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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 likely be observed in the Earth's atmosphere. Changing wind patterns, for example, may effect the long distance dispersal of microorganisms. 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 studies. 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 become increasingly recognized. For example, the infections of the western Atlantic scallop reefs by the fungus Aspergillus fumigatus, transported by African dust demonstrate such potential (2). If climatic changes alter wind patterns and subsequently 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 speciation 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 3,260 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, allowing 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 air mass back trajectory data.

Objectives

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

Methods

Field Sampling:

Sampling at SPL was performed prospectively by efforts already engaged in research at the site. Cloud water was sampled on a weather laboratory staff by placing free-floating snow or rainwater into the path of incoming snow and collecting using a sterile tool. Samples were shipped frozen to DRI in Las Vegas and stored at -20°C prior to analysis.

Sample Analysis:

Snow and cloud water was seeded with total and viable cells determined by flow cytometry (Cytomix Model MoBios 2000). Cell viability was assessed by fluorescing heterogeneous plate count (RNA and DNA) using a broad-spectrum DNA probe (e.g. Cy3) and a broad-spectrum RNA probe (e.g. Cy5), on a flow cytometer. DNA content was assessed using two-color DNA content analysis by flow cytometry, after staining with 7-aminoactinomycin (DAPI). High-resolution DNA content analysis was performed by flow cytometry with DNA content analysis using two-color DNA content analysis by flow cytometry, after staining with 7-aminoactinomycin (DAPI).

Results

Figure 1. Flow cytometric data indicating total “dissolved” total cells, viable cells “T.L.D.C.” and macronucleating organisms “Melt.” CHV, CH, SCC, CH, and SS indicate sample names: Cloud No.1, Big Grove, Cloud No.2, Big Grove, Snow, Cloud Collector, Cloud No.3, and Snow, respectively.

Figure 2. Phagocytic placement of snow and cloud isolates. Scale bar on the tree indicates percent difference in 16S rRNA gene sequences.

Figure 3. Back trajectory data indicating the path of an air mass that was sampled on 11/11/18. Data provided by G. Mahler, SPL.

Figure 4. Proposed mechanisms for sample collection, analysis and data reduction in 16S rRNA gene sequence analysis.

Figure 5. Flow cytometric data indicating total “dissolved” total cells, viable cells “T.L.D.C.” and macronucleating organisms “Melt.” CHV, CH, SCC, CH, and SS indicate sample names: Cloud No.1, Big Grove, Cloud No.2, Big Grove, Snow, Cloud Collector, Cloud No.3, and Snow, respectively.

Conclusions

- Cell viability as determined by flow cytometry was collared with culturable results.
- Bacterial DNA appears to be degraded (fragmented), as evidenced by much stronger PCR amplification of shorter fragments using SSO primers and short read data.
- Possible inhibition of amplification as demonstrated by unexpected “spiked” PCR attempts.
- A large sample volume may be needed to obtain a full length 16S rRNA gene.

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References