Diversity and metabolic and genomic characteristics of prokaryotes in deep granitic rock independent of photosynthesis

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Granite is a main constituent of the upper terrestrial crust, which potentially harbor one of the largest microbial habitats throughout the Earth' s history. Microbial investigations of deep granitic aquifers require drilling to avoid contamination from surface microorganisms. Although drilling from underground facilities is commonly practiced to avoid the contamination, the construction of underground facilities tends to cause mixing of shallow and deep groundwater. In the granitic basement where the Mizunami underground research laboratory (URL) was constructed, a highly fractured domain (HFD) and a sparsely fractured domain (SFD) were horizontally drilled from underground tunnels at 300 meter below ground level (mbgl). Based on previous biogeochemical site-characterizations, it was revealed that groundwater mixing is not evident in the SFD borehole (SFDB), and that sulfate reduction was biologically mediated in the HFD borehole (HFDB) before the construction. The purpose of this study is to reveal the diversity and metabolic and genomic features of microbial communities inhabiting the deep granitic environment at the Mizunami URL.
First of all, microbial communities inhabiting the SFD borehole (SFDB) were characterized. As the underground drilling introduced O_2 in the aquifer, the influence of drilling was clarified by monitoring temporal shifts over 4 years. Immediately after drilling, aerobic β-proteobacterial species were dominant, while the phylum Nitrospirae became dominant after 3 years, the close relatives of which were detected exclusively from deep subsurface environments. One-week incubation of the Nitrospirae-dominated community with ^{13}C-labeled bicarbonate and 1% H_2 and subsequent single-cell imaging with nanometer-scale secondary ion mass spectrometry (NanoSIMS) demonstrated that the assimilation of ^{13}C-labeled bicarbonate. From these results, it is implied that the granitic aquifer hosts microbial communities isolated from the photosynthetic ecosystem.
Secondly, microbial communities found in the HFDB were microbiologically and hydrogeochemically investigated. The HFDB groundwater was dominantly colonized by archaea suspected to mediate anaerobic methane oxidation (AOM), because of their phylogenetic relationship with anaerobic methanotrophic archaea subtype-2d (ANME-2d). To demonstrate whether AOM is mediated by the subsurface archaea, statistical analyses of microbial distributions and environmental factors, metabolic activity measurements of AOM and metagenomics-enabled genomic reconstruction were performed. The correlation between archaeal abundance and sulfate concentration was statistically validated. Two-week incubation of microbial cells with ^{13}C-labeled methane demonstrated anaerobic oxidation of methane (AOM) linked to sulfate reduction. A draft genome of the subsurface archaea contained functional genes required for AOM. In addition, the subsurface archaea were dominantly found in SFDB groundwater outflowing during drilling, which excludes the possibility that facility construction and underground drilling artificially stimulated the growth of the subsurface archaea. It is therefore concluded that a microbial ecosystem energetically dependent on methane does exist in the deep granitic environment.
The deep granitic biosphere revealed in this study is strongly suggested to be nearly independent of photosynthesis-derived organic matter. However, this inference is incositent with the dominance of Parcubacteria, many members of which are reported to thrive near-surface environemnts supplied with photosynthetic organic matter. Phylogenetic analysis demonstrated that Parcubacteria lineages detected from the Mizunami groundwater were novel and distantly related to those from the near-surface biosphere. As Parcubacteria is positioned at the root of a universtal tree of life recently resolved by

genomic advancement, it is likely that the early life might be hosted in the granitic biosphere before the emergence of phototrophic prokaryotes. It is futher suggested that the deep granitic environment has been a stable microbial habitat, even when meteorite bombardment was frequent on Earth before 3.5 biollion years ago.

Keywords: Deep biosphere, Anaerobic oxidation of methane, Candidate phyla radiation

