

An Inner Membrane Cytochrome Required Only for Reduction of High Redox Potential Extracellular Electron Acceptors

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ABSTRACT Dissimilatory metal-reducing bacteria, such as *Geobacter sulfurreducens*, transfer electrons beyond their outer membranes to Fe(III) and Mn(IV) oxides, heavy metals, and electrodes in electrochemical devices. In the environment, metal acceptors exist in multiple chelated and insoluble forms that span a range of redox potentials and offer different amounts of available energy. Despite this, metal-reducing bacteria have not been shown to alter their electron transfer strategies to take advantage of these energy differences. Disruption of *imcH*, encoding an inner membrane *c*-type cytochrome, eliminated the ability of *G. sulfurreducens* to reduce Fe(III) citrate, Fe(III)-EDTA, and insoluble Mn(IV) oxides, electron acceptors with potentials greater than 0.1 V versus the standard hydrogen electrode (SHE), but the *imcH* mutant retained the ability to reduce Fe(III) oxides with potentials of ≤ -0.1 V versus SHE. The *imcH* mutant failed to grow on electrodes poised at +0.24 V versus SHE, but switching electrodes to -0.1 V versus SHE triggered exponential growth. At potentials of ≤ -0.1 V versus SHE, both the wild type and the *imcH* mutant doubled 60% slower than at higher potentials. Electrodes poised even 100 mV higher (0.0 V versus SHE) could not trigger *imcH* mutant growth. These results demonstrate that *G. sulfurreducens* possesses multiple respiratory pathways, that some of these pathways are in operation only after exposure to low redox potentials, and that electron flow can be coupled to generation of different amounts of energy for growth. The redox potentials that trigger these behaviors mirror those of metal acceptors common in subsurface environments where *Geobacter* is found.

IMPORTANCE Insoluble metal oxides in the environment represent a common and vast reservoir of energy for respiratory microbes capable of transferring electrons across their insulating membranes to external acceptors, a process termed extracellular electron transfer. Despite the global biogeochemical importance of metal cycling and the ability of such organisms to produce electricity at electrodes, fundamental gaps in the understanding of extracellular electron transfer biochemistry exist. Here, we describe a conserved inner membrane redox protein in *Geobacter sulfurreducens* which is required only for electron transfer to high-potential compounds, and we show that *G. sulfurreducens* has the ability to utilize different electron transfer pathways in response to the amount of energy available in a metal or electrode distant from the cell.

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Geobacter sulfurreducens is a model dissimilatory metal-reducing anaerobe able to completely oxidize organic compounds inside the cell and transfer the resulting electrons to terminal acceptors beyond the outer membrane (1, 2). Extracellular electron acceptors utilized by *G. sulfurreducens* include chelated transition metals, particulate Fe(III) and Mn(IV) oxides (2), and electrodes poised at oxidizing redox potentials (3). *Geobacter* representatives are abundant in anoxic metal-reducing habitats, including aquatic sediments (2), Fe(III)-rich petroleum-contaminated sites (4), zones where U(VI) reduction is stimulated by organic acid addition (5), subsurface aquifers where Fe(III) reduction releases arsenic into drinking water (6, 7), and on electrodes used to produce electrical energy (8, 9). Despite their contribution to global biogeochemical processes and emerging biotechnological applications, the molecular mechanism for electron transfer across the inner membrane of *Geobacter* is not known, and there is no respiratory protein-based marker for monitoring

the activity of these ubiquitous metal-reducing bacteria in their natural environment.

Part of the difficulty in studying *Geobacter* stems from the diversity of redox proteins potentially utilized by these organisms for respiration. *Geobacter* genomes typically encode 60 to 90 multiheme *c*-type cytochromes, few of which are conserved between all species (10). This is in stark contrast to another well-studied metal-reducing family, the *Shewanellaceae*. This group of facultative anaerobes encodes a single inner membrane NapC/NirT family tetraheme cytochrome (CymA) (11) that passes electrons to outer membrane conduits comprised of two decaheme *c*-type cytochromes (12–14). Synthesis of all proteins involved in the *Shewanella* pathway is simply induced by a shift to anaerobic conditions, rather than the presence of metals, and deletion of the CymA inner membrane cytochrome eliminates growth with all extracellular electron acceptors (11, 15). In contrast, *G. sulfurreducens* exhibits a complex transcriptional response to different

extracellular electron acceptors (16–19), and no single deletion eliminates electron transfer to all electron acceptors (20, 21).

Despite this evidence for complexity, published models of the *Geobacter* electron transport chain invoke a single *Shewanella*-like route from the quinone pool to an array of outer surface proteins able to interact with soluble compounds, which then are suggested to pass electrons further to extracellular proteins interacting with larger acceptors (22–24). If such models are true, mutants defective in reduction of soluble metals, such as Fe(III) citrate, should also be defective in utilization of all insoluble acceptors, such as Fe(III) oxides. Such a hypothesis is challenged by directed mutant (20, 25) and transposon mutagenesis (21) studies that continue to find *G. sulfurreducens* mutants defective in electron transfer to only a subset of extracellular acceptors. The array of *Geobacter* mutant phenotypes argues against a simple single pathway, as well as models where different proteins are required based on solubility (chelates versus oxides) or metal content (Fe versus Mn) of the acceptor.

