Metal Reduction and Protein Secretion Genes Required for Iodate Reduction by *Shewanella oneidensis*

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**ABSTRACT** The metal-reducing gammaproteobacterium *Shewanella oneidensis* reduces iodate (IO$_3^-$) as an anaerobic terminal electron acceptor. Microbial IO$_3^-$ electron transport pathways are postulated to terminate with nitrate (NO$_3^-$) reductase, which reduces IO$_3^-$ as an alternative electron acceptor. Recent studies with *S. oneidensis*, however, have demonstrated that NO$_3^-$ reductase is not involved in IO$_3^-$ reduction. The main objective of the present study was to determine the metal reduction and protein secretion genes required for IO$_3^-$ reduction by *Shewanella oneidensis* with lactate, formate, or H$_2$ as the electron donor. With all electron donors, the type I and type V protein secretion mutants retained wild-type IO$_3^-$ reduction activity, while the type II protein secretion mutant lacking the outer membrane secretin GspD was impaired in IO$_3^-$ reduction. Deletion mutants lacking the cyclic AMP receptor protein (CRP), cytochrome maturation permease CcmB, and inner membrane-tethered c-type cytochrome CymA were impaired in IO$_3^-$ reduction with all electron donors, while deletion mutants lacking c-type cytochrome MtrA and outer membrane β-barrel protein MtrB of the outer membrane MtrAB module were impaired in IO$_3^-$ reduction with only lactate as an electron donor. With all electron donors, mutants lacking the c-type cytochromes OmcA and MtrC of the metal-reducing extracellular electron conduit MtrCAB retained wild-type IO$_3^-$ reduction activity. These findings indicate that IO$_3^-$ reduction by *S. oneidensis* involves electron donor-dependent metal reduction and protein secretion pathway components, including the outer membrane MtrAB module and type II protein secretion of an unidentified IO$_3^-$ reductase to the *S. oneidensis* outer membrane.

**IMPORTANCE** Microbial iodate (IO$_3^-$) reduction is a major component in the biogeochemical cycling of iodine and the bioremedialation of iodine-contaminated environments; however, the molecular mechanism of microbial IO$_3^-$ reduction is poorly understood. Results of the present study indicate that outer membrane (type II) protein secretion and metal reduction genes encoding the outer membrane MtrAB module of the extracellular electron conduit MtrCAB are required for IO$_3^-$ reduction by *S. oneidensis*. On the other hand, the metal-reducing c-type cytochrome MtrC of the extracellular electron conduit is not required for IO$_3^-$ reduction by *S. oneidensis*. These findings indicate that the IO$_3^-$ electron transport pathway terminates with an as yet unidentified IO$_3^-$ reductase that associates with the outer membrane MtrAB module to deliver electrons extracellularly to IO$_3^-$.

**KEYWORDS** *Shewanella oneidensis*, iodate, iodine, metals, reduction

Iodine is a biologically active element commonly found in freshwater and marine environments in the forms of iodide (I$^-$; −1 oxidation state) and iodate (IO$_3^-$; +5 oxidation state) (1). IO$_3^-$ is more thermodynamically stable than I$^-$, yet I$^-$ is the predominant form in the environment, potentially indicating that microbial IO$_3^-$

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reduction is a major component of the iodine biogeochemical reaction network (1–3). In marine environments, microbial IO$_3^-$ reduction is the primary mechanism for IO$_3^-$ reduction to I$^-$ (4–8). Microbial IO$_3^-$ reduction has also attracted interest as a component of alternative strategies for remediation of waters and sediments contaminated with radioactive iodine released to the environment (9). The nuclear waste product and radioactive isotope $^{129}$I is produced during uranium and plutonium fission reactions and displays a half-life of $1.6 \times 10^7$ years (10). Following the 2011 Fukushima nuclear reactor catastrophe, westerly winds deposited a large portion of the radioactive iodine in the Pacific Ocean, where radioactive IO$_3^-$ and I$^-$ were the predominant $^{129}$I forms (11–13). Radioactive iodine is also found in contaminated groundwater at the U.S. Department of Energy Savannah River and Hanford sites (9, 13, 14). Despite the human health concerns surrounding the fate and transport of radioactive iodine in the environment, the molecular mechanism of microbial IO$_3^-$ reduction remains poorly understood (15).

