

Indigenous Microbial Populations Dominantly Thriving in Deep Terrestrial Crust

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The terrestrial crust composed of granite is one of the largest, but least-understood, habitats of microbial life on Earth. This is mainly because it is technically challenging to collect microbiological samples without drilling contamination. In comparison to drilling from the land surface, short underground drilling significantly lowers the extent of contamination. However, the construction of underground facilities has tremendous impacts resulted from the intrusion of shallow groundwater into the granitic basement. The Mizunami underground research laboratory (URL) was constructed in the granitic basement, and the hydrologic disturbance caused by the underground construction was not evident in a sparsely fractured aquifer with low hydraulic conductivities. We drilled an underground borehole called 09MI21 and monitored the dynamics of microbial community structures over 4 years. The 09MI21 borehole was sectioned by a multi-packer system, and the interval #2 and #4 (09MI21-2 and -4) were annually monitored over four years immediately after drilling in 2009. The number of microbial cells in groundwater was determined by microscopic observations. For microbial community structure analysis, microbial cells were collected from groundwater by filtration and subjected to DNA extraction. 16S rRNA gene sequences were obtained by Sanger sequencing for Mizunami samples. Phylotypes were determined by grouping of sequences with >97% similarity and phylogenetically characterized by constructing a neighbor joining tree and calculating bootstrap values of branching points using the maximum likelihood algorithm. The total cell numbers were monitored from 2009 to 2012 and ranged from 10^4 to 10^5 cells/ml and decreased with time. 16S rRNA gene sequence analysis of 09MI21-2 and -4 resulted in 128 phylotypes after phylotype assignment with 97% similarity. Immediately after drilling in 2009, both 09MI21-2 and 4 were dominantly colonized by betaproteobacterial phylotypes. The dominant betaproteobacterial phylotypes were closely related to *Hydrogenophaga* spp. known to oxidize H₂ under aerobic conditions. After 2011, a phylotype classified within Nitrospirae phylum was stably dominant over two years. The phylogenetic relationships of Nitrospirae phylotypes were further analyzed by comparing their unambiguously aligned sequences against sequences from environmental clones and cultured species. One of four Nitrospirae clades was dominant in 09MI21-2 and 09MI21-4, and the dominant phylotype has been also detected in deep granitic groundwater from underground boreholes at Grimsel Test Site (GTS) in Switzerland. The most dominant phylotype formed a firm cluster with environmental sequences from hot crustal fluids from South Africa Gold Mine and Yellow Stone National Park rather than from soils and surface sediments. It is most likely that bacteria represented by the Nitrospirae phylotype are widespread and abundant in the terrestrial crust at depth, one of the vastest habitats of life from the early Earth history.

Keywords: subsurface microbiology, granitic basement, 16S rRNA gene sequencing, Mizunami underground research laboratory, Grimsel Test Site, deep biosphere