

[S9-2]

Archaeal Nitrification in Marine Sediment of East Sea

Sung-Keun Rhee*, Soo-Je Park, and Byoung-Joon Park

Department of Microbiology, Chungbuk National University

Members of the archaea have been detected in most moderate and cold environments such as forest and agricultural soils [1]. Considering their abundance and broad distribution, non-extremophilic Crenarchaeota are likely to play important roles in global organic and inorganic matter cycles [3, 4]. Recently, an ammonia-oxidizing archaeon of MG I has been isolated and verified to contain the *amoA* gene and oxidize ammonia [5]. Only one strain of non-extremophilic archaea was isolated from an aquarium sediment. The diversity and abundance of archaeal 16S rRNA and putative ammonia monooxygenase α -subunit (*amoA*) genes were comparatively analyzed to study genetic potential for nitrification of ammonia-oxidizing archaea (AOA) in the surface layers (0~1 cm) of four marine sediments of the East Sea, Korea. After analysis of a 16S rRNA gene clone library, we found various archaeal groups that include the crenarchaeotal group (CG) I.1a (54.8%) and CG I.1b (5.8%), both of which are known to harbor ammonia oxidizers (Fig. 1). Notably, the 16S rRNA gene of CG I.1b has only previously been observed in terrestrial environments. The 16S rRNA gene sequence data revealed a distinct difference in archaeal community among sites of marine sediments. Diverse *amoA* sequences were retrieved from sediment environments [2]. Most of the obtained *amoA* sequences were not closely related to those of the clones retrieved from estuarine sediments and marine water columns (Fig. 2). Furthermore, clades of unique *amoA* sequences were likely to cluster according to sampling sites. Using real-time PCR, quantitative analysis of *amoA* copy numbers showed that the copy numbers of archaeal *amoA* ranged from 1.1×10^7 to 4.9×10^7 per gram of sediment and were more numerous than those of bacterial *amoA*, with ratios ranging from 11 to 28. In conclusion, diverse CG I.1a and CG I.1b AOA inhabit surface layers of marine sediments and AOA, and especially, CG I.1a are more numerous than other ammonia-oxidizing bacteria.

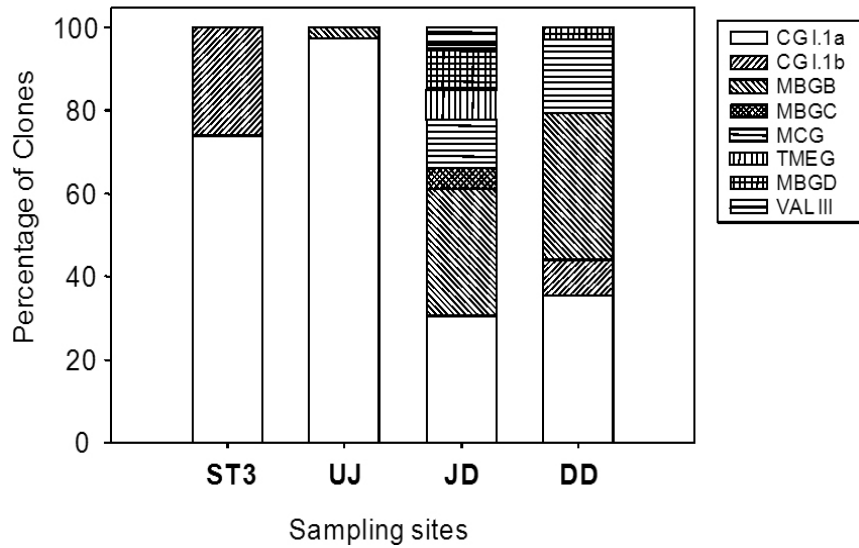


Fig. 1. Diversity and abundance of archaea from marine sediments. Relative abundance of archaeal groups of marine sediments is shown in the bar chart.

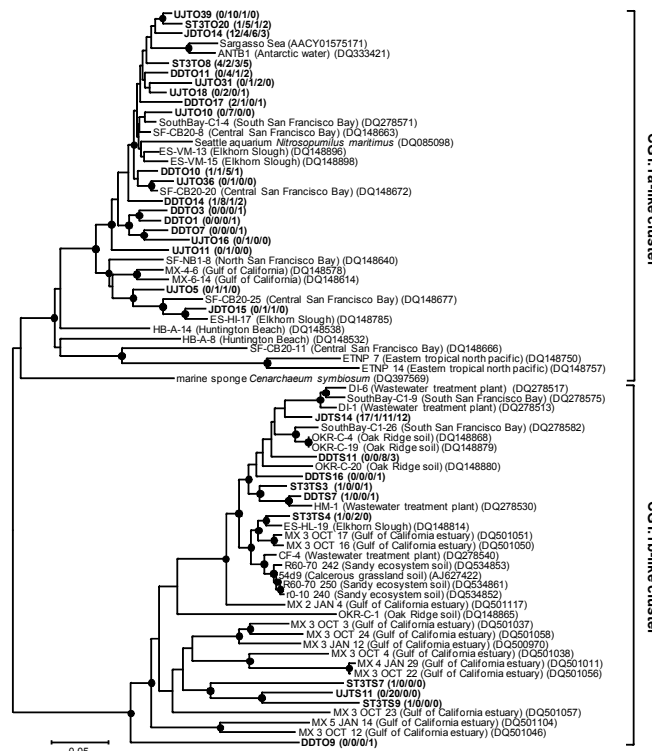


Fig. 2. Phylogenetic tree constructed from archaeal *amoA* gene sequences that were obtained from marine sediments. Phylotype names refer to the name of the sampling site (ST3, UJ, JD, and DD). The letters O and S that are written before the digit in the clone name indicate clones from a library produced using PCR primer set A or B, respectively. Numbers in parentheses following the clone names indicate the number of times that the sequences were found in the clone libraries from the four sampling sites ST3, UJ, JD, and DD, respectively. Clones from this study are indicated in boldface. The reference sequences were chosen to show the diversity of the sequences and to indicate the closest relatives to the sequences found in our study. The scale bar represents five substitutions per 100 nucleotide positions. Significant bootstrap values ($\geq 50\%$; 1000 replicates) are indicated by filled circles at branch points.

References

1. DeLong EF (1992) Archaea in coastal marine environments. *Proc Natl Acad Sci U S A* 89:5685-5689.
2. Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc Natl Acad Sci U S A* 102:14683-14688.
3. Hallam SJ, Mincer TJ, Schleper C, Preston CM, Roberts K, Richardson PM, DeLong EF (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. *PLoS Biol* 4:e95.
4. Karner MB, DeLong EF, Karl DM (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* 409:507-510.
5. Konneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437:543-546.