Application of Metagenomic-Guided Proteomics to Assess Degradation of RDX in Pure Cultures and Groundwater from Contaminated Sites

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Background/Objectives. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a soluble, nonvolatile cyclic nitramine explosive that has been widely used in military and civilian applications. RDX is also a common groundwater contaminant and a possible human carcinogen. RDX is biodegradable under both aerobic and anaerobic conditions, and numerous RDX-degrading isolates have been reported. Yet, our understanding of the roles of these known RDX degraders in the environment, the prevalence of RDX degraders in natural or engineered systems, as well as their associated RDX-degrading microbial communities, genes and proteins in response to engineered interventions, is still developing. A better knowledge of in situ RDX degradation could potentially guide the isolation of novel RDX degraders and the development of suitable biomarkers for monitoring intrinsic or engineered RDX bioremediation.

This study used proteomics to evaluate degradation of RDX by pure cultures of *Pseudomonas fluorescence* IC and *Gordonia* KTR with and without an amendment with RDX. Additionally, samples of groundwater from two contaminated sites were analyzed to evaluate if the RDX degradation was attributed to naturally occurring microorganisms with the use of metagenomic sequencing and discovery proteomics.

Approach/Activities. Metagenomic guided proteomics provided basis to evaluate expression of proteins involved in RDX degradation in pure culture of RDX degraders. Findings from this experiment informed on up and down regulation of RDX degradation proteins. Further, shotgun proteomic and metagenomic analyses were used to evaluate natural attenuation of RDX contaminated sites. The goal of this study was to confirm that the naturally occurring microbial communities and the resulting RDX- degrading peptides are present within the plume. The goal of the metagenomic analysis was to develop a comprehensive listing of microbial species involved in the degradation of RDX as well as other organisms that might be influential in syntrophic degradation. This information will help to evaluate the role of continued microbial activity to support ongoing attenuation of residual RDX. The metagenomic information may help improve understanding of the effect of the microbial biofilms on the aquifer.

Results/Lessons Learned. The primary results identify a variety of peptides that may serve as biomarkers of degradation of RDX. The discovery proteomics tool applied during this work aids accurate detection of specific peptides in environmental samples as well as has the potential to provide a wealth of new information on protein function and activities within the subsurface. The data presented demonstrate validity of a step wise approach in which 16S sequencing and gene specific sequencing aid targeted proteomic in detection of key peptides involved in the biodegradation of RDX.