IO$_3^-$-reducing microorganisms include the facultative anaerobe *Shewanella oneidensis*, which reduces a wide range of terminal electron acceptors, including oxidized forms of iron, manganese, nitrogen, sulfur, uranium, plutonium, technetium, and iodine (16–18). *S. oneidensis* also transfers electrons to a variety of extracellular electron acceptors, including Fe(III), Mn(III), and Mn(IV) oxides (19, 20). To transfer electrons to external Fe(III) oxides, *S. oneidensis* employs a variety of novel respiratory strategies, including (i) direct enzymatic reduction via decaheme c-type cytochromes associated with the extracellular electron conduit (EEC) located on the surface or surface extensions of the *S. oneidensis* outer membrane (21–23), (ii) extracellular electron transfer via endogenous or exogenous electron shuttling compounds (24–26), and (iii) nonreductive Fe(III) solubilization by organic ligands to produce more readily reducible soluble organic Fe(III) complexes (27–29).

Previous findings for other IO$_3^-$-reducing microorganisms indicated that nitrate (NO$_3^-$) reductase may catalyze the reduction of IO$_3^-$ as an alternative electron acceptor (30–32). However, neither assimilatory nor dissimilatory NO$_3^-$ reductases are required for IO$_3^-$ reduction by *S. oneidensis* (33). The molecular mechanism of IO$_3^-$ reduction by *S. oneidensis* has yet to be examined. The electron transport pathways of *S. oneidensis* consist of upstream dehydrogenases linked via the menaquinone pool and the inner membrane-tethered c-type cytochrome CymA to downstream terminal reductase complexes, including the metal-reducing EEC (19, 34, 35). The *S. oneidensis* EEC is comprised of outer membrane β-barrel protein MtrB (and essential cysteine residue C42) (36) and decaheme c-type cytochromes MtrA and MtrC (34, 37–40). MtrC is translocated to the outside face of the outer membrane through GspD, the outer membrane secretin of the type II protein secretion system (21, 41, 42). Other proteins essential for electron transport to external metal oxides include the c-type cytochrome maturation permease CcmB (43) and the cAMP receptor protein (CRP), required for anaerobic respiratory gene expression in *S. oneidensis* (44).

Although NO$_3^-$ reductase is not required for IO$_3^-$ reduction by *S. oneidensis*, identification of metal reduction and protein secretion genes involved in this process will aid in development of biomarkers to examine the potential for microbial IO$_3^-$ reduction, a prominent process in iodine cycling in natural and contaminated environments such as the $^{129}$I-contaminated Hanford and Savannah River sites. Likewise, such biomarkers could be used to track $^{129}$I cycling in a pump-and-treat system currently treating contaminated groundwater at Hanford. Formation of I$^-$ could lead to increased adsorption onto organic material, such as granular activated carbon (GAC) in a fluidized bed reactor (FBR) that is part of the pump-and-treat process, or by organic matter in environmental systems such as those found at Savannah River. The main objective of the present study was to test the hypothesis that the *S. oneidensis* metal reduction and protein secretion pathways required for Fe(III), Mn(III), and Mn(IV) oxide reduction are also involved in IO$_3^-$ reduction. The experimental strategy to test the hypothesis included (i) construction of additional *S. oneidensis* gene deletion mutants lacking metal reduction and protein secretion pathway components and (ii) tests of the
battery of metal reduction and protein secretion pathway mutants for IO$_3^-$ reduction activity.

