

In-situ and laboratory high-pressure cultivations of deep microbiome at two Japanese underground facilities

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Underground Facility: New Frontier for Deep Microbiome

It remains enigmatic whether the doubling time of subsurface microbiome tends to range from thousands to millions of years based on biogeochemical profiles [1]. In contrast, laboratory-based activity measurements are considered to overestimate metabolic rates, given technical difficulties in reproducing subsurface conditions such as high pressure and extremely low energy fluxes.

In this study, in-situ cultivation was performed for granite-hosted microbiome in a 300-m deep borehole horizontally drilled at Mizunami Underground Laboratory. Previously, sulfate reduction rates of 1–5 nM yr⁻¹ have been biogeochemically estimated, which agree with well-characterized deep biospheres [2]. Unexpectedly, the amendment of 0.1 M sulfate without the addition of any energy sources resulted in doubling of originally dominant microbial populations of *Nitrospira* and *Ignavibacteria* within 7 days. It is also unexpected that the production of hydrogen sulfide was not observed during the microbial growth.

The Horonobe Underground Laboratory was constructed in tertiary sedimentary rocks, from which methane is economically produced for local communities. Laboratory high-pressure incubations were conducted for 215-m deep groundwater where anaerobic methane-oxidizing archaea subtype 2d (ANME-2d) with multi-heme cytochromes containing 50 or more heme-binding motifs were dominant [3]. ¹³C-labelled methane was oxidized without adding electron acceptors such as amorphous Fe(III), nitrate and sulfate. Although suspended particles containing Fe(III) are suspected to serve as an electron acceptor, the production of aqueous Fe(II) was not evident. Low-pressure incubations led to negligible oxidation of ¹³C-labelled methane coupled to substantial production of aqueous Fe(II). These results highlight pressure-dependent metabolic activities of subsurface microbiome.

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[1] Hehler & Jørgensen (2013) *Nature Rev. Microbiol* 11, 83-94. [2] Suzuki et al. (2014) *PLOS One*, id. 0113063. [3] Hemsdorf et al. (2017) *ISME J*, 11,1915-1929.