Abstract

Discovery of a Novel Subsurface Syntrophic Niche : Methoxylated Aromatic Compounds Degradation
(深部地下圏でメトキシ芳香族化合物を分解する新規共生細菌の発見)
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Deep subsurface environments provide large habitats for a variety of functionally active microbial communities, which contain 20% of the total biomass of microorganisms on earth, therefore understanding of their ecological roles is very important in terms of biogeochemical cycle. Especially, methane is important material, major component of natural gas, amounts of which are equal to 11% of global total energy demand, estimated 40% of which are produced by deep subsurface microorganisms. Methoxylated aromatic compounds (MACs, -OCH_3) are derived from lignin and occur in large quantities on Earth, especially in coals and sedimentary organic matter (*e.g.*, kerogen) in deep subsurface environments, indicating that microbial MACs degradation methane production might play important role in methane formation and carbon cycling in the deep subsurface. The chemically inert O-methyl groups prevent most microorganisms from directly utilizing aromatic compounds containing phenyl-methyl ether, so demethylation reaction is first step and necessary of complete biodegradation of aromatic compounds, therefore understanding of microbial MACs degradation methane production is essential for elucidation of carbon cycling in the deep subsurface environments. Homoacetogens and methanogens are anaerobically capable of degrading MACs, but most are derived from surface environments, mechanisms of MACs degradation methane production in deep subsurface environments are unclear. This study aims to investigate microbial MACs degradation coupled with methane production in subsurface environment.

In chapter 2, isolation and characterization of novel syntrophic MAC degrading bacterium were demonstrated. An anaerobic thermophilic, rod-shaped bacterium possessing a unique non-lipid sheathed-like structure enveloping a single-membraned cell, designated strain NRmbB1^T was successfully isolated from the deep subsurface oil field located in Yamagata Prefecture, Japan. Growth occurred with 40-60˚C (optimum, 55˚C), 0-2% (2%), NaCl and pH 6.0-8.5 (8.0). Fermentative growth with various sugars was observed. Glucose-grown cells generated acetate, hydrogen, pyruvate and lactate as the main end products. Syntrophic growth occurred with glucose, pyruvate and 3,4,5-trimethoxybenzoate in the presence of a hydrogen/formate-scavenging partner, and growth on 3,4,5-trimethoxybenzoate was only observed under syntrophic condition. Based on 16S rRNA gene phylogeny, strain NRmbB1^T belongs to a distinct order-level clade in the class *Clostridia* of the phylum *Firmicutes*, sharing low similarity with other isolated organisms (*i.e.*, 87.5% for top hit *Moorella thermoacetica* DSM 2955^T). In total, chemotaxonomic, phylogenetic and genomic characterization revealed that strain NRmbB1^T represents a novel species of a new genus. In addition, we also propose the associated family and order as *Koleobacteraceae* fam. nov and *Koleobacterales* ord.nov., respectively.

In chapter 3 focuses on phylogenetic relationship of members of the phylum *Firmicutes*. *Firmicutes* is the second most abundant phylum, and its members are widely distributed across the globe and ecosystems. Besides, the phylum contains various members of major clinical pathogens as well as industrially useful microbes, thereby *Firmicutes* members have gained attention and interest in the environmental, medical and biotechnological research and industry fields. Nonetheless, evolutionally relationships and phylogenetic placements of this phylum are quite complicated and unclear. Indeed, 16S rRNA gene-based phylogenetic trees for the phylum *Firmicutes* are often not consistent with those constructed based on genome sequences (i.e., ribosomal protein sequences). This study compared phylogenetic trees constructed using various genetic marker (16S rRNA gene, 23S rRNA gene, ribosomal proteins and conserved proteins). The phylogenetic trees appeared that the phylogenetic placement in the 23S rRNA gene tree was congruent with both ribosomal proteins tree and conserved proteins tree, and these trees are well consistent with the GTDB taxonomy, while 16S rRNA gene tree was incongruent with these trees and exhibited very low bootstrap values for the branches. Our results showed that 23S rRNA gene is much more suitable as a universal marker gene for evaluating phylogenetic relationship of the phylum *Firmicutes* members than 16S rRNA gene.

In chapter 4, syntrophic MACs degradation system was elucidated through cultivation, genomic analysis and thermodynamics. Cultivation experiments showed that strain NRmbB1 could grow with MAC as the sole carbon/energy source only in the presence of a formate- and hydrogen-scavenging methanogenic archaeon. Genome analysis revealed that the strain harbored a unique MAC-degrading methyltransferase system with a hydrogenase and corresponding energy conservation pathways and indicated that the strain seems to convert MAC into hydrogen and formate. Thermodynamics revealed that MAC-degradation coupled with formate and hydrogen reduction is endergonic, this result was congruent with the results of the cultivation experiments.

Taken togeghter, this study isolated a novel anaerobic bacterium from deep subsurface oil reservoir and proposed *Koleobacterales* ord. nov., *Koleobacteraceae* fam. nov., and *Koleobacter methoxysyntrophicus* gen. nov. sp. nov. for this strain. Furthermore, this study discovered that the bacterium is capable of syntrophically degrading MAC with hydrigen/formate-scavenging methanogens and proposed novel MAC degrading pathway from its genome. All known MAC-degraders can grow alone, indicating that the bacterium represents a novel MAC-degrading niche. There are few research involved in MAC utilizing microorganism in deep subsurface, and mechanism of MAC degradation coupled with methane production in deep subsurface is unclear. This study indicated demethylation of MAC in deep subsurface environments was occurred by co-culture (syntophic bacteria and hydrigen/formate-scavenging methanogen), this is the first discovery in the world. Furthermore, our findings also indicated that MAC degrading syntrophic bacterium may play a key role in methane formation (e.g., coal bed methane) in deep subsurface environments.