

Multi-depth and Two Different Pore-size Exploration of Subsurface Biosphere Inhabiting the Methane-rich Deep Aquifers at Suwa Basin, Japan.

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Subsurface biosphere consists of diverse uncultured prokaryotes. Especially, ultra-small prokaryotes (< 0.22- μ m) are important as they include putative deep branches of the tree of life, such as DPANN (an acronym of the first reported groups: *Diapherotrites*, *Parvarchaeota*, *Aenigmarchaeota*, *Nanoarchaeota* and *Nanohaloarchaeota*) and Patescibacteria (formerly CPR) [1]. Many studies have attempted to constrain metabolic activities of subsurface lineages including ultra-small fraction by metagenomics. However, estimated metabolic capacity is required to be demonstrated to better understand their contribution to biogeochemical cycles and adaptation to habitats. In this context, we conducted multi-depth sampling (10 – 1000 meters below ground level, mbgl) of deep aquifers at the tectonic boundaries by two different pore-size filtration (0.1- μ m and 0.3- μ m) and performed geochemical analyses to understand *in-situ* physiology.

Here, we investigated terrestrial deep aquifers at Suwa Basin, Japan (36.0°N, 138.1°E), which is located on Itoigawa-Shizuoka Tectonic Line. The high concentrations of ¹⁴C-dead methane and organic carbon [2] plausibly support metabolic activity of subsurface biosphere. The groundwater samples were obtained at two depths of three wells (10 – 100 mbgl) and seven hot springs (max. 1000 mbgl). The samples were filtrated by 0.3- μ m and 0.1- μ m filters, sequentially. The microbial compositions were analyzed by 16S rRNA gene sequencing. We also conducted water chemistry analyses and isotope measurements ($\delta^{13}\text{C}$, δD , $\delta^{15}\text{N}$, $\Delta^{14}\text{C}$).

The microbial composition on 0.3- μ m filters of well samples indicated the proliferation of aerobic methanotrophic and methylotrophic community which is plausibly supported by biogenic methane and organic compounds dissolved in the aquifers. While metabolic capacity is similar among 10 – 100 mbgl samples, the dominant lineages were different depending on location. Although chemolithoautotrophs were commonly abundant on 0.3- μ m filters of hot spring samples, compositions are drastically different at each site. The community structures

on 0.1- μ m filters of all samples are quite different from corresponding 0.3- μ m filters and enriched in DPANN and Patescibacteria. In this presentation, the diversity of microbial communities will be discussed with heterogeneity in geochemical conditions of deep aquifers on the pull-apart basin.

[1] Nakai et al.(2020), *Microbes Environ.* 35, ME20025.

[2] Urai et al.(2022), *ACS Earth Space Chem.* 6, 1689-1697.