Cometabolic Degradation of 1,4-Dioxane by a Novel Gram-Negative Propanotrophic Bacterial Isolate

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Background/Objectives.
As a widely used stabilizer for some chlorinated solvents, 1,4-dioxane (dioxane) has been detected worldwide as a contaminant in surface water and aquifers. Unfortunately, its hydrophilic nature and structural stability preclude effective removal by conventional physical and chemical treatments. Bioremediation, when applicable, is thus the most economical and eco-friendly alternative to mitigate the large and dilute plumes formed by dioxane. A number of bacterial strains have been isolated or identified due to their capability of degrading dioxane via metabolism or cometabolism. A majority of these strains are Gram-positive Actinomycetes belonging to the genera of *Rhodococcus*, *Pseudonocardia*, and *Mycobacterium*. In situ application of these Gram-positive Actinomycetes is limited due to their slow growth and clump forming that hurtles their subsurface distribution in the contaminated aquifers. In this study, we isolate and report the first Gram-negative bacterial strain DD4 that is capable of degrading dioxane with propane as the primary substrate.

Approach/Activities.
An active sludge sample was obtained from a local wastewater treatment plant in northern New Jersey. Prior to the enrichment, 2.0 g of sludge (wet weight) was washed three times with sterile phosphate buffer solution to remove dissolved natural organic carbon sources. The washed sample was suspended in 20 mL NMS in a 120-mL serum bottle supplemented with appropriate amount propane and dioxane as carbon sources, and incubated on a rotary shaker at 160 rpm and 30 ^{o}C. The supernatant of the culture was transferred and refreshed biweekly. Degradation of propane and dioxane was monitored during the enrichment. After two months of incubation, the final enrichment culture exhibiting fast propane and dioxane removal rates was diluted and plated onto R2A agar plates. After incubation at 30 ^{o}C overnight, morphologically distinct colonies were obtained. Individual colonies were transferred to 20 mL of NMS amended with propane and dioxane to verify the dioxane co-metabolism. A bacterial strain grown on propane and co-metabolize dioxane was selected and characterized by physiological and biochemical tests, which was subsequently designated as strain DD4 by 16S rRNA gene sequencing analysis. Further, microcosms were prepared using the mixture of three groundwater samples collected from the source zone area of a dioxane-impacted site located in southern CA. DD4 was inoculated with the initial concentration of 1.25 µg total protein/mL. To distinguish the inhibitory effects of co-occurring contaminants (e.g., 1,1-DCE) and other factors, a parallel treatment was prepared with NMS medium spiked with 10 mg/L dioxane. Abiotic controls for both treatments was conducted using killed DD4 cells.

Results/Lessons Learned.
DD4 can effectively degrade dioxane when fed with propane. Such degradation activity can be sustained by repeated amendment of propane with little or no clumps formed. DD4 can survive in relatively cold environment at temperature as low as 10 ^{o}C, and tolerate the salinity as high as 3% (as NaCl, w/v%). Degradation of propane and dioxane were both completely inhibited by the exposure of acetylene, suggesting the involvement of monooxygenase which is under molecular characterization in our lab. Microcosm assays with field samples demonstrate DD4 can remove dioxane from 10.4±0.1mg/L to below 30 µg/L within 8 days of incubation and overcome the

inhibition of the environmental factors (e.g., 1,1-DCE), suggesting the suitability of DD4 as a potent inoculum candidate for in situ bioaugmentation.