RESEARCH LETTER

Identification of a molecular signature unique to metal-reducing *Gammaproteobacteria*

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Abstract

Functional genes required for microbial (dissimilatory) metal reduction display high sequence divergence, which limits their utility as molecular biomarkers for tracking the presence and activity of metal-reducing bacteria in natural and engineered systems. In the present study, homologs of the outer membrane beta-barrel protein MtrB of metal-reducing *Gammaproteobacteria* were found to contain a unique N-terminal CXXC motif that was missing from MtrB homologs of nonmetal-reducing *Gammaproteobacteria* and metal- and nonmetal-reducing bacteria outside the *Gammaproteobacteria*. To determine whether the N-terminal CXXC motif of MtrB was required for dissimilatory metal reduction, each cysteine in the CXXC motif of the representative metal-reducing gammaproteobacterium *Shewanella oneidensis* was replaced with alanine, and the resulting site-directed mutants were tested for metal reduction activity. Anaerobic growth experiments demonstrated that the first, but not the second, conserved cysteine was required for metal reduction by *S. oneidensis*. The ability to predict metal reduction by *Gammaproteobacteria* with unknown metal reduction capability was confirmed with *Vibrio parahaemolyticus*, a pathogen whose genome encodes an MtrB homolog with an N-terminal CXXC motif. MtrB homologs with an N-terminal CXXC motif may thus represent a molecular signature unique to metal-reducing members of the *Gammaproteobacteria*.

Introduction

Dissimilatory metal-reducing bacteria occupy a central position in a variety of environmentally important processes, including the biogeochemical cycling of carbon and metals, the bioremediation of radionuclides and organohalides, and the generation of electricity in microbial fuel cells (Lovley & Coates, 1997; Thamdrup, 2000; Lovley *et al.*, 2004; Logan, 2009). Metal-reducing bacteria are scattered and deeply rooted throughout both prokaryotic domains (Lonergan *et al.*, 1996; Vargas *et al.*, 1998). Functional genes required for microbial metal reduction display high sequence divergence, which limits their use as molecular biomarkers to examine fundamental ecological principles and environmental parameters controlling metal reduction in both natural and engineered systems. A variety of *c*-type cytochromes, for example, are key components of the electron transport systems of many metal-reducing bacteria (Weber *et al.*, 2006; Richter *et al.*, 2012), yet their widespread occurrence in nonmetal-reducing bacteria and high sequence divergence limit their utility as molecular biomarkers for tracking the presence and activity of metal-reducing bacteria as a functional group. The gene encoding the eukaryotic-like citrate synthase (*gltA*) in the *Geobacteraceae* family has received attention as a molecular biomarker for tracking the presence and activity of metal-reducing *Geobacteraceae* in subsurface environments (Bond *et al.*, 2005; Wilkins *et al.*, 2011). However, *gltA* is found only in members of the *Geobacteraceae* family, thus limiting its application as a molecular biomarker for metal-reducing bacteria outside the *Geobacteraceae* family.

The large *γ-proteobacteria* class within the phylum *Proteobacteria* (Williams *et al.*, 2010) was selected as a bacterial group to search for molecular signatures unique to metal-reducing bacteria outside the *Geobacteraceae* family. The large number of genera (over 250) and complete or nearly complete genomes (over 200) in the *γ-proteobacteria* class (Williams *et al.*, 2010) facilitates nucleotide sequence comparisons of genes in both metal- and

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nonmetal-reducing bacteria, potentially aiding in the identification of molecular signatures unique to metal-reducing *γ-proteobacteria*. The *γ-proteobacteria* class includes *Shewanella oneidensis*, a gram-negative, facultative anaerobe that reduces a wide range of metals, including Fe(III) and Mn(IV) as terminal electron acceptor (Myers & Nealson, 1988; Venkateswaran *et al.*, 1999). *Shewanella oneidensis* employs a number of novel respiratory strategies for dissimilatory metal reduction, including (1) localization of *c*-type cytochromes on the cell surface (or along extracellular nanowires) where they may deliver electrons to external metals (Myers & Myers, 1992; DiChristina *et al.*, 2002; Gorby *et al.*, 2006); (2) nonreductive dissolution of metal oxides to form more readily reducible organic metal complexes (Taillefert *et al.*, 2007; Fennessey *et al.*, 2010; Jones *et al.*, 2010); and (3) delivery of electrons to external metals via endogenous or exogenous electron shuttles (Hernandez *et al.*, 2004; Marsili *et al.*, 2008; Roden *et al.*, 2010).

Shewanella oneidensis contains an electron transport chain that consists of IM-localized primary dehydrogenases, menaquinone, and CymA, a menaquinol-oxidizing *c*-type cytochrome that functions as a central branch point in electron transport to Fe(III), Mn(IV), nitrate (NO_3^-), nitrite (NO_2^-), dimethyl sulfoxide (DMSO), and fumarate (Myers & Myers, 1997). CymA transfers electrons to the periplasmic *c*-type cytochrome MtrA (Schuetz *et al.*, 2009), which interacts with outer membrane (OM)-localized protein complexes composed of transmembrane β-barrel protein MtrB (Beliaev & Saffarini, 1998; Myers & Myers, 2002) and decaheme *c*-type cytochrome MtrC (Shi *et al.*, 2006; Ross *et al.*, 2007). Purified MtrC reduces Fe(III) (Hartshorne *et al.*, 2007; Eggleston *et al.*, 2008), and in proteoliposomes, purified MtrB, MtrC, and MtrA form a lipid-embedded 'porin–cytochrome' complex (Richardson *et al.*, 2012) that transfers electrons from internal reduced methyl viologen to external Fe(III) substrates (Hartshorne *et al.*, 2009; White *et al.*, 2013).

Previous nucleotide sequence analyses indicated that the N-terminus of *S. oneidensis* MtrB contained a unique CXXC motif (Beliaev & Saffarini, 1998). The identification of a CXXC motif in *S. oneidensis* MtrB was unusual because CXXC motifs are generally not found in OM β-barrel proteins, most likely to avoid protein-folding problems caused by redox-reactive cysteines during passage across the intermembrane space in eukaryotes or the periplasmic space in bacteria (Tamm *et al.*, 2004; Schleiff & Soll, 2005; Denoncin *et al.*, 2010). The identification of an unusual CXXC motif in the N-terminus of MtrB led us to hypothesize that this motif may represent a molecular signature unique to metal-reducing *γ-proteobacteria*. To test this hypothesis, nucleotide sequence analyses were carried out to correlate dissimilatory metal reduction capability with the presence of MtrB homologs containing an N-terminal CXXC motif. Site-directed mutational analyses were performed to determine whether the N-terminal CXXC motif of MtrB was required for metal reduction by the representative metal-reducing *γ-proteobacterium S. oneidensis*. The ability to predict dissimilatory metal reduction by a *γ-proteobacterium* with unknown metal reduction capability was then tested with *Vibrio parahaemolyticus*, a human pathogen whose genome encodes an MtrB homolog with an N-terminal CXXC motif.

