified in the 5 and 35 cm sample only.

Results from AlaskanPDB:

- DNA polymerase, ATP deaminase, and b-lactamase were found at all three depths.
- Upper layers had many protein sequences uniquely similar to AlaskanPDB.

Results from AMO_DB:

- DNA polymerase and alpha-amylase (from Acidobacteria spp) were found at all depths.
- At 35 cm depth, many other proteins were also identified such as peptidases, b-lactamase, CoA carboxylase, Methylmalonyl-CoA carboxylase.

All layers showed evidence of cellular motility and celulose growth. Many enzymes that are involved in glucose-utilizing metabolic pathways were identified.

**Figure 5.** Cystal protein extraction methodology

**Figure 6.** Net CH4 flux rate in (A) microcosm and (B) full-core thawing experiment. (A) Five grams of active layer, AL, and permafrost layer, PL, were incubated aerobically and anaerobically at 4°C for 24 and 12 hours with or without amendments. All AL microcosms showed CH4 consumption whereas all PL microcosms produced CH4 at a very low concentration. CH4 consumption by AL was estimated to be 41.4 ± 0.344 ml per gram fresh weight at 4°C. A core was completely thawed and incubated at 4°C whereas the control was just thawed down to -1°C. All data were recorded in the first 15 days from the fully thawed core (data points omitted for visualisation purpose). Rate of CH4 release into the headspace decreased with time, and eventually the overall system switched to CH4 uptake. Both experimental setups indicated that the whole system was CH4 sink for ambient CH4 even when the top few tens cm of PL is thawed.

**Conclusions:**

- Alpha-proteobacteria, belonging to Type I methanotrophs, play an important role in CH4 oxidation in Canadian Arctic cryosols.
- Native cryosol layers yielded protein identification of no co-metabolising indigenous microorganisms as well as the insufficiency of species in the database. Microorganisms become more metabolically active upon thawing although the activity level remained low.
- Incubation at higher temperature and with additional carbon sources selectively revealed the methanotrophic organisms and significantly increased the number of identifiable proteins.
- Proteins involved in CH4 cycling and other microbial pathways have yet to be identified despite this increased biomass.
- The large percentage of unidentified proteins pointed out the complexity of the microbial community including cryotolerant microorganisms and the need for in-depth sequencing of the cryosol from this region and the development of a customized database with informative phylogenetic data.

Proteome profiling strategy

Proteins were extracted from the cryospheric samples using either the SDS–TCA or phenol/chloroform protocol (as shown in the flowchart). Protein extracts were analyzed via mass spectrometry using LTQ-Orbitrap for identification. Proteins were separated on an Biohpic resin column using a 24 h MuDPiT setup. The raw spectra were matched to the various databases (as described in the grey box) using either DBDigger or SEQUEST. Proteins were identified by database searching using the search algorithm with a small number of false positives. The proteins were grouped into functional categories.

**Proteome profile of microcosms**

Microcosm experiment was set up to investigate the effect of acute Adamant amendment on microbial community and protein expression. 2.5 g of AL cryosol was amended with acetate (2 mM). Sterilized water of same volume was added to the control sample for the enriched water sample. Microcosms, in duplicates, were incubated with filtered 18 ppm of CH4 at 2°C for 16 days without shaking.

**Proteome profile of thawed permafrost core**

A 1-m long core was completely thawed to 4°C in cold room (B) and 5 g of samples were weighed out to characterize the variation in the microbial composition and protein expression across depth after prolonged thawing. A 1-m long core was completely thawed to 4°C in cold room (B) and 5 g of samples were weighed out to characterize the variation in the microbial composition and protein expression across depth after prolonged thawing.

**Proteome profile of enrichment cultures**

A 0.1 g cryosol was incubated with different medium and incubated at 10°C in order to selectively cultivate microbial consortia. Proteins extracted from high cell density cultures were analyzed and data processing is in progress.

**Future work:**

- Qualitative and quantitative biodiversity assessments of cryospheric samples in various incubation experiments to complement the protein data.
- Expansion of databases to include a wider diversity of organisms, especially fungi and algae.
- Construct databases for specific functional groups, for example, methanogens and nitrate reducers.
- Apply proteome profiling strategy to more samples.

