

IEH Undergraduate Intern Mentoring Opportunity

Deadline: **February 22, 2013**

Selections Announced: **mid-March, 2013**

Name/Title/Institution(s) of senior mentor(s): Brad Tebo, EBS Division Head, EBS

Name/Title/Institution(s) of frontline mentor(s): Kati Geszvain, Senior Research Associate, EBS

Project Title: Investigating the regulation of Mn(II) oxidation in *Pseudomonas putida* GB-1

Context for Project:

Bacterial manganese (II) oxidation has a profound impact on the biogeochemical cycling of Mn, one of the most common transition metals in the Earth's crust, and on the availability of trace metals that adsorb to the surfaces of solid Mn (III, IV) oxides. The Tebo lab employs the genetically tractable Mn oxidizing bacterium *Pseudomonas putida* GB-1 to study the molecular mechanisms by which bacteria oxidize Mn(II) to Mn(IV). This has allowed us to identify two enzymes responsible for the oxidation reaction and regulatory pathways that affect Mn(II) oxidase activity. The details of how these pathways regulate the oxidase enzymes remain unknown.

Brief Description:

Expression of the Mn(II) oxidase genes *mnxG* and *mcoA* is regulated by the transcription factor MnxR. A strain lacking the MnxR gene ($\Delta mnxR$) fails to oxidize Mn(II), due to lack of expression of the oxidase genes. However, a substantial amount of *mcoA* transcript remains in the $\Delta mnxR$ strain. This suggests that a negative regulator prevents translation of the *mcoA* transcript in this strain. The student's research will involve performing genetic screens to identify this putative negative regulator and to investigate the pathways regulating Mn(II) oxidation in *P. putida* GB-1.

Proposed Outcomes/Broader Impact:

Understanding how Mn(II) oxidation is regulated will help us to understand the role this function plays in the physiology of the cell. For example, if starvation response pathways are identified as regulating oxidation, this would suggest that Mn(II) oxidation plays a role in acquiring nutrients under starvation conditions. Also, understanding regulation will facilitate the use of Mn(II)-oxidizing organisms in bioremediation applications.

Proposed timeline (within a 10 week span):

Make media and learn how to work with *P. putida* GB-1 – 1 week

Generate random transposon insertions in $\Delta mnxR$ and screen for restored Mn(II) oxidation – 2 weeks

Screen mutants for motility defects – 1 week

Map the sites of transposon insertion – 2 weeks

Characterize the phenotypes of the mutants – 1 week

Plasmid complementation and/or generation of in-frame deletions, if possible – 3 weeks

Intern academic experience and skill set should include: I would prefer to work with a biology major that has an emphasis on Genetics or molecular biology. This project is fairly flexible, with approaches that can be tailored to the experience of the student; therefore I would be willing to work with a younger student.