Genomic and molecular ecological studies on thermophilic hydrogenogenic carboxydotrophs

Kimiho OMAE

Summary

The microbial hydrogenogenic carbon monoxide (CO) oxidizing activity achieved by hydrogenogenic carboxydotrophs with a membrane associated CO-oxidizing and H_2-producing machinery, the carbon monoxide dehydrogenase (CODH)/energy converting hydrogenase (ECH) complex, is considered as an important metabolic process in microbial community as well as biotechnological application. However, previous isolation-based studies biased in hydrothermal environments, which revealed ~20 isolates, have resulted in the limited information about CO metabolism and ecology of thermophilic hydrogenogenic carboxydotrophs.

First, to reveal novel CO metabolism of thermophilic hydrogenogenic carboxydotrophs, I performed *de novo* sequencing and feature analysis of the *Calderihabitans maritimus* KKC1 genome, which is the first isolate from marine sediment and the phylogenetically novel bacterium (Chapter 2). This analysis revealed that *C. maritimus* KKC1 harbored six CODH gene clusters, which is the highest number encoded in a single genome, including three known CODH gene clusters, CODH–acetyl-CoA synthase (Wood-Ljungdahl pathway), CODH–ECH (energy conservation) and CooF–CODH–flavin adenine dinucleotide-nicotinamide adenine dinucleotide oxidoreductase (reducing power production), while the other three had novel genomic contexts. One of the three novel CODH genes was associated with 2-oxoglutarate:ferredoxin oxidoreductase, which is a CO_2-fixing enzyme in reverse tricarboxylic acid (RTCA) cycle, and expected to comprise novel CO metabolism where CO is incorporated via RTCA cycle. From these results, it is predicted that unexplored hydrogenogenic carboxydotrophs might possess the novel CO metabolism and the exploration of these microbes in environments is important for understanding the diversity of CO metabolisms.

To explore more thermophilic hydrogenogenic carboxydotrophs in wide variety of environments, culture-independent method, which is high-throughput and avoids cultivation bias, is desired. But design of CODH-targeted universal primers was difficult due to the sequence diversity. Therefore, in Chapter 3, I constructed a reference database of these microorganisms with revealing distribution of CODH–ECH gene clusters upon

16S rRNA phylogeny to enable exploration of thermophilic hydrogenogenic carboxydotrophic isolates by microbial community analysis. This analysis identified 71 genomes of bacteria and archaea including the 46 overlooked potential hydrogenogenic carboxydotrophs, whose hydrogenogenic CO oxidizing activities have never been reported, from ~140,000 prokaryotic genomes, and expanded the estimation for diversity of these microorganisms to four phyla, 26 genera, and 43 species. By microbial community analysis using this reference data, I showed that potential thermophilic hydrogenogenic carboxydotrophs in phylum Firmicutes widely distributed in hydrothermal environments with small relative abundance.

While microbial community analysis can easily evaluate the 71 hydrogenogenic carboxydotrophic isolates, we cannot define hydrogenogenic carboxydotroph by the 16S rRNA gene sequence. In Chapter 4, I designed new primers for PCR amplification of CODH genes of CODH–ECH gene clusters (CODHech genes) to directly evaluate the diversity of thermophilic hydrogenogenic carboxydotrophs including unknown species by culture-independent way. My primer design strategy dividing target CODHech genes of Firmicutes members based on phylogenetic subclades provided the six new primer sets, four of which effectively amplified the CODHech genes. Amplicon sequencing with these primers in two hot spring sediments in combination with CO-enrichment successfully identified at least six lineages of CODHech genes which might be derived from rare thermophilic hydrogenogenic carboxydotrophs possibly including unknown species. To our knowledge, this is the first time that the CODHech genes are identified by CODH-targeted primers in environments.

These studies expanded our knowledge on metabolic and phylogenetic diversity and distribution of thermophilic hydrogenogenic carboxydotrophs. When correctively considering these results, it is predicted that thermophilic hydrogenogenic carboxydotrophs including still unknown species with novel CO metabolism are widely distributed in hydrothermal environments. In the future work, application of the culture-independent techniques, which I developed and validated in hydrothermal environments, to vast variety of environments including lake or marine sediments, soils and composts will pave the way for revealing diversity and distribution of these microorganisms.