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Extracellular Organic Disulfide Reduction by *Shewanella oneidensis*

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Background: Thiol/disulfide redox recycling by *Shewanella oneidensis* has been shown to facilitate the reduction of Fe(III) minerals such ferrihydrite and smecitite clays. Thiol-mediated Fe(III) mineral dissolution is driven by enzymatic disulfide reduction, whereby reduced thiols act as a conduit for electron transfer to minerals surfaces. Currently, the mechanism of extracellular disulfide reduction is poorly understood.

Materials and Methods: Experiments were conducted to identify the genes involved in the extracellular reduction of disulfide bonds. We employed transposon mutagenesis to isolate a *S. oneidensis* mutant impaired in organic disulfide reduction. Mutant screening was performed using the cell-impermeable disulfide compound 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). The reduction of the disulfide bond in DTNB produces 2-nitro-5-thiobenzoate (TNB) which is a yellow-colored product that can be quantified spectrophotometrically. After isolating a mutant deficient in DTNB reduction, the location of the transposon insertion was identified, and genetic complementation was carried out to restore the loss of disulfide reduction activity.

Results and Discussion: The screening of 9,348 transposon mutants resulted in the isolation of a mutant strain that lost ~90% of its DTNB reduction activity. Genome sequencing of mutant strain revealed that the transposon was inserted into the *dsbD* gene which encodes for an oxidoreductase involved in cytochrome *c* maturation. Complementation of the mutant strain with the wild-type *dsbD* partially restored DTNB reduction activity. Because DsbD catalyzes a critical step in the assembly of multi-heme *c*-type cytochromes, we further investigated the role of extracellular electron transfer cytochromes in organic disulfide reduction. The results indicated that mutants lacking proteins in the Mtr system were severely impaired in their ability to reduce DTNB. These findings provide new insights into extracellular organic disulfide reduction and the enzymatic pathways of thiol-mediated Fe(III) mineral dissolution.