

## Microbial protein synthetic activities in deep underground granites revealed by meta-proteomic analysis

\*Nao Masuda<sup>1</sup>, Akito Ishikawa<sup>2</sup>, Yohey Suzuki<sup>2</sup>, Kazuyoshi Endo<sup>2</sup>

1. Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 2. Department of Earth and Planetary Science, Graduate School of Science, The University of Tokyo

Deep underground environments represent one of the extreme environments with little molecular oxygen, high pressure, and few nutrient resources. Recent geochemical, microbiological, and molecular analyses made it clear that microorganisms do exist in such environments. In particular, granite, which is the most abundant rock type in the deep underground environment, with a maximum age of 4 billion years ago, may have been a stable habitat for microbial life throughout the history of the Earth. Thus, understanding the ecology of microorganisms in the deep granite environment would be important for understanding the history of life evolution. In addition to geochemical analysis, 16S rRNA and metagenomic analyses have been carried out to characterize microbial communities inhabiting deep granite environments.

Metagenomic analysis is a method that can directly analyze the genomes of microorganisms in the environment without relying on cultivation, however, interpretation of the data is limited to discussion based on the existence of genes. It has been unclear whether the proteins they encode are actually expressed in the environment.

In the present study, aiming at clarifying the ecology of microorganisms in a deep underground granite environment, we performed metaproteomic analysis on the microorganisms collected by filtering the groundwater 300 m underground at the Mizunami Underground Research Laboratory located in Gifu prefecture, central Japan. Metaproteomic analysis is a method for directly identifying the proteins that exist in the samples, an approach which allows us to know the genes that are actually expressed in the environment. As a result, a total of 961 proteins (from 151 prokaryote species), and 995 proteins (from 153 prokaryote species) were identified from the 1st and the 2nd samples, respectively (179 species in total). For each protein, the function and its expression level were determined. By summarizing the expression levels of proteins expressed in each species, we were able to discriminate the species that are actively synthesizing proteins in this environment from those that are not. These results were not clear from the 16S rRNA analysis or other genomic analysis alone, indicating the importance of performing proteomic analysis.

Focusing on the function of proteins, those that are essential for survival are well expressed in most species, while only a limited number of proteins involved in metabolic pathways are expressed. This may suggest that microorganisms in the underground granite environment generally live with a reduced level of activity. The expression levels of the proteins are the highest in the species of Nitrospirae among all taxa, and in particular, genes involved in sulfur metabolism and ATP synthesis are well expressed. This suggests that Nitrospirae are very active in sulfur metabolism and sulfur respiration. In addition, proteins involved in methane metabolism are also well expressed in Euryarchaeota (including ANME-2d). This result is consistent with the previous genomic study (Ino et al., 2018) suggesting that ANME-2d mediates anaerobic oxidation of methane in a granite environment.

Keywords: Microorganisms, Metaproteomic analysis, Deep underground environment, Groundwater