

Biomarker Preservation Potential of Subsurface Ecosystems. T.C. “Outsider” Onstott¹, R.L. Harris¹, B. Sherwood Lollar², K.A. Pedersen³, F.S. Colwell⁴, S.M. Pfiffner⁵, T.J. Phelps⁵, T.L. Kieft⁶ and C. Bakermans⁷ ¹Dept. of Geosciences, Princeton University, Princeton, NJ, USA 08544 (tullis@princeton.edu; rlh6@princeton.edu), ²Dept. of Earth Sciences, University of Toronto, Toronto, Ontario, Canada M5S 3B1 (bslollar@chem.utoronto.ca), ³Microbial Analytics Sweden AB, Mölnlycke, Sweden (kap@micans.se), ⁴College of Earth, Ocean, and Atmospheric Sciences, Oregon State University, Corvallis, OR, USA 97331 (rcolwell@coas.oregonstate.edu), ⁵Center for Environmental Biotechnology, University of Tennessee, Knoxville, TN, USA 37996 (pfiffner@utk.edu; tphelps@utk.edu), ⁶Dept. of Biology, New Mexico Tech, Socorro, NM 87801 (tkieft@nmt.edu), ⁷Altoona College, The Pennsylvania State University, Altoona, PA, USA (cub21@psu.edu)

Introduction: During the past 30 years investigations of the continental and marine deep subsurface biosphere have revealed a biomass abundance and diversity that rivals that comprises a significant fraction of Earth’s total biosphere. The utilization of physical and chemical tracers during coring was critical in the discovery of the deep biosphere because the tracers were able to distinguish biomarkers that represented indigenous subsurface prokaryotes from those introduced by drilling. The data used to characterize the abundance, diversity and long term activity includes: 1) cellular counts; 2) DNA/RNA/protein/ATP analyses; 3) phospholipid fatty acid (PLFA)/diglyceride fatty acid (DGFA)/Archaeal lipid analyses; 4) stable isotopic analyses of gases, aqueous species, biominerals and authigenic minerals; and 5) textures and composition of biodegraded glass and petroleum.

Biomass: The prokaryote cellular abundance in the subsurface ranges from 10^9 cells/gram to below the limit of detection by microscopic techniques (~6 cells/gram) and PLFA and qPCR of 16S rRNA (~ 10^2 cells/gram). Cellular abundance slowly diminishes with depth, but spikes in cellular abundance occur often at the interfaces between organic-rich shale and organic-poor sandstone. Although cellular abundance tends to be higher in sedimentary rocks, values as high as 10^{5-8} cells/gram have been reported for ash flow tuffs and metamorphic rocks. Cellular abundances on fracture surfaces are $\sim 10^5/\text{cm}^2$ [1] and in ice are $\sim 10^{2-9}/\text{cm}^3$.

In Cretaceous Period sandstone and shale in the San Juan Basin of New Mexico the PLFA, indicative of active bacterial communities, concentrations ranged from 3,000 to 0.1 pmoles/gram and diminished with depth up to 200 meters. The DGFA, indicative of recently dead and inactive bacteria, concentrations ranged from 0.2 to 8,000x pmoles/gram (equivalent to $\sim 5 \times 10^3$ to $\sim 2 \times 10^8$ cells/gram) and increased with depth. The absence of DGFA from the thermal aureole of a 3.3 myr basaltic intrusion not only illustrated the thermal lability of these biomarkers but also implied that the DGFA was greater than the intrusion age [2].

Biodiversity: Culture based analyses, nonculture based 16S rRNA/18S rRNA amplicon, microscopic observations, and metagenomic/metatranscriptomic

analyses have revealed that the active subsurface biosphere is dominated by Bacteria and to a lesser extent Archaea. These analyses have also revealed the presence of micro-Eukaryotes (e.g. fungi and protists) and multicellular meiofauna (e.g. nematodes) [3]. Viruses are present at 10x the abundance of prokaryotes and are active as well.

Subsurface Microbial Activity: The signatures of subsurface metabolic activity during the thousands of millenia of isolation in the subsurface are preserved in the biodegradation signatures of petroleum deposits (anaerobic alkane-degrading bacteria), in the C and H isotopic composition of CH_4 (methanogenic and methanotrophic Archaea and methanotrophic bacteria) [4], and in the C isotopic composition of carbonate mineralized fractures (both heterotrophic and autotrophic microorganisms). The fossilized remains of subsurface microbial biofilms have been reported in fracture-filling calcite [5]. Trace fossils ranging in size from tens to hundreds of microns of chemolithotrophic bacteria have been reported in volcanic glass. These features have also been found in Archean pillow basalt metamorphosed to greenschist facies [6]. Reduction spheroids and carbonate concretions created by subsurface anaerobic bacteria have been reported ranging in size from millimeters to meters.

Implications for Mars Sample Return: If life emerged on the surface of Mars it may have succumbed to a Gaian bottleneck [7] of a rapidly diminishing atmosphere. The subsurface biosphere whether sheltered in sedimentary or igneous rocks in sub-freezing saline pore water [8] would have continued to grow and evolve and their remains preserved in freshly excavated rock today.

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