Chemical speciation analysis of the brown and black precipitates observed in the gill chamber of the vent shrimp *Rimicaris kairei*

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The hydrothermal vent shrimp *Rimicaris* dominates the megafaunal community in many hydrothermal vent ecosystems in the globe, including Mid Atlantic Ridge, the Central Indian Ridge (CIR), and the Caribbean Mid-Cayman Rise. *Rimicaris* harbors numerous strains of episymbiotic bacteria in its gill chamber and depends on them for most of the nutrition as adults. Probably due to the metabolism of this symbiotic bacteria, the tissues in the gill chamber were found to be encrusted with brown or black iron precipitates. The precipitates are removed by molting, but in a few days after molting, *Rimicaris* will be covered with precipitates again. Although *Rimicaris* relies on episymbiotic bacteria nutritionally, it is thought that the precipitates originated from episymbiotic bacteria disturb their swimming activity, nutrient uptake by the host. Thus, the relationship between *Rimicaris* and its epibionts seems to be different from general mutualistic relationship. To understand this symbiotic relationship correctly, it is necessary to clarify the relationship among the host, episymbiotic bacteria, and the precipitates. In this study, iron chemical speciation analysis based on XAFS were performed to black and brown precipitates in the gill chamber of *Rimicaris kairei* in order to clarify the chemical environment in the gill chamber.
Specimens of *Rimicaris kairei* were collected during the YK16-E02 cruise at two hydrothermal vent site, Edmond and Kairei on the CIR. Each tissues were fixed in 99.5 % ethanol, and embedded in Epoxy resin(Epofix, Struers), and both sides polished thin section with a thickness of about 50 μm were obtained. Thin section was subjected to μ-XRF element analysis and Fe K-edge XANES analysis at KEK PF BL-4A to investigate the element distribution and the speciation of iron around the bacteria. Fitting was carried out using a software REX 2000 (Rigaku) using ferrihydrite, goethite, magnetite, $FePO_4$, pyrite, pyrrhotite as reference materials.
By observing the freeze-dried samples by scanning electron microscope (JSM-6100, JEOL) and thin section samples by polarized microscopy, it was observed that the brown precipitates forms crusts of about 50 μm in thickness and covers entirely the inside of the branchiostegite, where black precipitates occur as submicron size microparticles in organic filmy tissues around filamentous bacteria. From the XANES fitting, it was suggested that the brown precipitates contain ferrihydrite, goethite, $FePO_4$, while the black precipitates contain ferrihydrite, goethite, $FePO_4$, and pyrrhotite. It was revealed that iron exists as Fe^{3+} in the brown precipitate, but in the black precipitate, iron exist as a mixture of Fe^{2+} and Fe^{3+}. It is estimated that the brown precipitates were formed in the aerobic environment and the black precipitates were formed in the relatively anaerobic environment. Overall, our suggested that the gill chamber environment was diverse even among the same species. We will perform next-generation sequencing of microbes in the gill chamber, and we need to investigate in detail the precipitation conditions of each mineral and the environment in which the host can grow.

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