Tools for the Characterization and Manipulation of Reductive Dehalogenases for Bioremediation of Chlorinated Solvents

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Background/Objectives. Reductive dehalogenase (RDase) enzymes are key players in the anaerobic bioremediation of halogenated contaminants, such as chlorinated solvents, pesticides, and disinfection byproducts. RDases cleave the carbon-halogen bond of these organohalides facilitating their detoxification and removal from the environment. The enzymatic characterizations of new RDases have been greatly impeded due to the challenge of isolating them from their host organisms. Heterologous expression could address this issue by using a model host such as *Escherichia coli* to produce the enzymes. However, the RDase family has been notoriously difficult to study in heterologous hosts due to their complex cofactor requirements (vitamin B_{12} and [4Fe-4S] clusters), and their oxygen sensitivity.

Approach/Activities. In this work, we describe the optimization of a heterologous expression system for RDases in *E. coli*; this work was done through lab-scale trials. This system addresses the bottleneck of cofactor delivery by incorporating a vitamin B_{12} uptake (Btu) system to enhance cofactor incorporation. This expression system allows for the purification and isolated characterization of RDases, as well as the manipulation of the RDase structure. We have used this expression system to analyze how natural differences in the active sites of chloroalkane dechlorinating RDases affect their activity on different substrates.

Results/Lessons Learned. This work will highlight the utility of this novel expression system in the study of RDases for remediation purposes. In studying the isolated enzymes, activity on alternative substrates can be identified and previously unknown byproducts can be detected. We have also compared highly similar enzymes and identified varying dechlorination activity that results from a couple of amino acid differences. This knowledge is particularly important for more effective bioremediation strategies by recruiting organisms with the RDase that will have the desired activity on target contaminants. Further, we will discuss the potential for modifying RDases for more desirable activity. With the ability to modify the RDase sequence, there is the capability of designing or evolving enzymes to target different types of organohalide contaminants.