

## Search for microbial genetic markers associated with methane leakage from gas hydrates in the deep sea

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This paper describes the search for the genetic markers in surface sediment of sea floor to develop the in-situ detection instrumentation technology for methane leakage. Methanotrophs consume methane as their sole energy and carbon source and are distributed at methane producing sites. Aerobic methanotrophic bacteria use soluble methane monooxygenases (sMMO) or particulate methane monooxygenases (pMMO) to convert methane to methanol. The anaerobic oxidation of methane mediated by anaerobic methanotrophs (ANME) and sulphate-reducing bacteria plays a critical role in anoxic sediments of cold seeps. Methanotrophs are ubiquitous in soil, fresh water and the open ocean but have not been well characterized in deep-sea hydrocarbon seeps and gas hydrates, where methane is unusually abundant. In the last few decades, cultivation-independent molecular methods have been applied widely to investigate microbial diversity and quantify predominant organisms in natural microbial community. Methanotroph diversity has been studied in different environments using the polymerase chain reaction (PCR). Our goal in this study was to compare the composition of bacterial and archaeal communities associated with gas hydrates with those of bacterial and archaeal communities in normal marine sediments in the Nankai Trough of Japan in order to identify specific DNA markers (16S RNA and pmoA genes) for methane leakage in the deep sea. The development of in-situ detection of DNA markers associated with methane leakage is now ongoing with the application of a totally integrated and automated system for in-situ gene analysis of microbes (IISA-gene system).

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