Project Number: 1020499

Project Title: The Role of Melanin Production by *Shewanella oneidensis* MR-1 in Relation to Metal and Radionuclide Reduction and Immobilization.

Principal Investigator: Charles E. Turick

Research Objective

This project address how the bacterial metabolite pyomelanin accelerates solid phase metal reduction by dissimilatory metal reducing bacteria. This physiological trait offers a means of using subsurface bacteria for detoxificaiton of metal and actinide contaminated environments, especially when metals and actinides are sorbed onto solid minerals. Pyomelanin may provide a means by which bacteria can transfer electrons to solid phase contaminants, thereby decreasing toxicity as well as mobility of contaminants in the environment. The overall hypothesis of this project is: Melanin production occurs during growth with tyrosine and contributes to electron transfer in the genus *Shewanella*. Specific goals of this project include: 1) Quantify the role of pyomelanin on solid phase metal reduction, 2) Determine environmental conditions that contribute to pyomelanin production, 3) Determine the role of pyomelanin on reduction of solid phase contaminants.

Research Progress and Implications

Overview. This report summarizes the first 2 years of research where we have quantified the role of pyomelanin on solid phase metal reduction and determined the biochemical pathway responsible for pyomelanin production. In general, this work was accomplished through biochemical assays that targeted the specific enzyme responsible for pyomelanin production and confirmed with directed mutagenesis. Quantification of enhanced electron transfer was achieved using electrochemical techniques developed in our lab that incorporate whole bacterial cells.

Progress. In order to establish the role of tyrosine catabolism in pyomelanin production and hence, hydrous ferric oxide (HFO) reduction, *S. oneidensis* MR-1 cultures were grown in a minimal medium with tyrosine in the presence or absence of the competitive inhibitor sulcotrione. Cultures with sulcotrione and tyrosine had significantly less pigmentation compared to tyrosine supplemented cultures without sulcotrione. Subsequent resting cell studies with HFO and H_2 (as electron donor) with washed cell suspensions from these cultures demonstrated enhanced HFO reduction capacities compared to cultures grown with tyrosine and sulcotrione, no tyrosine or sulcotrione only. The addition of sulcotrione alone did not affect HFO reduction rates.

Through directed mutagenesis we have shown that pyomelanin production in *S. oneidensis* MR-1 is a result of incomplete tyrosine catabolism, resulting in the excretion of the partial breakdown product homogentisic acid. This precursor to pyomelanin is auto oxidized and self polymerizes outside the cell to form pyomelanin. Tyrosine concentrations in the growth medium (including from cryptic growth) contribute to

increased levels of pyomelanin production, which results in higher rates of solid phase metal reduction. Two mutant strains were produced, a pyomelanin deficient strain (*Mela*$^-$) and a pyomelanin over-producer (*Hmga*$^-$). HFO reduction was enhanced with the pyomelanin over-producer and diminished with the pyomelanin deficient strain when compared to the wild type strain (MR-1) (Fig. 1). Addition of purified pyomelanin enhanced HFO reduction capacity by the pyomelanin deficient strain (Fig. 1).

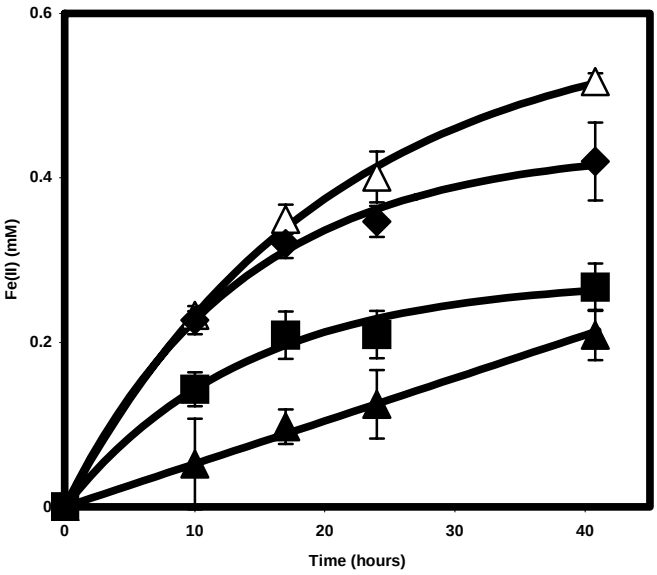

Figure 1. HFO reduction by 3 strains of *S. oneidensis*. Resting cell studies of cells pregrown in LBSM + 300 mg/l tyrosine demonstrated increased HFO reduction efficiency by the pyomelanin over-producing strain (*Hmga*$^-$) (♦) relative to MR-1(■) and the pyomelanin deficient mutant (*Mela*$^-$) (▲), which demonstrated the least amount of HFO reduction. Addition of pyomelanin (20μg/ml) to resting cell cultures of the pyomelanin deficient mutant (Δ) demonstrated drastically increased levels of HFO reduction. In a parallel study where tyrosine was omitted from the medium, HFO reduction rates did not vary significantly between these strains (data not shown).