Materials and methods

Bacterial strains and cultivation conditions

Bacterial strains and plasmids used in this study are listed in Table 1. For genetic manipulations, all *Escherichia coli* and *S. oneidensis* strains were cultured at 30 °C in Luria–Bertani medium (10 g L^{-1} NaCl, 5 g L^{-1} yeast extract, 10 g L^{-1} tryptone). For aerobic and anaerobic growth experiments, all *S. oneidensis* strains were cultured in a defined salts medium (M1) supplemented with 20 mM

Table 1. Strains and plasmids used in this study

	Features	Source
Strains		
Shewanella oneidensis		
MR-1	Wild-type strain	ATCC
Δ*mtrB*	In-frame deletion mutant	This study
C42A	Site-directed mutant	This study
C45A	Site-directed mutant	This study
C42A plus *mtrB*	C42A complemented with wild-type *mtrB*	This study
Escherichia coli		
β2155 λ pir	*thrB1004 pro thi strA hsdS lacZ_M15* (F9 *lacZΔM15 lacI*q *traD36 proA*1 *proB*1) Δ*dapA::erm pir::*RP4 Km^R	Dehio & Meyer (1997)
XL10 Gold	Km^R electrocompetent	Agilent
Vibrio parahaemolyticus	Wild-type strain RIMD 2210633	ATCC
Vibrio harveyi	Wild-type strain BB120	ATCC
Plasmids		
pKO2.0	4.5-kb γR6K, *mob*RP4 *sacB* Gm^R *lacZ*	Burns & DiChristina (2009)
pBBR1MCS	Cm^R lacZ	Kovach *et al.* (1995)
pKO2.0-*mtrB*	pKO2.0 with in-frame deletion of *mtrB*	This study
pKO2.0 + *mtrB*	pKO2.0 containing wild-type copy of *mtrB*	This study

lactate as carbon/energy source (Myers & Nealson, 1988). *Vibrio parahaemolyticus* and *V. harveyi* were tested for anaerobic metal reduction activity in marine broth (Difco) growth medium. Bacterial growth experiments were carried out in a B. Braun Biostat B batch reactor with automatic feedback control of pH, temperature, and dissolved O_2 concentration. Electron acceptors were synthesized as previously described (Saffarini *et al.*, 1994; Blakeney *et al.*, 2000; Taratus *et al.*, 2000; Payne & DiChristina, 2006; Neal *et al.*, 2007) and added at the following final concentrations: NO_3^-, 10 mM; NO_2^-, 2 mM; Fe(III) citrate, 50 mM; amorphous MnO_2, 15 mM; trimethylamine-*N*-oxide (TMAO), 25 mM; $S_2O_3^{2-}$, 10 mM; fumarate, 30 mM; and DMSO, 25 mM. Gentamycin was supplemented at 15 μg mL^{-1}. For the growth of *E. coli* β2155 λ pir, diaminopimelate was amended at 100 μg mL^{-1}.

Analytical procedures

Cell growth was monitored by direct cell counts via epifluorescence microscopy and by measuring terminal electron acceptor depletion or end product accumulation. Acridine orange-stained cells were counted (Zeiss AxioImager Z1 Microscope) according to the previously described procedures (Burnes *et al.*, 1998). Cell numbers at each time point were calculated as the average of 10 counts from two parallel yet independent anaerobic incubations. NO_2^- was measured spectrophotometrically with sulfanilic acid-*N*-1-naphthyl-ethylenediamine dihydrochloride solution (Montgomery & Dymock, 1962). Fe(III) reduction was monitored by measuring HCl-extractable Fe(II) production with ferrozine (Stookey, 1970). Mn(IV) concentration was measured colorimetrically after reaction with benzidine hydrochloride as previously described (Burnes *et al.*, 1998). Mn(III)-pyrophosphate concentration was measured colorimetrically as previously described (Kostka *et al.*, 1995). $S_2O_3^{2-}$ concentrations were measured by cyanolysis as previously described (Kelly & Wood, 1994). Growth on O_2, TMAO, DMSO, and fumarate was monitored by measuring increases in cell density at 600 nm. Control experiments consisted of incubations with cells that were heat-killed at 80 °C for 30 min prior to inoculation.

Nucleotide and amino acid sequence analyses

Genome sequence data for *S. oneidensis* MR-1, *S. putrefaciens* 200, *S. putrefaciens* CN32, *S. putrefaciens* W3-18-1, *S. amazonensis* SB2B, *S. denitrificans* OS217, *S. baltica* OS155, *S. baltica* OS195, *S. baltica* OS185, *S. baltica* OS223, *S. frigidimarina* NCIMB400, *S. pealeana* ATCC 700345, *S. woodyi* ATCC 51908, *S.* sp. ANA-3, *S.* sp. MR-4, *S.* sp. MR-7, *S. loihica* PV-4, *S. halifaxens* HAW-EB4, *S. piezotolerans* WP3, *S. sediminis* HAW-EB3, and *S. benthica* KT99 were obtained from the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) or the Department of Energy Joint Genome Institute (DOE-JGI, http://jgi.doe.gov). MtrB homologs in the NCBI databases were identified via BLAST analysis (Altschul *et al.*, 1997) using *S. oneidensis* MtrB as the search query. Multiple alignments of MtrB homologs were generated with CLUSTALW (http://www.ebi.ac.uk/Tools/clustalw2/index.html) (Chenna *et al.*, 2003). β-Barrel architecture of the MtrB homologs was predicted using the program PRED-TMBB (Bagos *et al.*, 2004). LOGO diagrams were generated using the CLUSTALW alignment files (Crooks *et al.*, 2004).

