Title: Outer Cell Surface Components Essential for Fe(III) Oxide Reduction by Geobacter metallireducens

Running Title: Fe(III) Oxide Reduction by Geobacter metallireducens

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Abstract

Geobacter species are important Fe(III) reducers in a diversity of soils and sediments. Mechanisms for Fe(III) oxide reduction have been studied in detail in Geobacter sulfurreducens, but a number of the most thoroughly studied outer surface components of G. sulfurreducens, particularly c-type cytochromes, are not well conserved among Geobacter species. In order to identify cellular components potentially important in Fe(III) oxide reduction in Geobacter metallireducens, gene transcript abundance was compared in cells grown on Fe(III) oxide or soluble Fe(III) citrate with whole-genome microarrays. Outer-surface cytochromes were also identified. Deletion of genes for c-type cytochromes that had higher transcript abundance during growth on Fe(III) oxides and/or were detected in the outer-surface protein fraction identified six c-type cytochrome genes, that when deleted removed the capacity for Fe(III) oxide reduction. Several of the c-type cytochromes which were essential for Fe(III) oxide reduction in G. metallireducens have homologs in G. sulfurreducens that are not important for Fe(III) oxide reduction. Other genes essential for Fe(III) oxide reduction included a gene predicted to encode a NHL-repeat containing protein, and a gene potentially involved in pili glycosylation. Genes associated with flagella-based motility, chemotaxis, and pili had higher transcript abundance during growth on Fe(III) oxide, consistent with the previously proposed importance of these components in Fe(III) oxide reduction. These results demonstrate that there are similarities in extracellular electron transfer between G. metallireducens and G. sulfurreducens, but that the outer-surface c-type cytochromes involved in Fe(III) oxide reduction are different.

Introduction
The mechanisms for electron transfer to Fe(III) oxide in Geobacter species is of interest because Geobacter species play an important role in Fe(III) reduction in a wide diversity of soils, aquatic sediments, and subsurface environments (28). Furthermore, an understanding of the mechanisms for Fe(III) oxide reduction is expected to provide insights into other important types of extracellular electron transfer in Geobacter, such as electron transfer to electrodes (24) and interspecies electron transfer (35, 44). Initial studies on the mechanisms for Fe(III) oxide reduction in Geobacter species were conducted with G. sulfurreducens because it was the first Geobacter species for which a genetic system was developed (9). However, G. metallireducens is a more effective Fe(III) oxide reducer than G. sulfurreducens and has other environmentally significant physiological properties not found in G. sulfurreducens, such as the ability to anaerobically oxidize aromatic hydrocarbons (28), including benzene (51). Therefore, understanding how Fe(III) oxides are reduced in G. metallireducens aids in understanding the physiology of this important model organism and provides the opportunity to find conserved mechanisms for Fe(III) oxide reduction in Geobacter species. A genetic system has recently been developed for G. metallireducens, which now makes such studies feasible (46).

Initial studies suggested that like G. sulfurreducens (38), G. metallireducens requires type IV pili for Fe(III) oxide reduction (46). The pili of G. sulfurreducens posses metallic-like conductivity (32), which is distinct from the electron hopping/tunneling associated with other known forms of biological electron transport (30). Measurements of the conductivity of the pili of G. metallireducens have not been reported, but the PilA sequence of G. metallireducens is 76% similar to the G. sulfurreducens PilA sequence. The G. sulfurreducens pili are decorated with the multi-heme c-type cytochrome OmcS (21), which is required for Fe(III) oxide reduction (34). The spacing of OmcS on pili is too great to contribute to conduction of electrons along the
length of the pili (21, 31) and multiple lines of additional evidence rule out this possibility (21, 29, 31, 32). Therefore, it has been proposed that the role of OmcS is to facilitate electron transfer from the pili to Fe(III) oxides (25). However, there is no homolog of OmcS in G. metallireducens, in line with the overall poor conservation of outer surface c-type cytochromes in Geobacter species (7).

