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

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Bacteria of the genus *Shewanella* grow by transferring electrons to soluble and insoluble metals for energy production. When *Shewanella* transfer electrons to toxic metals and radionuclides, these compounds become less mobile and toxic in the environment. A better understanding of the biomolecular mechanisms involved in the electron transfer to insoluble metals by *Shewanella* offers potential in developing new strategies for bioremediation of contaminated sites.

The pigment pyomelanin is particularly important in this process, and is produced by *Shewanella oneidensis* MR-1. In the presence of excess pyomelanin, *S. oneidensis* MR-1 reduces the insoluble Fe(III)-oxide, hydrous ferric oxide (HFO), at a greater rate than without additional pyomelanin. This is accomplished because, under anaerobic conditions, pyomelanin serves as a soluble electron shuttle to insoluble minerals.

The overall hypothesis of this work is that pyomelanin production in the genus *Shewanella* plays a significant role as a mechanism of metal and radionuclide reduction and immobilization, and its production can be manipulated with the addition of proper nutrients. By understanding the role and regulation of pyomelanin production in microorganisms, remediation of metal- and radionuclide-contaminated environments may be accelerated. We are concentrating our studies on *S. oneidensis* MR-1, the type organism of this genus.

To date we have demonstrated the inhibition of pyomelanin production by *S. oneidensis* MR-1 using sulcotrione [2-(2-chloro-4-methane sulfonylbenzoyl)-1,3-cyclohexanedione], a competitive inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase (4HPPD) that is responsible for pyomelanin production in MR-1. Using the suicide vector system (pDS3.1) developed in our lab we generated a 700 bp deletion in the gene *melA*, which encodes for 4PHPPD. The *melA*$^-$ mutant was unable to produce pyomelanin. In addition, we have generated a pyomelanin-overproducing mutant through a deletion in the gene *hmgA*, which encodes for homogentisic acid oxidase. Both homogentisic acid oxidase and 4PHPPD are involved in tyrosine catabolism. Our studies demonstrated that HFO reduction decreased but was not completely halted with sulcotrione-treated cells or in the absence of *melA*. The amount of HFO reduction increased with the pyomelanin overproducing mutant. The addition of pyomelanin to the pyomelanin deficient mutant increased HFO reduction. Our results also show that pyomelanin production is a function of tyrosine concentration in the growth medium and plays a significant role in HFO reduction but is not the only mechanism involved in this process.