In-frame gene deletion mutagenesis and genetic complementation analysis

mtrB was deleted from the *S. oneidensis* genome via application of a *Shewanella* in-frame gene deletion system (Burns & DiChristina, 2009). Regions corresponding to *c.* 750 bp upstream and downstream of *mtrB* were independently PCR-amplified and subsequently joined using overlap-extension PCR. Primers for *mtrB* deletion are listed in Table 2. The resulting fragment was cloned into suicide vector pKO2.0, which does not replicate in *S. oneidensis*. This construct (designated pKO-*mtrB*) was

Table 2. Primers used for in-frame gene deletion mutagenesis, site-directed mutagenesis, and DNA sequencing

Deletion mutagenesis primers	
MtrBD1	GACTGGATCCCTCCTCTAAGAGTCCAATGGCTGGC
MtrBD2	CAGCATCAGCATTTGTGCGGTGTAGCCTGTGTTGG CTAATAACGCTAGAGT
MtrBD3	ACTCTAGCGTTATTAGCCAACACAGGCTACACCGC ACAAATGCTGATGCTG
MtrBD4	GACTGTCGACACATTTAGCCAAGCCCTAAGCCGT
MtrBDTF	CAGAGCAAGTCGAAGCCACCTTAG
MtrBDTR	CCATCGGTACTATGGCAAACAGAGC
Site-directed mutagenesis primers	
C42A-Sense	GTGAAATTATCCGCATGGAGCGCAAAAGGCTGCG TCGTTGAAACG
C42A-Anti	CGTTTCAACGACGCAGCCTTTTGCGCTCCATGCG GATAATTTCAC
C45A-Sense	GCATGGAGCTGTAAAGGCGCAGTCGTTGAAACGG GCACA
C45A-Anti	TGTGCCCGTTTCAACGACTGCGCCTTTACAGCTCC ATGC
Sequencing primers	
MtrB-SeqF	GATCACTCTAGCGTTATTAGCCAAC
MtrB-SeqR	GTTGCTTGAACCTGCTGTTATC
MtrB cloning primers	
MtrB-CompF	GACTGGATCCGGTTCTAACCATCCAT
MtrB-CompR	GACTGTCGACCAGAGGCGGGCTTTT

mobilized into wild-type MR-1 via conjugal transfer from *E. coli* donor strain β2155 λ pir. *S. oneidensis* strains with the plasmid integrated into the genome were selected on solid LB medium containing gentamycin (15 μg mL^{-1}). Single integrations were verified via PCR with primers flanking the recombination region. Plasmids were resolved from the genomes of single integrants by plating on solid LB medium containing sucrose (10% w/v) with NaCl omitted. In-frame deletions were verified by PCR and direct DNA sequencing (GeneWiz, South Plainfield, NJ). Genetic complementation of Δ*mtrB* was carried out by cloning wild-type *mtrB* into broad-host-range cloning vector pBBR1MCS (Kovach *et al.*, 1995) and conjugally transferring the recombinant vector into Δ*mtrB* via biparental mating procedures (DiChristina *et al.*, 2002).

Site-directed mutagenesis

Single amino acid mutations in MtrB (C42A or C45A) were constructed using the Quickchange Lightning site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA). The *mtrB* gene and regions *c.* 750 bp upstream and downstream were PCR-amplified as a single fragment and subsequently cloned into pBBR1MCS. Mutagenesis primers C42A-sense, C42A-antisense, C45A-sense, and C45A-antisense (Table 2) were used in mutagenesis PCR according to the manufacturer's instructions. The resulting PCR products were subsequently transformed into XL10 Gold Kan^R competent cells (Agilent Technologies). Correct amino acid mutations (C42A or C45A) were verified by direct DNA sequencing using primers MTRB-SeqF and MTRB-SeqR (Table 2). The mutated *mtrB* constructs were subsequently cloned into suicide vector pKO2.0 and were 'knocked in' to the native chromosomal position. Nucleotide sequence changes were verified by PCR and DNA sequencing of *S. oneidensis* 'knock-in' transformants. Genetic complementation of mutant C42A was carried out by cloning wild-type *mtrB* into broad-host-range cloning vector pBBR1MCS (Kovach *et al.*, 1995) and conjugally transferring the recombinant vector into mutant C42A via biparental mating procedures (DiChristina *et al.*, 2002).

Results and discussion

Identification of N-terminal CXXC motifs in MtrB homologs within the genus *Shewanella*

Recent proteoliposome studies indicated that electrons are transferred from internal reduced methyl viologen to external Fe(III) substrates by a porin–cytochrome complex composed of *S. oneidensis* β-barrel protein MtrB and decaheme cytochromes MtrA and MtrC (Richardson *et al.*, 2012; Richter *et al.*, 2012; Shi *et al.*, 2012b). *Shewanella oneidensis* MtrB was predicted to contain a 55-amino-acid N-terminus followed by 28 β-sheets that form a transmembrane β-barrel domain (White *et al.*, 2013). MtrB homologs with high sequence similarity were identified in the genomes of 22 metal-reducing members of the genus *Shewanella* (Supporting Information, Table S1, Fig. S1), but not in the genome of nonmetal-reducing *S. denitrificans* (Brettar *et al.*, 2002). Multiple sequence alignment of the 22 *Shewanella* MtrB homologs indicated that each consisted of a 46- to 82-amino-acid N-terminus followed by a C-terminus with 25–30 β-sheets (Table S1, Fig. S1). The N-terminus of all 22 *Shewanella* MtrB homologs contained a CKXC motif corresponding to amino acid positions 42–45 in *S. oneidensis* MtrB (Fig. 1, Table S1, Fig. S1). The *S. oneidensis* genome also contains three additional MtrB paralogs (MtrE, DmsF, and SO4359) (Gralnick *et al.*, 2006) with lower overall amino acid sequence similarity to MtrB (43–55% and *e*-values

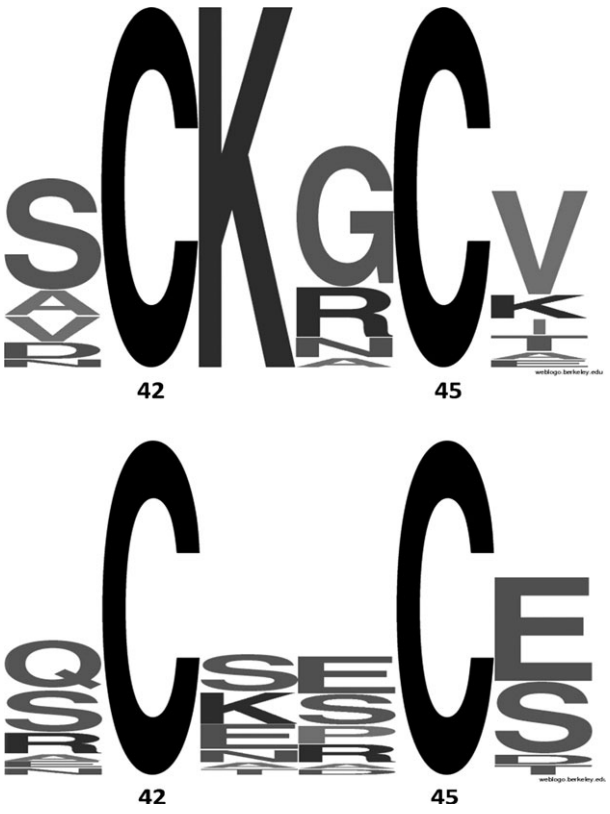

Fig. 1. LOGO diagrams comparing the amino acids in the N-terminal CXXC motifs of MtrB homologs identified in the genomes of 22 metal-reducing *Shewanella* strains (top panel) and 20 CXXC-containing MtrB homologs in γ-*proteobacteria* outside the genus *Shewanella* (bottom panel) (corresponding to amino acid positions 42–45 of *Shewanella oneidensis* MtrB). Strain designations are listed in Table S1.

ranging from 1e−38 to 4e−127). Each of the three additional MtrB paralogs also contained a conserved N-terminal CKXC motif (Table S2, Fig. S2).

