Evaluation of Sub-Zero Hydrogen Peroxide Treatment For In Situ Biological Decontamination of Subsurface Ice Probes

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In recent years psychrophiles from extreme environments, such as those from sub-glacial lakes in Antarctica, have been the focus of increasing interest from both basic science and biotechnology perspectives. International laws, as well as scientific integrity, place severe restrictions on the technologies and methods of sample collection. It is imperative that the sample and the sampled site remain pristine and biologically uncontaminated by organisms brought to sampling depths from the surface. This presents a significant challenge to our current capabilities for in situ biological decontamination in these extreme environments. To prevent biological contamination of sub-glacial waters, we need new capabilities to decontaminate the sampling probe before it enters the sub-glacial lake. We have initiated studies of the use of the oxidative agent hydrogen peroxide as a practical way of biologically decontaminating probes in situ. In this method, the sampling probe will be biologically decontaminated during the descent phase through the ice and before the probe enters the liquid water environment. A study was conducted in which bacterial spores and the representative protein, the enzyme RNase, were subjected to hydrogen peroxide treatment at sub-zero temperatures. Results of the RNase study show that 25% hydrogen peroxide at 0°C inactivates the enzymatic properties of RNase almost immediately upon exposure. SDS-PAGE analysis of the enzyme during hydrogen peroxide treatment shows complete degradation of the protein after four hours. In addition, the results of spore inactivation experiments show that 20% hydrogen peroxide in the liquid phase at –20°C is effective in preventing the germination of *Bacillus subtilis* spores although exposure times of 140-160 hours may be required. Our results suggest that the use of a strong oxidizing agent, such as hydrogen peroxide, to envelope the probe may be sufficient, with proper incubation time, to sterilize the sampling probe prior to deployment into the sub-glacial lakes of Antarctica.