

Methanogenic archaeal diversity and characterization of a novel methanol utilizing methanogen in a natural gas field

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Previous geochemical studies indicated that methane deposited in dissolved-in-water type natural gas fields in Japan is mostly biogenic due to biological degradation of organic matter in sediment. The present study is aimed to better understand the diversity of methanogens and the formation process of methane in those biogenic gas fields. We investigated the phylogenetic diversity of methanogenic archaea and characterized a novel methanogen isolated thereof.

A sediment sample was obtained from one of the sand separators for commercial gas-water-producing wells. Phylogenetic analysis of the archaeal community in the sediment was conducted based on 16S rRNA gene clone libraries constructed using archaeal specific primers. The sample was also incubated with H₂-CO₂, acetate, or methanol to determine the methanogenic activity.

Based on the gene cloning analysis, the archaeal library was dominated by the clones closely related to *Methanobacterium aarhusense*, *Methanocalculus pumilus* and *Methanolobus zinderi*. Using a culture-based method, we observed that H₂-CO₂ or methanol was converted to methane. In particular, a *Methanolobus*-like methanogen was observed in the enrichment culture with methanol, indicating the presence of viable methylotrophic methanogens in the moderately high temperature gas field (45°C). To date, three new species have been validated within the genus *Methanolobus*, all of which have been taken from deep subsurface environments. We isolated and characterized the methylotrophic methanogen from the enrichment to find that the optimum growth temperature was 40-45°C and methanol was the only substrate for the isolate to produce methane. These results suggest that active methanol-degrading methanogens are present as well as hydrogenotrophic methanogens, and that methanol is one of the primary substrates for methanogenesis in this natural gas field.