The identification of N-terminal CXXC motifs in the MtrB homologs of all 22 metal-reducing *Shewanella* strains was unusual because CXXC motifs are generally not found in transmembrane β-barrel proteins, most likely to avoid protein-folding problems caused by the redox-reactive cysteines during passage across the intermembrane space or periplasm (Tamm *et al.*, 2004; Schleiff & Soll, 2005; Denoncin *et al.*, 2010). CXXC motifs are generally found in cytoplasmic and periplasmic proteins where they carry out a diverse array of functions such as catalyzing disulfide bond exchanges, binding transition metals, or acting as the redox-sensing module of transcriptional activators (Ritz & Beckwith, 2001; Green & Paget, 2004; Antelmann & Helmann, 2011). Transmembrane β-barrel proteins found in the mitochondria and chloroplast of higher eukaryotes and the OM of gram-negative bacteria are generally involved in active ion transport or passive nutrient uptake (Schulz, 2000). *Shewanella oneidensis* MtrB appears to function as a structural sheath facilitating interaction and electron transfer from MtrA to MtrC in a transmembrane porin–cytochrome complex (Hartshorne *et al.*, 2009; Firer-Sherwood *et al.*, 2011a, b; White *et al.*, 2013). The N-terminal CXXC motif of the *Shewanella* MtrB homologs may facilitate such electron transfer via as yet unknown molecular interactions.

Identification of N-terminal CXXC motifs in MtrB homologs outside the genus *Shewanella*

Nine MtrB homologs displaying amino acid sequence similarity to *S. oneidensis* MtrB had been previously reported in bacterial genomes outside the genus *Shewanella*, including metal- and nonmetal-reducing *Acidobacteria* and α-, β-, γ-, and δ-*proteobacteria* (Hartshorne *et al.*, 2009). Four additional MtrB homologs were subsequently identified in the MtrAB modules of Fe(II)-oxidizing α- and β-*proteobacteria* (Shi *et al.*, 2012a, b). The rapid expansion of sequenced bacterial genomes has resulted in a sharp increase in the number of proteins displaying similarity to *S. oneidensis* MtrB. As of July 2013, the list of MtrB homologs identified outside the *Shewanella* genus numbered 52 (Table S3, Fig. S3), including one each from the phyla *Acidobacteria* and NC10 group, and 50 from the α-, β-, γ-, and δ-*proteobacteria*. The 52 MtrB homologs facilitated amino acid sequence analysis of MtrB homologs in bacteria that cross phylogenetic and phenotypic lines, including metal- and nonmetal-reducing strains.

Literature searches were conducted to determine the dissimilatory metal reduction capability of the host strains harboring each of the 52 MtrB homologs (Table S3). Correlations between the similarity of the 52 MtrB homologs and the ability of the corresponding host strains to catalyze dissimilatory metal reduction were not observed. The 52 MtrB homologs found outside the *Shewanella* genus were subsequently ranked according to *e*-value, ranging from the MtrB homolog of the metal-reducing γ-*proteobacterium Ferrimonas balearica* (*e*-value of 7.00e-145) to the MtrB homolog of the metal-reducing δ-*proteobacterium Geobacter metallireducens* (*e*-value of 0.28). CLUSTALW analyses of the 52 MtrB homologs (Table S3) indicated that N-terminal length varied from 4 to 132 amino acids, while the number of C-terminal β-sheets varied from 22 to 32 sheets. MtrB homologs of the γ-*proteobacteria Ferrimonas*, *Aeromonas*, and *Vibrio* were represented in 20 of the top 21 MtrB homologs, and each of the 20 *Ferrimonas*, *Aeromonas*, and *Vibrio* homologs contained an N-terminal CXXC motif (Fig. 1, Table S3). The threshold *e*-value for MtrB homologs containing an N-terminal CXXC motif was 4.00e-43 displayed by the MtrB homolog of *V. vulnificus* YJ016. *Ferrimonas* and *Aeromonas* species are facultatively anaerobic γ-*proteobacteria* capable of dissimilatory metal reduction (Knight & Blakemore, 1998; Martin-Carnahan & Joseph, 2005; Nolan *et al.*, 2010), while *Vibrio* species have not been previously examined for dissimilatory metal reduction activity. Of the top 21 MtrB homologs, only the MtrB homolog of the γ-*proteobacterium Nitrosococcus halophilus* Tc4 lacked an N-terminal CXXC motif (Table S3). *N. halophilus* Tc4 is a nitrifying chemolithotroph that obligately respires oxygen as terminal electron acceptor (Campbell *et al.*, 2011). These results indicate that N-terminal CXXC motifs are found in MtrB homologs of γ-*proteobacteria* capable of dissimilatory metal reduction, while N-terminal CXXC motifs are missing from the MtrB homolog of an obligately aerobic, nonmetal-reducing γ-*proteobacterium*.

The remaining 29 MtrB homologs were found in one *Acidobacterium*, one NC10 group strain, and 27 α-, β-, γ-, and δ-*proteobacteria* (Table S3). None of the remaining 29 MtrB homologs contained an N-terminal CXXC motif. α- and β-*Proteobacteria* were represented in 18 of the 29 MtrB homologs lacking an N-terminal CXXC motif, including the MtrB homologs of the Fe(II)-oxidizing β-*proteobacteria Dechloromonas aromatica, Gallionella capsiferriformans,* and *Sideroxydans lithotrophicus* (Emerson & Moyer, 1997; Chakraborty *et al.*, 2005; Hedrich *et al.*, 2011). CXXC motifs were also missing from the N-terminus of PioB, the MtrB homolog of the Fe(II)-oxidizing α-*proteobacterium Rhodopseudomonas palustris* (Jiao & Newman, 2007), and from the MtrB homolog of the γ-*proteobacterium Halorhodospira halophila*, a sulfur-oxidizing anoxygenic phototroph