RESULTS

Effect of electron donor on IO$_3^-$ reduction activity by the S. oneidensis wild-type strain. A set of anaerobic incubations with batch cultures of the S. oneidensis wild-type strain was carried out to determine the optimum IO$_3^-$ concentration that avoided IO$_3^-$ or produced I$^-$ toxicity and maximized the IO$_3^-$ reduction activity of the S. oneidensis wild-type strain at cell densities of 10$^8$ ml$^{-1}$. IO$_3^-$ concentrations of 500 $\mu$M inhibited IO$_3^-$ reduction activity, while 250 $\mu$M was the optimum initial IO$_3^-$ concentration (Fig. 1). The IO$_3^-$ reduction activities of the S. oneidensis wild-type strain with lactate and formate as electron donors were similar (512 and 455 nmol h$^{-1}$ mg of protein$^{-1}$, respectively), while the IO$_3^-$ reduction activity with H$_2$ as the electron donor was approximately 4-fold lower (120 nmol h$^{-1}$ mg of protein$^{-1}$) (Table 1). The extents of reaction (of the initial 250 $\mu$M IO$_3^-$ starting concentration) for IO$_3^-$ reduction by the S. oneidensis wild-type strain with lactate and formate as electron donors were similar (512 and 455 nmol h$^{-1}$ mg of protein$^{-1}$, respectively), while the IO$_3^-$ reduction activity with H$_2$ as the electron donor was approximately 4-fold greater than the extent of reaction with H$_2$ as the electron donor (16%) (Table 1).

IO$_3^-$ reduction activity of S. oneidensis EEC mutant strains. The IO$_3^-$ reduction activity of the S. oneidensis EEC mutant strains was determined with either lactate, formate, or H$_2$ as the electron donor. The ΔmtrB and mtrB-C42A site-directed mutant strains were severely impaired in IO$_3^-$ reduction activity with lactate as the electron donor (3% and 7% of the wild-type rate, respectively, and 2% and 7% of the wild-type extent of reaction, respectively). In contrast, the ΔmtrB mutant strain retained wild-type IO$_3^-$ reduction activity with formate or H$_2$ as the electron donor (107% and 145% of the wild-type rate, respectively) (Fig. 2; Table 1). The IO$_3^-$ reduction activity of the ΔmtrB mutant strain was restored to wild-type rates by providing a wild-type copy of mtrB in trans. With lactate as the electron donor, the ΔmtrB/mtrB transconjugant strain reduced IO$_3^-$ at a rate almost 2-fold higher (179% of the wild-type rate) than that of the wild-type strain and displayed an extent of reaction approximately 143% of that of the wild-type strain (Fig. 2B; Table 1). In contrast, the mtrB-C45A site-directed mutant reduced IO$_3^-$ at near wild-type rates (96% of the wild-type rate) and displayed a near wild-type extent of reaction (102% of the wild-type extent of reaction). The ΔmtrA mutant was also severely impaired in IO$_3^-$ reduction activity with lactate as the electron donor (20% of the wild-type rate) and displayed a significantly lower extent of reaction (16% of the wild-type extent of reaction) (Fig. 2A; Table 1). However, with formate as the electron donor, the ΔmtrA mutant reduced IO$_3^-$ at near wild-type rates (82% of the wild-type rate) and displayed a near wild-type extent of reaction (79% of the wild-type rate) (Fig. 2A; Table 1).
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<tr>
<th>Condition or strain</th>
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<td>60 ± 3 (102)</td>
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aValues represent means of triplicate samples; errors represent 1 SD. ND, not determined.

bReaction rate was calculated from the first 4-h anaerobic incubation (lactate and formate) or 8-h incubation (H₂) values.

cExtent of reaction is reported as the percentage of IO₃⁻ reduced to I⁻ upon completion of the 24-h incubation period, after which further IO₃⁻ reduction was minimal.

