

## Monitoring of the abundance and diversity of Microbial community in deep subsurface groundwater

Fumiko Watanabe[1], Tatsuo Miyoshi[2], # Takeshi Naganuma[1]

[1] School of Biosphere Sci., Hiroshima Univ., [2] Biosphere Science, Hiroshima Univ

<http://home.hiroshima-u.ac.jp/hubol/>

The Environment of deep subsurface is thought to be rather stable. Environmental parameters such as water temperature, pH, ORP show only little variation. Previous studies observed the microbial abundance from  $10^3$  to  $10^6$  cells  $\text{ml}^{-1}$  in the groundwater at Tono area. However, only limited data sets have been collected for a long period. Variation in the microbial diversity has not characterized, either.

Groundwater samples were weekly collected from the borehole KNA-6 in the Tono mine at the depth of 158-160 m (sedimentary layer) and 163-200 m (granite) below ground water level over the period from July 2001 to December 2002. Total counts were determined by epifluorescence microscopy. ATP contents were measured by the luciferine-luciferase reactions. Bacterial 16S rDNA sequences were amplified and analyzed to construct a phylogenetic tree.

Total counts in the Tono groundwater varied within the range of  $1.41 \times 10^4$  to  $2.54 \times 10^5$  cells  $\text{ml}^{-1}$  in the sediment layer and from  $7.32 \times 10^3$  to  $2.98 \times 10^5$  cells  $\text{ml}^{-1}$  in the granite rock. ATP contents also varied within the range from 1.63 to 132.67 pM in the sediment layer and from 0 to 116.33 pM in the granite rock. These variations may provide

the evidence for temporal fluctuation in the microbial abundance in the relatively stable subsurface. There were no correlations between the microbial abundance and environmental parameter.

The DGGE band patterns suggest that the microflora in the granite rock have more substantial variation than in the sedimentary layer.

Certain DGGE bands (16S rDNA) were related to sulfur-oxidizing bacteria and ammonia oxidizing bacteria from the KNA-6 groundwater. These microorganisms may serve as the primary producers in the Tono subsurface biosphere.