



NASA ASTROBIOLOGY INSTITUTE ANNUAL REPORT YEAR



[July 2001 – June 2002]

Project Report: Delivery of Organic Materials to Planets

Michigan State University
Executive Summary
Principal Investigator: Michael Thomashow

Bacteria are the most ancient, simplest, and most numerous of Earth's life forms. Their versatility enables them to live in even the subfreezing temperatures of the permafrost, the perennially frozen soil that is characteristic of arctic and some subarctic regions. Astrobiologists are identifying and analyzing the ways in which bacterial genes and proteins effect the remarkable adaptability that allows these primitive microorganisms to flourish in hostile environments on Earth and, possibly, in space.

Low temperatures are a predominant environmental characteristic of interstellar space, asteroids, meteors, and, of course, our Solar System, including most of the planets and their satellites. An understanding of the effects that low temperatures have on the responses and evolution of biological organisms is, therefore, integral to our knowledge of astrobiology. Toward this end, we are exploring multiple aspects of microbial adaptation to low temperatures. One major line of investigation – Genomic and Proteomic Analysis of Permafrost Bacteria – is to conduct genomic and proteomic analyses of bacteria that have been isolated from the Arctic and Antarctic permafrost. Our basic objectives include (1) identifying genes and proteins that enable permafrost bacteria to inhabit subfreezing environments, and (2) determining how genome expression in the permafrost bacteria is affected the by low temperatures and other environmental conditions associated with the permafrost, and by those conditions that “hitchhiker” bacteria might encounter during travel through space on natural objects or spacecraft.

In a second major line of investigation – Bacterial Adaptation to Low Temperature – we are directly examining, through “test–tube evolution” experiments, how bacteria genetically adapt to low temperatures. The fundamental objective is to understand how an organism, with a given complement of genes, can cross niche barriers that are defined by decreasing temperatures. Finally, in a “Field Truth” investigation – Indigenous Bacteria of Arctic and Antarctic Permafrost – we are exploring the microbial ecology of the permafrost environment and the physiological state of the resident microbial community. This objective is being accomplished by determining the phylogenetic diversity of the bacterial permafrost population and the metabolic activities present in permafrost soils.

Our first year as members of the NASA Astrobiology Institute has been rewarding and exciting. In this “getting up to speed” year, we have successfully

attracted graduate students and postdoctoral researchers to the research projects being conducted and have begun to generate results that provide important foundations for our overall research goals.

In the Genomic and Proteomic Analysis project, we completed a phenotypic, or visible-properties, survey of 20 permafrost bacterial isolates and chose two, *Exiguobacterium* 255-15 and *Psychrobacter* 273-4, for complete genome sequencing; that is being accomplished in collaboration with the Department of Energy (DOE) Joint Genome Institute. The strains were isolated from permafrost soils that were isolated from 2 million to 3 million and 20,000 to 40,000 years old, respectively; they were determined to have genomes that contain about 2.5 million base pairs (2.5 Mb) (determined by pulse field gel electrophoresis) and to be capable of growth at low temperature (-2.5°C) and low water activity (5 osmolar salt); these are distinctive characteristics that one would anticipate for microbes inhabiting the permafrost. DNA sequencing representing a 10-fold coverage of the *Exiguobacterium* isolate has been obtained, and similar sequencing of the *Psychrobacter* strain should be accomplished shortly. We should, therefore, soon be in a position to determine the complete informational content of both of these permafrost bacteria. Not only will this be an important milestone for our particular project, but it will be significant for microbial genome sequencing in general, for no genome sequence has yet been published for a psychroactive bacterium.

The phenotypic survey experiments have also provided tantalizing indications that both the *Exiguobacterium* and *Psychrobacter* strains chosen for detailed genomic studies are able to sense and respond to low temperature. We have tested both strains for their abilities to utilize 96 different carbon sources (using Biolog plates) and have found that the metabolism profiles differ with growth at 24°C and 4°C. In addition, we have found that *Exiguobacterium* strain is very hard to lyse when grown at 24°C, but very easy to lyse after growth at 4°C. And with the *Psychrobacter* strain, exposure to low temperature (both 4°C and -10°C) results in an increase in ice nucleation activity, a response that may have an important role in freezing tolerance. Indeed, long-term freezing experiments (freezing periods of up to 12 months) indicate that *Psychrobacter* that have been grown at low temperature (4°C) survive freezing better than those grown at higher temperatures. Additional progress in the Genomic and Proteomic Analysis project includes the development of two-dimensional liquid chromatography methods to produce polypeptide profiles for both soluble and membrane proteins for the *Exiguobacterium* strain and a demonstration of transfer of a wide host range plasmid from *Escherichia coli* to the *Psychrobacter* strain; the latter is a critical first step in the development of a transposon mutagenesis procedure to identify genes that are responsive to changes in the environment and are that required for life at low temperature.