In this report, we describe how redox potential explains many of these discrepancies, through discovery of ImcH, an inner membrane *c*-type cytochrome in *G. sulfurreducens*. The *imcH* mutant reduced insoluble Fe(III) oxides yet could not reduce insoluble Mn(IV) oxides or chelated Fe(III). Because of the low electron-accepting potential of Fe(III) oxides relative to these other compounds, poised electrodes were used to investigate the role of redox potential. A switch from high to low potential induced respiration of the *imcH* mutant but only when electrodes were poised at a sufficiently low redox potential of -0.1 V (versus the standard hydrogen electrode [SHE]). These experiments are consistent with different pathways being used by *G. sulfurreducens* for transfer of electrons out of the quinone pool to low- versus high-potential acceptors and suggests a mechanism for sensing the redox potential of extracellular objects. These findings also provide a molecular explanation for recent electrochemical evidence supporting at least two separate electron transfer pathways out of *Geobacter* electrode-grown cells (26). Since Fe(III) naturally occurs in up to 15 different oxide or oxyhydroxide forms (27), spanning more than half a volt of redox potential (28, 29), multiple electron transfer pathways could be utilized by *Geobacteraceae* in response to the energy available in environmentally relevant metals and provide an explanation for cytochrome diversity in these organisms.

RESULTS

ImcH is an inner membrane multiheme *c*-type cytochrome. In a modified transposon mutagenesis protocol (21), 18 independent mutants containing insertions in the locus GSU3259 which were unable to grow with soluble Fe(III) citrate as the electron acceptor were found (Fig. 1A). The protein disrupted in these mutants was predicted to contain up to three transmembrane helices (depending on processing of a putative signal-anchor), a region of NapC/NirT homology, and up to 7 *c*-type heme binding motifs (Fig. 1B). Since NapC/NirT-family proteins typically transfer electrons from the quinone pool to periplasmic acceptors (11, 30) and no such enzyme has been described for *Geobacter* spp., this protein was targeted for further study. By fusing a C-terminal polyhistidine tag to genomic GSU3259, we confirmed that the protein was expressed even during growth with the non-metal acceptor fumarate and colocalized with succinate dehydrogenase in membranes separated via sucrose gradient centrifugation (Fig. 1C). This was

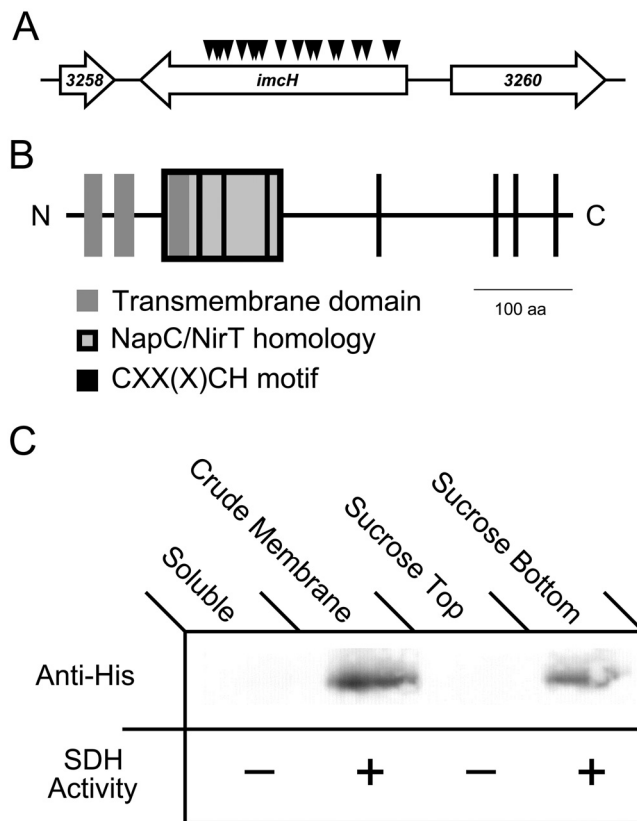


FIG 1 Domain structure and inner membrane localization of ImcH. (A) The monocistronic *imcH* gene was interrupted 18 times using transposon mutagenesis (black triangles). All transposon mutants in this gene failed to reduce soluble Fe(III) citrate. (B) ImcH has three putative transmembrane helices (gray bars), seven putative *c*-type heme motifs (black bars), and a region of homology to NapC/NirT-like quinone oxidoreductases (gray box). The first putative *c*-type heme motif is CXXXCH. (C) Sucrose-gradient separated ImcH-His \times 6 membrane fractions, tested for succinate dehydrogenase (SDH) activity as a marker of inner membranes, and probed using an anti-6 \times His antibody. ImcH was detected only in fractions with SDH activity.

consistent with proteomic surveys which reported that the native protein is localized to the inner membrane (18), as well as software predictions (PSORTb, v 3.0.2) (31). Based on these results, GSU3259 was named *imcH* (inner membrane cytochrome *H*).