The only other G. sulfurreducens outer-surface c-type cytochrome known to be essential for Fe(III) oxide reduction is OmcB, which appears to be embedded in the outer membrane (37) and is speculated to facilitate electron transfer from the periplasm to the outer surface (26). There is no homolog to omcB in G. metallireducens, although another c-type cytochrome is found in a syntenous location (1). The only c-type cytochromes known to be involved in Fe(III) reduction that are well conserved between G. sulfurreducens and G. metallireducens are PpcA and MacA, which are located in the periplasm (1, 6, 23).

Comparing gene expression during growth on insoluble electron acceptors versus growth on soluble electron acceptors has been a productive strategy for identifying components involved in extracellular electron transfer in G. sulfurreducens (14, 36). Here we report on components of G. metallireducens likely to be important in electron transfer to Fe(III) oxides identified from gene expression, protein localization, and gene deletion studies.

Materials and methods

Bacterial strains and growth conditions

Geobacter metallireducens (ATCC 53774 and DSM 7210) was routinely cultured under strict anaerobic conditions with 10 mM acetate provided as the sole electron donor as previously
described (27). Either Fe(III) citrate (56 mM) or Fe(III) oxide (100 mM) were provided as the sole terminal electron acceptor for Fe(III) reduction studies. Samples of Fe(III) oxide cultures were dissolved in 0.5 N HCl and Fe(II) concentrations were measured using the ferrozine assay as previously described (2).

For genetic manipulations Fe(III) citrate (56 mM) was provided as the electron acceptor along with the addition of ferrous ammonium sulfate (500 μM) and yeast extract (0.1%) to liquid medium and agar plates (46). Genetic manipulations were carried out in an anaerobic chamber containing N₂/CO₂/H₂ (in percent, 83/10/7) atmosphere and at a temperature of 30°C. *Escherichia coli* was cultivated with Luria-Bertani medium with or without antibiotics (43). All bacterial strains and plasmids are listed in Table S2.

**SDS-PAGE and protein identification**

The loosely bound surface proteins fraction of *G. metallireducens* was isolated during mid-exponential growth as previously described (34). Cells grown with Fe(III) oxide provided as the electron acceptor were treated with equal volumes of TPE and oxalate solution prior to cell fractionation. The outer membrane protein fraction was isolated using a previously described method (20). Loosely bound and outer membrane protein fractions were combined, and protein concentration was determined with the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL). *c*-*type cytochromes were identified by separation with SDS-PAGE, and stained for heme as previously described (45). Equal amount of proteins were loaded in each lane. Differentially expressed *c*-type cytochrome bands from the Tris-Tricine polyacrylamide gel were excised and sent to the Laboratory for Proteomic Mass Spectrometry at the University of Massachusetts.
Medical School for liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analysis.

DNA Microarrays

Total RNA for microarray analysis was extracted from quadruplicate cultures of *G. metallireducens* cells grown with acetate (10 mM)-Fe(III) citrate (55 mM) or acetate (10 mM)-Fe(III) oxide (100 mM) during exponential growth using methods previously described (17). RNA samples were purified with the RNeasy MinElute Clean-Up kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions, and treated with the TURBO DNA-free DNase (Ambion, Austin, TX, USA). The RNA samples were tested for genomic DNA contamination by PCR amplification of the 16S rRNA gene (49). The concentration and quality of the RNA samples were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). All RNA samples had A260/280 ratios, of 1.8–2.0, indicating high purity (3). cDNA was generated with the TransPlex Whole Transcriptome Amplification Kit (Sigma-Aldrich, St-Louis, MO, USA) according to manufacturer’s instructions.

