Enhancing the Bioremediation of *Bacillus subtilis* using Trehalose

Lucas Delahanty, Class of 2010

Bioremediation is the process by which microorganisms reduce, prevent, or repair the effects of environmental contaminants. My project attempts to use the benign soil bacteria *B. subtilis* to fight groundwater hexavalent chromium pollution. *B. subtilis* naturally reduces hexavalent chromium to the significantly less toxic trivalent form, but current methods of bioremediation (with all bacteria used) have limits due to the destructive nature of reactive oxygen species generated during this process. Previous methods have generally tried to provide bacteria with optimal nutrition for growth in order to compensate for the damage sustained. Our novel approach aims to insert the trehalose producing genes OtsB and OtsA (together as the OtsBA operon) into a plasmid, which *B. subtilis* can then take up and use to generate its own trehalose. Trehalose is a compatible solute that has been proven to protect organisms against a plethora of different conditions and damaging effects, including the oxidative damage that has been implicated in the limits of bioremediation. By enabling *B. subtilis* to produce trehalose, we should be able to surpass that ceiling.

I spent my summer constructing the pHCMC02-OtsBA and pHCMC04-OtsBA plasmid shuttle vector constructs, sub-cloning them into *E. coli*, and finally transforming them into *B. subtilis*. After much difficulty I was able to successfully transform the *B. subtilis*, and began preliminary chromate stress tests. Due to contamination issues I was unable to complete the stress tests before my fellowship ended, but the initial results were promising. It appears that the strains containing the OtsBA constructs were able to reduce more chromate than non-overexpressers, especially the pHCMC04-OtsBA strain, which contains a xylose inducible promoter. The pHCMC02 plasmid contains a weak constitutive promoter. The research for concrete data that shows trehalose is indeed being produced and that it is aiding the *B. subtilis* continues. Hopefully one day the research will lead to cleaner and safer drinking water across the world, and help to unlock the barely tapped potential trehalose could have as a beneficial biochemical agent across many fields of science.

Faculty Mentor: Peter Woodruff

Funded by the INBRE Post-Bac Fellowship