

## **Use Of Amino Acid Racemization To Investigate The Metabolic Activity Of "Dormant" Microorganisms In Siberian Permafrost**

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Permafrost occupies a significant part of North America and Eurasia, and accounts for around 20% of Earth's land surface. Permafrost represents a temperature-stable environment that allows the prolonged survival of microbial lineages at subzero temperatures. Microorganisms from ancient permafrost have been revived and isolated in pure cultures. Permafrost is a unique environment serving as a "natural gene bank", with many species frozen in time (i.e. preserved in an unchanging evolutionary state). Permafrost presents a golden niche for future biotechnology, and is also a unique environment for studying longevity and survivability microorganisms (pro- and eukaryotes). Permafrost, alone among cold environments, offers a sedimentary column in which, in one borehole made in the thick permafrost, we can observe in the preserved genetic material the history of biological evolution during the last several hundred thousand or maybe even a few million years. A thorough study of the phylogenetic relationships of organisms at each depth, as well as comparisons between different depths of permafrost, using molecular evolution techniques, will give us a unique window into the process of evolution of microbial communities over geologic time.

The longevity of (micro)organisms in cold environments is of great interest to astrobiology since cryospheres are common phenomena in the solar system, particularly on satellites, comets and asteroids, and on some of the planets. Recent data from the Mars Global Surveyor mission suggest the possibility of permafrost or perhaps even liquid water under the Martian surface. The probability of finding life on Mars, if it exists, is probably higher in such environments. In addition, the evaluation of the possibility of transfer of living organisms between planets via impact ejecta needs the information on the maximum time over which microorganisms in cold environments can remain dormant and subsequently revive and reproduce. Our strategy for the search for extraterrestrial life or its remnants is based on studying the most probable environments in which life (extant or extinct) may be found, and determining the maximum period of time over which such life could be preserved. The terrestrial permafrost, inhabited by cold adapted microbes, can be considered as an extraterrestrial analog environment. The cells and their metabolic end-products in Earth's permafrost can be used in the search for possible ecosystems and potential inhabitants on extraterrestrial cryogenic bodies. The study of microorganisms (or their remnants) that were buried for a few million years in permafrost provides us with a unique opportunity to determine the long-term viability of (micro)organisms.

We have analyzed the degree of racemization of aspartic acid in permafrost samples from Northern Siberia (Brinton et al. 2002, *Astrobiology* 2, 77), an area from which microorganisms of apparent ages up to a few million years have previously been isolated and cultured. We find that the extent of aspartic acid racemization in permafrost cores increases very slowly up to an age of approximately 25,000 years (around 5 m depth). The apparent temperature of racemization over the age range 0-25,000 years, determined using measured aspartic acid racemization rate constants, is -19 C. This apparent racemization temperature is significantly lower than the measured environmental temperature (-11 to -13 C), and suggests active recycling of D-aspartic acid in Siberian permafrost up to an age of around 25,000 years. This indicates that permafrost organisms are capable of repairing some molecular damage incurred while they are in a "dormant" state over geologic time.