

The microbial community structure in Sagara oil reservoir

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The recent microbiological studies reported that active microbial habitat for methanogen, sulfate reducers (*Archaeoglobus*, *d-Proteobacteria*, gram positives), fermenters (*Thermococcus*, *Thermotogales*, gram positives etc.) and other heterotrophs (*g-Proteobacteria* etc.) are in subsurface petroleum oil reservoirs. However, microbial distribution at vertical distances in depth has not been demonstrated since the samples in previous studies are only to use oil and the formation water. Here, we show the vertical profile of microbial community structure in Japanese terrestrial oil reservoir by a combination of molecular ecological analyses and culture dependent studies.

The sequential WRC (Whole Round Core) samples (200 mbsf) were recovered from a drilling project for Sagara oil reservoir, Shizuoka Prefecture, Japan, conducted in Jan. -Mar. 2002. The lithology of the core samples was composed of siltstone, sandstone, or partially oil containing sand. The major oil components were gasoline, kerosene and light oil, that is a unique feature observed in the Sagara oil reservoir.

The direct count of DAPI-stained cells suggested that the biomass was relatively constant, 1.0×10^8 cells/g through the core of the non-oil layers, whereas the oil-bearing layers had quite higher population density at a range of 1.0×10^8 - 3.7×10^8 cells/g. The vertical profile of microbial community structures was analyzed by the sequence similarity analysis, phylogenetic analysis and T-RFLP fingerprinting of PCR-amplified 16S rDNA. From bacterial rDNA clone libraries, most of the examined rDNA were similar with the sequence of genera *Pseudomonas*, *Stenotrophomonas* and *Sphingomonas* within *g-Proteobacteria*. Especially, *Pseudomonas stutzeri* was predominantly present in all oil-bearing layers. From archaeal rDNA clone libraries, all rDNA clone sequences were phylogenetically associated with uncultured soil group in Crenarchaeota. In addition, culture dependent analysis also show the dominant species of oil reservoir is *Pseudomonas stutzeri* that can degrade petroleum and we could not cultivate autotrophs and fermenters.

These results strongly suggest the absence of sulfate reducers, sulfur dependent fermenters and methanogens that have been previously detected as dominant microbial components in other oil reservoir environments. The absence of organisms that depend on reductive components indicate the low flux of reductive gasses and oil from mother rock of Sagara oil reservoir.