DETECTION OF MICROBES IN THE SUBSURFACE

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If experience on Earth is somewhat parallel to that of Mars the most likely place to detect evidence for life is in the subsurface sediments that have been protected from the intense ultraviolet irradiation and the peroxide-like oxidants that probably have destroyed the organic carbon molecular fossils that might have existed in the sediments. The experience of expanding the known biosphere on Earth with the detection of an active and diverse microbial community in the deep subsurface sediments has relevance to the search for evidence of life on Mars. A collaborative program, Deep Probe, was organized by F. J. Wobber of the Department of Energy and C. F. Fliermans of the Savannah River Laboratory to recover sediments from the deep subsurface. Extraordinary precautions were utilized to control contamination from surface soils and drilling fluids and a successful program to recover the sediments from as deep as 1,000 feet was achieved. The cores from the sediments were recovered through a paring device which removed the outer surface and immediately transferred them to an anaerobic sterile chamber from which they were distributed to the cooperating universities and national laboratories for analysis. Essentially, a microbiota was recovered from the aquifers that contained between 10^2 to 10^6 organisms/g dry wt. They showed diverse metabolic propensities that were clearly different from organisms from surface soils and sediments. The organisms were primarily bacteria with gram positive cell walls dominating the clay formations. These areas showed the least metabolic activities and microbial biomass. The aquifers showed a more heavily gram negative community with a higher biomass. The aquifers were aerobic and strictly anaerobic; facultatively anaerobic and strictly aerobic bacteria were recovered. The details of the findings for the Deep Probe will be published by the research groups shortly.

The point for MRSR is that the subsurface contained microbial life where it was not expected. Microorganisms were detected by their metabolic activity and growth. However, these techniques depend on the ability to detect and grow each type of microorganism. How does one detect all the organisms present in a subsurface sample? Using ultrasensitive detection techniques for "signature biomarkers" it is possible to both determine the biomass (based on universally distributed biomarkers) and the community structure (based on the detection of biomarkers restricted to subsets of the community). Living cells create unique molecules that have distinctive half lives in soils and subsurface sediments. In the subsurface, it was important to determine if the non-culturable microbiota were viable or potentially viable as contrasted with the accumulation of molecular fossils. Bacteria create urionic acid containing polysaccharide exopolymers that are degraded slowly, but they may persist when cells are no longer viable. The cell walls of bacteria contain monomers such as muramic acid, N-glucuronic acid, ketodeoxyoculonic acid, and the lipipolysaccharide-Lipid A. These unusual components can persist in soils after the death of cells. The cytoplasm contains the DNA, RNA, enzymes, and ATP, all of which can persist after cell death and lysis. The membranes contain lipids, and one class of these lipids, the polar phospholipids, are rapidly degraded on the death of the cells by external and internal phospholipases. The phospholipases form neutral lipids from the polar lipids. Petroleum which is a residue of biological material is clearly lipid, but it contains no phospholipids.

The detection of phospholipids offers a mechanism to determine the presence of viable or potentially viable microbes in subsurface sediments. Phospholipids are formed from fatty acids which, in the microbial world, have a sufficiently diverse structure and asymmetric distribution that they can be used as "signatures" of different groups of microbes. Thus, it is possible to quantitatively define the microbial community structure by the patterns of the polar lipid fatty acids (and ethers if the Archaebacteria are included). The development of high resolution chromatographic separations by capillary gas chromatography, or supercritical fluid chromatography of electron withdrawing derivatives for detection by mass spectrometry of the negative ions has provided 100 attomolar sensitivities with no loss in resolution for fatty acids and lipid amines from environmental samples. The lipids also contain information about the community nutritional status as some specific fatty acids or endogenous lipid storage polymers accumulate during periods of stress. Since the techniques involve the isolation and separation of individual "signature" components it is possible to define specific metabolic activities using ^{13}C or ^{15}N labeled precursors. Recently, it has been shown that the tedious procedures of extraction, fractionation, derivatization, and analysis can possibly be developed into a rapidly automated system. The system is based on the manipulation of supercritical fluid extraction of sedimentary samples with fractionation of the lipids based on the differences in polarity. The lipid samples can be trans-esterified in the gas phase and separated chromatographically prior to analysis by tandem mass spectrometry.

The search for evidence of microbial life in the deep subsurface of Earth has implications for the MRSR program. If suitably protected environments can be found on Mars then the instrumentation to detect biomarkers could be used to examine the molecular details. Finding a lipid in Martian soil would represent possibly the simplest test for extant or extinct life. A device that could do a rapid extraction possibly using the supercritical fluid technology under development now with detection of the carbon content would certainly indicate a sample to be returned.