Whole-genome microarray hybridizations were carried out by Roche NimbleGen, Inc (Madison, WI, USA). Quadruplicate biological and triplicate technical replicates were conducted for all microarray analyses. Cy3-labeled cDNA was hybridized to oligonucleotide microarrays based on *G. metallireducens* genome and resident plasmid sequences (accession number NC007515 and NC007517 at GenBank). The microarray results were analyzed with Array 4 Star (DNASTAR, Madison, WI, USA). A gene was considered differentially expressed only if the *P* value determined by Student’s t-test analysis was less than or equal to 0.01. Microarray data has been deposited with NCBI GEO under accession number GSE40316.
RT-qPCR

Microarray results were confirmed with RT-qPCR. The Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and the ABI 7500 Real-Time PCR System were used to amplify and to quantify PCR products. Each reaction consisted of forward and reverse primers at a final concentration of 200 nM, 5 ng of cDNA, and 12.5 μl of Power SYBR Green PCR Master Mix (Applied Biosystems). Primer pairs were designed with amplicon size of 100 to 200-bp for the following: Gmet 0557, Gmet 2896, Gmet 0909, Gmet 0930, Gmet 1868, and Gmet 2029. Expression of these genes was normalized with proC expression, a gene shown to be constitutively expressed in Geobacter species (15). Relative levels of expression of the studied genes were calculated by the $2^{-\Delta\Delta CT}$ method (22). Sequences from all primers used for RT-qPCR are listed in Table S3.

Deletion mutants construction and complementation

Primers used for construction of mutants are listed in Table S3. All mutants were made by replacing the gene of interest with a spectinomycin resistance gene as previously described (46). All restriction digestions were carried out according to manufacturer’s instructions. PCR were done with the JumpStart Taq DNA polymerase (Sigma-Aldrich). Briefly, primer pairs were used to amplify by PCR flanking regions of approximately 500 bp downstream and upstream of the target genes using G. metallireducens genomic DNA as template. PCR products were digested with the AvrII (CCTAGG) (NEB, Beverly, MA) restriction endonuclease, ethanol precipitated, and ligated with the T4 DNA ligase (NEB). The ligation reaction was loaded onto a 1% agarose gel, and a 1 kb band was purified using the Qiaquick Gel Extraction Kit (Qiagen)
and cloned into pCR2.1 TOPO cloning vector resulting in pCR2.1up5′+3′dn. Sequences of the cloned products were verified by Sanger sequencing. The spectinomycin resistance cassette was digested with XbaI (TCTAGA) (NEB) from pUC19-Sp′loxP (46) and the recombinant plasmid pCR2.1up5′+3′dn was digested with AvrII. The spectinomycin resistance cassette was cloned into pCR2.1up5′+3′dn to complete the construction of the mutant alleles. Plasmids bearing mutant alleles were linearized by digesting with either KpnI (GGTACC) (NEB) or XhoI (CTCGAG) (NEB) and concentrated by ethanol precipitation. The linearized plasmids were electroporated into *G. metallireducens* as described previously (46). Replacement of wild type alleles by mutant alleles in *G. metallireducens* was verified by PCR. Deletion mutants made in this study were complemented by amplifying the respective genes with their native ribosome binding site (RBS) using *G. metallireducens* genomic DNA as template. The resulting PCR products were digested and cloned under the control of a constitutive lac promoter into pCM66 (33).

**Results and Discussion**

In order to gain insight into which genes coding for outer cell surface proteins might be important for insoluble Fe(III) oxide reduction in *G. metallireducens*, gene transcript abundance was compared in cells grown on Fe(III) oxide or Fe(III) citrate (Table S1). The microarray analysis revealed 792 genes differentially expressed at 2-fold change and 95% confidence. 437 of these genes were up-regulated with growth on Fe(III) oxide, whereas 355 were down-regulated. Additional focus was placed on genes with higher transcript abundance during growth on Fe(III) oxide based on the assumption that genes more highly expressed during growth on Fe(III) oxide are likely to play an important role in this process.
Cytochromes

The microarray analysis revealed 23 genes coding for c-type cytochromes that had higher transcript abundance in cells grown on Fe(III) oxide (Table 1). In order to identify additional cytochromes that might have roles in Fe(III) oxide reduction, proteins were isolated from the outer surface protein fraction and stained for heme because cytochromes localized on the outer surface of the cell have the potential to directly interact with extracellular electron acceptors. Heme-staining of outer-surface proteins separated by SDS-PAGE revealed numerous protein bands with a stronger signal in preparations of cells grown on Fe(III) oxide versus cells grown on Fe(III) citrate, six of which could be identified via liquid-chromatography/mass spectrometry (Fig. 1). Four of the genes identified (Gmet 0679, Gmet 0825, Gmet 0909, and Gmet 2896) were genes that had higher transcript abundance in cells grown on Fe(III) oxide than in Fe(III) citrate-grown cells (Table 1).

