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Abundance, diversity, function and composition of microbial community on oceanic Mn crusts from Takuyo-Daigo Seamount

Shouta Nitahara[1*], Shingo Kato[1], Akihiko Yamagishi[1]

[1]Department of Molecular Biology, Tokyo U

Background and Purpose

Ferro-manganese oxide deposits are often observed on the seafloor. Rocks covered with these ferro-manganese oxide deposits are called Mn crust or Mn nodule. Oceanic Mn crusts grow slowly (1-10 mm/ Myr), and are observed mainly on old seamounts. These Mn crusts are expected to cover 70 % of seafloor at water depth of 5 km to 6km (Rona, 2003).

Microbes on Mn crusts may play a role in geochemical cycling between hydrosphere and lithosphere, and in accumulation of ferro-manganese oxides. However, little is known about microbes on oceanic Mn crust. In this study, our purpose is to clarify composition, diversity, abundance and function of microbes and spatial distribution in each depth on Mn crust in Takuyo-Daigo seamount by 16S rRNA gene and *amoA* gene analyses.

Method

We collected Mn crusts and nodules, sediments and ambient seawater from Takuyo-Daigo Seamount on NT09-02 cruise in Feb 2009. The water depth of each sampling point was 1200 m, 1419 m, 2209 m and 2991 m, respectively. DNA was extracted from each sample. We amplified 16S rRNA gene and *amoA* gene by PCR with prokaryote-universal and archaea-specific primer sets and archaeal and bacterial *amoA* specific primer sets. We constructed the clone libraries and determined the nucleotide sequences of clones in the libraries. The microbial community compositions were determined by phylogenetic analyses. And diversities of microbial community were determined by statistical analysis. We also estimated the copy number of 16S rRNA genes and *amoA* genes of bacteria and archaea by quantitative PCR.

Result and Discussion

Bacterial and archaeal cell numbers were estimated to be 10^7 to 10^8 cells/g in the ferro-manganese samples, respectively. Archaea dominated in three of four Mn crust samples (50˜83 % of total cell numbers). The copy numbers of bacterial and archaeal *amoA* gene were 10^6 copy/g in both samples. In contrast, the copy numbers of bacterial and archaeal *amoA* genes were 10^7 copy/g and 10^6 copy/g, respectively.

Phylotypes closely related to *Nitrosospira* and Marine crenarchaeota Group I (MGI) were detected from four Mn crust samples. These groups include ammonia-oxidizing chemoautotrophs. Furthermore, *amoA* genes, encoding an ammonia-oxidizing enzyme, were also detected from Mn crust samples. These results suggest that these putative ammonia-oxidizing microbes play a role as primary producers in the microbial ecosystem of the Mn crust.

Few phylotypes (1-8 species) were shared between the solid samples (Mn crust and sediment) and seawater sample of the same depth, as shown by comparative analysis. Phylotypes of MGI detected from the solid samples and seawater sample were separated into distinct clusters in the phylogenetic tree. Furthermore, Phylotypes of MGI in the solid samples formed several distinct clusters. The detected *amoA* genes in the solid samples and seawater samples were also separated into distinct clusters in the phylogenetic tree. These results suggest that several subgroups of phylotypes of MGI, which distincted from those in seawater sample, exist on Mn crusts.

The detected archaeal *amoA* genes were related to *amoA* genes of uncultured crenarchaeaota (95 -98 %) and to that of cultivated *Nitrosopumilus maritimus* (79-83 %). The detected bacterial *amoA* genes were most closely related to *amoA* gene from cultivated species of *Nitrosolobus multiformis* and *Nitrosospira* sp. (78 % respectively). These results suggest that uncultivated and novel ammonia oxidizing bacteria and archaea exist on the Mn crusts.

Keywords: Ferro-manganese crust, 16S rRNA gene, amoA gene, ammonia oxidizing bacteria