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Long distance microbial transport in air: Global change implications

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Abstract
The first manifestations of global change will most likelv be observed in the Earth's atmosphere. Changing wind patterns, for example, may effect the long distance dispersal of microor-g anisms. The overall objective of this research is to correlate molecular assessments of microbial community structure from cloud water and snow samples, obtained from DRI's Storm Peak Laboratory atop Mt. Werner in Colorado, with atmospheric data and calculated air mass back trajectories. Our activities for summer of 2009 will be a focused proof-of-concept exercise to determine if intact microbial DNA and viable cells can be recovered from cloud water and alpine snow samples. Specific methods employed will include DNA extraction and PCR amplification of the bacterial 16s rRNA gene, community fingerprinting (T-RFLP), flow cytometric cell counting, and dilution plate counting.

Long Distance Microbial Transport in Air: Global Change Implications

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Introduction

The most direct and immediate manifestations of global change will likely occur in the atmosphere. Whereas, it has long been understood that microorganisms can be transported long distances in the air (1), the microbiology of continental air masses is a relatively new area of research with significant potential importance to global change study. While most of the microbial burden in the atmosphere is likely to be represented by harmless soil bacteria, the potential for the transport of foreign pathogens has been recently recognized. For example, the infections of the western Atlantic's coral reefs by the fungus *Aspergillus sydowii*, transported by African dust demonstrate such potential (2). If climatic changes alter wind patterns and subsequently the long distance dispersal of microorganisms, the potential for rapid spread of pathogens will be altered. As the longevity of droughts and temperatures increase due to global change, the introduction of fungal species to new areas by forest fires is becoming more prevalent (3, 4).

Storm Peak Laboratory (SPL), Steamboat Springs Colorado, is operated by the Desert Research Institute and is a unique platform for atmospheric microbial studies. Located at 3,200 m on Mt. Werner and with a very long uninterrupted fetch to the west, the Storm Peak site enables time-extended observations of free tropospheric and in-cloud conditions. The lab focuses on a variety of atmospheric phenomena including cloud nucleation and snow formation, utilizing state of the art instrumentation to sample aerosols and calculate back trajectories of air masses. Here we report on molecular characterizations of snow and associated cloud water samples collected at SPL in association with climate and airmass back trajectory data.

Figure 1. Storm Peak Laboratory, Steamboat Springs, CO.

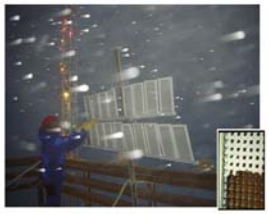

Figure 2. A demonstration of cloud water harvest technique used by SPL staff. Photo Gary Franc, University of WY

Objectives

1. Determine whether or not airborne microorganisms co-vary by origin of air mass (e.g. marine vs. continental) and with the passage of weather systems.
2. Determine if microbial population density and diversity vary between snow and cloud water.
3. Determine physiological condition (e.g. viable vs. dead) of cells in cloud water.

Methods

Field Sampling:
Sampling at SPL was performed in conjunction by others already engaged in research at the site. Cloud water was harvested by laboratory staff by placing fine mesh nylon screens (ethanol-disinfected) into the path of incoming clouds and collected using sterile tools. Samples were shipped frozen to DRI in Las Vegas and stored at -80 °C prior to analysis.

Sample Analysis:
Snow and cloud water was melted and total and viable cell counts determined by flow cytometry (AATI, MicroPro 3000). Cell viability was assessed by heterotrophic plate counts (R2A and ½ R2A medium) after serial 10-fold dilution. Microbial diversity was examined using SSU rRNA-based techniques including T-RFLP (terminal restriction fragment length polymorphism) and DNA library construction. DNA sequencing was performed by Functional Biosciences (Madison, WI). DNA sequence analysis included manipulations using Sequencher, MEGA, and Arb software packages.