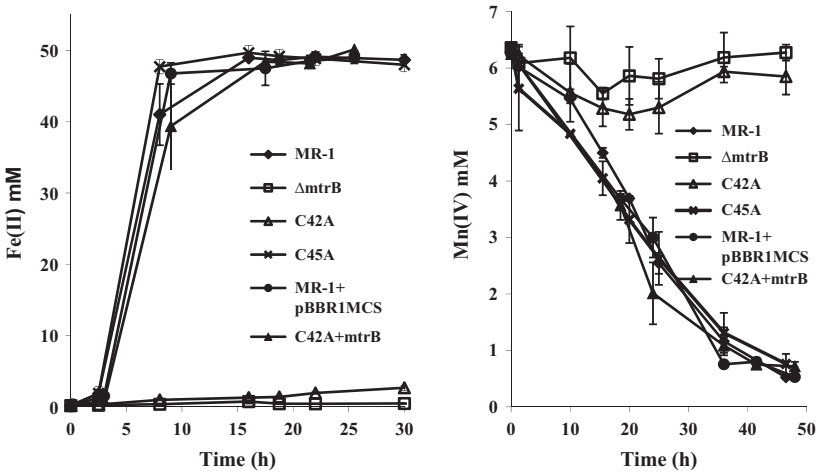

Fig. 2. Dissimilatory metal reduction activity of strains *Shewanella oneidensis* wild-type, wild-type containing pBBR1MCS, Δ*mtrB*, C45A, C42A, and C42A complemented by wild-type *mtrB* with either Fe(III) (left panel) or Mn(IV) (right panel) as terminal electron acceptor. Values are the means of two parallel but independent anaerobic incubations; error bars represent standard deviations. Some error bars cannot be seen due to small standard deviations.

(Challacombe *et al.*, 2013). Three of the 29 MtrB homologs lacking an N-terminal CXXC motif were found in metal-reducing bacteria, including the β-*proteobacterium Rhodoferax ferrireducens* (Finneran *et al.*, 2003) and the δ-*proteobacteria Geobacter* sp. M21, *G. metallireducens* and *G. uraniireducens* (Shelobolina *et al.*, 2008). These results indicate that MtrB homologs of metal-reducing γ-*proteobacteria* contain an N-terminal CXXC motif that is missing from MtrB homologs of nonmetal-reducing γ-*proteobacteria* and from all bacteria outside the γ-*proteobacteria*, including those catalyzing dissimilatory metal reduction or oxidation reactions.

The first conserved cysteine in the N-terminal CXXC motif of MtrB is required for dissimilatory metal reduction by *S. oneidensis*

To determine whether the N-terminal CXXC motif of MtrB was required for dissimilatory metal reduction, the N-terminal CXXC motif of *S. oneidensis* MtrB was selected for site-directed mutational analysis, and the resulting CXXC mutants were tested for dissimilatory metal reduction activity. *S. oneidensis* mutant strain C42A was unable to reduce Fe(III) or Mn(IV) as terminal electron acceptor (i.e. displayed metal reduction-deficient phenotypes identical to Δ*mtrB*; Fig. 2), yet retained wild-type respiratory activity on all nonmetal electron acceptors, including O_2, NO_3^-, NO_2^-, $S_2O_3^{2-}$, fumarate, DMSO, and TMAO (Fig. S3). *S. oneidensis* mutant strain C45A, on the other hand, displayed wild-type reduction activity of all electron acceptors, including Fe(III) and Mn(IV) (Figs 2 and S3). The involvement of C42 in metal reduction activity was confirmed via restoration of wild-type metal reduction activity to C42A transconjugates provided with wild-type *mtrB* on pBBR1MCS (Fig. 2). These findings indicate that the first, but not the second, cysteine in the N-terminal CXXC motif of MtrB is required for dissimilatory metal reduction by *S. oneidensis*.

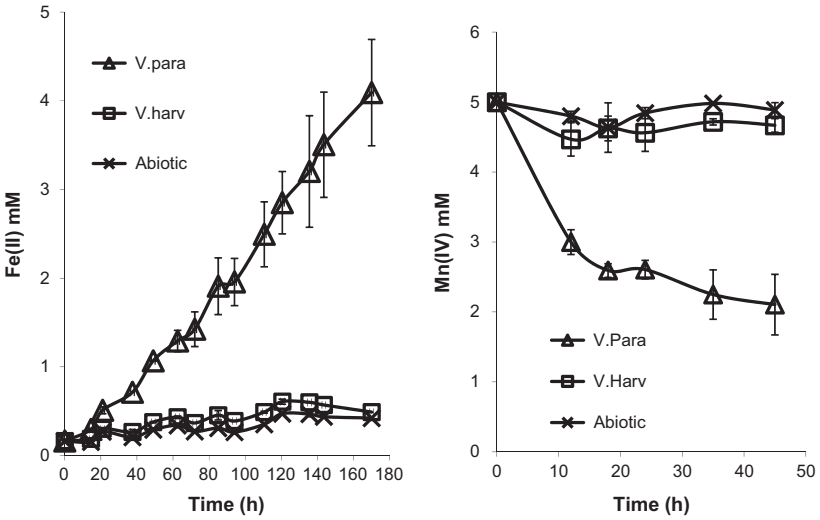

Fig. 3. Dissimilatory metal reduction activity of *Vibrio parahaemolyticus* and *V. harveyi* wild-type strains with either Fe(III) (left panel) or Mn(IV) (right panel) as terminal electron acceptor. Values are the means of two parallel but independent anaerobic incubations; error bars represent standard deviations. Some error bars cannot be seen due to small standard deviations.

These findings also indicate that overlapping MtrB function is not provided by the MtrB paralogs MtrE, DmsF, and SO4359 or that these paralogs are expressed under metal-reducing conditions different than those employed in the present study (Myers & Myers, 2002; Gralnick *et al.*, 2006).

The involvement of C42 in metal reduction by *S. oneidensis* and the absence of the corresponding N-terminal CXXC motif in MtrB homologs of metal-reducing *Rhodoferax* and *Geobacter* species indicate that the molecular mechanism of metal reduction by γ-, β-, and δ-*proteobacteria* differs in at least one fundamental aspect. The biochemical function of C42 in metal reduction by *S. oneidensis* is currently unknown. Based on the participation of CXXC motifs in metal binding, redox sensing, and disulfide bond formation (Ritz & Beckwith, 2001; Green & Paget, 2004; Antelmann & Helmann, 2011), potential roles for C42 include the binding of metals or cofactors required for electron transport by the MtrCAB complex, sensing redox conditions via sulfur redox chemistry, or enhancing MtrB interaction with other cysteine-containing metabolites and proteins via heterologous disulfide bond formation. Current work is focused on examining these possibilities during metal reduction by *S. oneidensis*.

