

## Molecular characterization of groundwater microbial communities in a deep Neogene sedimentary rock

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Diversity and distribution of archaeal and bacterial 16S rRNA gene sequences in deep aquifers of mid to late Miocene hard shale located in the northernmost part of the Japanese archipelago were investigated. A major fault in the NW-SE direction runs across the studied area. Three and one groundwater samples were collected from the boreholes on SW and NE sides of the fault, respectively, at different depths of 296, 374 and 625 m below ground level (mbgl) on the SW side and 458 mbgl on the NE side. Neutral and weakly saline properties of the groundwaters were observed. The acridine orange-stained total counts were in the orders of  $10E+05$ - $10E+06$  cells ml<sup>-1</sup> and  $10E+03$  cells ml<sup>-1</sup> in the HDB-6/-11 and HDB-10. Libraries of PCR-amplified 16S rRNA gene clones, total 204 and 203 for archaea and bacteria, respectively, were constructed. Phylogenetic analyses showed that the libraries from the HDB-6/-11-aquifers were generally coherent but quite different from the libraries from the HDB-10-aquifer. All the archaeal clone libraries from the HDB-6/-11-aquifers were predominated by a single sequence closely related to the archaeon *Methanoculleus chikugoensis*, and the corresponding bacterial libraries were mostly dominated by the sequences related to Bacteroidetes, Firmicutes and Delta-Proteobacteria. In contrast, the libraries from the HDB-10-aquifer were dominated by uncultured environmental archaeal clones with no methanogen sequences and by Beta-Proteobacterial clones with no sequences related to Bacteroidetes and Delta-Proteobacteria.