

Microbial ecology in deep subsurface granites: Insights from groundwater meta-proteomics

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Deep underground environments represent one of the extreme environments with little molecular oxygen, high pressure, and very few nutrient resources, but is presumed to harbor a large biomass. In particular, granite, which is the most abundant rock type in the deep underground environment, may have been a stable habitat for microbial life throughout the history of the Earth. Thus, ecology of microorganisms in the deep granite environment would be important for understanding the history of life as well as the interactions between microbial ecosystems and their biogeochemical environments.

To investigate the microbial ecology in a deep underground granite environment, the present study has been conducted at the Mizunami Underground Research Laboratory (URL) in central Japan. Metagenomic analysis is underway to obtain comprehensive information of the ecology of the microorganisms in the granite of Mizunami URL, but its interpretation is still limited to discussions based on the existence of genes. Therefore, it has been unclear whether the proteins they encode are actually expressed in the environment. In the present study, we performed metaproteomic analysis for microorganisms collected by filtering the groundwater at 300 m deep from the surface, and identified proteins that are undoubtedly expressed by the microorganisms based on the analysis performed in duplicate. For each protein, the function and its expression level were determined.

For the three species, one archaeon (ANME-2d) and two bacteria (Nitrospirae, Parcubacteria) that indicated the highest levels of protein expression among all those species, their ecology has been characterized in detail by Gene Ontology and KEGG pathway analyses. As a result, it was demonstrated that ANME-2d actively mediates anaerobic methane oxidation in the granite environment. This observation confirmed the results of the previous study based on genomic analysis [1]. It was also strongly suggested that the Nitrospirae actively mediate catabolic sulfate reduction. The ecology of Parcubacteria remained unclear, but we observed that at least some enzymes in the glycolysis, gluconeogenesis, and the pentose phosphate pathways are expressed.

[1] Ino et al. (2018), *The ISME Journal* 12, 31-47.