# Isolation of Psychrotolerant Microorganisms from Cold, Deep-Subsurface Waters

Corien Bakermans
*Center for Genomic and Evolutionary Studies on Microbial Life at Low Temperatures*
*Michigan State University*
*106 Plant Biology Bldg, East Lansing, MI 48824*
*UNITED STATES*
bakerm16@msu.edu

Rudolph E. Sloup, Michael F. Thomashow, James M. Tiedje
*Center for Genomic and Evolutionary Studies on Microbial Life at Low Temperatures*
*Michigan State University*
*UNITED STATES*

Lisa M. Pratt, Tullis C. Onstott
*Indiana University-Princeton University-University of Tennessee Astrobiology Initiative*
*UNITED STATES*

Timo Ruskeeniemi
*Nuclear Waste and Environmental Geology*
*Geological Survey of Finland*
*FINLAND*

Cold, deep-subsurface environments are important analogs to the Martian subsurface. Our goal is to isolate, identify, and characterize microbes that are capable of inhabiting cold, deep-subsurface environments on Earth, such as are accessible from the Kinross/Echo Bay Lupin gold mine, Nunavat Territory, Canada. We isolated aerobic, heterotrophic, psychrotolerant bacteria from 4 separate boreholes that sampled waters with a range of depths (890 or 1130 m), temperature (9 to 12°C), and salinity (3 to 33 ppt). The adaptation of microbial communities to low temperatures was assessed by incubating aliquots of borehole waters aerobically on low-nutrient agar at a variety of temperatures (0, 4, 20, 30, or 37°C) and counting the number of colonies that appeared. For all samples, counts were maximal at incubation temperatures of 0, 4, and 20°C; and decreased as incubation temperature increased (to 30 and 37°C). Forty-seven isolates were selected from the plates incubated at ≤20°C for further analysis. RFLP analysis of 16S rRNA genes revealed 19 phylotypes (for 40 isolates)—only 1 phylotype was common to all samples. Non-saline samples appear to host a more diverse community of organisms (about two times more phylotypes and isolates present) than saline samples. The high number ($5\_10^2$ to $5\_10^4$ cfu/ml) and diversity of culturable organisms present supports the idea that large diverse communities persist in deep subsurface waters. In addition, these data suggest that the majority of the culturable microbes from the deep subsurface waters are psychrotolerant not mesophilic; hence, the community is well adapted to its low-temperature environment.