The function of c-type cytochromes whose genes had higher transcript abundance during growth on Fe(III) oxide and/or were identified as outer-surface cytochromes was analyzed by constructing mutant strains in which one of the cytochrome genes was deleted. Six of the c-type cytochrome deletion mutants were unable to grow with Fe(III) oxide as the sole electron acceptor, but could grow on Fe(III) citrate (Table 2; Fig. S1). In each case complementation of the deletion mutants with expression of the appropriate gene in trans partially restored the capacity for Fe(III) oxide reduction (Table 2; Fig. S1).

Gmet 2896, which was one of the genes required for Fe(III) oxide reduction, had higher transcript abundance during growth on Fe(III) oxide (Table 1) and the Gmet 2896 protein was
localized outside the cell (Fig. 1). Gmet 2896 is predicted to encode a tetraheme c-type cytochrome in the same family as OmcE of *G. sulfurreducens* with 44% amino acid sequence identity (7), which is also found on the outer surface and is predicted to contain four hemes. Gmet 2896 appeared in a band located around 14 kDa (Fig. 1) even though its predicted molecular weight is 24.9 kDa. This suggests that Gmet_2896 might be processed after translation in a manner similar to OmcZ of *G. sulfurreducens* (20, 36). Long initial lag periods in growth on Fe(III) oxide (34) and current production (14) have suggested a role for OmcE in extracellular electron transfer in *G. sulfurreducens*, but the ability of this strain to adapt for growth on Fe(III) oxide (34) and current production (32) have demonstrated that OmcE is not essential for these functions.

Like Gmet 2896, Gmet 0930 had higher transcript abundance in Fe(III) oxide-grown cells (Table 1) and is required for Fe(III) oxide reduction (Table 2; Fig. S1). Gmet 0930 is in the same family as the gene for OmcZ (20) of *G. sulfurreducens* (7). Unlike the Gmet 0930 protein, OmcZ is not required for Fe(III) oxide reduction (36). However, it is also an outer-surface protein (19) and is required for optimal current production (36, 40). Its localization at the interface between anode biofilms and electrodes suggests that it facilitates electron transfer to electrodes in *G. sulfurreducens* (19).

Gmet 0909, was the most highly up-regulated gene coding for a c-type cytochrome in cells grown on Fe(III) oxide (Table 1) and the Gmet 0909 protein was detected outside the cell (Fig 1). Along with Gmet 0534, Gmet 0909 is the only cytochrome gene with higher transcript abundance during growth on Fe(III) oxide that is conserved across all sequenced *Geobacter* species (7). Numerous attempts to construct a strain in which Gmet 0909 was deleted failed, suggesting that this mutation might also be required for growth on Fe(III) citrate.
The proteins of Gmet 0557 and Gmet 0558 were not detected in the outer surface, but both of these genes, which were predicted to be in the same operon, had much higher transcript levels in cells grown on Fe(III) oxide (Table 1), and are required for Fe(III) oxide reduction (Table 2; Fig S1). Both proteins are predicted to be localized either in the periplasm or in the extracellular fraction (Table 1). Gmet 0557 is predicted to have 4 heme binding sites, whereas Gmet 0558 is predicted to have between 23-27 heme binding sites. The closest homolog to Gmet 0557 in *G. sulfurreducens* is OmcP (GSU2913) with 59% amino acid sequence identity. The closest homolog to Gmet 0558 in *G. sulfurreducens* is OmcO (GSU2912) with 67% amino acid sequence identity. Neither OmcP nor OmcO are essential for Fe(III) oxide reduction by *G. sulfurreducens*, although transcript levels were higher for both genes during growth on Fe(III) oxide compared with Fe(III) citrate (Aklujkar et al., submitted for publication).

