

## **Arctic Microbes: Population Abundance and the Effects of a Warming Environment**

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### **Background**

The effects of global warming are most profound in the Arctic. They include an increased rate of sea-ice decline, melting permafrost, and migration changes in plants and animals. In addition, an increase is expected in existing and invasive microorganisms, which can have adverse effects on the local food chain. For example, Bradley *et al.* (2005) discussed the impact of a warming climate on free-living bacteria and parasites; an increase of such bacteria may promote diseases affecting marine fish, caribou, fox, lemming, sea otters, and other arctic species. Alaska Natives in rural villages rely on subsistence living of the previously listed Arctic animals as a major component of their diet (Barnhardt and Kawagley 2010). New diseases amongst subsistence foods will directly impact the health of the village communities devoted to subsistence living. Microbial pathogens influenced by a warming climate can also be zoonotic, meaning the disease is transmissible from animals to humans. An important health concern in the Arctic is the increase of rabid Arctic fox coming in from the ice potentially infecting both domestic animals and humans (Hueffer *et al.* 2013).

### **Significance**

Arctic warming is occurring rapidly (Moritz *et al.* 2002; ACIA 2005) increasing the length in unfrozen conditions, in which the majority of microbial activity occurs (Wallenstein *et al.* 2007). Changes associated with these unfrozen conditions (snow conditions, ice layers, summer temperatures, and nutrient cycling) can lead to bacterial and viral proliferations (ACIA 2005).

The role of bacteria in high latitude environments such as the Alaskan Arctic region has been given much attention in recent years (Anderson 2010; Campbell *et al.* 2010; Hansen *et al.* 2007; Lee *et al.* 2012; Rinnan and Bååth 2009). For example, Alaskan permafrost is thawing, releasing carbon sources utilized as a nutrient for soil microbiota (Boddy *et al.* 2008; Heal *et al.* 1981). An inventory of tundra microbial communities (Heal *et al.* 1981) provided a significant advanced understanding of arctic microorganisms. It is hypothesized that Arctic warming will continue to affect arctic microorganisms. It is speculated that changes within the arctic microbial composition will be followed by changes in arctic vegetation, which will ultimately affect arctic animals and the subsistence lifestyle of people in the Arctic. These hypotheses will be tested via measurements of microbial community composition and abundance in Arctic soils seasonally and annually.

### **Research Designs and Methods**

Soil Sample Collection: Organic layer, topsoil, and permafrost soil samples are taken from the Barrow Environmental Observatory (BEO) (Figure 1 and 2). The BEO is a 7,466-acre, specifically zoned Scientific Research District to facilitate field research activities in an accessible yet natural tundra ecosystem. Samples are collected in duplicate to have a working sample and an archived sample. The organic layer samples are collected from organic layers at 2 cm depth (Lee *et al.*, 2012). Topsoil samples are collected around 10 cm depth. Permafrost soil samples will be collected from the uppermost of the permafrost horizon (Figure 3). The actual depth of each sample is measured as well as temperature (air and soil) and soil moisture levels. Soil samples are kept frozen during transportation and stored at -80 °C before analysis. Samples are collected an average of 6 months per year (pending weather and safe transport across

seasonally frozen tundra lakes) from the same BEO designated region for 4 years. During each sample collection, we collect samples from three sites all within 10 m.



Figure 1. Collecting soil samples in the field. Photo by Theresa Vertigan.

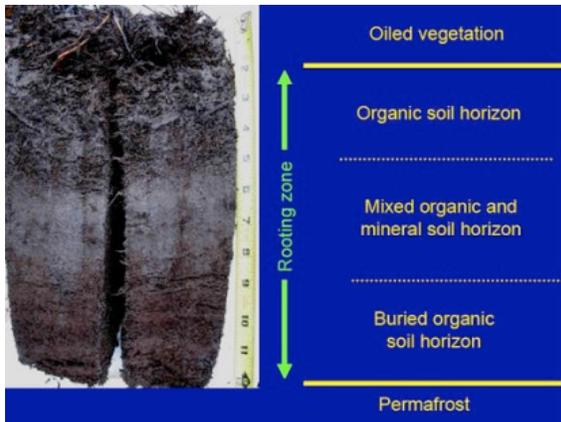


Figure 2. Tundra core sample (imagearcade.com)



Figure 3. Drill hole showing permafrost. Photo by Moema Umann

Medium Preparation: For culturing, 6 different types of solid agar are prepared. These solid agar mixtures include: Brain Heart Infusion (BHI), Tryptic Soy Agar (TSA), Czapek-Dox Agar (CZ), Sabouraud Agar (SDA), Eosin Methylene Blue (EMB), and Nutrient Agar (NA). After mixing, mixtures are placed in an autoclave for sterilization (250 F for 15 minutes) and are placed on

plates after cooling. Twenty plates of each medium are prepared and sealed after solidification occurs. The solid agar plates are stored in the refrigerator until use is needed. For the culturing process, 5 different types of liquid agar (BHI, TSB, CZ, SDB, and NB) are prepared, sterilized, and placed in test tubes. Test tubes are sealed and placed in refrigerator for storage.

Bacteria Culturing: Bacteria is grown directly from the soil on different agar mediums. Soil from a classified hole layer is extracted in 0.25 g amounts and added to 2.5 mL of agar broth (BHI, TSB, CZ, and SDB, separately). The soil-agar mixture is then incubated at 4 °C for 10 minutes, and corresponding plates are labeled. Two plates containing the solid medium are created for each medium, one for incubation at 10 °C and one for incubation at 24 °C. After incubation, 100 microliters is added to each plate. Using the lawn process, the liquid mixture is spread evenly throughout the plate with a sterilized loop and placed in their respective incubation temperatures for 24 hours or until growth is present.

