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

Charles E. Turicck² and Alex Belayev²

²Biotechnology Section, SRNL, 999W, Aiken, SC. 29808. (803)-819-8407. Fax:(803)-819-8416. Email: Charles.Turicck@srnl.doe.gov

²Environ. Microbiol. Group, PNNL, P.O. Box 999 MSIN 77-50, Richland, WA 99352. (509)-376-4183. Fax: (509)-376-9650. Email: Alex.Belayev@pnnl.gov

**Abstract**

Melanin in the genus *Shewanella* plays a significant role in the reduction and immobilization of a variety of metals and radionuclides. *Shewanella* species are known to reduce iron, uranium, and actinides in the environment through microbially induced ferro- and uranium reduction. Melanin is produced by *Shewanella* species as a byproduct of the degradation of aromatic compounds, and functions as a protective pigment in the environment and as a surface-associated molecule that facilitates electron transfer to metals. The pigment melanin is a particularly attractive target for remediating metal and radionuclide contaminated sites.

**Inhibition Studies**

**Objectives**

- Determine the impact of Fe(III) oxide reduction by melanin produced by *S. oneidensis* MR-1.
- Prevent melanin production with enzyme inhibition.
- Develop a mutant deficient in melanin production by deletion of the melA gene.
- Develop a melanin-expressing producer by deletion of the 4HPPD gene.
- Determine the rate and degree Fe(III) oxide reduction when melanin production is prevented and when it is over produced.
- Relate the results to previous, similar studies using minimal or nutrient rich growth media.

**Mutagenesis studies**

**Gene organization of the melA locus on the *S. oneidensis* MR-1 chromosome.**

![Gene organization of the melA locus on the *S. oneidensis* MR-1 chromosome.](image)

**Conclusions**

Melanin production is prevented by inhibition of 4-hydroxyphenylpyruvate dioxygenase activity and by deletion of the melA gene, which encodes the 4-hydroxyphenylpyruvate dioxygenase enzyme.

Melanin production is dependent on electron donation to metals that are growing in rich nutrient media.

A decrease in the level of melanin results in decreased Fe(III) oxide reduction when cells are grown in rich nutrient media.

In the absence of melanin, metal reduction is still possible, indicating the presence of other mechanisms of metal reduction.

Melanin production is a result of the tyrosine concentration in nutrient rich media, which causes a decrease in melanin production.

Melanin may be produced by *S. oneidensis* MR-1 as a byproduct of standard growth media.

In previous Fe(III) oxide reduction studies with *Shewanella* that incorporated either rich nutrient media (such as tryptone soy broth) or minimal media (such as LBSM), melanin may have contributed to a portion of metal reduction reported.