In order to more precisely characterize the role of pyomelanin as a surface associated electron shuttle, electrochemical studies were designed for whole bacterial cells. Cyclic voltammetry comprises electrochemical techniques that provide information of the electrochemical activities of materials by sweeping the potential back and forth at predetermined rates. Cyclic voltammetry demonstrated redox activity of whole cell suspensions was a function of pyomelanin content. Pyomelanin increased the formal potential of redox couples of the wild type by 82mV relative to the melanin minus strain. To confirm the effect of pyomelanin on electron transfer, pyomelanin addition to the melanin-minus mutant resulted in a shift of the redox potential in the positive direction relative to the same strain without pyomelanin. The melanin minus strain with supplemental pyomelanin demonstrated a redox couple similar to that of the wild type and the pyomelanin overproducing strain. The pyomelanin over-producer demonstrated 2 redox couples, suggesting that pyomelanin effects existing electron transfer mechanisms and when present in excess, provides an addition mechanism for electron transfer for this strain. Pyomelanin alone demonstrated redox activity similar to that of the second redox couple of *HmgA*$^-$ but had a higher formal potential. The difference in redox couples between the pyomelanin overproducer and pyomelanin alone may be a result of pyomelanin integration onto the bacterial membrane.

The second phase of the research involves the role of pyomelanin on U(VI) and Cr(VI) when sorbed to minerals. To date we have evidence that both U(VI) and Cr(VI) are reduced on the surface of minerals by soluble (cell-free), reduced pyomelanin. We will also examine the role of melaninized and non-melaninized bacterial cells on solid-phase metal and actinide reduction.

Implications. To date we have shown that partial tyrosine catabolism has potential to increase solid phase metal reduction rates in pyomelanin-producing, dissimilatory metal reducing bacteria. Related research in our lab has shown that pyomelanin production is common to subsurface bacteria and stimulation of pyomelanin production in uranium contaminated soils increases redox activity in the soil effluents as well as immobilizing uranium, relative to controls.

Planned Activities

Ongoing research activities involve understanding electron transfer and metal sequestration properties of pyomelanin. We are examining solid-phase Cr(VI) and U(VI) reduction by cell-free and cell-associated pyomelanin Solid-phase reduction of Cr(VI) and U(VI) is being examined with the minerals goethite and diaspore. Because diaspore is isostructural with goethite with similar surface charge and hydrophobicity, it allows us to differentiate between pyomelanin reduction and Fe(II) influenced processes. This work is being conducted jointly at SRNL and the National Synchrotron Light Source Facility and is planned to conclude in September 2007.

Information Access

Publications

C. E. Turick, F. Caccavo Jr, and L. S. Tisa. Pyomelanin is Produced by *Shewanella algae* BrY and Effected by Exogenous Iron. Submitted, 2006.

C. E. Turick, A. S. Knox, C. L. Leverette, and Y. G. Kritzas. In-situ Uranium Stabilization by Microbial Metabolites. Submitted, 2006.

Invited Talks

C. E. Turick, A. Beliaev, A. A. Ekechukwu, D. A. Lowey. April, 2006. Electron transfer at the microbe/mineral interface is mediated by the extracellular pigment pyomelanin. American Chemical Society National Meeting, Atlanta, GA.

C. E. Turick, A.S. Knox, T. Poppy, A. Maloney, Y.G. Kritzas and C. L. Leverette. September, 2006. Electrochemically-active metabolite production accelerates metal reduction and immobilization in a variety of bacterial genera. American Chemical Society National Meeting, San Francisco, CA.

C. E. Turick and A. S. Knox. November, 2006. Microbial melanin production enhances in-situ uranium immobilization. American Chemical Society Regional Meeting, Augusta, GA.

Poster Presentations.

C. E. Turick, Y. G. Kritzas. 2005. Microbial Metabolite Sharing Accelerates Insoluble Metal Reduction by a Variety of Bacterial Genera. General Meeting of the American Socieity for Microbiology, Atlanta, GA.

C. E. Turick, A. Beliaev, T. E. Poppy, A. M. Maloney, B. A. Zakrajsek. 2005. The Role of Melanin Production by *Shewanella oneidensis* MR-1 on Hydrous Ferric Oxide Reduction. General Meeting of the American Socieity for Microbiology, Atlanta, GA.