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Cultivating approach for understanding symbiont-host linkage of invertebrates in deep-sea chemosynthetic ecosystem

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Chemoautotrophic ecosystem are supported by chemoautotrophs, which are organisms that are capable of fixing carbon by using chemical energy obtained from the oxidation of reduced compounds blowing up from hydrothermal vents, such as sulfuric compounds, methane, and hydrogen. Several benthic invertebrates are well known to be hosts of chemoautotrophic bacteria in cells of specialized tissues and on the surface and obtain nutrition from the bacteria. The behaviors of the symbiont and organisms have been estimated by evidences from the results by field works, phylogenetic analyses, and characterization of isolates related to the symbionts, named 'field work based approach'. However, the approaches are limited by unclear causal associations between environmental and physiological factors and their behaviors. We, therefore, developed a rearing tank system with feed-back control system to provide hydrogen sulfide, which is strong reducing and toxic gas, in order to characterize the deep-sea organisms and their symbionts by 'cultivating approach'.

Using rearing tank system newly developed, we first examined the effect of low concentration H_2S on the episymbiotic bacterial communities of a deep-sea crab, *Shinkaia crosnieri*, by comparison of with/without H_2S feeding. The H_2S concentrations in tank were successfully maintained between 5 to 60 micro mol. per litter for 80 days with the exception of brief periods of mechanical troubles. The survivals of the crab were 44 individuals against initial 60 individuals (73.3%) for 84 days cultivation. On the other hand, the values were three individuals against initial 30 individuals (90.0 %)without the feeding. Even if additional effects of a trouble be considered, the survivals with H_2S feeding seemed to be less than that without the feeding. Busy setae were observed at few days after the continuous feeding of H_2S. Furthermore, white biofilm caused and increased on the surfaces of tank wall and of sea sand in the feeding unit. According to real-time PCR analysis, the copies of partial 16S rDNA of the episymbiont with feeding were three-orders of magnitude larger than those without the feeding. Based on a phylogenetic analysis of episymbiont, several phylotypes were detected in *alphaproteobacteria*, *ganmaproteobacteria*, *epsironproteobacteria* and *flavobacteria*, from the crab with H_2S feeding. The symbiont-related phylotypes would be grouped into four different groups; *gammaproteobacteria* in marine epibiont group I, *Sulfurovum*-affiliated *epsilonproteobacteria*, *Osedax mucofloris* endosymbiont-affiliated *epsilonproteobacteria*, and *flavobacteria* closely related to CFB group bacteria epibiont of *Rimicaris exoculata*. Based on the analysis of the biofilm, several phylotypes belong into the above results of epibionts. Growth of these bacteria would be dependent on only chemical and physical conditions except of pressure, and physiological host-symbiont interaction might not be necessary to be significant factor. On the other hand, marine epibiont group I in *ganmaproteobacteria*, which has been detected in epibiont phylotypes, was not detected. This fact implies a possibility that the marine epibiont group I tightly bind to their host by somewhat physiological interaction. Interestingly, one of major clades of phylotypes were *Sulfimonas*-affiliated bacteria, and closely related to endosymbiont of a snail, *Alviniconcha* sp. type II that was collected from Vienna Woods site, Manus Basin, where Iheya North field in Okinawa Trough, where *S. crosnieri* was collected, located approximately 3,700 km of geographical distance of Vienna Woods site. The significant growth factors of these bacteria would be also chemical and physical conditions, but not geographical factors. The results demonstrate possibility and clue that the behaviors are able to be discussed against chemical and physical factors, which distinguished from geographical and physiological factors.

Keywords: rearing tank, symbiont-host linkage, *Shinkaia crosnieri*, feed-back control, semiconductor gas sensor