

Recent trials of laboratory culture with chemosynthetic organisms

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Chemosynthetic ecosystem is dominated by the organisms what has symbiotic bacteria in their body. Deep-sea bivalve *Bathymodiolus septemdirum* 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 with hydrogen sulfide (H₂S). 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. septemdirum* as healthy condition. 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 ¹³C labeled inorganic carbon into their body.

Individuals of *B. septemdirum* 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 and in March of 2013. The samples are collected around Myojin-Sho submarine volcano on the Izu-Ogasawara Ridge. 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 H₂S 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 H₂S source. Therefore, we prefer to confirm the bacteria have been hopefully maintained more than three months in our chemostat system or not. For this purpose, carbon isotope labeling experiments were carried out to clarify the existence of symbiotic bacterial activity. The carbon isotope will be taken into organic matter of *B. septemdirum* if the symbionts are active after laboratory culture. We have compared the carbon isotopic uptake between under H₂S positive and under H₂S negative (control) conditions, respectively. Meantime, dissolved oxygen (DO) of each cultivation was monitored to check health and activity of individual bivalves. The results show the labeled ¹³C signals were detected on the organic matters of both gills and foot especially under H₂S positive condition. Surprisingly, the activity was much positive even the individuals were kept for fourteen months in the chemostat system.

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