

Abstract

Shewanella oneidensis MR-1 is a facultatively anaerobic gamma-proteobacterium that possesses diverse respiratory capacities, including the ability to reduce Cr(VI) to Cr(III). Chromate is a widespread anthropogenic pollutant and is found at unacceptable levels in subsurface sediments and groundwater at U.S. Department of Energy (DOE) sites. In situ microbial bioreduction of Cr(VI) to Cr(III) may serve as a potential strategy for detoxification and immobilization of chromate. However, effective bioremediation depends on an understanding of the genetic pathways underlying heavy metal resistance and biotransformation. Towards this goal, previous studies employing genomic profiling and proteomic analyses were performed to characterize the dynamic molecular response of MR-1 to acute chromate challenge. A gene encoding a putative azoreductase (SO3585) was substantially upregulated at both the mRNA and protein levels in response to acute chromate exposure. Protein database searches using the derived SO3585 primary sequence revealed approximately 28% sequence identity with *Pseudomonas putida* ChrR and *Escherichia coli* YieF, two soluble flavoproteins that have been demonstrated to exhibit chromate reductase activity. SO3585 contains the conserved structural domain characteristic of NADPH-dependent FMN reductases. We have created an in-frame deletion of *so3585* in MR-1 using a *cre-lox*-based recombination system and have characterized the phenotype of the resulting mutant in the presence of varying concentrations of chromate under aerobic conditions. The *so3585* deletion mutant resembled the wild-type strain in terms of growth; however, the mutant was able to reduce chromate at a substantially faster rate compared to the wild-type strain. Based on its genomic proximity and co-regulated expression profile, we predict that SO3585 functions in a complex together with the proteins SO3586 (glyoxalase family) and membrane-associated SO3587 (hypothetical protein). Studies are underway to purify SO3585, to determine whether it interacts with SO3586 and SO3587 and can reduce Cr(VI).

EXPERIMENTAL

- S. oneidensis* MR-1 and mutant strains were grown in Luria-Bertani (LB) medium in presence or absence of different concentrations of added metal at 30°C under aerobic conditions. Growth was monitored using a Bioscreen C microbiological culture system (Growth Curves USA). Cr(VI) reduction was measured spectrophotometrically at wavelength 540 nm using the 1,5-diphenylcarbazide (DPC) method (Park, C. H., M. Keyhan, S. Wilemga, S. Fendorf, and A. Matin. 2000. Purification to homogeneity and characterization of a novel *Pseudomonas putida* chromate reductase. *Appl. Environ. Microbiol.* 66:1788-1795).
- An in-frame deletion of the *so3585* (FIG. 1) in the MR-1 chromosome was created using a *cre-lox*-based recombination system (Deneef VJ, Klappenbach JA, Patrauchan MA, Florizone C, Rodrigues JL, Tsou TV, Verstraete W, Ellis LD, Tiedje JM. 2006 Genetic and genomic insights into the role of benzoate-catabolic pathway redundancy in *Burkholderia xenovorans* LB400. *Appl Environ Microbiol.* 72(1):585-593).
- Our previous transcriptomic profiling and whole-cell proteomic analyses of MR-1 under chromate stress conditions identified a putative azoreductase (SO3585) as playing a potential role in the cellular response to Cr(VI) stress. Microarray experiments are described in detail in the publication below. Both the transcripts and encoded proteins were found to be up-regulated at high levels under Cr conditions (S.D. Brown, M.R. Thompson, N.C. VerBerkmoes, K. Chourey, M. Shah, J. Zhou, R.L. Hettich, and D.K. Thompson. 2006. Molecular dynamics of the *Shewanella oneidensis* response to chromate stress. *Molecular and Cellular Proteomics*, in press).
- The full-length *so3585* gene was cloned into the pTrcHis-TOPO[®] vector (Invitrogen), and a recombinant 6xHis-tagged SO3585 was overexpressed in *E. coli* host cells. Expression of the recombinant protein was verified by Western blot analysis using an antibody against an N-terminal peptide containing the HisG epitope.

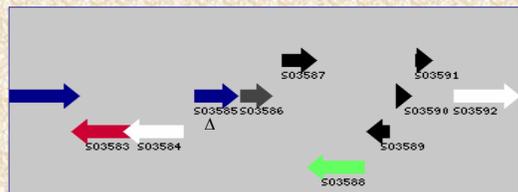


FIG. 1

RESULTS

TABLE 1. Transcriptome Profiling under Conditions of Cr(VI) Challenge

ORF	Gene Product	Time=	5 min ^a	30 min ^a	60 min ^a	90 min ^a	3 h ^b	24 h ^c
so3585	azoreductase, putative		5.6	60.9	28.2	30.1	52.6	0.7
so3586	glyoxalase family protein		3.8	26.4	16.1	13.1	14.1	0.5
so3587	hypothetical protein		3.7	17.5	10.4	14.2	3.2	0.9

- ^aRelative gene expression (fold induction) 5, 30, 60, and 90 min post-1 mM chromate addition. No Cr(VI) reduction measured.
- ^bRelative gene expression (fold induction) 3 h post-0.3 mM chromate addition. Cells actively reducing Cr(VI).
- ^cRelative gene expression (fold induction) 24 h post-0.3 mM chromate addition. Complete Cr(VI) reduction.