dThe values in parentheses are in comparison with the wild-type rates (percent) within each set of lactate, formate, or H₂ values.
With H₂ as the electron donor, the ΔmtrA mutant reduced IO₃⁻/H₂O₂ at rates over 2-fold higher than the wild-type strain (212% of the wild-type rate) and displayed an extent of reaction almost 2-fold higher than that of the wild-type strain (197% of the wild-type extent of reaction). With lactate as the electron donor, the ΔmtrA/mtrA transconjugant strain reduced IO₃⁻/H₂O₂ at a rate nearly 2-fold greater than that of the wild-type strain (164% of the wild-type rate) and displayed a higher extent of reaction (141% of the wild-type extent of reaction) (Fig. 2B; Table 1).

Conversely, with lactate as the electron donor, the ΔmtrC, ΔomcA, and ΔmtrCΔomcA EEC mutant strains reduced IO₃⁻ at near wild-type rates with lactate (76%, 69%, and 110% of the wild-type rate, respectively) and displayed near wild-type extents of reaction (82%, 75%, and 112% of the wild-type extent of reaction) (Fig. 2A; Table 1). In a similar fashion, with formate as the electron donor, the ΔmtrC, ΔomcA, and ΔmtrCΔomcA mutant strains reduced IO₃⁻ at near wild-type rates (125%, 121%, and 142% of the wild-type rate, respectively) and displayed near wild-type extents of reaction (113%, 116%, and 130% of the wild-type extent of reaction, respectively). With H₂ as the electron donor, the ΔmtrC, ΔomcA, and ΔmtrCΔomcA mutant strains also reduced IO₃⁻ at near wild-type rates (142%, 87%, and 139% of the wild-type rate, respectively) and displayed near wild-type extents of reaction (147%, 98%, and 112% of the wild-type extent of reaction, respectively) (Fig. 2A; Table 1).

IO₃⁻ reduction activity of additional S. oneidensis c-type cytochrome and crp mutants. With lactate as the electron donor, ΔcymA and ΔccmB c-type cytochrome mutant strains were also severely impaired in IO₃⁻ reduction activity (both 10% of the wild-type rate) and displayed significantly lower extents of reaction (3% and 8% of the wild-type extent of reaction, respectively) (Fig. 3A; Table 1). With formate as the electron donor, the mutant strains were also impaired in IO₃⁻ reduction activity (25%
and 22% of the wild-type rate, respectively) and displayed significantly lower extents of reaction (25% and 20% of the wild-type extent of reaction, respectively). With H₂ as the electron donor, the ΔcymA and ΔccmB mutant strains were also impaired in IO₃⁻ reduction activity (56% and 55% of the wild-type rate, respectively) and displayed significantly lower extents of reaction (28% and 16% of the wild-type extent of reaction, respectively). The ΔcymA/cymA and ΔccmB/ccmB transconjugant strains recovered near wild-type rates of IO₃⁻ reduction (80% and 85% of the wild-type rate, respectively), and displayed near wild-type extents of reaction (98% and 101% of the wild-type extent of reaction, respectively) with lactate as the electron donor (Fig. 3B; Table 1). With lactate, formate, and H₂ as electron donors, the Δcrp mutant strain was severely impaired in IO₃⁻ reduction activities (15%, 27%, and 38% of the wild-type rate, respectively) and displayed significantly lower extents of reaction (11%, 28%, and 46% of wild-type extent of reaction, respectively) (Fig. 3A; Table 1), while the IO₃⁻ reduction activity of the ΔcymA/cymA transconjugant strain was partially restored to wild-type rates with lactate as the electron donor (54% of the wild-type rate and 85% of wild-type extent of reaction) (Fig. 3B; Table 1). The IO₃⁻ reduction activities of the transconjugant strains with formate or H₂ as the electron donor were not determined.

