A Novel Putative Propane Monooxygenase Initiating Metabolism of 1,4-Dioxane

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Background/Objectives.
1,4-Dioxane (dioxane) is a groundwater contaminant of emerging concerns given its carcinogenic potential and widespread occurrence. To date, there are more than 10 bacterial species that have been isolated to be capable of utilizing dioxane as the sole carbon and energy source. However, tetrahydrofuran (THF) monooxygenase is so far the only group of bacterial enzymes that have been well characterized and studied due to their critical role in initiating the oxidation of dioxane and other cyclic ethers. The gene cluster *thmADBC* encodes four components of the THF monooxygenase. Recent studies demonstrated *thmADBC* is absent in many newly isolated dioxane degraders (e.g., *Mycobacterium dioxanotrophicus* PH-06), which represents a knowledge gap to uncover novel enzymes/genes involved in the biodegradation of dioxane.

Approach/Activities. In this study, the genome of *Mycobacterium dioxanotrophicus* PH-06 was sequenced using PacBio RS II long-read sequencing technique to unveil a presence of existence of a novel putative propane monooxygenase gene cluster (*prmABCD*), which is phylogenetically disported from any known *thmADBC* genes. The transcription of this putative monooxygenase gene cluster in PH-06 was evaluated using reverse transcription PCR (RT-PCR) and Reverse transcription quantitative PCR (RT-qPCR) analysis to discern its relation with the metabolism of dioxane and other substrates of interest. Further, the complete MO gene cluster was cloned and transformed into a heterologous host to inevitably elucidate the catabolic capability of its encoded enzyme complex in initiating dioxane degradation. Degradation intermediates of dioxane by this enzyme were characterized in the medium extracts of the transformant cells by GC/MS.

Results/Lessons Learned.
Similar to other soluble di-iron monooxygenase (SDIMO) genes, this annotated putative propane monooxygenase gene cluster in PH-06 consists of four gene components, encoding the large and small hydroxylase subunits, the coupling protein, and the reductase. The closest homologs of this *prmABCD* gene cluster include monooxygenase genes found in (hydrocarbon-degrading) *Rhodococcus wratislaviensis* IFP2016 and *Mycobacterium chubuense* NBB4, classified as a group-6 SDIMO gene. RT-PCR analysis revealed the polycistronic transcription of all four components of *prmABCD* are induced when PH-06 is fed with dioxane, THF, or propane in comparison with glucose, pyruvate, and other common degradation products. Dioxane biotransformation capability was confirmed with *Mycobacterium smegmatis* mc^2-155 cells heterologously expressing the PH-06 *prmABCD* gene cluster. The expression clones harboring *prmABCD* sustain their ability to degrade dioxane after induction. Detection of 2-hydroxyethoxyacetic acid (HEAA) in the expression clones proves α-hydroxylation as the function of the PH-06 Prm to initiate the cleavage of cyclic ether compounds. The identification of this novel catabolic gene advances our understanding of dioxane metabolic pathways and enables development of molecular biomarkers to assess bioremediation potential at contaminated sites.