METHANOGENIC ACTIVITY AND MICROBIAL DIVERSITY IN A HIGH-TEMPERATURE BIODEGRADED OIL RESERVOIR

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Introduction

Recently, microbial ecological research in petroleum reservoirs is of great interest in regard to the microbial enhanced oil recovery (MEOR). The microbiological and biogeochemical studies have indicated that methanogens are distributed over petroleum reservoirs worldwide and produce methane in situ. However, the microbial methanogenic processes in such environments are still poorly understood. In the present study, we investigated the methanogenic community and activity in a high-temperature petroleum reservoir by a combination use of geochemical analyses, radioisotope tracer experiments, culture-dependent and -independent analyses.

Results

The formation water, crude oil, and gas samples were obtained from a commercial oil producing well (depth, 938 m; temperature, 54˚C) in Yamagata, Japan. Carbon isotopic composition of gas components and saturated hydrocarbon composition of crude oil were determined by GC-C-IRMS and GC-FID, respectively. To measure methanogenic potential, formation water samples were collected in N_2-filled sterilized glass bottles. To determine the metanogenic pathway in the reservoirs, radioisotope tracer technique was applied to microcosms comprising of the formation water and oil with a volume ratio of 100:1. Some portion of formation water was fixed with ethanol and used for total cell counts with DAPI staining. Phylogenetic analysis of archaeal and bacterial communities in the formation water was conducted based on 16S rRNA gene clone libraries constructed using archaeal- and bacterial-specific primers. Cell density of culturable methanogens was measured by serial dilution with methanogenic substrates, and the cultured ones were phylogenetically identified by direct sequencing of their 16S rRNA genes.

In the geochemical analysis, the predominance of isoprenoid over straight-chain alkanes were observed in this study, indicating that the oil has been partially biodegraded. Radiotracer experiments using [^{14}C]-labelled bicarbonate, acetate and methanol clearly revealed that microbial methanogenesis was active in situ and its pathway was mainly hydrogenotrophic followed by methylotrophic. We also observed the culturable methanogens with the order of 10^3 cells per ml in the formation water, which is approximately 1% of the total cell count (3.2×10^5 cells/ml). Based on 16S rRNA gene sequencing analysis, the methanogens cultured in this study were closely related to *Methanothermobacter thermautotrophicus*, a thermophilic hydrogenotrophic methanogen, with 99% of sequence similarity. In the original formation water, *Methanothermobacter thermautotrophicus* was also predominant and occupied 90% of the archaeal 16S rRNA gene clone library constructed. We successfully isolated a methylotrophic methanogen *Methermicoccus shengliensis* strain AmaM with methanol, which we later found to also use methoxlated aromatic compounds as substrates (Mayumi et al., 2016). Bacterial community diversity is also low. Most of the bacterial clones (62% of total clones) were moderately related to *Syntrophus gentianae*, an anaerobe syntrophically oxidizing benzoate (94% of sequence similarity). *Syntrophaceae* have been frequently retrieved from low temperature anaerobic hydrocarbon biodegrading microbial communities in previous studies (Jones et al., 2008,

Gray et al., 2011), suggesting that the organisms may contribute to anaerobic oxidation of organic compounds and supply hydrogen to the methanogen in the oil field.

Conclusions

A high temperature oil reservoir, with clear geochemical evidence for oil biodegradation, harbors active thermophilic hydrogenotrophic methanogens. Meanwhile, methylotrophic methanogens may also contribute significantly to the methanogenesis in this oil field.

References

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