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# Evaluating snow microbial assemblages

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# Evaluating Snow Microbial Assemblages

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## Abstract

Psychrophiles are organisms that grow optimally below 20°C (1). The US Great Basin is home to many mountain peaks with an abundance of alpine snow environments perfect for psychrophilic habitation. 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 no 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 as the depth of the snow increased. By creating a clone library and utilizing DNA sequencing technology we were able to obtain 16S and 18S rRNA 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 eukaryl 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 the psychrophilic community by performing denaturing gradient gel electrophoresis (DGGE) on samples from all three sites.
5) Identify microbial members of the Mt. Conness psychrophilic community by generating a 16S and 18S rRNA gene clone library and sequencing representative genes.

## Results

### Snow sampling

We hiked up to an elevation of 3,292 m to Rock 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 visually by the presence of pigmentation in the snow (Fig. 4). At least one depth profile and multiple surface samples were collected during both PCT and WP trips. 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 pigment present (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.

Fig. 1. WP depth profile sampling

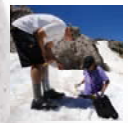

Fig. 2. PCT depth profile sampling

Fig. 3. Map showing sampling sites

### Snow filtering

The snow samples were processed for DNA extraction using a peristaltic pump to pump the melted snow through a 0.1 mm mesh screen and then through 0.2 µm polyethersulfone filters (Fig. 5a). Samples for pigment analysis were filtered through 0.45 µm glass fiber filters and the filters were extracted in acetone and analyzed by Jeremy Memmott, in the laboratory of Dr. Chris Fritsen. to another lab.

Fig. 4. Pigmented snow surface sample sites; a) PCT 1B b) PCT 2B

Fig. 5. Snow filtering; a) isolating cells for DNA extraction; b) DAPI slide preparation

### Microscopy

Samples for microscopy were fixed with formalin (3.7%), stained with 4',6-diamidino-2-phenylindole (DAPI) and filtered through 0.2 µm polycarbonate membrane filters using a vacuum pump (Fig. 5b). 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 (600X). Optical sections were taken in two sets, under ultraviolet conditions to fluoresce the DAPI and CY5 conditions to fluoresce any chlorophyll-pigmented cells in order to create overlaid micrographs.

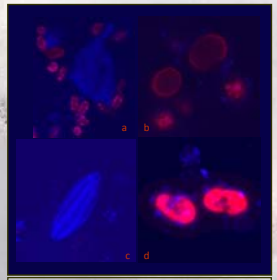

Fig. 6. Confocal microscope micrographs; a) PCT 1B surface; b, c, d) WP2 surface.

When examining the PCT 1B surface sample an abundance of elliptical cells were observed (Fig. 6 a) compared to the WP2 surface sample which revealed an abundance of more 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 deposited on the snow from some other location (Fig. 6 a).

### Chlorophyll measurements

Pigment analysis was performed to assess the amount of photosynthetic life in snow samples using standard methods of pigment extraction and fluorometric determination (3).

| Site Description | Starting Depth (cm) | Ending Depth (cm) | Chlorophyll (ug/L) |
|---|---|---|---|
| WP 2 Pond 1 Mat | 0 | 0 | 0.10 |
| WP 2 Pond 2 | 0 | 0 | 0.02 |
| WP 2 Surface | 0 | 0 | 5.85 |
| WP 2 Sub surface 2 | 0 | Nd | 1.68 |
| WP 2 | 0 | 8 | 2.15 |
| WP 2 | 8 | 18 | 2.85 |
| WP 2 | 18 | 28 | 1.98 |
| WP 2 | 28 | 48 | 1.05 |
| WP 2 | 48 | 68 | 0.31 |
| WP 2 | 68 | 88 | 0.11 |
| WP 1 Top ¾" | 0 | 2 | 0.69 |
| WP 4 Grab Sample | 0 | 3 | 400.53 |

Table 1. Chlorophyll concentration measurements of multiple WP sample sites.

### 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 sequence 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 gene 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 polyacrylamidegel and run overnight at 71 V at 70°C.