Prediction of dissimilatory metal reduction activity by γ-*proteobacteria* with unknown metal reduction capability

As described above, 20 of the top 21 MtrB homologs were identified in the genera *Ferrimonas*, *Aeromonas*, and *Vibrio* (Table S3). Although *Ferrimonas* and *Aeromonas* species are known to catalyze dissimilatory metal reduction (Knight & Blakemore, 1998; Nakagawa *et al.*, 2006; Nolan *et al.*, 2010), the dissimilatory metal reduction capability of *Vibrios* is not well studied. The ability to predict dissimilatory metal reduction by a γ-*proteobacterium* with unknown metal reduction capability was tested with *V. parahaemolyticus*, a pathogen whose genome encodes an MtrB homolog with an N-terminal CXXC motif. A CSEC motif was identified in the N-terminus of the *V. parahaemolyticus* MtrB homolog VP1218 (87QD1_VIBPA; Table S3). Subsequent anaerobic incubations demonstrated that *V. parahaemolyticus* reduced Fe (III) and Mn(IV) as terminal electron acceptors (Fig. 3), while *V. harveyi*, a *Vibrio* control strain lacking the MtrB homolog, was deficient in Fe(III) and Mn(IV) reduction activity (Fig. 3).

Results of the present study indicate that MtrB homologs of metal-reducing γ-*proteobacteria* contain an N-terminal CXXC motif that is missing from the MtrB homologs of *Acidobacteria* and NC10 group strains, nonmetal-reducing γ-*proteobacteria*, and all α-, β-, and δ-*proteobacteria*, including those catalyzing dissimilatory metal reduction or oxidation reactions. The N-terminal CXXC motif of MtrB is required for dissimilatory metal reduction by the representative metal-reducing γ-*proteobacterium S. oneidensis*, and the ability to predict dissimilatory metal reduction by a γ-*proteobacterium* with unknown metal reduction capability was confirmed with *V. parahaemolyticus*, a pathogen whose genome encodes an MtrB homolog with an N-terminal CXXC motif. MtrB homologs with N-terminal CXXC motifs may thus represent a molecular signature unique to metal-reducing members of the γ-*proteobacteria*, with the potential for further development as a biomarker for tracking the presence and activity of metal-reducing γ-*proteobacteria* in natural and engineered systems.

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References

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W & Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.

Antelmann H & Helmann JD (2011) Thiol-based redox switches and gene regulation. *Antioxid Redox Signal* **14**: 1049–1063.

Bagos PG, Liakopoulos TD, Spyropoulos IC & Hamodrakas SJ (2004) PRED-TMBB: a web server for predicting the topology of beta-barrel outer membrane proteins. *Nucleic Acids Res* **32**: W400–W404.

Beliaev AS & Saffarini DA (1998) *Shewanella putrefaciens mtrB* encodes an outer membrane protein required for Fe(III) and Mn(IV) reduction. *J Bacteriol* **180**: 6292–6297.

Blakeney MD, Moulaei T & DiChristina TJ (2000) Fe(III) reduction activity and cytochrome content of *Shewanella putrefaciens* grown on ten compounds as sole terminal electron acceptor. *Microbiol Res* **155**: 87–94.

Bond DR, Mester T, Nesbo CL, Izquierdo-Lopez AV, Collart FL & Lovley DR (2005) Characterization of citrate synthase from *Geobacter sulfurreducens* and evidence for a family of citrate synthases similar to those of eukaryotes throughout the *Geobacteraceae*. *Appl Environ Microbiol* **71**: 3858–3865.

Brettar I, Christen R & Hofle MG (2002) *Shewanella denitrificans* sp. nov., a vigorously denitrifying bacterium isolated from the oxic-anoxic interface of the Gotland Deep in the central Baltic Sea. *Int J Syst Evol Microbiol* **52**: 2211–2217.

Burnes BS, Mulberry MJ & DiChristina TJ (1998) Design and application of two rapid screening techniques for isolation of Mn(IV) reduction-deficient mutants of *Shewanella putrefaciens*. *Appl Environ Microbiol* **64**: 2716–2720.

Burns JL & DiChristina TJ (2009) Anaerobic respiration of elemental sulfur and thiosulfate by *Shewanella oneidensis* MR-1 requires *psrA*, a homolog of the *phsA* gene of *Salmonella enterica* serovar Typhimurium LT2. *Appl Environ Microbiol* **75**: 5209–5217.

Campbell MA, Chain PSG, Dang HY, El Sheikh AF, Norton JM, Ward NL, Ward BB & Klotz MG (2011) *Nitrosococcus watsonii* sp. nov., a new species of marine obligate ammonia-oxidizing bacteria that is not omnipresent in the world's oceans: calls to validate the names '*Nitrosococcus halophilus*' and '*Nitrosomonas mobilis*'. *FEMS Microbiol Ecol* **76**: 39–48.

Chakraborty R, O'Connor SM, Chan E & Coates JD (2005) Anaerobic degradation of benzene, toluene, ethylbenzene, and xylene compounds by *Dechloromonas* strain RCB. *Appl Environ Microbiol* **71**: 8649–8655.

Challacombe J, Majid S, Deole R *et al.* (2013) Complete genome sequence of *Halorhodospira halophila* SL1. *Stand Genomic Sci* **8**: 206–214.

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG & Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res* **31**: 3497–3500.

Crooks GE, Hon G, Chandonia JM & Brenner SE (2004) WebLogo: a sequence logo generator. *Genome Res* **14**: 1188–1190.

Dehio C & Meyer M (1997) Maintenance of broad-host-range incompatibility group P and group Q plasmids and transposition of Tn5 in *Bartonella henselae* following conjugal plasmid transfer from *Escherichia coli*. *J Bacteriol* **179**: 538–540.

Denoncin K, Vertommen D, Paek E & Collet JF (2010) The protein-disulfide isomerase DsbC cooperates with SurA and DsbA in the assembly of the essential beta-barrel protein LptD. *J Biol Chem* **285**: 29425–29433.

DiChristina TJ, Moore CM & Haller CA (2002) Dissimilatory Fe(III) and Mn(IV) reduction by *Shewanella putrefaciens* requires *ferE*, a homolog of the *pulE* (*gspE*) type II protein secretion gene. *J Bacteriol* **184**: 142–151.

Eggleston CM, Voros J, Shi L, Lower BH, Droubay TC & Colberg PJS (2008) Binding and direct electrochemistry of OmcA, an outer-membrane cytochrome from an iron reducing bacterium, with oxide electrodes: a candidate biofuel cell system. *Inorg Chim Acta* **361**: 769–777.

Emerson D & Moyer C (1997) Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. *Appl Environ Microbiol* **63**: 4784–4792.

Fennessey CM, Jones ME, Taillefert M & DiChristina TJ (2010) Siderophores are not involved in Fe(III) solubilization during anaerobic Fe(III) respiration by *Shewanella oneidensis* MR-1. *Appl Environ Microbiol* **76**: 2425–2432.

Finneran KT, Johnsen CV & Lovley DR (2003) Rhodoferax ferrireducens sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III). *Int J Syst Evol Microbiol* **53**: 669–673.

