

Thiotrophic and methanotrophic symbionts of hydrothermal vent mussels in the Suiyo Seamount and the Okinawa Trough

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Mussels inhabiting hydrothermal vents depend on microbial carbon assimilation via chemoautotrophy (mostly thioautotrophy), methanotrophy or both. A goal of mussel symbiosis study is to simultaneously link the trophic function and phylogenetic identity of the symbionts in vivo in order to confirm the type of symbiosis. Two mussel species of the genera *Myrina* and *Bathymodiolus* were collected from western Pacific back-arc hydrothermal vents at the Suiyo Seamount (SSM) and Mid-Okinawa Trough (MOT), Japan, respectively. Three functional genes were examined to characterize the symbioses in these mussels, namely *cbbL*, *atpS* and *pmoA* encoding the catalytic subunits of the enzymes ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39), ATP sulfurylase (EC 2.7.7.4) and particulate methane monooxygenase (EC 1.14.13.25), respectively. The 16S rRNA gene sequence analysis showed that each mussel species harbors a monospecific endosymbiont. The bulk gill DNA of the *Myrina* SSM-mussel showed PCR amplifications of *atpS* and *cbbL*, indicating a thioautotrophic endosymbiont. In contrast, the bulk gill DNA of the *Bathymodiolus* MOT-mussel showed the amplification of *pmoA*, in addition to the previously detected *cbbL* (Elsaied, H., and T. Naganuma. 2001. *Appl. Environ. Microbiol.* 67:1751-1765). This methanotrophic *cbbL*-bearing endosymbiont showed a distinct phylogenetic lineage within the branch of gammaproteobacterial methanotrophs. Fluorescence in situ hybridization demonstrated simultaneous occurrence of *cbbL* and *pmoA* in the MOT-mussel endosymbiont. This is the first molecular and visual evidence for a novel methanotrophic bacterial endosymbiont that bear the autotrophic CO₂ fixation-related gene *cbbL*.