

## Microbial World in the Subsurface of Tokyo

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Studies in the past 20 years have revealed that a huge biosphere exist in terrestrial (as well as marine) subsurface. Subsurface microbes have attracted attention of geomicrobiologists in that many of them are not culturable and may have specific strategy for the life in subsurface. Our research group, Geomicrobiology group, aims to elucidate chemical and microbial processes in a geological system especially in the area around Tokyo. That is, from the lower part, a large field of natural gas consisting of biogenic methane in the deep zone (~2,500 m), aquifer with abundant groundwater flow (~500 m), thick layers of ancient marine clay spread around central Tokyo in the shallow subsurface (~50 m). Interesting results have been obtained in each zone.

The mechanism of gas field formation (when, where and who?) is not clear yet. In the formation water of the gas field (~1100 m), various methanogens including hydrogenotrophic methanogens such as *Methanobacterium*, and aceticlastic methanogens such as *Methanosaeta*, were detected (1). This showed the possibility that methanogens are producing methane in the deep gas field at present.

Groundwater from the deep aquifers at depths <500 m can be obtained from the artesian wells in this area. Therefore, this area is very suitable to examine microbial ecology in deep subsurface. This highly reduced groundwater samples (ORP: -200 to -500 mV) which were proved to be 300-24,000 years old, contained  $10^3$ - $10^4$  cells/ml of total bacterial cells (2). Clone analyses using 16S rRNA gene revealed that clones which did not belong to any known phylum were dominant in some wells. These clones were very close to the sequence obtained from another deep mine groundwater in Japan, suggesting that this group is typical in deep groundwater although their physiology is yet to be clarified.

Clay sediments have very limited space (pore size of < 1 $\mu$ m) and water flow. Holocene clay sediments at the shallow part (~50 m) were also investigated to know if there exist living microbes in this kind of environment and if they exist, what they are doing. DNA-based clone analysis revealed that many clones were close to the sequences obtained from marine environments including those belonging to the candidate groups, JS1, ANME-I, and Marine Benthic Group-C, which are typically found in marine sediments (3). These were unexpected because, although the sediments are ancient marine origin, present concentrations of chloride in interstitial water indicated that seawater has been almost replaced by surface water. DNA information might include information from remnant dead cells or DNA trapped in the sediments from the time of sedimentation. Further studies are ongoing to reveal the active microbial components by various methods including RNA-based

analysis and activity measurements using radio-isotope ( $^{14}\text{C}$ ) and stable-isotope ( $^{13}\text{C}$ ,  $^{15}\text{N}$ ) tracers. Approximately  $10^4$  cells/g of SFDA-positive cells with esterase activity were found. Preliminary results indicated occurrence of denitrification, methanogenesis and anoxic methane oxidation in the clay sediments. Slow rates of methanogenesis with generation time of 1 month were observed. Methanogens belonging to *Methanobacterium* were proved to be involved in slow methanogenesis.

### References

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- [2] Machida et al. IAH Book of Selected Papers. In press.
- [3] Takeuchi et al. Geomicrobiology Journal. 2009. 26: 104-118.