Title

Exploring active chemolithoautotrophic microorganisms thriving at deep-sea hydrothermal vent chimney structures in the Mid-Okinawa Trough by using RNA-based microbial community analysis and a new culture method.

Hisashi Muto

Summary

Since the discovery of a deep-sea hydrothermal field in 1977, hydrothermal chimneys have attracted much attention as a research target due to the diversity of their microbial communities, their potential as an analogue for the sub-seafloor environment and as a mineral resource. Physiological ecology and phylogeny of chimney microorganisms have been studied using a combination of culture-dependent and culture-independent methods, but there are some challenges due to methodological issues. For example, it is technically difficult to extract microbial RNA, which is essential for analyzing microbial activity in deep-sea hydrothermal chimneys using culture-independent methods, and biogeographical studies that require numerous isolates remain laborious because there is no consideration of the solid media incubation technique for efficient isolation of deep-sea hydrothermal vent living microorganisms.

In chapter 2, I assessed RNA extraction methods for deep-sea vent chimneys that had complex mineral compositions. Mineral-RNA adsorption experiments were conducted using mock chimney and *Escherichia coli* total RNA solution and showed that detectable RNA significantly decreased possibly due to adsorption onto minerals. This RNA decrease was prevented by adding sodium tripolyphosphate (STPP), deoxynucleotide triphosphates (dNTPs), salmon sperm DNA, and NaOH. The addition of STPP was also effective for RNA extraction from the mixture of *E. coli* cells and mock chimney minerals when TRIzol reagent and the RNeasy column were used. A combination of STPP, TRIzol reagent, the RNeasy column, and sonication resulted in the highest RNA yield from a natural chimney sample. This new method may extend analytical methods for microbial communities within deep-sea hydrothermal vent chimneys, and thus may further our understanding of microbial activities in deep-sea hydrothermal fields.

In chapter 3, first, a simple and efficient solid media cultivation method for hydrogen- / sulfur-oxidizing microorganisms that are dominant in deep-sea hydrothermal vents was investigated. Combination of pouch bags, oxygen absorbent, and H_2/CO_2 mixture gas, I succeeded in solid media cultivation of hydrogen- / sulfur-oxidizing microorganisms, including that had never reported colony formation on solid media. And

I found that the colony formation ratio was higher when gellan gum was used as a gelling agent than agar was used and that the colony formation ratio was further improved by adjusting the CO_2 concentration in the gas phase. The optimized solid media cultivation method succeeded in culturing a variety of microorganisms, including E*psilonproteobacteria* and *Aquificae,* which are dominant hydrogen- / sulfur-oxidizing microorganisms living in the deep-sea hydrothermal vent chimneys. Second, I conducted a comparative transcriptome analysis of *Nitratiruptor* sp.SB155-2 to determine why the colony formation ratio of *Epsilonproteobacteria* living in deep-sea hydrothermal vents varied depending on the gelling agents and gas phase conditions during solid media cultivation and identified genes whose transcript levels varied with media properties, gelling agents, and CO_2 ratio in the gas phase. These results indicated that strain SB1 may change its energy-obtaining pattern in response to the physical properties of the culture medium. In addition, it was shown that the change in colony formation ratio by gelling agent and CO_2 ratio in the gas phase could be derived from the stress response and activity change of rTCA cycle.

These studies will help expand our knowledge of the physiological ecology, phylogeny, and biogeography of microorganisms living in deep-sea hydrothermal fields. Future works on the transcriptomic, proteomic, metabolomic analysis and efficient isolation of deep-sea hydrothermal chimney living microorganisms will also help us to further understand spatial, and temporal microbial diversity and activities of deep-sea hydrothermal vent living microorganisms.