Microbial abundance and species composition in the Tono subsurface biosphere based on lipid biomarker analysis

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Recent studies estimate that half the living carbon on earth may exist in the subsurface environment. It is important to understand the microbial abundance and diversity in the subsurface environment to evaluate the influence on hydrogeological and geochemical processes. The classical microbiological approach, such as direct count method, was conducted. However, this may not be satisfactory for extreme environmental samples such as this subsurface one. Therefore, lipid biomarker analysis was also used to determine microbal biomass and community structure.

Groundwater samples were collected from a borehole MIU-4 in the Tono area at 5 depths of 82.5, 95.0, 315.0, 584.0, and 754.5 m (drilling depth). Chances for microbial contamination during sampling were carefully minimized. Total and viable counts were determined by epifluorescence microscopy. Viable counts were based on 1) intact membrane cells, 2) ETS-active cells, and 3) esterase-active cells. Groundwater samples and sedimentary-, granite-rock samples were used for the lipid analysis. To minimize any contamination, the centers of the rock samples were taken carefully by subcoring system under anaerobic and sterilized condition.

Total counts stained by SYBR Green I were from 1.0 x 104 to 1.1 x 105 cells ml-1 in the groundwater samples. Viability, based on cell membrane integrity, was found to range from 16.6 to 100%, viability of CTC-active cells were lower number of the detection limit to 29.4%, viability of FDA-active cells were lower number of the detection limit to 51.5%. CTC-active cells were not detected from the groundwater at 584.0 m and 754.5 m deep. Abundance of live microorganisms in the groundwater samples, based on phospholipid analysis, was estimated to be from 0.7 x 104 to 2.8 x 104 cells ml-1 (conversion expression: 1 mol LPO4 = 7.47 x 1015 cells of Escherichia coli), showing the lowest viability at the depths of 584.0 m and 754.5 m as shown by CTC and FDA counts. Species composition was inferred from fatty acid composition. Upper three sampling depths and lower two sampling depths showed different clusters of microorganisms, based on fatty acid composition. The microbial biomarker at upper sampling depths showed dominance by different types of sulfate-reducing bacteria (SRBs). The biomarker on lower sampling depths was found to be a unique biomarker, characterized by membraneflluidity. This implies microbial habitat segregation due to various adaptations to physico-chemical parameters in the subsurface habitats.

Abundant microorganisms were measured in the sedimentary rock samples (1.1 x 105 - 1.6 x 108 cells g-1), and different types of SRBs biomarkers were detected by phospholipid analysis. However, the microbial abundance was very low in the granite rock samples. This implies that microbial populations in the crystalline rocks may be small.