

Bacterial endosymbioses of hydrothermal vent mussels and cold seep tubeworms

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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*. 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*.

Gutless tube-dwelling worms of pogonophorans (also known as frenulates) and vestimentiferans depend on primary production of endosymbiotic bacteria. The endosymbionts include thiotrophs that oxidize sulfur for autotrophic production and methanotrophs that oxidize and assimilate methane. Although most of the pogonophoran and vestimentiferan tubeworms possess single thiotrophic 16S rRNA genes (16S rDNA)

related to gamma-proteobacteria, some pogonophorans are known to bear single methanotroph species or even dual symbionts of thio- and methanotrophs. The vestimentiferan *Lamellibrachia* sp. L1 show symbiotic 16S rDNA sequences of alpha-, beta-, gamma- and epsilon-proteobacteria, varying among specimens, with RuBisCO form II gene (*cbbM*) sequences related to beta-proteobacteria. An unidentified pogonophoran from the world's deepest cold-seep at 7326 m deep in Japan Trench hosts a symbiotic thiotroph based on 16S rDNA with the RuBisCO form I gene (*cbbL*). In contrast, a shallow water pogonophoran (*Oligobrachia mashikoi*) in coastal Japan Sea has a methanotrophic 16S rDNA and thiotrophic *cbbL*, which may suggest the feature of type X methanotrophs. These observations demonstrate that pogonophoran and vestimentiferan worms have higher plasticity in bacterial symbioses than previously suspected. 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.