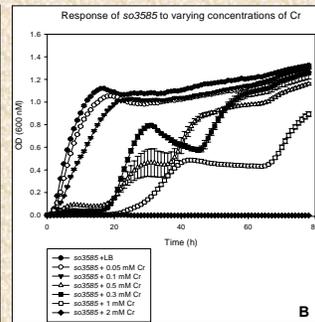
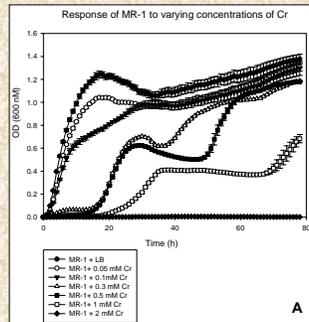


FIG. 2. Growth of MR-1 (A) and deletion mutant Δ so3585 (B) in LB in the absence or presence of different chromate concentrations.

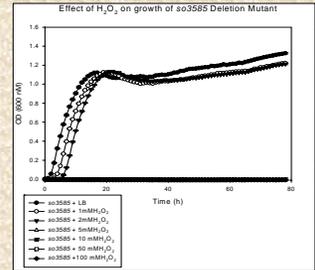
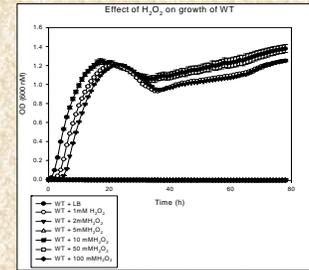
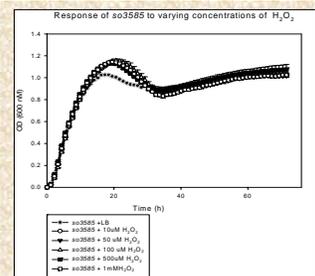
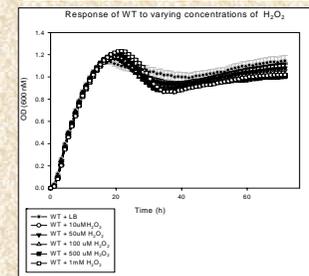


FIG. 4. Growth of MR-1 and deletion mutant Δ so3585 in LB in the absence or presence of different H₂O₂ concentrations.

Mean (K₂CrO₄/Control)

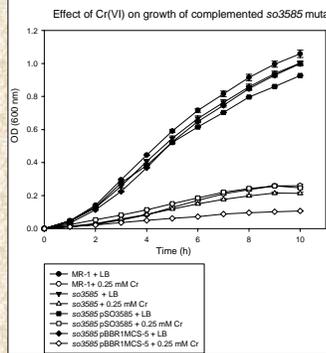


FIG. 3. Restoration of growth phenotype in complemented mutant Δ so3585 in LB amended with 0.250 mM chromate.

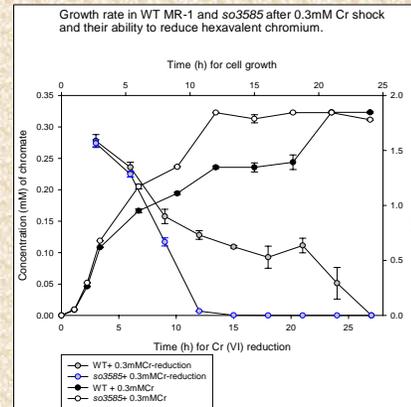


FIG. 5. Growth and Cr(VI) disappearance patterns for *S. oneidensis* MR-1 and the *so3585* deletion mutant. No abiotic conversion of chromate was detected in the LB broth-only control (data not shown).

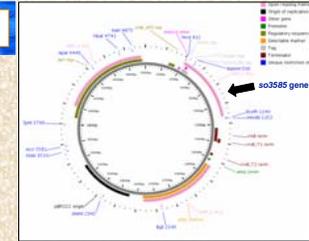


FIG. 6. Cloning of the MR-1 *so3585* gene into the pTrcHis expression vector (Invitrogen).

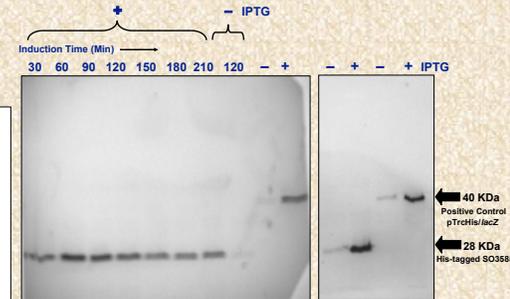


FIG. 7. Western blot analysis of recombinant His-tagged SO3585 overexpressed in *E. coli* Top10 cells using the anti-HisG HRP conjugate antibody. The pTrcHis vector alone (negative control) showed no Ab binding (result not shown).

SUMMARY

- We created an MR-1 mutant strain harboring an in-frame deletion of *so3585* (annotated as encoding a putative azoreductase) and characterized the mutant phenotypes compared to MR-1.
- Interestingly, the *so3585* mutant was able to reduce Cr(VI) at a substantially faster rate compared to the WT strain (Fig. 5).
- A preliminary pull-down experiment failed to show any interactions between members of the complex and future studies will examine a different bait protein and growth conditions.
- Future studies will focus on the purification and biochemical characterization of the putative azoreductase.

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- Project web site: http://compbio.ornl.gov/shewanella_chromium_stress/