Gmet 0557 and Gmet 0558 are located in the same operon as Gmet 0556, which is predicted to encode a NHL repeat-containing protein localized in the extracellular matrix (50), and had higher transcript abundance in cells grown on Fe(III) oxide (Table S1). Gmet 0556 is predicted to contain conserved immunoglobulin-like fold domains, which are considered to play a possible role in cell adhesion in other microorganisms (4). The homolog to Gmet 0556 in *G. sulfurreducens*, GSU2914, was also more highly expressed with growth on Fe(III) oxide compared with Fe(III) citrate (Aklujkar et al., submitted for publication). Furthermore, the homologous gene in *G. uraniireducens*, Gura 3430, was more highly expressed during growth in sediments in which insoluble Fe(III) was expected to be the electron acceptor, compared to cells grown with fumarate as the electron acceptor (16). Deletion of Gmet 0556 produced a strain that could not reduce Fe(III) oxide, but was capable of reducing soluble Fe(III) citrate (Table 2; Fig.
Expressing Gmet 0556 in trans restored the capacity for Fe(III) oxide reduction (Table 2; Fig. S2). Further functional analysis of this protein seems warranted.

Like Gmet 0557 and Gmet 0558, Gmet 1867 and Gmet 1868 are both located in the same operon, had higher transcript levels in cells grown on Fe(III) oxide (Table 1), and their proteins were not detected in the outer-surface proteins. Both Gmet 1867 and Gmet 1868 are required for Fe(III) oxide reduction (Table 2; Fig. S1). Localization of Gmet 1867 is unclear whereas Gmet 1868 is predicted to contain 7-8 heme-binding sites, and Gmet 1868 is predicted to have 4. The closest homolog to Gmet 1867 and Gmet 1868 in *G. sulfurreducens* are respectively GSU1786 (57% amino acid sequence identity) and GSU1787 (69% amino acid sequence identity). Neither of these genes have been previously reported to be involved with Fe(III) oxide reduction in *G. sulfurreducens*, although GSU1787 had higher transcript abundance with growth on Fe(III) oxide compared with Fe(III) citrate (Aklujkar et al., submitted for publication).

Several *c*-type cytochrome deletion mutants exhibited no phenotype on Fe(III) oxide or Fe(III) citrate (Table 2). Gmet 0910 and Gmet 0913 have no homologs in *G. sulfurreducens* (7).

None of the *G. sulfurreducens* homologs for Gmet 0534, Gmet 0571 Gmet 0580, Gmet 0581, Gmet 0679, Gmet 0825, Gmet 0912, Gmet 1866, Gmet 2470 and Gmet 2839 have been found to be essential for optimal Fe(III) oxide reduction (Aklujkar et al., submitted for publication).

**Extracellular Polysaccharide Genes**

A number of genes annotated as contributing to polysaccharide biosynthesis had higher transcript abundance in Fe(III) oxide-grown cells (Table 3). The most highly expressed were Gmet 2028 through Gmet 2032. Genes coding for homologs of Gmet 2028 (GSU1983), Gmet
2029 (GSU1984), Gmet 2030 (GSU1985), Gmet 2031 (GSU1986), and Gmet 2032 (GSU1987) all had higher transcript abundance in *G. sulfurreducens* when grown on Fe(III) oxide compared with Fe(III) citrate (Aklujkar et al., submitted for publication). Furthermore, homologs of Gmet 2030 (Gura 1669), Gmet 2029 (Gura 1670), Gmet 2028 (Gura 1670), Gmet 2003 (Gura 2342) and Gmet 0458 (Gura 1672) were up-regulated in *G. uraniireducens* grown on sediments in which insoluble Fe(III) was expected to be the electron acceptor, compared to cells grown with fumarate as the electron acceptor (16), suggesting conserved functions for these proteins in the process of extracellular electron transfer among *Geobacter* species. It has recently been proposed that another gene (*xapD*; GSU1501) involved in an extracellular polysaccharide network in *G. sulfurreducens* contributes to insoluble Fe(III) reduction, biofilm formation and c-type cytochrome anchoring (41, 42). The homologue of *xapD* in *G. metallireducens* (Gmet_1403) was not differentially expressed on Fe(III) oxide compared to ferric citrate.