**IO₃⁻ reduction activity of *S. oneidensis* type I, II, and V protein secretion mutants.** With lactate as the electron donor, the type I (ΔtolC) and the type V (ΔSO3800) protein secretion mutants reduced IO₃⁻ at near wild-type rates (97% and 113% of the wild-type rate, respectively) and displayed near wild-type extents of reaction (104% and 109% of the wild-type extent of reaction, respectively) (Fig. 4; Table 1). With formate as the electron donor, the mutants similarly reduced IO₃⁻ at near wild-type rates (96% and 129% of the wild-type rate, respectively) and displayed near wild-type extents of reaction (118% and 92% of the wild-type extent of reaction, respectively). With H₂ as

![Graph](https://via.placeholder.com/150)

**Figure 3** IO₃⁻ reduction activity of *S. oneidensis* wild-type (MR-1) and crp mutants with IO₃⁻ as the electron acceptor and lactate, formate, or H₂ as the electron donor and their complemented strains with pBBRcymA, pBBRccmB, and pBBRcrp, respectively (A), and with IO₃⁻ as the electron acceptor and lactate as the electron donor (mutant strains normalized to wild-type levels) (B). Values are means of triplicate samples from anaerobic incubations. Error bars represent SDs. Some error bars cannot be seen due to small SDs.
the electron donor, the type I and type V protein secretion mutants reduced IO₃⁻ at near wild-type rates (117% and 139% of the wild-type rate, respectively) (Fig. 4; Table 1) and displayed near wild-type extents of reaction (101% and 124% of the wild-type extent of reaction, respectively). However, with lactate, formate, and H₂ as electron donors, the ΔgspD type II protein secretion mutant was severely to partially impaired in IO₃⁻ reduction activity (30%, 45%, and 56% of the wild-type rate, respectively) and displayed significantly lower extents of reaction with all three electron donors (27%, 40%, and 55% of the wild-type extent of reaction, respectively).

**DISCUSSION**

The molecular mechanism of microbial IO₃⁻ reduction is poorly understood. Under NO₃⁻-reducing anaerobic conditions, microorganisms, including the phytoplankton *Navicula* and the bacteria *Pseudomonas* sp. strain SCT, *Agrobacterium*-related strain DVZ35, and *Escherichia coli*, reduce IO₃⁻ to I⁻, which led to the hypothesis that NO₃⁻ reductase reduces IO₃⁻ as an alternative terminal electron acceptor (6, 7, 30, 32, 45, 46). The IO₃⁻-reducing NO₃⁻ reductase hypothesis was recently brought into question, however, by findings with *S. oneidensis*, which demonstrated that NO₃⁻ and IO₃⁻ reduction activities were not inhibited by the presence of saturating levels of the competing electron acceptor and that NO₃⁻ reductase-deficient deletion mutants retained wild-type IO₃⁻ reduction activity (33).

*S. oneidensis* also reduces external metal oxides via EEC-mediated electron transfer either at the outside face of the outer membrane or via outer membrane extensions (i.e., nanowires) (19, 35, 47). The *S. oneidensis* EEC (MtrCAB) is composed of outer membrane β-barrel protein MtrB, which forms a ternary complex with decaheme c-type cytochromes MtrC and MtrA (48, 49). *S. oneidensis* mutants lacking MtrCAB display Fe(III), Mn(III), and Mn(IV) reduction-deficient phenotypes (39, 47, 50, 51). In addition, methyl viologen-reduced proteoliposomes containing only the *S. oneidensis* MtrCAB complex transfer electrons to external soluble and solid Fe(III) substrates (20, 52, 53). In the present study, ΔmtrA and ΔmtrB deletion mutants were severely impaired in IO₃⁻ reduction activity with lactate as the electron donor, indicating that MtrA and MtrB also function as critical components of the lactate-dependent IO₃⁻ reduction pathway. However, IO₃⁻ was reduced at wild-type rates with formate or H₂ as the electron donor, indicating that the electron transport pathway components required for IO₃⁻ reduction are electron donor dependent.

