海底表層堆積物におけるメタン生成古細菌の分離とメタン関連微生物の群集構造
Isolation of methanogenic archaea and distribution of methanogenic and methanotrophic archaea in subseafloor sediment

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Shallow gas hydrates are estimated to be buried around Japan, especially in Japan Sea. The methane trapped in those hydrates are produced by biogenic (microbial) or thermogenic system. But the relationship between shallow gas hydrates and the methanogens are yet to be confirmed. So this study focuses on isolation and diversity of methanogenic and methanotrophic archaea.
Sediment samples were collected from the subseafloor (with or without specific structure) by the MBARI push corer, during an environment assessment cruise. Samples were collected from the top, middle, bottom of the recovered sediments of each push core. The samples were stored in different temperature for the microbiological cultivation experiment and microbiological diversity analysis, respectively.
For the methanogenic archaea isolation, cultivation was carried out by enrichment culture using methanogen medium. The cultures were cultivated by 15℃ and 30℃, respectively. We successfully isolated several methanogenic archaea from the surface sediment. The result of the 16S rRNA gene sequence analysis showed the isolated strains identified as one of the order of the methanogen, Methanomicrobiales.
For the methanogenic and methanotrophic archaea diversity analysis, DNA was extracted from the sediment samples, using ISOIL kit. The methane related functional gene, the *mcr*A gene of methanogenic and methanotrophic archaea was choosen as the target gene. The genes were amplified by PCR method. The PCR products were purified by FastGene Gel/PCR Extraction Kit. The purified products were analyzed by clone library method. The result of the clone library analysis indicated that specific structure of the surface of the subseafloor have specific methanogenic and methanotrophic archaea structure.
This study was conducted as a part of the shallow methane hydrate exploration project of METI.

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