Subculturing: After bacteria growth is present, plates are removed from incubation and analyzed for different bacteria types. These bacteria are labeled and 2 new, corresponding plates (matching mediums) are created for each bacteria type. Then, the bacteria is plated using the streaking method, and are incubated again. After bacteria growth is present on plates, the plates are removed and are subcultured two more times. Then, 20 mL of liquid NB are added to 10 flasks, and are labeled to correspond with each agar medium and incubation temperature.

Bacteria is then added to each flask, by touching a sterilized loop to the bacteria column and mixing the loop into the NB. The flasks are then incubated in an orbital mixer at 10°C overnight (flasks rotate gently in mixer). Plates of solid NA are labeled to a corresponding NB flask. After liquid in flasks is cloudy, they are removed from the mixer, and 100 microliters are added to the center of each plate. The liquid is spread evenly throughout the plate using a sterilized, L-shaped

spreader. Plates are then incubated and subcultured two more times after growth is present (Figure 4).

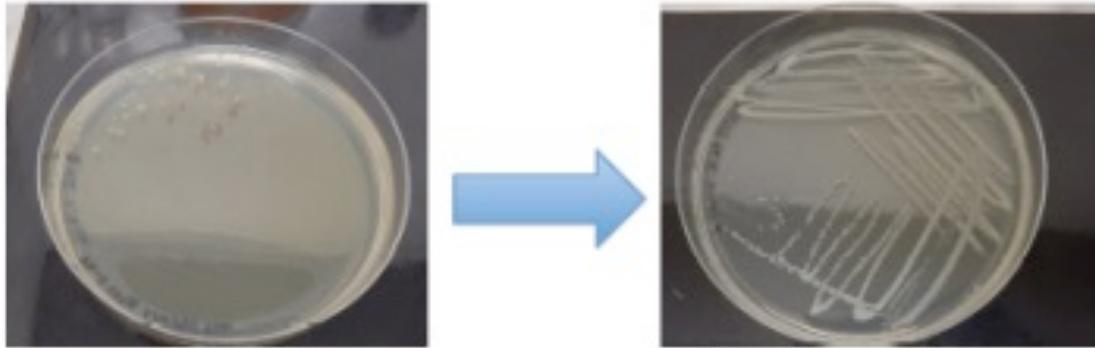


Figure 4. Permafrost sample cultured and then isolated onto a separated plate.

DNA Manipulation: DNA is extracted from soil using the procedure included in the PowerMax Soil DNA Isolation Kit (Mo Bio Laboratories Inc.). The concentration and purity of the extracted DNA will be measured using a Nanodrop spectrophotometer. DNA will then be sequenced using the Oxford Nanopore MinIon (Figure 5) and analysis will be completed through the University of Alaska Fairbanks or other source. DNA will also be extracted from pure cultures (Figure 5). Extracted DNA will be sequenced for 16S ribosomal DNA. Isolates will be compared to known DNA sequences using National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST). The sequences run through NCBI BLAST will determine the closest related operational taxonomic unit (OTU) with a 95% threshold of similarity.

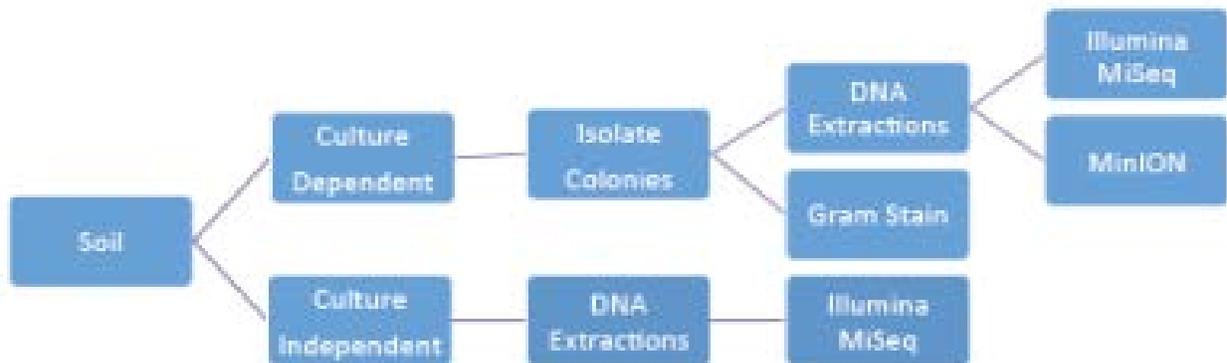


Figure 5. Flow chart for DNA sequencing and analysis for both culture dependent and culture independent samples.

### **Preliminary Results**

Initial results from one isolate map to *Pseudomonas brassicacearum*, a gram-negative soil bacteria, with known interactions with plants, has been indicated as both a growth promoter or pathogenic in different instances (Ortet *et al.*, 2011 and Tiantian *et al.*, 2012). This result reinforces the need for further research into microbial dynamics associated with changes in the Arctic.

### **Acknowledgements**

We would like to thank the support provided by the National Institute of General Medical Sciences of the National Institutes of Health (NIH) under three linked awards number RL5GM118990, TL4GM1188992, AND 1UL1GM118991. This work is solely the responsibility of the authors and does not necessarily represent the official view of the National Institutes of Health. We would like to thank the University of Alaska Fairbanks Biomedical Medicine and Student Learning (BLaST) program for providing our initial funding under the NIH grants listed above. We would also like to thank our current supporter, the National Science Foundation TCUP program: Grant No. 1622418. Special thanks goes to Ian Herriot from the University of Alaska Fairbanks for assistance in our initial sequence analysis.

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