Firer-Sherwood MA, Bewley KD, Mock JY & Elliott SJ (2011a) Tools for resolving complexity in the electron transfer networks of multiheme cytochromes *c*. *Metallomics* **3**: 344–348.

Firer-Sherwood MA, Ando N, Drennan CL & Elliott SJ (2011b) Solution-based structural analysis of the decaheme cytochrome, MtrA, by small-angle x-ray scattering and analytical ultracentrifugation. *J Phys Chem B* **115**: 11208–11214.

Gorby YA, Yanina S, McLean JS *et al.* (2006) Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *P Natl Acad Sci USA* **103**: 11358–11363.

Gralnick JA, Vali H, Lies DP & Newman DK (2006) Extracellular respiration of dimethyl sulfoxide by *Shewanella oneidensis* strain MR-1. *P Natl Acad Sci USA* **103**: 4669–4674.

Green J & Paget MS (2004) Bacterial redox sensors. *Nat Rev Microbiol* **2**: 954–966.

Hartshorne RS, Jepson BN, Clarke TA, Field SJ, Fredrickson J, Zachara J, Shi L, Butt JN & Richardson DJ (2007) Characterization of *Shewanella oneidensis* MtrC: a cell-surface decaheme cytochrome involved in respiratory electron transport to extracellular electron acceptors. *J Biol Inorg Chem* **12**: 1083–1094.

Hartshorne RS, Reardon CL, Ross D *et al.* (2009) Characterization of an electron conduit between bacteria and the extracellular environment. *P Natl Acad Sci USA* **106**: 22169–22174.

Hedrich S, Schlomann M & Johnson DB (2011) The iron-oxidizing proteobacteria. *Microbiology* **157**: 1551–1564.

Hernandez ME, Kappler A & Newman DK (2004) Phenazines and other redox-active antibiotics promote microbial mineral reduction. *Appl Environ Microbiol* **70**: 921–928.

Jiao Y & Newman DK (2007) The pio operon is essential for phototrophic Fe(II) oxidation in *Rhodopseudomonas palustris* TIE-1. *J Bacteriol* **189**: 1765–1773.

Jones ME, Fennessey CM, DiChristina TJ & Taillefert M (2010) *Shewanella oneidensis* MR-1 mutants selected for their inability to produce soluble organic-Fe(III) complexes are unable to respire Fe(III) as anaerobic electron acceptor. *Environ Microbiol* **12**: 938–950.

Kelly DP & Wood AP (1994) Synthesis and determination of thiosulfate and polythionates. *Method Enzymol* **243**: 475–501.

Knight V & Blakemore R (1998) Reduction of diverse electron acceptors by *Aeromonas hydrophila*. *Arch Microbiol* **169**: 239–248.

Kostka JE, Luther GW & Nealson KH (1995) Chemical and biological reduction of Mn(III)-pyrophosphate complexes – potential importance of dissolved Mn(III) as an

environmental oxidant. *Geochim Cosmochim Acta* **59**: 885–894.

Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop RM & Peterson KM (1995) Four new derivatives of the broad-host-range cloning vector PBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* **166**: 175–176.

Logan BE (2009) Exoelectrogenic bacteria that power microbial fuel cells. *Nat Rev Microbiol* **7**: 375–381.

Lonergan DJ, Jenter HL, Coates JD, Phillips EJP, Schmidt TM & Lovley DR (1996) Phylogenetic analysis of dissimilatory Fe(III)-reducing bacteria. *J Bacteriol* **178**: 2402–2408.

Lovley DR & Coates JD (1997) Bioremediation of metal contamination. *Curr Opin Biotechnol* **8**: 285–289.

Lovley DR, Holmes DE & Nevin KP (2004) Dissimilatory Fe(III) and Mn(IV) reduction. *Adv Microb Physiol* **49**: 219–286.

Marsili E, Baron DB, Shikhare ID, Coursolle D, Gralnick JA & Bond DR (2008) *Shewanella* secretes flavins that mediate extracellular electron transfer. *P Natl Acad Sci USA* **105**: 3968–3973.

Martin-Carnahan A & Joseph SW (2005) Genus I. *Aeromonas* stanier 1943. *Bergey's Manual of Systematic Bacteriology*, Vol. 2 (Garrity GM, Brenner DJ, Krieg NR & Staley JT, eds),pp. 557–578. Springer Verlag, New York, NY.

Montgomery HAC & Dymock JF (1962) Rapid determination of nitrate in fresh and saline waters. *Analyst* **87**: 374–378.

Myers CR & Myers JM (1992) Localization of cytochromes to the outer-membrane of anaerobically grown *Shewanella putrefaciens* MR-1. *J Bacteriol* **174**: 3429–3438.

Myers CR & Myers JM (1997) Cloning and sequence of *cymA* a gene encoding a tetraheme cytochrome *c* required for reduction of iron(III), fumarate, and nitrate by *Shewanella putrefaciens* MR-1. *J Bacteriol* **179**: 1143–1152.

Myers CR & Myers JM (2002) MtrB is required for proper incorporation of the cytochromes OmcA and OmcB into the outer membrane of *Shewanella putrefaciens* MR-1. *Appl Environ Microbiol* **68**: 5585–5594.

Myers CR & Nealson KH (1988) Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Science* **240**: 1319–1321.

Nakagawa T, Lino T, Suzuki KI & Harayama S (2006) *Ferrimonas futtsuensis* sp. nov. and *Ferrimonas kyonanensis* sp. nov., selenate-reducing bacteria belonging to the *Gammaproteobacteria* isolated from Tokyo Bay. *Int J Syst Evol Microbiol* **56**: 2639–2645.

Neal AL, Dublin SN, Taylor J, Bates DJ, Burns L, Apkarian R & DiChristina TJ (2007) Terminal electron acceptors influence the quantity and chemical composition of capsular exopolymers produced by anaerobically growing *Shewanella* spp.. *Biomacromolecules* **8**: 166–174.

Nolan M, Sikorski J, Davenport K *et al.* (2010) Complete genome sequence of *Ferrimonas balearica* type strain (PAT (T)). *Stand Genomic Sci* **3**: 174–182.

Payne AN & DiChristina TJ (2006) A rapid mutant screening technique for detection of technetium [Tc(VII)] reduction-deficient mutants of *Shewanella oneidensis* MR-1. *FEMS Microbiol Lett* **259**: 282–287.

Richardson DJ, Butt JN, Fredrickson JK *et al.* (2012) The porin-cytochrome' model for microbe-to-mineral electron transfer. *Mol Microbiol* **85**: 201–212.

Richter K, Schicklberger M & Gescher J (2012) Dissimilatory reduction of extracellular electron acceptors in anaerobic respiration. *Appl Environ Microbiol* **78**: 913–921.

