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

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The deep terrestrial subsurface is known to harbor microbial life that is energetically dependent on organic matter and/or H_2. Recent studies have provided indications that anaerobic oxidation of methane (AOM) is microbiologically mediated in these environments as well as in the deep oceanic crust. As methane and sulfate are abundant in deep terrestrial aquifers, a substantial portion of subsurface microbial biomass may harvest energy from AOM. In this study, groundwater was collected from two adjacent boreholes drilled into highly and sparsely fractured domains at a 300-m depth stage of the Mizunami underground research laboratory in central Japan. The highly fractured domain was associated with groundwater dominantly colonized by Archaea implicated in AOM and bacteria of the candidate phyla OD1 and OP3, none of which were detected in the sparsely fractured domain where groundwater is enriched in H_2 (~10-100 nM) and depleted in sulfate (<5 μM). We detected ^{13}C-enriched dissolved inorganic carbon from microbial cells incubated with $^{13}CH_4$ in groundwater with and without the molybdate inhibition of dissimilatory sulfate reduction, indicating trace oxidation of methane (TOM) by methanogens and AOM, respectively. Although a syntrophic partnership among methanotrophs and the bacteria needs further investigation, our results demonstrate that deep methanotrophy is coupled to sulfate reduction in one of the largest microbial habitats on Earth.