Novel Group-6 Propane Monooxygenases in Charge of 1,4-Dioxane Biodegradation in Psychrophilic Propanotrophic Consortia

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Background/Objectives. In situ bioaugmentation for 1,4-dioxane (dioxane) remediation using laboratory isolates is restricted by the low temperatures (4~14 °C) at impacted aquifers. Available dioxane degraders are largely mesophilic since they were isolated at room temperature or above, yielding lower growth and dioxane biodegradation activity at aquifer-relevant temperatures. In this present study, two propanotrophic consortia were enriched and characterized given their ability to cometabolize dioxane at 14 °C. The diversity of soluble di-iron monooxygenases (SDIMOs) are profiled using amplicon-based sequencing to reveal the key enzymes that participate in dioxane cometabolism.

Approach/Activities. Enrichments were prepared with activated sludge samples collected from a local wastewater treatment plant. After washing with PBS, sludge samples were incubated at 14 °C in two different culture media, ammonia mineral salts (AMS), and nitrogen mineral salts (NMS). Propane consumption and dioxane removal were monitored by GC-FID. Amplicon-based sequencing was used to target the 16S rRNA gene and the SDIMO genes. Heterologous expression was used to validate the catalytic function of selected Group-6 SDIMOs.

Results/Lessons Learned. After successive rounds of enrichment, steady dioxane degradation and propane consumption was observed in both consortia. 16S rRNA amplicon sequencing revealed several genera in both consortia associated with dioxane degradation and cold tolerance. Additionally, several genera were observed in only the NMS consortia associated with the use of nitrate as the nitrogen source. Furthermore, analysis of the SDIMO amplicon sequencing reveals the presence of three SDIMO groups, 3, 5, and 6, all associated with propane consumption. Particularly, four group-6 SDIMOs are present at high abundances of 72.39% and 70.62% in the AMS and NMS consortia, respectively. Their dominance in combination with the expression of these genes in heterologous hosts confirms their role in dioxane breakdown. This work has uncovered consortia capable of tackling dioxane contamination at environmentally relevant temperatures, and lays the groundwork for uncovering the key differences between SDIMOs in mesophiles and psychrophiles.