

Physiological and molecular ecological studies on carbon monoxide oxidizers in aquatic environments

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Summary

Prokaryotes called carbon monoxide (CO) oxidizers utilize CO as an energy source using the key enzyme CO dehydrogenase (CODH). CODH is divided in two types: nickel-containing CODH (Ni-CODH) which is sensitive to oxygen, and aerotolerant molybdenum-containing CODH (Mo-CODH). CO oxidizers are considered to play an important role as a sink for atmospheric CO. Moreover, particular CO oxidizers with Ni-CODH (hydrogenogenic CO oxidizers) would consume toxic levels of CO and provide H₂ to surrounding microbial communities. However, knowledge about the physiological role of CO oxidation and the ecological role of CO oxidizers remain poorly understood because of the limited number of isolates, and insufficient understanding of diversity as well as ecological features of CO oxidizers in the environment.

In chapter 2, I isolated a hydrogenogenic CO oxidizer *Parageobacillus* sp. G301 and performed genomic and physiological analysis of it in order to expand the number of isolates and to gain insight into the versatility of CO metabolism of G301. Genomic analyses showed that G301 was the sole isolate which possessed both genes encoding Ni-CODH (*cooS*) and Mo-CODH (form I *cox*). Genome-based prediction and culture experiments implied that G301 couple CO oxidation using Ni-CODH with hydrogen evolution in anoxic conditions, and couple CO oxidation using Mo-CODH with the reduction of O₂ and nitrate in oxic conditions and anoxic conditions with nitrate, respectively. These indicated a novel CO metabolism to perform either of Ni-CODH-mediated and Mo-CODH-mediated CO oxidation according to the available electron

acceptors. Furthermore, comparative genomic analysis showed that there was no significant genomic difference between CO oxidizers and non-CO oxidizers in the genus *Parageobacillus*, implying the possibility that *codh* genes might be an optional genetic element which improve fitness under limited conditions.

In chapter 3, I focused on CO oxidizers with Mo-CODH which are dominant in oxic water column, and are thus suitable for ecological analyses. In order to reveal the diversity of prokaryotes which have form I *cox* (potential *cox*-containing CO oxidizers: *pcox*-CO oxidizers), I investigated metagenome-assembled genomes (MAGs) with form I *cox* from a catalog of MAGs originated from the ocean which was reported in a previous literature. I identified 1,297 MAGs of *pcox*-CO oxidizers which were classified into 234 species-level genome clusters in nine phyla. Metagenomic read mapping analysis showed that 34 species-level genome clusters were dominant in oceanic regions characterized by geographic locations and environmental conditions. The 34 species-level genome clusters exhibited distinct distribution patterns, indicating the species-level niche separation.

In chapter 4, I performed ten-month long observation in Osaka Bay to identify *pcox*-CO oxidizers which could contribute to CO oxidation in coastal surface water and to gain insight into their ecology. CO depletion was observed in culture experiments using surface seawater sampled from Osaka Bay, suggesting microbial CO oxidation. 16S rRNA amplicon sequencing analysis showed that there were eight amplicon sequence variants (ASVs) which were affiliated to four species-level genome clusters to which *pcox*-CO oxidizers belonged. The dynamics of ASVs varied among species-level genome clusters. One of the eight ASVs cooccurred with two ASVs which were not affiliated to species-level genome clusters to which *pcox*-CO oxidizers belonged. The two cooccurred ASVs was classified in genera in phylum Bacteroidota which were known to correlate

with phytoplankton, implying the possibility that there might be interaction between *pcox*-CO oxidizers and phytoplankton and/or other prokaryotes.

In these studies, I not only expanded the taxonomic and physiological diversity of isolates of CO oxidizers, but also gained insight into the physiological role of CO oxidation. Moreover, I expanded our knowledge about the diversity of *pcox*-CO oxidizers in the ocean, and unveiled the differences in biogeographic distribution patterns as well as temporal dynamics between species, both of which would lead to deeper understanding of the ecological role of CO oxidizers. Collectively, it is assumed that diverse CO oxidizers utilize CO in their specific niches. In the future works, isolation of novel CO oxidizers as performed for G301 from the vast variety of environments and under wider range of temperature would lead to the discovery of novel CO oxidizers, which could provide further insight into the physiological role of CO oxidation. In addition, the combination of species-level ecological characterization of *pcox*-CO oxidizers performed here and multifaceted ‘omics’ approaches (e.g. metatranscriptome and metaproteome) as well as microcosms as an experimental approach would enable more in-depth understanding of ecological role of CO oxidizers.