Japan Geoscience Union Meeting 2013
(May 19-24 2013 at Makuhari, Chiba, Japan)

MIS23-08 Room:103 Time:May 24 11:15-11:30

Coordination of NanoSIMS and cell sorting to reveal microbial metabolic activity in sediment of the South Pacific Gyre

Yuki Morono[1*], Motoo Ito[1], Terada Takeshi[2], Fumio Inagaki[1]

[1]Kochi Institute for Core Sample Research, JAMSTEC, [2]Marine Works Japan, Ltd

The South Pacific Gyre (SPG) is characterized as the most oligotrophic open ocean environment. The sediment is rich in oxygen but poor in energy-sources such as reduced organic matter, and hence harbors very low numbers of microbial cells in relatively shallow (˜20 meters below the seafloor) subseafloor sediment (D'Hondt et al., 2009; Kallmeyer et al., 2012). In such an energy-limited sedimentary habitat, only a small size of microbial community persists living functions with extraordinary low oxygen-consumption rate (Roy et al., 2012). However, because of the current technological limitation, deeper habitats of the SPG remain largely unknown.

During IODP Expedition 329, sediment samples recovered from whole sedimentary column down to the sediment-basement interface were successfully recovered, providing an unprecedented opportunity to tackle some technological challenges to clarify if indigenous life is present, and if any, what is the microbiological and biogeochemical characteristics in such extreme environments.

To evaluate small biomass in the SPG sedimentary habitat accurately, we made modification on a cell separation technique. Cell recovery ratio was monitored with an image-based cell enumeration technique (Morono et al., 2009). The control samples were prepared by mixing E. coli cells in sterilized sediment. Increasing sediment volume resulted in lower recovery of microbial cells. Cell recovery rates in the SPG sediment samples, which contain small zeolitic mineral grains, were generally lower than those in other oceanographic settings (i.e., organic-rich continental margin sediments). To gain cell recovery rate, we examined multiple density gradient layers. After multiple modifications, we cold increase cell recovery rate up to 80-95%. In addition, cell enumeration using flow cytometry showed consistent numbers with microscopy-based cell count.

We then used the above-mentioned technique for deciphering eco-physiology of microbial life in the SPG sediments. During Expedition 329, we have initiated incubation with stable isotope-labeled substrates such as bicarbonate, glucose, amino acids, acetate, and ammonium (Morono et al., 2011) under the (micro-)aerobic condition. One of the critical technological challenges in this project is to harvest low concentrations of sedimentary microbial cells for the single-cell-based microbiological analysis. Using a new cell separation technique and sorting, we successfully sorted enough number of microbial cells in small spots on the membranes (i.e., 10^3 to 10^5 cells per spot). Preliminary results from NanoSIMS analysis showed incorporation of substrates after 1.5-years incubation of microbial cells in subseafloor sediments of the SPG.

Keywords: NanoSIMS, Subseafloor biosphere, South Pacific Gyre, Stable Isotope Probing