Engineering Bacteria for Superior Chromate Bioremediation

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Chromate [Cr(VI)] is a wide-spread environmental contaminant, and is particularly abundant at DOE waste sites. An attractive way to minimize its toxicity is through reduction to Cr(III) using bacteria. The long-term objective of this project is to engineer bacteria with superior capacity for chromate (and certain nuclide) remediation under field conditions. Several improvements are needed both at the enzymatic as well as cellular levels to make bacteria efficient agents of chromate bioremediation [1–4]. A critical one is minimizing chromate toxicity to remediating bacteria. In support of our previous findings that much of this toxicity arises from reactive oxygen species (ROS) generation during chromate reduction, we now show that "tight" [4,5] two-electron chromate reductases can out-compete one-electron reducers of this compound, generating much less ROS and thus constitute a safer pathway for this reduction. Studies with appropriate mutants and cloned genes demonstrate that loss or overproduction of the "tight" enzymes enhances or decreases, respectively, chromate toxicity in *P. putida* and *E. coli*. Thus, initial steps at generating strains more suitable for chromate reduction have been achieved using the principle of maximizing flow of chromate through the "safer" pathways of its reduction. Further improvements are being attempted, using the DNA shuffling approaches and appropriate screens developed in this laboratory. Initial results indicate isolation of shuffled products encoding higher turnover number chromate reductases.

To assess what effects enzyme engineering can have besides the intended improvements, it is important to know the biological role of the enzyme involved. We show [6] that the cellular role of "chromate reductases" is safe disposal of quinonoid compounds, which are abundant in the environment and in metabolic products. The "safe" chromate reductases reduce quinones by simultaneous two-electron transfer, avoiding formation of highly reactive semiquinone intermediates from one-electron reducers of quinones, and producing quinols that promote tolerance of oxidative stress. A *P. putida* strain over-expressing one of these enzymes (ChrR) recovers more quickly than wild-type in H_2O_2-amended media, whereas a *chrR* mutant takes much longer than the wild type. These growth phenotypes correlate with intracellular indicators of oxidative stress: protein carbonylation (oxidation) analyzed by Western analysis; and cellular H_2O_2 stress analyzed by FACS and the H_2O-responsive dye, H_2DCFDA. Thus, enhancing the activity of ChrR will not only increase the *P. putida* chromate reduction rate but also augment its capacity to withstand oxidative stresses of polluted sites.

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