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Evaluating snow microbial assemblages

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Abstract

Psychrophiles are organisms that grow optimally below 20°C (1). The Great Basin is home to many mountain peaks with an abundance of alpine snow environments, perfect for psychrophilic habitats. We analyzed samples from three different locations, Wheeler Peak, Pacific Crest Trail, and Mount Conness, characterizing and comparing the psychrophilic communities at varying depth intervals in the snow. Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) showed its notable difference in community structure with depth, but there was a distinct difference when comparing different snow environments (i.e. shaded vs. full sun exposure). The chlorophyll concentration decreased in the snow with depth increased. By creating a clone library and utilizing DNA sequencing technology we were able to obtain 16S and 18S rDNA gene sequences from samples collected from Mount Conness, which allowed us to identify microbes living in the ecosystem. This information enabled us to produce bacterial and eukaryotic phylogenetic trees, giving us a clear look into the diversity of this psychrophilic community. Out of seventy bacterial results, there were fifty-three β-Proteobacteria, thirteen Sphingobacteria, and only three Actinobacteria, with one unclassified bacteria as well. These results will guide us in our future plans for experimentation.

Aims and Methods

1) Collect surface samples and depth profiles from Wheeler Peak (WP) and Pacific Crest Trail (PCT) on the back side of Mount Lincoln, and process previously collected samples from Mount Conness (MC).
2) Use confocal microscopy and fluorescent nucleotide staining to visualize the microbial life in our samples.
3) Measure chlorophyll concentrations at different depths of the snow.
4) Characterize psychrophilic community by performing denaturing gradient gel electrophoresis (DGGE) on samples from all three sites.
5) Identify morphological organisms of the Mt. Conness psychrophilic community by generating 16S and 18S rRNA gene clone library and sequencing representative gene.

Results

Snow sampling

We hiked up to an elevation of 3,720 m to Roch Glacier on Wheeler Peak and to an elevation of 2,406 m on Pacific Crest Trail. Mt. Conness samples were obtained on a previous expedition in 2009 (Fig. 3). Sampling sites were identified via ArcGIS data and GPS coordinates of pigmentation in the snow (Fig. 4). At least one depth profile and multiple surface samples were collected during both PCT and WP sites. Depth profiles were taken in intervals of 10 cm to a depth of 1 m. Surface sample depths varied depending upon the depth of the pigmentation (2-10 cm). The snow samples were transported back to the laboratory in insulated coolers and allowed to melt naturally overnight at ambient temperature to avoid cell deterioration.

Snow filtering

The snow samples were processed for DNA extraction using a peristaltic pump to pump the melted snow through a 0.2 mm mesh screen and then through 0.2 μm polyvinylidene filters (Fig. 5). Samples for pigment analysis were filtered through a 0.2 μm glass fiber filters and the filters were extracted in acetone and analyzed by Jeremy Memmott in the laboratory of Dr. Chris Frolen to another lab.

Microscopy

Samples for microscopy were fixed with formalin (3.75%), stained with 0.5 diamidino-2-phenylindole (DAPI) and filtered through 0.2 μm polycarbonate membrane filters using a vacuum pump (Fig. 6). The filters were then attached to glass microscope slides with immersion oil and covered with a cover slip.

Slides were examined using a confocal microscope (TCS). Optical sections were taken in two sets, under ultraviolet light to fluorescence the DAPI and CY5 conditions to fluorescence any chloro-pigmented cells in order to create a overlay micrographs.

When examining the PCT surface sample an abundance of elliptical cells were observed (Fig. 6 a) compared to the WP surface sample which revealed an abundance of spherical cells surrounded by a halo of DAPI-stained bacteria (Fig. 6 b, d). We also viewed what appeared to be the skeleton of a diatom, which was likely leached on the snow from some other location (Fig. 6 c).

Chlorophyll measurements

Sampling sites were identified via ArcGIS data and GPS coordinates of pigmentation in the snow (Fig. 4). At least one depth profile and multiple surface samples were collected during both PCT and WP sites. Depth profiles were taken in intervals of 10 cm to a depth of 1 m. Surface sample depths varied depending upon the depth of the pigmentation (2-10 cm). The snow samples were transported back to the laboratory in insulated coolers and allowed to melt naturally overnight at ambient temperature to avoid cell deterioration.

Denaturing gradient gel electrophoresis (DGGE)

DGGE is a technique that can be used to separate DNA fragments independent of size (2). Unique 16S and 18S rRNA gene sequences have different melting (denaturing) points that are sequence-dependent. A denaturing gradient of urea and formamide is produced, and allows for the separation of different DNA sequences in a sample. 16S and 18S rRNA gene sequences were amplified from each snow sample using polymerase chain reaction (PCR). The DNA was then loaded onto a polyacrylamide gel and run overnight at 7V, 7°C.

Gene Sequencing Results

Samples collected from Mount Conness were subjected to DNA extraction, PCR, and these products were cloned into Escherichia coli to create a clone library. The clone library was taken to the Nevada Genomics Center for sequencing. Bacteria 16S rRNA gene sequences

Taxonomic classification of the bacterial 16S rRNA gene sequences was assigned using the Ribosomal Database Project II. The sequences assigned to different taxa are shown in a hierarchical order (Fig. 8). An abundance of β-Proteobacteria, 55 of the total sequences was revealed, including 24 unclassified Deltabacteriaceae.

Eukaryal 18S rRNA gene sequences

Comparisons revealed that the most closely related form of Chlorophyta algae observed (Fig. 10). A variety of heterotrophic protists was observed. We also found a diverse array of fungi, and protozoa affiliated with the algal phylum Chromista. In addition we detected sequences affiliated with two kinds of mucocellular organisms including mites and rotifers (Fig. 9).

Future Directions

This work contributes to ongoing research in Dr. Murray’s laboratory where she is studying microbial communities associated with snow fields as part of the NASA by World Program. The snow samples discussed here will be further characterized and compared between all three snow field locations to generate a better understanding of the diversity and community structure of snow-associated microbial assemblages. This work is also part of a larger effort to develop life detection technologies in icy habitats and understand the requirements of life in such locations.

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Literature Cited


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