Genetic diversity of the CO2-fixing enzyme RuBisCO in deep-sea microorganisms

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Phylogenetic information of deep-sea microorganisms has been accumulated mainly based on the 16S rDNA sequences. In understanding the microbial contribution to the deep-sea primary production, the 16S rDNA-based phylogeny will be better complemented by the genes that encode the enzymes relevant to the carbon fixation. The genes that encode the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO, E.C. 4.1.1.39) represent such an enzyme involved in the autotrophy. The phylogenetic diversity of the RuBisCO genes of deep-sea microorganisms was analyzed as a part of this study. Bulk genomic DNA was isolated from 4 types of samples collected from 6 deep-sea areas. The area included the Mid-Atlantic Ridge, Loihi Seamount (Hawaii), and 4 various deep-sea habitats around Japan. The 4 types of samples were hydrothermal vent water, chimney fragment, sediment and symbiont-bearing tissues of the vent mussel Bathymodiolus sp. (phylum Mollusca) and the seep tubeworm Lamellibrachia sp. (phylum Vestimentifera).

The RuBisCO genes that encode both forms I and II large subunits (cbbL and cbbM) were amplified by PCR from the DNA of the collected samples, cloned and sequenced. From each amplified product, 50 clones were recovered and sequenced to be grouped into operational taxonomic units (OTUs). A total of 35 OTUs were recorded from the total 350 cbbL-carrying clones; and a total of 24 OTUs were recorded from the total 250 cbbM-carrying clones. All the current OTUs have the characteristic RuBisCO amino acid motif sequences that exist in the known RuBisCOs. The recorded OTUs were related to different RuBisCO groups of proteobacteria, cyanobacteria, and eukarya. The diversity of the RuBisCO genes may be correlated with certain characteristics of the microbial habitats. The RuBisCO sequences from the symbiont-bearing tissues showed a phylogenetic relationship with those from the free-living ambient bacteria. Also, the RuBisCO sequences of known species of thiobacilli and those from widely distributed marine habitats were closely related to each others. This suggests that the thiobacilli-related RuBisCOs may distribute globally and contribute to the primary production of organic carbon in the deep-sea food chain.

Another part of this study based on the in situ hybridization technique was conducted to confirm the DNA analysis. This technique was performed on the biological samples, which include the methane seep tubeworm Lamellibrachia sp. and the hydrothermal vent mussel Bathymodiolus sp. Both of the two gutless animals depend on autotrophic endosymbiotic bacteria for their feeding. These endosymbiotic bacteria are found in the unique internal organ, trophosome, of the tubeworm Lamellibrachia sp. and the external organ, gill of the mussel Bathymodiolus sp. Histologically, the trophosome of the tubeworm consists of lobules, while the gill of the mussel consists of filaments, both containing endosymbiont-bearing cells called as bacteriocytes.

In case of the tubeworm Lamellibrachia sp., only, a single RuBisCO gene, cbbM was detected and localized bound to the periphery of the trophosome lobules at sites coincident with the location of coccoid cells. On the other hand, cbbL was not detected in the trophosome of the tubeworm Lamellibrachia sp. (phylum Vestimentifera). By contrast, RuBisCO cbbL was detected and localized at the peripheries of the gill filaments of the studied mussel Bathymodiolus sp. (phylum Mollusca). The in situ hybridization technique provided the first direct visual evidence for the sites of carbon fixation in the trophosome of the tubeworm and the gills of the mussel.