

## Molecular Mechanism of Microbial Iodate Reduction

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**Background/Objectives.** An attractive method for remediation of radioactive iodine-contaminated subsurface environments is liquid extraction and trapping volatile iodine gases via microbially-catalyzed iodine redox transformations, including microbial iodate ( $\text{IO}_3^-$ ) reduction, which is a major component of the iodine biogeochemical reaction network in iodate-contaminated subsurface environments.  $\text{IO}_3^-$  is postulated to be reduced by microbial nitrate reductase (NR), although this hypothesis has not been extensively investigated. However, previous gene deletion results have demonstrated that NR is not the primary enzymatic mechanism for iodate reduction by *Shewanella oneidensis*. In the present study, metal respiratory (*mtr*) pathway mutants of *S. oneidensis* were examined for  $\text{IO}_3^-$  reduction activity to identify the components shared by the metal, nitrate, and  $\text{IO}_3^-$  reduction systems of *S. oneidensis*.

**Approach/Activities.** Ten *S. oneidensis* *mtr* mutants were anaerobically cultivated in defined minimal medium (M1) containing lactate, formate or  $\text{H}_2$  as electron donor and 250 mM  $\text{IO}_3^-$  as electron acceptor at 30°C to determine iodate reduction activities. Chemical (EMS) or biological (Tn5) mutagenesis were used for selection of  $\text{IO}_3^-$  reduction-deficient mutants and mutated loci were identified by whole genome-sequencing. Mutated genes required for  $\text{IO}_3^-$  reduction were confirmed by in-frame gene deletion and  $\text{IO}_3^-$  reduction activity analyses. Methyl halide transferase (MHT) genes of *S. oneidensis* were individually expressed in *E. coli* to identify MHT gene responsible for production of volatile organoiodine compounds such as iodomethane.

**Results/Lessons Learned.** Ten *mtr* or protein secretion mutants of *S. oneidensis* were anaerobically cultivated with lactate as the electron donor. *DmtrA* and *DmtrB* mutants could not reduce  $\text{IO}_3^-$ , but *mtrC*, *omcA* and *omcA-mtrC* mutants retained  $\text{IO}_3^-$ -reduction activity. Also, type II protein secretion mutant *DgspD* did not reduce  $\text{IO}_3^-$ . These results indicate that *mtr* and type II protein secretion pathway components are involved in  $\text{IO}_3^-$ -reduction. However, when formate or  $\text{H}_2$  was used for electron donor, *mtr* mutants of *S. oneidensis* displayed wild-type  $\text{IO}_3^-$ -reduction activity, while *DgspD* could not reduce  $\text{IO}_3^-$ . This result indicates that *S. oneidensis* employs separate electron donor-dependent *mtr* pathways for  $\text{IO}_3^-$  reduction.