

## Confirmation of chemosynthetic activities of *Bathymodiolus septemdierum* through laboratory culture

NAGAI, Yukiko<sup>1\*</sup>; TOYOFUKU, Takashi<sup>1</sup>; NOMAKI, Hidetaka<sup>1</sup>; CHIKARAISHI, Yoshito<sup>1</sup>; WATSUJI, Tomoo<sup>1</sup>; IKUTA, Tetsuro<sup>1</sup>; TAKAKI, Yoshihiro<sup>1</sup>; YOSHIDA, Takao<sup>1</sup>; SHIGENO, Shuichi<sup>1</sup>; INOUE, Koji<sup>2</sup>; KONISHI, Masaaki<sup>1</sup>

<sup>1</sup>Japan Agency for Marine-Earth Science and Technology (JAMSTEC), <sup>2</sup>University of Tokyo, <sup>3</sup>KITAMI Institute of Technology

Submarine volcanic activities circulate seawater between bottom water and interstitial water around hydrothermal vent. The heated water absorb carbon dioxide, methane, sulfur dioxide, hydrogen sulfide (H<sub>2</sub>S) and others originated from magmas. Chemosynthetic ecosystem is distributed around these thermal vent. Many volcanic activates has been found around the Japanese archipelagos. Chemosynthetic biology earn living energy by the organisms what has symbiotic bacteria in their body with these volcanic gases. Deep-sea bivalve *Bathymodiolus septemdierum* have been hosting some sulfur oxidizing bacteria in their gills. The bacteria have ability to synthesize such organic compounds as sugars from inorganic carbon source. Many questions have been still remaining about the emergence and maintenance mechanisms of such symbiotic relationship between host animal and bacteria. Even though the development of laboratory culture techniques of such chemosynthetic bivalves are very useful approach to understand the detailed ecology and for further experiments, the technique is not developed very well. Our research group try to set chemostat water bath up with hydrogen sulfide to keep *B. septemdierum* as live. We try to use the culture system to evaluate the bivalves can keep their symbiotic bacteria to make much longer life time in laboratory. The activity of symbiotic bacteria has been tested by the uptake ability of <sup>13</sup>C labeled inorganic carbon into their body.

Individuals of *B. septemdierums* are captured during dive series of ROV Hyper-dolphin system of two cruises of R/V Natsushima operated by Japan Agency for Marine-Earth Science and Technology (JAMSTEC) in April of 2012, in March of 2013 and in April of 2014. The samples are collected around Myojin-Sho submarine volcano on the Izu-Ogasawara Ridge (1224-1285m depth). Collected samples were kept under 4 °C water tank in an on-board low-temperature room till the end of cruise. Then, the individuals are immediately transfer to on-land laboratory water tank after cruise to avoid the unfavorable environment. The water tank has been designed as chemostat system with H<sub>2</sub>S supply to maintain symbiotic bacteria of deep-sea chemosynthetic animals. The individuals are cultured in this system for three months and fourteen months respectively. Here, previous study shows the symbiotic bacteria disappeared within three months without H<sub>2</sub>S source. Therefore, we prefer to confirm the bacteria hopefully maintained more than three months in our chemostat system or not. For the purpose, carbon isotope labeling experiments were carried out to clarify the symbiotic bacterial activity. The carbon isotope will be taken by *B. septemdierums* if the symbionts are active. We compare the carbon isotopic uptake between under H<sub>2</sub>S positive and under H<sub>2</sub>S negative (control) conditions. Meantime, dissolved oxygen (DO) of each cultivation was monitored to check health and activity of individuals. The results show the labeled <sup>13</sup>C was taken into organic matter in both gills and foot especially under H<sub>2</sub>S positive condition. The isotopic measurement of compound specific carbon isotopes in fatty acid show positive result. By this result, we consider the labeled carbon should be incorporated to the cell membrane of host animal. We summarize the symbiotic bacteria have been maintained for fourteen months in the chemostat system.

Keywords: deep-sea biology, chemosynthetic ecosystem