

Co-occurrence and Metabolic Consequences of Candidate Bacterial Phyla and Anaerobic Methane Oxidizing Archaea in the Deep Crustal Biosphere

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The terrestrial crust is known to harbor deep microbial life energetically dependent on organic matter and/or H₂. Recent studies have provided fragmented pieces of evidence suggesting that anaerobic oxidation of methane (AOM) is microbiologically mediated in the terrestrial subsurface as well as the deep oceanic crust. As the abundance of methane is extremely common in the deep aquifers, the existence of subsurface microbial ecosystems capable of harvesting the energy from AOM can dramatically change our view of the Earth's biosphere. Here we show the integrated evidence of the anaerobic methanotrophy based on geochemical, stable isotopic, molecular phylogenetic and metagenomic data from the deep granitic aquifer. High-quality groundwater was collected from two adjacent boreholes drilled into highly and sparsely fractured domains at a 300-m deep stage of the Mizunami underground research laboratory (URL), central Japan. The highly fractured domain was associated with groundwater dominantly colonized by AAA (AOM associated Archaea) and the candidate phyla OD1 and OP3, neither of which were detected from the sparsely fracture domain with groundwater enriched with H₂ (~10-100 nM) and depleted in sulfate (<5 μM). Consistent with 16S rRNA gene sequences, methyl-coenzyme M reductase gene sequence analysis revealed the habitat segregation of AAA and methanogens corresponding to the fracture domains. As the strong correlation of AAA and sulfate was statistically indicated by canonical correspondence analysis (CCA), anaerobic methane oxidation coupled to sulfate reduction was experimentally demonstrated by the amendment of ¹³CH₄ of, and the subsequent detection of ¹³C-enriched dissolved inorganic carbon from, microbial cells incubated in groundwater with and without the molybdate inhibition of dissimilatory sulfate reduction. Heatmap of 16S rRNA gene abundance in the boreholes within the two domains over two years showed the highly correlated distributions of AAA and the candidate phylum OP3, the nearly completed genome of which has functional genes involved in sulfur metabolisms such as a potential reductase gene of nitrite and sulfite. Although the syntrophic partnership among AAA and the candidate phyla needs to be further investigated, our results clearly demonstrate that the deep methanotrophy coupled to sulfate reduction is microbiologically mediated, which leads to the reconsideration of the biomass production and the cycling of hydrogen, carbon and sulfur in one of the largest microbial habitats on Earth.

Keywords: Deep biosphere, Mizunami underground research laboratory, Anaerobic oxidation of methane