In the Bacterial Adaptation to Low Temperature project, *E. coli* is serving as the experimental organism. A total of 30 replicate lineages, representing populations with diverse thermal histories, have been adapted to 20°C for 2,000 generations, and efforts are in progress to examine adaptation to even lower temperatures (12°C-14°C). Significantly, initial analysis of the 2,000-generation-evolved populations indicates that adaptation to 20°C is frequently

associated with a loss of competitive fitness at 40°C. Thus, a trade-off associated with low-temperature adaptation may be a loss of fitness for growth at high temperatures. A genetic analysis of the evolved strains indicates that deletion events are more common than gene-duplication events in the low-temperature-evolved populations. Determining whether these changes in the genome contribute to the increase in competitiveness at low temperature is now an important goal.

Adaptation to freezing conditions is also being investigated as part of the Bacterial Adaptation project. The first question that we have addressed is whether there is a possibility that *E. coli* has the capacity to evolve an increased fitness in freezing conditions. The results to date suggest that it may. In particular, we have found that lines of *E. coli* that have been adapted for 20,000 generations to growth at 37°C have a greater kill-rate when subjected to daily freeze-thaw cycles than do non-adapted populations that have not been selected for greater fitness at high temperature. Thus, *E. coli* can display genetic variation in freezing tolerance suggesting that selection for increased freezing tolerance may result in the evolution of strains with increased fitness for freezing.

One of the objectives in our Indigenous Bacteria of Arctic and Antarctic Permafrost Project is to increase our understanding of the phylogenetic diversity of the bacteria that inhabit the Arctic and Antarctic permafrost and to assess how much of that diversity can be successfully captured upon culturing from the permafrost. In these studies, 16S rDNA (recombinant deoxyribonucleic acid) genes were amplified by polymerase chain reaction (PCR) using “universal” bacterial primers and template DNA isolated directly from permafrost soil samples. High throughput DNA sequencing was performed on the cloned PCR products to obtain 16S rDNA gene sequences.

The soils tested to date had either been kept frozen since initial isolation or had been incubated under either aerobic or anaerobic conditions at 10°C for 8 weeks. DNA sequences for over 2,000 ribosomal RNA (*rrn*) genes have been determined and compared with sequences in the Ribosomal Database Project (RDP) by automated software that we designed. The results indicate significant microbial diversity in all samples, including the Antarctic as well as the Arctic soils. Furthermore, microbial growth at 10°C was observed in most samples, indicating the presence of living microbes. Members of the following phylogenetic Divisions were found: Green_Non-Sulfur Bacteria, Leptospirillum-Nitrospira, Nitrospina, Flexibacter-Cytophaga-Bacteroides, Planctomyces and relatives, Proteobacteria, Planctomyces and relatives, Fibrobacter, and Acidobacterium. Among the Gram Positive Bacteria were members of the Thermotogales, Cyanobacteria, Anaerobaculum and two Divisions known only by environmental clones, OPB80 and WCHB1-31. To our knowledge, this is the highest resolution sampling of soil bacterial diversity in existence for any habitat.

Although the above results indicate which microbial groups are present and that some are able to grow in the permafrost soil when the temperature was raised to 10°C, they do not indicate whether the microbes are active at the in situ temperature and water activity. Hence, additional experiments were

undertaken in the tundra zone of the Kolyma Lowland near the East Siberian Sea coast (lat. 70°N, long. 160°E). Here, the permafrost sediments are up to 800 m thick and have a mean annual temperature of -9°C to -11°C. One sample investigated was a peaty-loam of Holocene age origin (2,920 ± 40 years old), located at a depth of 80 cm below the permafrost surface. Metabolic activity was determined by analysis of hydrogenotrophic and acetoclastic rates of $^{14}\text{CH}_4$ (methane) production. The results indicated methane formation from $\text{H}^{14}\text{CO}_3^-$ and $^{14}\text{CH}_3\text{CO}_2^-$ at temperatures down to -16.5°C.

From this sample, a methanogenic psychrophilic bacterium, *Methanosarcina* sp. JL01 (16S *rrn* sequence AF 519802), was isolated. The second sample was collected from the lenses of overcooled brines (170–300 grams per liter) situated at depths of 40–50 m within permafrost sandy soils of marine origin that date back to Wisconsin glaciation (100–120 thousand years B.P.). These so-called “cryopegs” are the only hydrologic system on Earth that are characterized by permanent temperatures below zero, by high salinity, and by isolation from external factors throughout their geologic history. The metabolic activity of the indigenous cryopeg microorganisms was detected down to -15°C as measured by monitoring the incorporation of [^{14}C]-labeled D-glucose into microbial cells. From this sample, a psychrophilic bacterium, designated *Psychrobacter* 2pS (16S *rrn* sequence AF177557), was isolated.

It is believed that extraterrestrial life is only possible in the presence of water. Most of the planets (and their satellites) in the Solar System are distinguished by low temperatures, at which water remains unfrozen only if the solute concentration is high. The evidence that there is free water on Mars implies that its water is saline. Thus, Martian free water may occur as brine lenses formed when Mars lost its atmosphere and became dry and cold. The Arctic cryopegs, together with the inhabiting microorganisms, may serve as a model for low-temperature exobiological niches on Mars and other planets and satellites in our Solar System.