

## Identification of the Iodate Terminal Reductase in Metal-Reducing Bacteria

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**Background/Objectives.** An attractive method for remediation of radioactive iodine-contaminated subsurface environments is liquid extraction and trapping of volatile iodine gases via microbially-catalyzed iodine redox transformation and methylation reactions. Microbial iodate ( $\text{IO}_3^-$ ) reduction is a major component of the iodine biogeochemical reaction network in iodine-contaminated subsurface environments. We previously discovered that the metal (but not nitrate) reduction pathway is required for  $\text{IO}_3^-$  reduction by the metal-reducing bacterium *Shewanella oneidensis*. The identity of the terminal  $\text{IO}_3^-$  reductase, however, remains unknown. In the present study, a variety of metal (*mtr*) reduction pathway paralog mutants of *S. oneidensis* were examined for  $\text{IO}_3^-$  reduction activity to identify potential candidate enzymes catalyzing the terminal  $\text{IO}_3^-$  reduction reaction by *S. oneidensis* and other metal-reducing members of the *Shewanella* genus.

**Approach/Activities.** Six *S. oneidensis mtr* paralog mutants (*DmtrA-DmtrDEF*, *DmtrA-DdmsEF*, *DmtrA-DSO\_4360*, *DmtrF*, *DdmsB* and *DSO\_4357-4358*) were constructed by in-frame gene deletion mutagenesis. The  $\text{IO}_3^-$  reduction activity of the six *mtr* deletion mutants was tested in batch liquid cultures containing defined minimal medium amended with either formate or lactate as electron donor and  $\text{IO}_3^-$  as anaerobic electron acceptor.

**Results/Lessons Learned.** The *mtr* deletion mutants *DmtrA* and *DmtrB* were unable to reduce  $\text{IO}_3^-$  with lactate as electron donor, but retained wild-type  $\text{IO}_3^-$  reduction activity with formate as electron donor. This result indicated that one of *mtr* paralogs may be required for  $\text{IO}_3^-$  reduction with formate as electron donor. Among the three *mtr* paralog mutants (*DmtrA-DmtrDEF*, *DmtrA-DdmsEF* and *DmtrA-DSO\_4360*), only *DmtrA-DdmsEF* mutant displayed a deficiency in  $\text{IO}_3^-$  reduction activity with formate as electron donor. This finding suggests that the DMSO reductase system is also involved in  $\text{IO}_3^-$  reduction. Subsequent  $\text{IO}_3^-$  reduction activity tests of three *mtr* paralog mutants (*DmtrF*, *DdmsB* and *DSO\_4357-4358*) demonstrated that *DdmsB* was unable to reduce  $\text{IO}_3^-$  while *DmtrF* and *DSO\_4357-4358* retained wild-type  $\text{IO}_3^-$  reduction activity. Together these results provide complementary genetic and phenotypic evidence that the *S. oneidensis* DMSO terminal reductase displays broad substrate specificity and reduces  $\text{IO}_3^-$  as an alternative terminal electron acceptor.