The 16S rRNA gene products displayed two banding patterns. Lanes 1-5 had similar banding patterns but lane 6 had a distinct pattern. The 18S rRNA gene products showed at least two patterns, one in lanes 7-9 and another in lane 11, however, the quality of the gel made it difficult to determine whether a third banding pattern was present in lane 10 (Fig. 7). This analysis will be repeated to see if sequence differences can be further resolved.

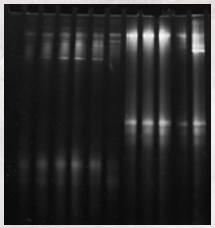


Fig. 7. DGGE
Lanes 1-7, 16S rRNA genes
Lanes 8-11, 18S rRNA genes
Lane 1, PCT 2A 0-10 cm
Lane 2, PCT 2A 20-30 cm
Lane 3, PCT 2A 20-30 cm
Lane 4 PCT 2A 40-60 cm
Lane 5 PCT 2A 60-80 cm
Lane 6 PCT 2B surface
Lane 7 PCT 2A 30-40 cm
Lane 8, PCT 2A 40-60cm
Lane 9, PCT 2A 60-80 cm
Lane 10, PCT 1B surface
Lane 11 PCT 2B surface

### 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.

### Bacterial 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, 53 of 70 total sequences, was revealed, including 24 unclassified Oxalobacteraceae.

Fig. 8. Bacterial 16S rRNA gene hierarchy

### Eukaryl 18S rRNA gene sequences

Chlamydomonas nivalis was the most dominant form of chlorophyte algae observed (Fig. 10). A variety of heterotrophic protozoans was observed. We also found a diverse array of fungi, and protists affiliated with the phylum Cilliophora. In addition we detected sequences affiliated with two kinds of multicellular organisms including mites and rotifers (Fig 9).

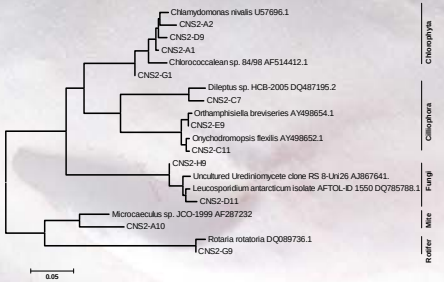


Fig. 9. Eukaryl 18S rRNA gene tree

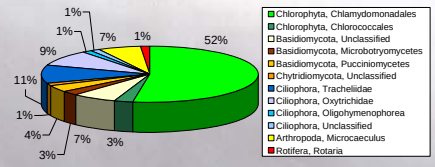


Fig. 10. Taxonomic affiliation of Mt. Conness snow eukaryal rRNA gene sequences

## Discussion

Differences in cell morphology observed with confocal microscopy suggest different species of algae were present, and that with the PCT samples possibly likely contained *Chlamydomonas nivalis.*

A halo of bacteria surrounding the PCT algal cells is likely bacteria being attached to a polysaccharide matrix surrounding the algal cells (4). The purpose of the association between algae and bacteria is unknown though the bacteria could be utilizing the algal polysaccharide as a carbon source.

Chlorophyll analysis of WP 2 depth profile showed that as the depth of snow increased the chlorophyll concentration decreased. WP grab sample was a particularly dense red algal patch, that showed an abundance of chlorophyll, as is to be expected.

After amplification a number of PCR products were lost and therefore we were not able to compare WP to PCT on the DGGE.

The different banding patterns in the DGGE gel suggest that PCT 2B and 2A have different bacterial and eukaryl community structures. This could be visually represented by the fact that the 2B surface was more heavily pigmented than 2A.

Mt. Conness bacterial library showed a dominance of Betaproteobacteria, in particular, sequences affiliated with uncultivated members of the Oxalobacteraceae. The role of these organisms in snow fields is unknown, but related sequences have been detected in Antarctic snow algal assemblages (5).

18S rRNA gene results showed the dominance of the red-pigmented *C. nivalis* snow algae. The remaining species present were diverse.

## 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 NAI Icy Worlds research 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.

## Acknowledgements

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