The potential role of the highly expressed Gmet 2029, Gmet 2030, Gmet 2031 and Gmet 2032 in Fe(III) oxide reduction was investigated by gene deletion. Only the loss of Gmet 2029 resulted in a *G. metallireducens* strain incapable of reducing Fe(III) oxide (Table 2; Fig. S3).

Gmet 2029 is predicted to encode a polysaccharide chain length determinant protein of the Wzz family. The Wzz proteins of several bacterial species (5, 10, 12, 13) are implicated in the length determination of the O polysaccharide, a major component of lipopolysaccharide (LPS). However, members of the Wzz protein family are found throughout the *Geobacteraceae* family including *G. sulfurreducens* (amino acid sequence identity = 39%) which produces a rough LPS without the O polysaccharide (48). The Wzz protein of *Pseudomonas aeruginosa* also appears to determine the chain length of polysaccharides involved in pilin glycosylation (11, 18). Thus, a
potential role of Gmet 2029 is modification of the pili known to play a role in long-range
electron transport.

Expression of Genes Previously Identified as Important in Fe(III) Reduction

In some instances gene expression patterns were consistent with previous observations of
G. metallireducens physiological differences between cells grown on Fe(III) oxide and Fe(III)
citrate.

For example, G. metallireducens grown on Fe(III) oxide produces flagella whereas cells
grown on Fe(III) citrate do not (8) and chemotaxis and motility are thought to be important in
Fe(III) oxide reduction (8, 46, 47). Genes encoding for flagella or chemotaxis proteins
represented a high proportion of the genes with the greatest increase in transcript abundance in
Fe(III) oxide-grown cells (Table 4). Gmet 0442, a gene coding for the flagellin protein FliC, was
the most highly up-regulated gene during growth on Fe(III) oxide.

The type IV pili of Geobacter sulfurreducens have metallic-like conductivity (32) and are
considered to be conduits for electron transfer to Fe(III) oxide (25, 39). G. metallireducens
produces type IV pili during growth on Fe(III) oxide, but not on Fe(III) citrate, which was
attributed to differences in the expression of the gene for the structural PilA protein (8).
Microarray results indicated a slight increase in pilA transcript abundance in Fe(III) oxide-grown
cells (1.7-fold; p-value = 0.19). The difference in transcript abundance for other genes
associated with pili functions was somewhat higher (Table 5). A strain of G. metallireducens in
which pilA was deleted was unable to reduce Fe(III) oxide, but retained the capacity for Fe(III)
citrate reduction, further suggesting the importance of pili in Fe(III) oxide reduction by G.
metallireducens (46).
Implications

These studies demonstrate that although *G. metallireducens* is closely related to *G. sulfurreducens* the outer surface c-type cytochromes that are essential for Fe(III) oxide reduction in these two species are distinct. The identification of a select few outer surface cytochromes from the over 91 putative c-type cytochrome genes in the *G. metallireducens* genome (1) generates a manageable list for future studies on mechanisms for electron transfer to Fe(III) oxide in this organism. Most important will be the development of antibodies or other reagents that will make it possible to determine the localization of the cytochromes as determining whether cytochromes required for Fe(III) oxide reduction are associated with the outer membrane, pili, or in the outer matrix will provide further insights into their functional role.

The identification of a NHL- repeat containing protein and of a Wzz family protein as being essential for Fe(III) oxide reduction demonstrates that there are still unknown components involved in extracellular electron transfer in *Geobacter* species and that the current understanding of this phenomenon is not complete.

