Japan Geoscience Union Meeting 2014
(28 April - 02 May 2014 at Pacifico YOKOHAMA, Kanagawa, Japan)

MIS22-10 Room:213 Time:April 29 16:45-17:00

Distribution of methanogenic and methanotrophic archaea in subseafloor sediment collected during UT12

IMAJO, Takumi[1*] ; KOBAYASHI, Takeshi[1] ; IMADA, Chiaki[1] ; TERAHARA, Takeshi[1] ; MATSUMOTO, Ryo[2]

[1]The graduate school of marine science and technology, TUMSAT, [2]Meiji University

Methane hydrate is now one of the most popular energy sources in the world, and various amounts are presumed to be buried around Japan's continental margins. Methane contained in methane hydrate in the deep sea sediment is produced by microbial or thermogenic system. In the microbial system, methanogenic and methanotrophic archaea play an important role in this environment. However, the studies on characteristics and abilities of these microorganisms are still underway in the Sea of Okhotsk. Therefore, this study focuses on isolation of the methanogenic archaea and analysis of community construction and diversity of these microorganisms.

Sediment samples were collected from the subseafloor by the piston coring, during UT12 (Umitaka-maru Gas Hydrate Research Cruise 2012). Samples were collected from each core sample at appropriate intervals. The samples were stored at 4 ℃ for the microbiological cultivation experiment use, and at -80 ℃ for the microbiological diversity analysis use, respectively.

For the isolation, cultivation was carried out by enrichment culture using H_2/CO_2 medium. The cultivation temperatures were 15 ℃ and 30 ℃, respectively. We successfully isolated several methanogenic archaea from the samples of the surface of the subseafloor. The result of the 16S rRNA gene sequence analysis showed that some of the strains were identified as closely related strains of *Methanogenium marinum*. In a previous literature, *M. marinum* was isolated from the cold marine sediment from the Scan Bay, Alaska. We also conducted the experiment to measure the methane productivity of our isolates by the range of the cultivation temperature.

For the analysis of community structure and diversity of methanogens, DNA was extracted from each sediment sample, using the ISOIL kit following the manufacturer's protocol. The 16S rRNA gene of methanogenic archaea and the mcrA gene of methanogenic and methanotrophic archaea were amplified by PCR. The PCR product was purified by FastGene Gel/PCR Extraction Kit following the manufacturer's protocol. The purified products were analyzed by T-RFLP method and clone library method. The results of the T-RFLP analysis showed that the various fragments were observed. Clone library sequencing analysis of mcrA genes indicated that some of them were identified as related sequences to *Methanogenium*. Also, results from T-RFLP method were used for MDS (Multi-Dimensional Scaling) analysis.

This experiment was supported by grants-in-aid for scientific research <KAKENHI>(Ryo Matsumoto, Meiji University).

Keywords: shallow gas hydrate, methanogenic archaea, methanotrophic archaea