In contrast to the lactate-dependent, IO₃⁻ reduction-deficient phenotypes displayed by the ΔmtrA and ΔmtrB mutant strains, the ΔmtrC mutant strain retained wild-type IO₃⁻ reduction activity regardless of electron donor, indicating that MtrC is not required for IO₃⁻ reduction by *S. oneidensis*. These findings differed from those of previous studies which demonstrated that MtrC is required for Fe(III), Mn(IV), Mn(III),
Tc(VII), U(VI), and flavin reduction by *S. oneidensis* (28, 39, 54–57). In addition, previous studies demonstrated that OmCA was required for Fe(III), Mn(IV), and Mn(III) reduction (39). In the present study, the Δ*omCA* and Δ*omCA* Δ*mtrC* double mutant strains retained wild-type IO$_3^-$ reduction activity with each electron donor, thus indicating that neither OmCA nor MtrC is required for electron transport to IO$_3^-$.

Previous studies with the *mtrB*-C42A and *mtrB*-C45A site-directed mutants demonstrated that cysteine at MtrB amino acid position 42 (but not at position 45) was required for Fe(III), Mn(IV), and Mn(III) reduction by *S. oneidensis* (36, 39). In a similar fashion, results of the present study demonstrated that the Δ*mtrB*-C42A mutant was severely impaired in IO$_3^-$ reduction activity, while the Δ*mtrB*-C45A mutant retained IO$_3^-$ at wild-type rates. Residues C42 and C45 comprise a conserved CXXC motif in MtrB homologs of metal-reducing gammaproteobacteria (36). The biochemical function of the CXXC motif of *S. oneidensis* MtrB is currently unknown but may involve MtrB maturation via disulfide bond formation or metal cofactor binding (36, 58, 59). The detection of a CXXC motif in MtrB homologs of gammaproteobacteria is diagnostic for microbial Fe(III) reduction (36). Future IO$_3^-$ reduction activity assays will be required to determine if the CXXC motif of MtrB homologs in gammaproteobacteria is also diagnostic for microbial IO$_3^-$ reduction. Such information will guide interpretation of *in situ* meta(omic) signals indicative of microbial IO$_3^-$ reduction in natural and contaminated environments such as the 129I-contaminated Hanford and Savannah River sites. These types of molecular signatures will be important for monitoring the 129I cycling in sites like Hanford, which may affect overall mobility of 129I in the oligotrophic aquifer. Diagnostic markers may also be used to monitor conversion of 129IO$_3^-$ to 129I$^-^-^-$ facilitates uptake of 129I on biofilm or GAC in FBRs.

*S. oneidensis* CcmB functions as the integral membrane component of the cytochrome c maturation complex (43). The Δ*ccmB* mutant strain was severely impaired in IO$_3^-$ reduction activity regardless of electron donor, which indicates that the c-type cytochrome pool is involved in anaerobic electron transport to IO$_3^-$.

Correspondingly, the Δ*cymA* mutant strain was also severely impaired in IO$_3^-$ reduction activity regardless of electron donor. Inner membrane-tethered tetraheme c-type cytochrome CymA functions as the central branch point in anaerobic electron transport by *S. oneidensis* (60, 61). CymA accepts electrons from the menaquinone pool for subsequent delivery to a variety of periplasmic or outer membrane localized terminal reductase complexes, including MtrA of the *S. oneidensis* EEC (61, 62). The IO$_3^-$ reduction-deficient phenotype of the Δ*cymA* strain indicates that the electron transport chain to IO$_3^-$ also includes CymA. The Δ*crp* mutant strain (lacking the cyclic AMP receptor protein) was also severely impaired in IO$_3^-$ reduction activity. CRP regulates expression of genes required for anaerobic respiration by *S. oneidensis* (44). The IO$_3^-$ reduction-deficient phenotype displayed by Δ*crp* indicates that IO$_3^-$ reduction gene expression is also regulated by CRP/cAMP levels in *S. oneidensis*.

