Application of Proteomics to Assess Degradation of RDX in Pure Cultures and Groundwater from Impacted 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. As such, RDX-impacted groundwater is found at many sites and is also a possible human carcinogen. RDX is biodegradable under both aerobic and anaerobic conditions, and several 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. Additional 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 shotgun proteomics to evaluate degradation of RDX by pure cultures of *Pseudomonas fluorescens* strain I-C and *Gordonia sp.* strain KTR9 with and without an amendment with RDX. Additionally, samples of groundwater from impacted sites were analyzed to evaluate if the RDX degradation was attributed to naturally occurring microorganisms with the use of transcriptomics, whole genome sequencing and proteomics used to estimate biodegradation rates for munitions contamination in groundwater.

Approach/Activities. Shotgun proteomics and transcriptomics provide a basis to evaluate expression of proteins involved in RDX degradation in pure cultures of RDX degraders. Findings from this experiment have informed on up and down regulation of RDX degradation proteins. Further, shotgun proteomics and metagenomics analyses will be used to evaluate natural attenuation of RDX-contaminated sites. This information will help to evaluate the role of continued microbial activity to support ongoing attenuation of residual RDX and to estimate biodegradation rates for munitions-impacted groundwater to facilitate cost effective transitions from active to passive treatment.

Results/Lessons Learned. The proteomics tool applied during this work supports accurate detection of specific peptides in environmental samples as well as having the potential to provide a wealth of new information on protein function and activities within the subsurface. The biomarkers from environmental samples collected from the RDX-impacted sites and the quantitative information will be used to estimate timeframes for RDX degradation. The application of quantitative proteomics and molecular tools will allow site remediation managers to make informed decisions on remedy selection and optimization at Navy sites.