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Microbial community analysis of hydrothermal plume in Suiyou Seamount caldera

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In deep sea hydrothermal system, hot fluid emitted from subsurface is supplied to sea water, and it makes plume. Various and reductive chemical components such as hydrogen, methane, ammonia, sulfur are included in the plume water, and the existence of the microbial population using it is expected. By clarifying distribution/dynamics of the microorganism which is related to the metabolism of such chemical components, it seems to become possible that not only emission of the chemical components directly to the ocean but also impact after the spouting to the ocean of organic substance supply are also estimated. In this study, the vertical distribution of microbial cells, which exists in the caldera plume water around the Suiyou Seamount area, was observed with Fluorescent in situ hybridization (FISH) and microscopic image analysis technique to clarify the dynamics of microbial population in the caldera.

Plume water for microbial analysis was sampled during NT-01-09 cruise and KR-01-15 cruise around Suiyou Seamount caldera. After one tenth volume of 38% nutrilized formalin was added to 100-200-mL of the samples, those were kept in the refrigater (4C) overnight to fix microbial cells. Some of the samples were filtrated onto PLL-filter with optimum cell numbers for microscopic observation, and then the PLL-filters were kept and brought back to the laboratoty under -80 C condition. The rest of the samples were also freezed and brought back to the laboratory under -80 C. These samples were used for 1. measurement of total cell number by Direct counting method using SYBR Green II, 2. measurement of the number of each microbial group such as Bacteria, Archaea, methane oxidizer, and sulfer oxidizer by FISH-DC method, and 3. Size and morphological analysis of each cell by digital image analysis.

 $0.6 \sim 1.5 \times 10^{5}$ microorganisms were measured in 1-mL of the plume water in the caldera. Number and size of the microorganism were the least in the subseabed, and it increased with going to the plume upper layer. 3×10^{4} cells were measured in 1-mL of the sea water in 1000-m layer in the caldera outside, and the cell population and size were being decreased in comparison with the microorganism in the caldera. The discrete value of FISH method using Bacteria probe in comparison with all microbial count by DAPI and SYBR Green II which are the DNA stain as a target also consisted for about 80% for the microorganism in the caldera. Generally, intracellular rRNA is made to be a target in the FISH method. Therefore, fluorescent intensity of microbial cells depends on rRNA contents, which strongly connected with microbial activities. Though fluorescent intensity of the cell could not be detected for the microorganism in the caldera caldera. These results indicate that the microorganism in the caldera actively proliferate.

All of detected microorganism were Bacteria in the plume water in the caldera, and the Archaea could not be detected. In the general marine environtment, alpha-proteobacteria, gamma-proteobacteria, and Cytophaga-Flavobacterium become predominant groups. However, it not dyed these bacteria by these probes, indicating that these bacteria are other microorganisms than alpha-proteobacteria, gamma-proteobacteria, and Cytophaga-Flavobacterium. Though the companion of methane oxidizer and sulfur oxidizer which shows paragenesis to the crab was detected, the proportion for all microbial population was little. By the announcement day, phylogenetic analysis and detection by the peculiar probe for novel Bacteria will be carried out in order to clarify the community structure of detected and unknown Bacteria.