The *S. oneidensis* genome encodes type I, II, and V protein secretion systems (39, 41, 42, 63). Δ*tolC* and ΔSO3800 deletion mutants retained wild-type IO$_3^-$ reduction activity regardless of electron donor, thus indicating that IO$_3^-$ reduction requires neither TolC-mediated type I protein secretion (for efflux of antibiotics, heavy metals, or toxic proteins) (64) nor SO3800-mediated type V protein secretion (an autotransporter-like serine protease involved in *S. oneidensis* adhesion to Fe(III) oxide surfaces [63]). In contrast, the IO$_3^-$ reduction-deficient phenotype displayed by the mutant with a deletion of gspD (encoding GspD, the outer membrane secretin of type II protein secretion) indicates that IO$_3^-$ reduction is linked to type II protein secretion in a manner similar to that of Fe(III), Mn(IV), and Mn(III) reduction. The type II protein secretion system is required for outer membrane localization of MtrC and OmCA (21, 65), and impairment of type II protein secretion results in mislocalization of MtrC and OmCA, with corresponding Fe(III), Mn(IV), and Mn(III) reduction-deficient phenotypes. The Δ*mtrC*, Δ*omCA*, and Δ*mtrC* Δ*omCA* mutant strains retained wild-type IO$_3^-$ reduction activity. These findings demonstrate that IO$_3^-$ reduction by *S. oneidensis* does not
require either of the EEC cytochromes MtrC and OmcA but does require type II protein secretion of an as-yet-unidentified IO\textsubscript{3}– reductase to the outside face of the outer membrane. In the current model of the lactate (MtrAB)-dependent S. oneidensis IO\textsubscript{3}– reduction system (Fig. 5), electrons originating from lactate dehydrogenase are trans-
ported via the menaquinone pool, CymA, and MtrAB to the terminal IO\textsubscript{3}– reductase that is translocated to the outside face of the outer membrane via type II protein secretion. Current work is focused on identification of the S. oneidensis IO\textsubscript{3}– reductase via comparison of the IO\textsubscript{3}–-reducing protein fractions harvested from the outside face of the outer membrane of S. oneidensis wild-type and ΔgspD mutant strains.

MATERIALS AND METHODS

Growth and cultivation conditions. S. oneidensis strains were routinely cultured aerobically at 30°C in lysogeny broth (LB) (10 g liter\textsuperscript{-1} of NaCl, 10 g liter\textsuperscript{-1} of tryptone, 5 g liter\textsuperscript{-1} of yeast extract). IO\textsubscript{3}– reduction rate experiments were conducted under anaerobic conditions in M1 minimal medium (66) amended with 20 mM lactate, 10 mM formate, or 2% H\textsubscript{2} gas as the electron donor and 250 μM IO\textsubscript{3}– as the anaerobic electron acceptor. When required for selection, gentamicin (20 μg ml\textsuperscript{-1}) was amended to the appropriate growth medium.

In-frame deletion mutagenesis of S. oneidensis genes. The genes crp and ccmB were deleted in frame from the S. oneidensis MR-1 genome following previously described procedures (67). Regions corresponding to ~750 bp upstream and downstream of crp and ccmB were PCR amplified with iProof ultrahigh-fidelity polymerase (Bio-Rad, Hercules, CA) (primers D1/D2 and D3/D4 [Table 2]) and subse-
quently joined using overlap extension PCR (primers D1/D4 [Table 2]). The resulting fragment was cloned into suicide vector pKO2.0, which does not replicate in *S. oneidensis* MR-1 via conjugation with *E. coli* donor strain E100D pir (Table 3) (68). *S. oneidensis* strains with the integrated plasmid were selected on LB agar containing gentamicin (15 μg ml⁻¹). Single-crossover integrations were verified using PCR with primers flanking the recombination region (TF/TR) and were resolved from the genomes by plating on LB agar lacking NaCl and containing sucrose (10% (wt/vol)). The in-frame deletion strains (Δcrp and ΔccmB) were verified by PCR with primers TF/TR (Table 2). Genetic complementation analysis of ΔmtrA, ΔmtrB, Δcrp, ΔcymA, and ΔccmB strains was carried out by cloning the wild-type gene into broad-host-range cloning vector pBBR1MCS (69) and conjugally transferring the recombinant vector into the respective mutant strains via biparental mating procedures (67).