**Presentation of related research @ AGU:**

- Gas fluxes in long-term thawing experiment (Poster # Monday, C138-0613)**
- Microbial biodiversity (Poster # Monday, C138-0620)
- Isotopic analysis of lipids (Talk: B140, Monday 4:00-4:15pm)
- Methanotrophs (Poster # Tuesday, B202-0400)
- In-situ gas fluxes (Talk: B42A, Thursday, 10:50-11:00)

Identifying active CH4-oxidizers in thawed Arctic perennialn through proteomics

1Maggie C. Lay1, Brandon Slackhouse1, Jonathan M. Mach2, Timonee Chooyu3, Robert L. Hettich1, Tatiana Vorobieva4, Susan Pfirr1 and Niles Lykins5, Nadia Myhrkoy1 and Tyle Whitty1 and T. C. Olszotta
1Princeton University; 2Oak Ridge National Laboratory; 3University of Tennessee; 4McGill University

The rate of CH4 release from thawed permafrost in the Arctic has been regarded as one of the determining factors for future global climate. It is uncertain how indigenous microorganisms would interact with such changing environmental conditions and hence impact their role on the fate of carbon compounds that are sequestered in the cryosphere. Multitudinous studies of sub-surface cryosol (top 5 cm) and microcosm experiments have provided growing evidence of effective methanotrophy (Fig. 2A&B).

Cryospheric samples corresponding to the active layer were sampled from a sparsely vegetated, ice-wedge polygon at the McGill Arctic Research Station at Axel Heiberg Island, Nunavut, Canada (N79°34', W96°45') before the onset of annual thaw (Fig. 1). Microscopy of 16S rRNA gene indicated the occurrence of methanotroph-containing bacteria in thawed cryosol in native cryosol including Bradyrhizobium, Methylocystaceae, and Alpha-Proteobacteria, and Methylobacterium sp. within the Verrucomicrobia (Fig. 3). The potential of methanotrophy is supported by preliminary analysis of metagenome data, which detected the presence of putative methane monooxygenase gene (MMO) sequences related to Bradyrhizobium sp. and Pseudomonas sp. (Fig. 4). Proteome profiling of native cryosol in general yielded minute traces of proteins, which likely hints at the dormant nature of the cryospheric microbial consortia. The lack of a specific database for permafrost posed an additional challenge to protein identification. Microbial community metabolism, including high Arctic microorganisms, at different temperatures, protein extraction and characterization identified 350 proteins from acetate-amended microbes, whereas only 33 proteins could be identified in the control set. Most of the identified proteins are involved in energy metabolism or post-translational modification of proteins. Although the activity of these enzymes was suppressed by the higher acetate concentration, other bacteria were activated. This was shown by at least a 5-fold increase in the number of identified proteins, which were primarily players in cellular energy metabolism. Among them, proteins belonging to the aromatic Fe-reducer, Geobacter sp. and to methane-oxidizers, Bradyrhizobium sp., Methylocystaceae sp. and Methylobacterium sp. appear dominant. This result indicates incubation experiment enhances microbial activities and causes significant shift in compositions of active community (Fig. 6).

In order to advance the database for better biodiversity and functional identification, we are currently using two characterization methods: 5) and consolidating metagenome data obtained from the same cryospheric samples. A depth profile (from active to permafrost layer) for methanotrophs is being determined by examining native cores, thawed cryosol as well as enrichment cultures. The protein profiles from these samples will be presented, which will be complemented by molecular studies.

Field site

McGill Arctic Research Station (79°34’N, 96°45’W)

Axel Heiberg Island

Canadian High Arctic

Poster number:

C138-0613

Mean annual precipitation around 640 mm

Mean annual air temperature -15.2°C (low -52°C and high 3°C)

Mean annual air temperature around 640 mm

DNA-derived methanotrophic communities

**Figure 4.** Number of sequences related to methane monooxygenase (MMO) retrieved in metagenome analysis of native cryosol at different depths. Functional identification was performed by comparing the metagenome data to various databases available on MG-RAST. Under the same criteria (max. e-value = 3×10^-10, min. identity = 50%, and min. alignment length = 50), the search against SEED returned with the greatest number of identifiable proteins.* Indicates the only result available for sample from 35 cm, which was obtained via the search against Sinbad. Caution has to be taken when interpreting this result because the presence of MMO genes does not indicate their expression in the environment and, hence, that the organisms hosting these genes are oxidizing CH4 in-situ.

**Proteome profiling strategy**

Proteins were extracted from the cryospheric samples using either the SDS–TCA or phenol/chloroform protocol (as shown in the flowchart). Protein extracts were analyzed via mass spectrometry using LTQ-Orbitrap for identification. Proteins were separated on an Biohpic resin column using a 24 h MuDPiT setup. The raw spectra were matched to the various databases (as described in the grey box) using either DBDigger or SEQUEST. Proteins were identified by database searching using the search algorithm with a small number of false positives. The proteins were grouped into functional categories.