The Role and Regulation of Melanin Production by *Shewanella oneidensis* MR-1 in Relation to Metal and Radionuclide Reduction/Immobilization

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The biotransformation of metals and radionuclides by subsurface bacteria offers considerable potential for environmental remediation of contaminated sites. The effectiveness of bioremedial technologies will increase, provided the biological mechanisms of metal reduction are better understood and manipulated for optimum benefit. Metal-reduction rates are accelerated by dissimilatory metal-reducing bacteria in the presence of humic compounds. This is a result of the redox cycling nature of humics as a function of their quinone moieties. *Shewanella algae* BrY and *S. oneidensis* MR-1 produce melanin, a pigment common to many microorganisms. Melanin is classified as a humic compound that has quinone moieties and redox cycling behavior. Under anaerobic conditions, melanin serves as a terminal electron acceptor and soluble electron shuttle to iron minerals. Melanin is also detected on the bacterial surface, which results in increased iron mineral reduction rates, by as much as tenfold.

Melanin production by *S. colwelliana* is a product of the *melA* gene, which encodes for the enzyme p-hydroxyphenyl pyruvate dioxygenase (pHPPD) and is part of the phenylalanine/tyrosine pathway. For melanin to be produced through this pathway, tyrosine is broken down to p-hydroxyphenyl pyruvate, then to homogentisic acid, which is then excreted from the cell. Outside the cell, homogentisic acid is autooxidized and self-polymerized to form quinone-rich melanin polymers. A nucleotide sequence similar to the *melA* gene of *S. colwelliana* was detected on the genome of *S. oneidensis* MR-1 via a BLAST search, revealing a 92.5% amino acid similarity to *melA*. Other genes responsible for melanin production, such as tyrosinase, were not detected on the *S. oneidensis* MR-1 genome.

Melanin production by *S. oneidensis* MR-1 may be an important mechanism for electron transfer to insoluble metals. To evaluate the role melanin plays in metal reduction, melanin production will be inhibited under various growth conditions with the triketone sulcotrione [2-(2-chloro-4-methane sulfonylbenzoyl)-1,3-cyclohexanedione], a pHPPD inhibitor. In addition, deletion mutants, deficient in melanin production, will be developed by deletion mutagenesis of the *melA* gene. The resulting cultures will be evaluated for soluble and insoluble metal-reduction capabilities. These results will determine the significance of melanin production as a mechanism for metal reduction. Since melanin production from *S. oneidensis* MR-1 requires extracellular homogentisic acid, the role of the Type II secretion system in melanin production will be determined using insertion mutagenesis techniques.