Anaerobic incubation conditions. Mutant strains were initially inoculated in liquid LB growth medium and incubated at 30°C for 24 h. Ten-milliliter subcultures at an initial optical density at 600 nm (OD600) of 0.02 were incubated at 30°C for 24 h. Subcultures were centrifuged at 4,000 rpm for 30 min.

### TABLE 2 Primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5′–3′)</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δcrp</td>
<td>D1 CTGATAGGATCTTCTTTATACCAACGTTCGGCC</td>
<td>BamHI (underlined)</td>
</tr>
<tr>
<td></td>
<td>D2 GGCTTAATACCGTCAAGTCTAATGTCGATTGTTATTGATTTAAGCC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D3 TTAGCTAAGGAGAAATCGACAGTTAGACTTCAGCTTGATTTAAGCC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D4 TCGATCTGCAGTCCATTATTGAGCCTCCTCA</td>
<td>SalI (underlined)</td>
</tr>
<tr>
<td>TF</td>
<td>GCGTAAATAAAACCTAAACGGAACT</td>
<td></td>
</tr>
<tr>
<td>TR</td>
<td>TAGCTAAGGATCTGTTGGGATT</td>
<td></td>
</tr>
</tbody>
</table>

| ΔccmB | D1 CTGATACCTGATACCTACTGCTAAGCATTGAAACC | Spel (underlined) |
|       | D2 GGCTTTGGTTATCTTTCTTTTTCATTTTATTGCCAAATCAGAACC | |
|       | D3 GCTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTG TTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTY  |
resuspended in 10 ml of M1 growth medium amended with 20 mM lactate, and incubated aerobi-
ically at room temperature for 8 h. The preconditioned cells were inoculated in 30-ml serum bottles at an ini-
tial OD_{600} of 0.1 in M1 growth medium amended with 250 μM IO_3^- and either 20 mM lactate or 10 mM
formate and incubated anaerobically via continuous sparging with 100% high-purity (hydrated) N_2 gas. For IO_3^- reduction activity assays with H_2 as the electron donor, the preconditioned cells were incubated
anaerobically via continuous sparging with high-purity (hydrated) anaerobic gas mix consisting of 2% H_2
and 98% N_2. Cultures were incubated at room temperature with gentle stirring under anaerobic
conditions maintained by continuous sparging with high-purity hydrated N_2 gas. At preselected time
points, OD_{600} was measured and IO_3^- concentrations were determined using the IO_3^-–triiodide forma-
tion method described below.

**Determination of IO_3^- concentrations via IO_3^-–triiodide formation with I^- at acidic pH.**
The extent of IO_3^- reduction was determined using the IO_3^-–triiodide method (33, 70). Culture samples were
added to 96-well 500-μl microtiter plates. Sodium citrate buffer (0.1 M, pH 3.3) and potassium iodide
solution (75 mM) were added to each well to initiate triiodide formation (IO_3^- + 5I^- + 6H^+ → 3I_3^- + 3H_2O).
Absorbance at 352 nm was measured with a UV spectrophotometer (Multiskan Go; Thermo Sci-
cific) after a 4-min reaction time. IO_3^- concentrations were determined from a previously generated
calibration curve.

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Y.J.T., J.K.M., and H.D.S. performed part of the experiments, developed part of the
protocol, and cowrote the manuscript. M.H.L., B.D.L., and T.J.D. developed the concept
and part of the protocol, coanalyzed all data, and cowrote the manuscript.

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