

# Microbial community predominant in hydrothermal plume and fluid at Suiyo-seamount

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Since the discovery of a deep-sea hydrothermal vent in 1970's, extremely hyper thermophiles and chemolithoautotrophic microbes, e.g. hydrogen, sulfur, methane, and manganese oxidizers, have been isolated and/or detected from hydrothermal fluid, hydrothermal plume, chimneys, and animals by using the cultivation and/or molecular biological techniques. Functions of several microbial species and qualitative microbial community structures have been investigated by these studies. Quantification of each microbial specimen and their activities become important to estimate microbiological impact to the earth, however, it is difficult to quantify microbial population and activities by the cultivation and molecular techniques.

Direct counting technique (DC) using fluorescent microscopy is one of the most useful techniques to quantify environmental microbial population. Our developed DC technique, which combined the DC with fluorescent in situ hybridization (FISH-DC), enables to quantify specific microbes in natural environment in a single cell level. In the Archaen Park project, we aimed to quantify the microbial population and microbial community structures in the hydrothermal fluid and hydrothermal plume obtained from Suiyo seamount using FISH-DC techniques. This study is a first molecular and cellular approach for both the qualification and quantification of microbial cells in hydrothermal systems.

## Microbes in the hydrothermal plume

Density of microbial cells varied from  $5.6 \times 10^4$  to  $1.1 \times 10^5$  cells ml<sup>-1</sup> throughout the water column in the Suiyo Seamount caldera. The apparent plume layer was assessed to be at a depth of 1,050 to 1,200 m inside the caldera by the cell density ( $1.0$ - $1.1 \times 10^5$  cells ml<sup>-1</sup>) and transmissivity. Two major phylogenetic sequences, named SUP05 and SUP01, were obtained by molecular analysis of the microbes in the apparent plume layer, and they were closely related to sulfur oxidizing microbes. Specific oligonucleotide probes for these groups were designed and applied to water samples from the Suiyo Seamount with the FISH-DC technique. 88-90% of Bacteria cells (52-58% of the total counts) were hybridized with the SUP05 probe and more than 98% of them were regarded as the SUP05 and SUP01 populations. Although growth and production rates remain to be defined, we conclude that this Suiyo Seamount caldera has functioned as a natural continuous incubator for these two phylotypes of Bacteria in such an aphotic deep-sea environment.

## Microbes in the hydrothermal fluid

Cell density of microbes in various hydrothermal fluid samples were estimated to be  $1.2 \times 10^4$ - $1.3 \times 10^6$  cells ml<sup>-1</sup>. Even in the hot hydrothermal fluids, whose temperature was over 300 C, more than  $1.2 \times 10^4$  microbial cells were detected in 1 ml fluid. Almost all of these microbes were assigned to Bacteria and few cells were determined to be Archaea. There is little correlation between the cell densities and chemical contents in the various hydrothermal fluids, showing that these microbes were immixed into the vein from surrounding sand. Interestingly, the cell density in 2002 samples apparently increased as compared to 2001 samples. These results implicated that the drilling provided reduced chemicals for the microbes in the sand through bore holes and widened the habitat of the microbes.