Potential microbial contribution to nitrogen cycling in the Tono subsurface

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Deep biosphere is characterized by unavailability of molecular oxygen (O2). While terrestrial and marine organisms respire aerobically, anaerobic respiration dominates in the subsurface using sulfate, nitrate, etc as electron acceptors (oxidizers). Nitrate-reducing bacteria (denitrifying bacteria), which are typical anaerobic respirers, were isolated and cultivated from the groundwater at Tono Geoscience Center, Gifu, Japan. In addition, nitrogen-fixing (N2-fixing) bacteria and nitrifying bacteria (ammonia-oxidizing bacteria) were detected. It is likely that these bacteria contribute to nitrogen cycling in the Tono subsurface via the following processes: N2 -(N-fixation)- Organic N -(degradation)- NH4 -(nitrification)- NO3 -(denitrification)- N2.

Groundwater samples were collected from a borehole TH-6 in the Tono area at the depths of 104, 132, 153 (sedimentary rocks) and 177 m (granite) below ground. Chances for microbial contamination were carefully minimized. Total counts were determined by epifluorescence microscopy. Bacterial 16S rRNA gene (16S rDNA) was sequenced and analyzed to construct a phylogenetic tree. Dot blot hybridization (DBH) technique was applied to detect the genes (narH and nir S) of the enzymes involved in denitrifiation. Isolation of denitrifying bacteria was conducted as well.

Total counts in the Tono groundwater were within the range of 4.4-8.2 million cells per ml, which is often seen for surface seawaters. Little tendency of cell count decrease with depth was shown.

The phylogenetic analysis of 16S rDNA suggested the occurrence of diverse denitrifiers and nitrifiers in the TH-6 borehole. N2-fixers such as Rhizobium spp. were also found from a nearby borehole (KNA-6) at almost the same depth. Although 16S rDNA of the denitrifiers were not recovered from the TH-6 samples, cultivation of various denitrifiers from the TH-6 samples was successful. Total 21 isolates were nitrate-reducers and 13 isolates were N2 gas-producers. The N2 gas-producers were shown to belong to the genera of Bacillus, Staphylococcus, Comamonas and Pseudomonas based on 16S rDNA. Pseudomonad strains were isolated from all the sampled depths. DBH of the denitrification-relevant genes, narH and nirS, showed the highest signal at the depth of 132 m below ground, which may indicate high denitrification activity at this depth.