Introduction to Biotechnology and Microbiology in the Study of Artic Microbes: Population Abundance and the Effects of a Warming Environment on Subsistence Living

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Subsistence Health Relevance of the Project: The effects of global warming are most profound in the Arctic, ranging from the 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.

Project Description: 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 2004). The purpose of the project is to assess arctic microbes from specific sites in Barrow, AK. To assess arctic microbes in Barrow, we will collect surface soil and permafrost core samples from the Barrow Environmental Observatory (BEO) (the BEO is a 7,466 acre, specially zoned Scientific Research District to facilitate field research activities in an accessible yet natural tundra ecosystem). To assess these samples, we developed the following specific aims:

Project Design and Methods:

Specific Aim 1 Culture-independent Assessment of Arctic Microbes, including the (1A) identification of arctic microbes, (1B) their abundance, and of microbial species, and (1C) how the abundance of and the composition of microbial species change over time.

Bacterial and Archaeal species will be identified from culture-independent assessment of permafrost and surface soil samples. Identification will involve isolating total DNA from environmental samples and then performing PCR using universal primers for bacterial or archaeal species. These PCR products will have variable regions, which will be identified through sequencing and aligning using the National Center for Biotechnology Information's basic local alignment search tool (BLAST). To determine the abundance of each bacterial species, we will use real time, qPCR (Triebenbach 2010), to calculate the relative quantity of DNA within each sample.

Soil Sample Collection: Surface soil and permafrost samples will be taken from six soil plots located on the Barrow Environmental Observatory (BEO). Within each soil plot, samples will be taken from two different soil layers. Surface soil samples will be collected from organic layers at 2 cm depth (Lee *et al*., 2012). Permafrost soil samples will be collected from the uppermost 10 cm of the permafrost horizon (~1.4-1.6 m below the soil surface) (Hansen 2007). Soil samples will be kept frozen during

transportation and stored at -20 °C before analysis. Samples will be collected Feb - Nov from the same BEO designated area for 5 years.

DNA Extraction: The previously described samples will be concentrated prior to extraction. DNA will be extracted from 1 mL of concentrated samples via a user-developed protocol for the isolation of bacterial DNA from soil using the QIAamp DNA Stool and QIAamp DNA Blood kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions for Gram-negative bacteria samples. DNA is stored at -80 °C until analysis.

PCR: To determine whether bacteria and or archaea are present in the permafrost or surface soil samples, we will use a similar system for detection and quantification of archeal communities from environmental samples as the one described by Takai. (Takai 2000). Bacterial DNA will be amplified via PCR for the 16S rRNA gene using universal primers (Kloos 1984). Archaea will be identified using universal domain specific primers for SSU rDNA as previously described (Takai 2000). All PCR reactions will use illustra PuRe Taq ready-to-go PCR beads (GE Healthcare, Pittsburgh, PA) and will be performed in a Thermal Cycler (Bio-Techne, Minneapolis, MN). Positive and negative controls will also be run alongside samples; *E. coli* DNA will be used for positive controls in 16S rRNA gene PCR reactions.

Sequencing: Microbial species will be identified via sequencing the PCR product and aligning using the National Center for Biotechnology Information's basic local alignment search tool (BLAST). DNA isolated from cultured microbes will be sequenced via Sanger sequencing at Elim Biopharmaceuticals (Hayward, CA). For genomic sequencing we will seek assistance from Dr. Jack Chen with the miSeq in the State Virology Lab with.

qPCR: Real-time quantitative PCR (qPCR) will be used to determine the quantity of bacterial and/or archeal DNA present (Takai 2000). We will use SYBR green with universal primers that will be designed for qPCR. qPCR will be performed at the UAF core lab (Fairbanks, AK).

Data Analysis: Sequenced genes and genomes will be deposited in Genbank. The change in species abundance (or the growth curves from heat stress experiments) will be analyzed in a two-way ANOVA with XLSTAT to compare the means of each month via Tukey multiple comparisons ($\alpha = 0.05$). The differences between species will be compared via a bonferroni multiple comparisons ($\alpha = 0.05$).

Specific Aim 2 Culture-dependent Assessment of Arctic Microbes: (2A) Bacteria will be cultured from samples, (2B) the effects a warming environment has on arctic microbes will be investigated and any advantages mutations will be identified (2C).

In addition to culture-independent methods, this study will assess some arctic microbes through culturing. To visualize cultured microbes, we will use the transmission electron microscopy at UAF. A culturable arctic microbe will allow us to perform serial and high shock temperature growth experiments to assess how a warming environment will affect these bacteria. The sequences of wild-type arctic bacteria compared to heat stressed bacteria will be compared to identify mutations that favored heat resistance.

Culturing: Using a variety of enriched media and growing conditions, we will culture bacteria. The cultured bacterial will be identified by isolating its DNA and following the culture-independent procedure described in Aim 1A. Potential microbial species previously identified in Arctic and Antarctic sea ice

include *Gammaproteobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria* for bacteria and *Methanomicrobiales*, M*ethanothermus*, and *Halobacteriales* for archaea (Collins 2010). Once culture methods are established, we will perform heat stress growth assays, to assess how microbes will be affected in a warming climate.

Microscopy: To determine morphology of cultured bacteria, each colony type will be Gram-stained using standard methods and photographed under a light microscope. For transmission electron microscopy, bacterial cultures will be grown overnight. The culture will then be transferred to a Formvar®-coated copper 200-mesh grid (SPI Supplies West Chester, PA), stained with phosphotungstic acid, and viewed on a JEOL JEM-1200EX TEM. Images will be collected with AMT Image Capture Software and an Orca (Hamamatsu) 12 bit 1024 by 1024 bit CCD camera at the Advanced Instrumentation Lab at UAF.

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