

Distribution and diversity of the genes involved in methane oxidation and sulfur cycling in the piston-cored sediments

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Piston-cored sediments were collected from the gas hydrate-bearing zones that are predicted in the off-Tokai area, central Japan. Vertical succession of microflorae in the sediments was investigated at the genomic DNA level, associated with sub-seafloor sulfate-methane interface (SMI). Targeted genes were *pmoA*, *amoA* and *mmoX* that are involved in methane oxidation; *dsr* for sulfate reduction; and, *soxB* for sulfide/sulfur oxidation. Presence/absence of each gene was examined by PCR for the samples from several sections (depths) of the piston-cores. When a gene was detected in a sample, 12 fragments of the PCR-amplified gene were randomly selected and analyzed, with the possibilities of yielding only one sequence from the 12 fragments and all different sequences. Very contrastive results were obtained. No *mmoX* was detected, and *pmoA* was detected in only a few samples. In contrast, *amoA* was detected in a number of samples with high genetic diversity. The *amoA* encodes ammonia-oxidizing enzyme, which also oxidizes methane. On the other hand, the genes involved in sulfur cycling, *dsr* and *soxB*, were detected in a number of samples with high genetic diversity. Particularly, *dsr* showed a clear vertical distribution in the piston-cores. The *dsr* in the upper layer was unsurprisingly related to that of Deltaproteobacteria. The *dsr* in the upper-to-middle layers was closely related to that known from the Guaymas Basin hydrothermal vent fields, where thermogenic hydrocarbons are present. The *dsr* in the lower layer formed a unique and novel group, which is likely specific to sub-SMI depths and thus relevant to unknown processes of anaerobic methane oxidation. Contrary, *soxB* showed little vertical distribution, and thus implied only weak association with SMI and related processes.