Horizontal distribution of anaerobic methanotrophs related with methane flux in cold-seep sediments off Naoetsu in Japan Sea

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Most of the methane in the subseafloor sediment is biologically consumed in anoxic sediments (Iversen and Jorgensen, 1985). Methanotrophic archaea is one of the responsible microbes for the anaerobic oxidation of methane (AOM) and they are classified into 3 groups (ANME-1,2,3) depending on their phylogenetic affiliation. As they have not been cultivated yet, we have little knowledge about their optimum conditions for the growth and preferred habitats in natural environments, e.g. temperature, salinity, and methane concentration. Enormous supply of subsurface methane has been reported through geological, geochemical and geophysical surveys at a seepage area off Naoetsu, eastern margin of the Japan Sea. In this study, we investigated the diversity, distribution, and quantification of ANME by molecular biological methods, and then we compared the population of ANME with geochemical and geophysical factors to clarify their preferred habitats.

Sediment samples were collected with push-core and piston-core samplers in 2006. The push-cores were collected with the ROV around the active methane seep site and microbial mats areas, while the piston cores were collected at the areas with unique geological features. Prokariotic DNA was extracted using subsamples of those cores and determined phylogenetic affiliation of genes encoding the alpha subunit of methyl-coenzyme M reductase (mcrA), which is supposed to be a key enzyme of AOM. Then the mcrA gene copy numbers were quantified by quantitative PCR.

Two types of mcrA gene, e.g. ANME-1 and ANME-2, were recovered from the sediment samples and both of them were detected in the same sample. The ANME-1-mcrA genes derived showed broad distribution in this area, while those from ANME-2 were often detected in shallower sediments. This indicated different adaptability of habitable environment for each ANME group.

The copy numbers of the mcrA gene in the sediment with a measurable methane concentration were apparently higher than in the sediment, which contained methane below the detection limits. The copy numbers of mcrA were abundant in the sulfate-depleted-surface. The depth of mcrA detected correlated with sulfate-depleted depth, which strongly related with methane flux. In addition, the sample with abundant mcrA gene copies has also tendency to own high copy numbers of 16S rRNA gene. These result indicated that methane flux strongly regulate not only the habitat of ANME community but also whole microbial population.