Ritz D & Beckwith J (2001) Roles of thiol-redox pathways in bacteria. *Annu Rev Microbiol* **55**: 21–48.

Roden EE, Kappler A, Bauer I, Jiang J, Paul A, Stoesser R, Konishi H & Xu HF (2010) Extracellular electron transfer through microbial reduction of solid-phase humic substances. *Nat Geosci* **3**: 417–421.

Ross DE, Ruebush SS, Brantley SL, Hartshorne RS, Clarke TA, Richardson DJ & Tien M (2007) Characterization of protein-protein interactions involved in iron reduction by *Shewanella oneidensis* MR-1. *Appl Environ Microbiol* **73**: 5797–5808.

Saffarini DA, DiChristina TJ, Bermudes D & Nealson KH (1994) Anaerobic respiration of *Shewanella putrefaciens* requires both chromosomal and plasmid-borne genes. *FEMS Microbiol Lett* **119**: 271–277.

Schleiff E & Soll J (2005) Membrane protein insertion: mixing eukaryotic and prokaryotic concepts. *EMBO Rep* **6**: 1023–1027.

Schuetz B, Schicklberger M, Kuermann J, Spormann AM & Gescher J (2009) Periplasmic electron transfer via the *c*-type cytochromes MtrA and FccA of *Shewanella oneidensis* MR-1. *Appl Environ Microbiol* **75**: 7789–7796.

Schulz GE (2000) Beta-Barrel membrane proteins. *Curr Opin Struct Biol* **10**: 443–447.

Shelobolina ES, Vrionis HA, Findlay RH & Lovley DR (2008) *Geobacter uraniireducens* sp. nov., isolated from subsurface sediment undergoing uranium bioremediation. *Int J Syst Evol Microbiol* **58**: 1075–1078.

Shi L, Chen BW, Wang ZM *et al.* (2006) Isolation of a high-affinity functional protein complex between OmcA and MtrC: two outer membrane decaheme c-type cytochromes of *Shewanella oneidensis* MR-1. *J Bacteriol* **188**: 4705–4714.

Shi L, Rosso KM, Zachara JM & Fredrickson JK (2012a) Mtr extracellular electron-transfer pathways in Fe(III)-reducing or Fe(II)-oxidizing bacteria: a genomic perspective. *Biochem Soc Trans* **40**: 1261–1267.

Shi L, Rosso KM, Clarke TA, Richardson DJ, Zachara JM & Fredrickson JK (2012b) Molecular underpinnings of Fe(III) oxide reduction by *Shewanella oneidensis* MR-1. *Front Microbiol* **3**: 50.

Stookey LL (1970) Ferrozine – a new spectrophotometric reagent for iron. *Anal Chem* **42**: 779–781.

Taillefert M, Beckler JS, Carey E, Burns JL, Fennessey CM & DiChristina TJ (2007) *Shewanella putrefaciens* produces an Fe(III)-solubilizing organic ligand during anaerobic respiration on insoluble Fe(III) oxides. *J Inorg Biochem* **101**: 1760–1767.

Tamm LK, Hong H & Liang BY (2004) Folding and assembly of beta-barrel membrane proteins. *Biochim Biophys Acta* **1666**: 250–263.

Taratus EM, Eubanks SG & DiChristina TJ (2000) Design and application of a rapid screening technique for isolation of selenite reduction-deficient mutants of *Shewanella putrefaciens*. *Microbiol Res* **155**: 79–85.

Thamdrup B (2000) Bacterial manganese and iron reduction in aquatic sediments. *Advances in Microbial Ecology*,(Schink B, Ed), pp. 86–103. Kluwer Academic Publishers, Dordrecht.

Vargas M, Kashefi K, Blunt-Harris EL & Lovley DR (1998) Microbiological evidence for Fe(III) reduction on early Earth. *Nature* **395**: 65–67.

Venkateswaran K, Moser DP, Dollhopf ME *et al.* (1999) Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov.. *Int J Syst Bacteriol* **49**: 705–724.

Weber KA, Achenbach LA & Coates JD (2006) Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nat Rev Microbiol* **4**: 752–764.

White GF, Shi Z, Shi L *et al.* (2013) Rapid electron exchange between surface-exposed bacterial cytochromes and Fe(III) minerals. *P Natl Acad Sci USA* **110**: 6346–6351.

Wilkins MJ, Callister SJ, Miletto M, Williams KH, Nicora CD, Lovley DR, Long PE & Lipton MS (2011) Development of a biomarker for *Geobacter* activity and strain composition; Proteogenomic analysis of the citrate synthase protein during bioremediation of U(VI). *Microb Biotechnol* **4**: 55–63.

Williams KP, Gillespie JJ, Sobral BWS, Nordberg EK, Snyder EE, Shallom JM & Dickerman AW (2010) Phylogeny of *Gammaproteobacteria*. *J Bacteriol* **192**: 2305–2314.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Multiple sequence alignments generated by CLUSTALW analysis of the N-termini of MtrB homologs identified in the genomes of 22 metal-reducing *Shewanella* strains.

Fig. S2. Multiple sequence alignments generated by CLUSTALW analysis of the N-termini of three CXXC-containing MtrB paralogs identified in the *Shewanella oneidensis* genome.

Fig. S3. Growth of *Shewanella oneidensis* MR-1 wild-type (●), Δ*mtrB* (Δ), C42A (□), and C45A (×) mutant strains with either O_2 (A), DMSO (B), TMAO (C), fumarate (D), nitrite (E), thiosulfate (F), or nitrate (G) as electron acceptor.

Table S1. Amino acid sequence identity (ID), similarity (Sim), expect-value (*e*-value), N-terminal CXXC motif (CXXC motif), number of amino acid residues in the N-terminus (N-term length), and number of β-sheets in the C-terminus (No. β-sheets) of the MtrB homologs identified in the genomes of 22 metal-reducing *Shewanella* strains.

Table S2. Amino acid sequence identity (ID), similarity (Sim), expect-value (*e*-value),108mm N-terminal CXXC motif (CXXC motif), number of amino acid residues in the N-terminus (N-term length), and number of β-sheets in the C-terminus (No. β-sheets) of the three MtrB paralogs identified in the genome of *Shewanella oneidensis* MR-1.

Table S3. Phylogenetic affiliation (Class), amino acid sequence identity (ID, %), similarity (Sim, %), expect-value (*e*-value), N-terminal CXXC motif, (CXXC motif) number of amino acid residues in the N-terminus (N-term length), number of β-sheets in the C-terminus (No. β-sheets), and reported dissimilatory metal reduction or oxidation activity of the host strain (metal redox) for 52 MtrB homologs displaying similarity to *Shewanella oneidensis* MtrB.