Acknowledgements

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References


Figure legend

Figure 1. c-type cytochrome content of outer membrane and loosely bound fractions from Fe(III) oxide and Fe(III) citrate cultures. Proteins (2 µg/lane) were separated by SDS-PAGE and stained for heme (A). Protein ladder was SeeBlue Plus2 Pre-Stained Standard (Invitrogen, Carlsbad, CA). Characteristics of identified c-type cytochrome (B). Subcellular localization predictions were done with PSORTb 3.0 (50).
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Table 1. Genes coding for c-type cytochrome up-regulated at least two-fold in *G. metallireducens* when Fe(III) oxide is the electron acceptor (P-value cutoff ≤ 0.01)

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<tr>
<td>Gmet 0825</td>
<td>cytochrome c, 11-12 heme-binding sites</td>
<td>Extracellular</td>
<td>2.5</td>
</tr>
<tr>
<td>Gmet 0930</td>
<td>cytochrome c, 6-8 heme-binding sites</td>
<td>Extracellular or periplasmic</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Subcellular localization predictions were done with PSORTb 3.0 (50).
<table>
<thead>
<tr>
<th>Strain</th>
<th>Fe(III) oxide (mM Fe(II)/day)</th>
<th>Fe(III) citrate (mM Fe(II)/hour)</th>
<th>Complementation Fe(III) oxide (mM Fe(II)/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type</td>
<td>6.1 ± 0.6</td>
<td>3.7 ± 0.2</td>
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<tr>
<td>c-type cytochrome mutants</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gmet 2896</td>
<td>0.0 ± 0.0</td>
<td>3.5 ± 0.2</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>Gmet 0930</td>
<td>0.0 ± 0.0</td>
<td>3.6 ± 0.2</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>Gmet 0557</td>
<td>0.0 ± 0.0</td>
<td>3.6 ± 0.1</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Gmet 0558</td>
<td>0.0 ± 0.0</td>
<td>3.1 ± 0.2</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Gmet 1867</td>
<td>0.0 ± 0.0</td>
<td>3.8 ± 0.2</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Gmet 1868</td>
<td>0.0 ± 0.0</td>
<td>3.9 ± 0.1</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Gmet 0534</td>
<td>5.7 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gmet 0571</td>
<td>4.9 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gmet 0580</td>
<td>5.3 ± 0.5</td>
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</tr>
<tr>
<td>Gmet 0581</td>
<td>6.4 ± 0.6</td>
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</tr>
<tr>
<td>Gmet 0679</td>
<td>5.7 ± 0.5</td>
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<tr>
<td>Gmet 0825</td>
<td>4.4 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gmet 0910</td>
<td>6.0 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gmet 0912</td>
<td>5.5 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gmet 0913</td>
<td>5.7 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gmet 1866</td>
<td>6.2 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gmet 2470</td>
<td>5.5 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gmet 2839</td>
<td>6.4 ± 0.5</td>
<td></td>
<td></td>
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<tr>
<td>Other mutant</td>
<td></td>
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</tr>
<tr>
<td>Gmet 0556</td>
<td>0.0 ± 0.0</td>
<td>3.9 ± 0.3</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>Polysaccharide-associated mutants</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gmet 2029</td>
<td>0.0 ± 0.0</td>
<td>3.8 ± 0.3</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Gmet 2030</td>
<td>5.9 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gmet 2031</td>
<td>6.1 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gmet 2032</td>
<td>6.2 ± 0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Each value is the mean and standard deviation of at least three replicates.*
