

論文内容の要旨

論文題目

Geochemical constraints on methanotrophic community in deep-sea hydrothermal and cold-seep environments

(海底熱水、冷湧水系における嫌氣的メタン酸化古細菌の分布とその地球化学的規制要因)

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Subseafloor environment is the largest known sink of methane on Earth, where most of the methane from deep sources is biologically oxidized by anaerobic methanotrophic archaea (ANME). The process to anaerobic oxidation of methane (AOM) is not fully understood that sulfate acts as the final electron acceptor according to the net reaction: $\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$. It is estimated that AOM in anoxic sediments eliminates >80% of the methane flux from the seafloor. Therefore, AOM plays a significant role not only for the biogeochemical carbon cycle in marine sediments but also for and global climate change.

ANMEs are classified into 3 groups (ANME-1, 2, 3) depending on their phylogenetic affiliations. They have been found in global methane-seep environments

and often reported as major microbial components. As they have not been cultivated yet, we have little knowledge about their optimum conditions for the growth and energy gain in natural environments, e.g. temperature, pH, salinity, and concentration of methane and sulfate. Therefore, it remains unclear what environmental factors control their preferential habitats. In this study, distribution and diversity of ANMEs in hydrothermal sediment and methane-seep field were investigated by using molecular biological methods in order to clarify their ecological niche, environmental limitation of their habitable zone, and regulation factors on AOM activities.

In CO₂-vented environment at Yonaguni Knoll IV hydrothermal field in the Okinawa Trough, the upper part of liquid CO₂/CO₂-hydrate bearing sediments represented acidic pH down to 4.5. The *in vitro* activity measurement of these sediments showed that pH optimum of methane-fueled sulfate reduction was lower than previously reported. Both microscopic observation and reverse transcription PCR analysis of 16S rRNA fragments confirmed the co-occurrence of ANME-2 and *Desulfosarcina/Desulfococcus* branch of sulfate reducer. In contrast to the active and diverse microbial population in surface sediments, microbial community in deeper sediments represented drastically low abundance and sulfate reduction rate. These results indicated that metabolically active and acidophilic microorganisms including ANME were mostly restricted in the upper part of the sediment possibly because of chemical property of liquid CO₂ or thermodynamic and kinetic constraints other than pH.

The diversity and distribution of ANMEs were investigated in methane hydrate

field off Joetsu in the Japan Sea. The sequence analysis of 16S rRNA genes showed the predominant population of ANME-1 and ANME-2 in the sediment cores, but their spatial distribution and abundance were found to be distinct. Quantitative PCR analysis of methyl-coenzyme M reductase gene (*mcrA*) showed that the members of ANME-1 predominated the sediments containing low sulfate concentrations, even below the sulfate-methane interface, indicating that they physiologically prefer the low level of sulfate, while ANME-2 inhabit shallow sediments containing relatively high concentrations of sulfate. Thus, the distributions of the ANMEs could be regulated by the adaptability of sulfate in the environments. The dominance of ANME-1 in deep part of the sediment was supported by the fact that net AOM reaction in low-sulfate sediment was thermodynamically and kinetically favorable. However, AOM is considered to proceed without direct coupling with sulfate reduction, because not cell aggregates but single cells were observed under the microscope. Considering thermodynamic energy obtained by sulfate-independent methane oxidation, acetate can be a likely candidate for mediator between AOM and sulfate reduction.

The adaptations of ANMEs to a wide range of variation in pH and sulfate concentration were found. Possible AOM reactions are also discussed taking into account the distribution of ANMEs, geochemical profile, and free energy yields in the environment. These results suggested the wide range of habitable environment of ANMEs and indicated a much more important role in biogeochemical cycles of carbon than previously assumed.