Molecular Analysis of Microbial Communities Associated with ATO Biodegradation

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Background/Objectives. The onset of insensitive munitions compounds (IMC) use in military activities has spurred researchers and practitioners to understand the environmental impacts of these compounds. Research is necessary to understand their impact which may be detrimental to the ecology and persistence in affected environments for unspecified durations. While IMCs categorize many specific compounds, 3-nitro-1,2,4-triazole-5-one (NTO) and more specifically, the biotransformation product, 3-amino-1,2,4,triazole-5-one (ATO) is the main focus of this study due to its underrepresentation in the literature and its ability to persist in soil. In this work, a microbial community capable of the biodegradation of ATO is examined under controlled culture conditions with clone libraries and novel qPCR assays. Examination of microbial communities involved in ATO biodegradation is necessary as literature does not indicate optimal conditions for bioremediation. Moreover, the mechanism of ATO degradation is not well understood.

Approach/Activities. Enrichment cultures were produced using a mineral medium inoculated with soil and ATO as a carbon and nitrogen source in order to identify biodegradation associated microorganisms. At various time points during the development of the enrichment culture over two years, clone libraries were performed to identify the predominate microbial community members, and novel qPCR assays were developed to track microbial growth (or disappearance) of these microbial community members.

Results/Lessons Learned. Enrichment cultures showed a complete mineralization of ATO. 16S rRNA gene sequencing of the enrichment culture identified more than two dozen microorganisms early on in the enrichment culture process. Over a couple of years, this culture has been reduced to fewer than one dozen bacterial strains while maintaining ATO mineralization. In the later stages of this culture with more stringent dilution and regrowth challenges, complete ATO degradation slowed from just a couple of weeks to more than a month. Many of these strains are closely relate to other azole degraders, which are hypothesized to play important roles in the ring cleaving step of ATO degradation. However, the physiological roles each microorganism plays at varying steps of the degradation process is not well known but is being studied. Understanding which strains are essential to the degradative pathway may facilitate development of biomarkers for ATO degradation genes in the environment, and biostimulation/bioaugmentation strategies for more effective and economical bioremediation.