Biochemical analysis and morphological characterization of deep-sea hydrothermal field endemic crab, *Shinkaia crosnieri*, associated with episymbionts

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Abstract

In this work, symbiotic related factors such as the ventral setae and the lectin-like proteins of the deep-sea endemic crab, *Shinkaia crosnieri*, as a model animal with episymbionts in extreme ecosystems, were studied to understand the host-episymbiotic association. A summary is given below.

We, human and many organisms from plants to animals on the earth, are symbiotic creatures harboring large numbers of both internal and external microbial partners. The hosts (animals as well as plants) with symbionts have developed systems to select and acquire specific partners. Deep-sea hydrothermal fields are highly productive environments with dense populations of various invertebrates. In the fields, primary production by photosynthesis is not performed; instead, chemoautotrophic bacteria produce organic matters using H_2S, H_2, CH_4 and CO in the vent effluents as energy sources. Most of the invertebrates inhabiting the hydrothermal fields and cold seeps have established endo- or epi-symbiotic relationships with specific chemoautotrophs in the vent environment, and are entirely dependent upon their symbionts (microbiome) for nutrition.

The deep-sea hydrothermal field galatheid crab, *S. crosnieri*, is one of the

dominant animal species colonizing hydrothermal vent fluids in the Okinawa Trough, Japan. The crab has soft and dense setae on the ventral surface of the body and harbor dense populations of chemolithoautotrophic and methanotrophic epibionts on the setae. The epibionts mainly consist of sulfur-oxidizing *Epsilonproteobacteria* and sulfur- or methane-oxidizing *Gammaproteobacteria*. These episymbiotic associations are assumed requiring coordination, important molecular communication, and recognition between partners. However, it is still unknown whether the host crab and symbiotic bacterial association are specific or less specific. The aim of this research was to comprehensively understand the mechanism of host-symbiont interaction of *S. crosnieri* in deep-sea hydrothermal fields.

In chapter 2, the morphology of the ventral setae of *S. crosnieri* and another crustacean without symbiont was compared. The morphological feature of *S. crosnieri* setae was specifically short setules, and the short setules on the seta made water fluid directly contact to the setal surface easily. These suggest the possibility that *S. crosnieri* has setae with short setules to enhance the productivity of symbiotic bacteria with supplying of nutrients such as H_2S and CH_4 to bacteria and/or to the efficient acquisition of symbiotic bacteria in the hydrothermal vent fluid.

In chapter 3, I have developed a lectin (carbohydrate-binding protein) activity assay suitable for rare samples, and made it possible to detect lectin activity on the conventional one-fifth scale. Also, I tried to purify the lectin-like protein in the serum of *S. crosnieri* with eight kinds of sugar affinity chromatography as well as ion exchange chromatography. The protein could not be purified, however, this study suggested that

there may be a different kind of lectin from deep-sea crustaceans, which may be much less specific to the target sugar.

In chapter 4, the lectin activity of the serum in *S. crosnieri* was detected for the first time, and its biochemical properties were analyzed. Non-strong binding was observed between the serum components and episymbiotic bacteria, suggesting low specificity for bacterial selection.

Finally, the findings in this study were summarized, as follows.

(1) *S. crosnieri* has the setae with significantly short setules.

(2) The setae with short setules are exposed to the fluid flow, which could assist supplying more fresh water and substrate to episymbiotic bacteria.

(3) *S. crosnieri* has lectins, one of the well-known host-derived symbiotic factor, circulating in the serum.

(4) The serum aggregated and inhibited the growth of one of the deep-sea hydrothermal field free-living bacteria, and contains some proteins recognizing some episymbiotic cells. The lectins in land organisms are much more selective, whereas the lectin from *S. crosnieri* is less selective.

Altogether, this study suggests that the priority for *S. crosnieri* is an efficient acquisition or enhancing the growth of episymbiotic bacteria rather than bacterial selection.