Results

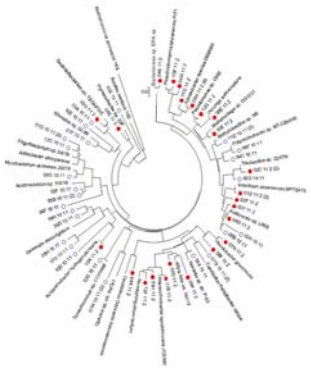

Figure 3. Phylogenetic placement of snow and cloud isolates. Scale bar on the tree indicates percent difference in 16S rRNA gene sequence.

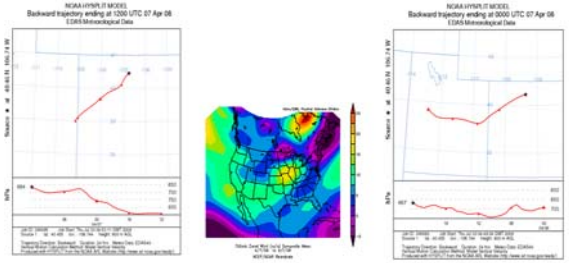

Figure 4. Back trajectory data indicating the path of an airmass that was sampled on 04/07/08. Data provided by G. Haller, SPL.

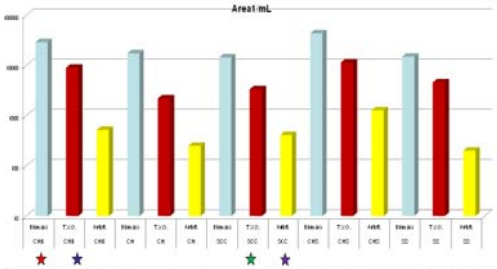

Figure 5. Flow cytometric data indicating total "biomass" (total cells), viable cells "T.V.O.," and autoflorescing organisms "Autofl." CHB, CH, SCC, CHS, and SS indicate sample names: Cloud H_2O Big Sieve, Cloud H_2O Big Screen, Snow Cloud Catcher, Cloud H_2O Small, and Snow Sieve, respectively.

Results Cont.

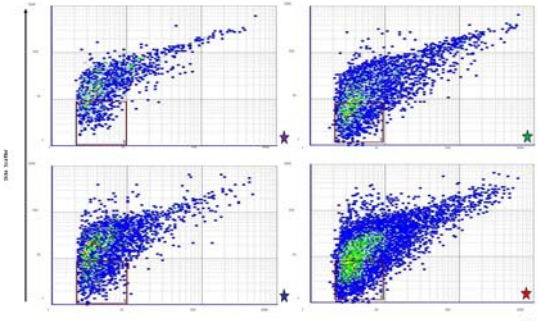

Figure 6. Flow cytometric cell counts from snow and cloud water samples collected at Storm Peak Lab. Preliminary results reveal that both snow and cloud water samples from April 2009 contained significant numbers of apparently viable cells.

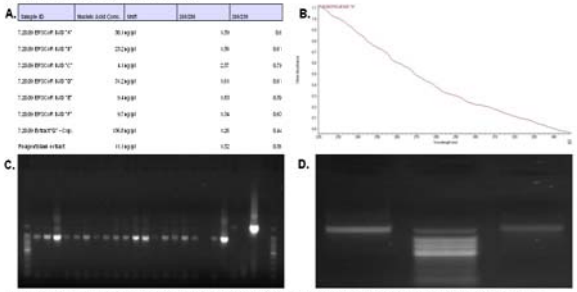

Figure 7. A, B. Nanodrop nucleic acid quantification and absorbance ratios indicating the quality of DNA. C, D. Electrophoresis of 1% agarose gels containing snow and cloud PCR products.

Conclusions

- Cell density as determined by flow cytometry is not consistent with culturable results.
- Bacterial DNA appears to be degraded (fragmented), as evidenced by much stronger PCR amplification of shorter fragments using 926R primers and nanodrop data.
- Possible inhibition of amplification as demonstrated by unamplified "spiked" PCR attempts.
- A larger sample volume maybe needed to obtain a full length 16S rRNA gene.

Acknowledgements

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