Table 3. Polysaccharide biosynthesis-associated genes up-regulated at least two-fold in *G. metallireducens* when Fe(III) oxide is the electron acceptor (P-value cutoff ≤ 0.01)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Annotation</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gmet 2030</td>
<td>periplasmic polysaccharide biosynthesis/export protein</td>
<td>20.9</td>
</tr>
<tr>
<td>Gmet 2029</td>
<td>polysaccharide chain length determinant protein, putative; Wzz family</td>
<td>14.3</td>
</tr>
<tr>
<td>Gmet 2032</td>
<td>TPR domain lipoprotein</td>
<td>12.3</td>
</tr>
<tr>
<td>Gmet 2028</td>
<td>polysaccharide biosynthesis protein, putative</td>
<td>11.8</td>
</tr>
<tr>
<td>Gmet 2031</td>
<td>glycosyltransferase domain protein</td>
<td>7.9</td>
</tr>
<tr>
<td>Gmet 2003</td>
<td>exopolysaccharide synthesis multitransmembrane protein H (exosortase); EpsH</td>
<td>4.9</td>
</tr>
<tr>
<td>Gmet 2023</td>
<td>polysaccharide deacetylase domain protein</td>
<td>4.7</td>
</tr>
<tr>
<td>Gmet 2013</td>
<td>polysaccharide deacetylase, putative</td>
<td>3.8</td>
</tr>
<tr>
<td>Gmet 0458</td>
<td>polysaccharide biosynthesis protein; CapD</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Table 4. Motility genes up-regulated at least 30-fold in *G. metallireducens* when Fe(III) oxide is the electron acceptor (P-value cutoff ≤ 0.01)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Annotation</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gmet 0442</td>
<td>flagellin FliC</td>
<td>94.5</td>
</tr>
<tr>
<td>Gmet 0719</td>
<td>conserved hypothetical protein</td>
<td>77.0</td>
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<tr>
<td>Gmet 0438</td>
<td>flagellar hook-associated protein FlgK</td>
<td>56.9</td>
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<tr>
<td>Gmet 0430</td>
<td>flagellar basal body rod protein FlgF</td>
<td>55.8</td>
</tr>
<tr>
<td>Gmet 0439</td>
<td>flagellar hook-filament junction protein FlgL</td>
<td>52.7</td>
</tr>
<tr>
<td>Gmet 0432</td>
<td>flagellar basal body P-ring formation protein FlgA</td>
<td>49.0</td>
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<tr>
<td>Gmet 0431</td>
<td>flagellar basal body rod protein FlgG</td>
<td>43.8</td>
</tr>
<tr>
<td>Gmet 3115</td>
<td>flagellar basal-body rod protein FlgB</td>
<td>43.1</td>
</tr>
<tr>
<td>Gmet 3112</td>
<td>flagellar M-ring mounting plate protein FliF</td>
<td>42.5</td>
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<tr>
<td>Gmet 3104</td>
<td>flagellar operon protein of unknown function DUF3766</td>
<td>39.5</td>
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<tr>
<td>Gmet 3098</td>
<td>flagellar biogenesis protein FlhO</td>
<td>39.1</td>
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<tr>
<td>Gmet 0427</td>
<td>flagellar biogenesis protein FlhF</td>
<td>37.6</td>
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<tr>
<td>Gmet 0444</td>
<td>flagellar filament cap protein FliD</td>
<td>32.1</td>
</tr>
<tr>
<td>Gmet 3101</td>
<td>flagellar basal body-associated protein FliL</td>
<td>31.0</td>
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</tbody>
</table>
Table 5. Pilus-associated genes up-regulated at least two-fold in *G. metallireducens* when Fe(III) oxide is the electron acceptor (P-value cutoff ≤ 0.05)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Annotation</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gmet 0967</td>
<td>Type IV pilus tip-associated adhesion PilY1-2</td>
<td>4.6</td>
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<tr>
<td>Gmet 1395</td>
<td>Type IV pilus biogenesis protein PilC</td>
<td>4.0</td>
</tr>
<tr>
<td>Gmet 0974</td>
<td>Type IV pilus assembly lipoprotein PilP</td>
<td>3.6</td>
</tr>
<tr>
<td>Gmet 3400</td>
<td>Twitching motility pilus retraction protein; PilT 3</td>
<td>3.4</td>
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<tr>
<td>Gmet 0975</td>
<td>Type IV pilus secretion lipoprotein PilQ</td>
<td>3.2</td>
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<tr>
<td>Gmet 0959</td>
<td>Type IV prepilin peptidase</td>
<td>2.0</td>
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</tbody>
</table>