Deletion of *imcH* eliminates electron transfer to soluble Fe(III). When the monocistronic *imcH* gene was replaced with a kanamycin resistance cassette, growth with fumarate as the electron acceptor was unaffected (Fig. 2A). The $\Delta imcH::Kan^r$ mutant retained the ability to attach to polystyrene plates in crystal violet biofilm assays ($92\% \pm 10.3\%$ of the wild-type level [$n = 4$]), and fumarate-grown mutant cells did not show differences in major *c*-type cytochromes commonly visualized by staining with 3,3',5,5'-tetramethylbenzidine.

Consistent with the transposon mutagenesis findings, the $\Delta imcH::Kan^r$ mutant was unable to grow with chelated Fe(III) as the electron acceptor (Fig. 2B) and could not reduce Fe(III) citrate in washed-cell assays (Fig. 2C). Complementation with *imcH* in *trans* restored the mutant's ability to reduce Fe(III) citrate (Fig. 2C).

Experiments were performed to test if the inability to reduce Fe(III) citrate was due to toxicity, rather than a defect in electron

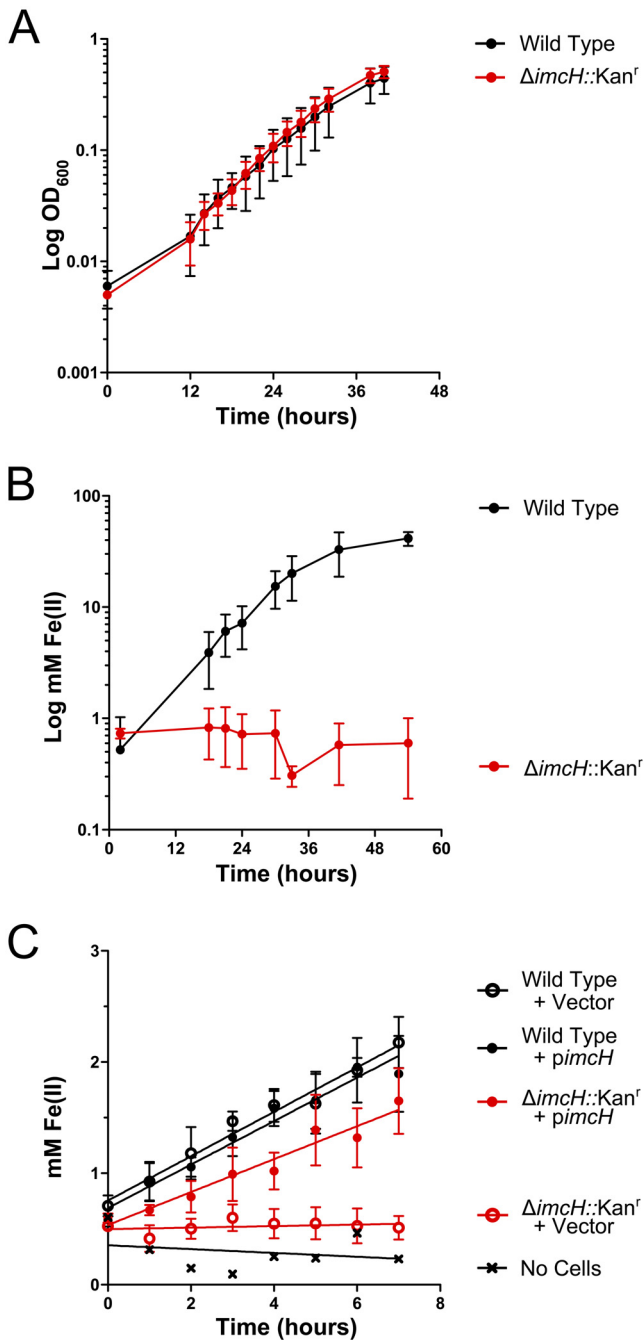


FIG 2 ImcH is required for electron transfer to soluble Fe(III). (A) Optical density of the wild type versus that of the *imcH* deletion mutant during growth with fumarate as the electron acceptor (\pm SD; $n = 5$). (B) Production of Fe(II) for the wild type versus the *imcH* deletion mutant during growth using Fe(III) citrate as the electron acceptor (\pm SD; $n = 3$). (C) Washed-cell assays showing complementation of the Fe(III) citrate reduction phenotype by expressing *imcH* in *trans*. Open circles represent strains carrying the empty vector, and filled circles indicate strains carrying the complementation vector (*pimcH*) (\pm SD; $n = 3$).

transfer. Wild-type and $\Delta imcH::Kan^r$ cells using fumarate as the electron acceptor grew at identical rates after addition of Fe(III) citrate. Wild-type cells immediately reduced added Fe(III), further supporting constitutive expression of *imcH*, while mutants

lacking *imcH* failed to reduce Fe(III) even after hours of incubation. Further assays demonstrated that $\Delta imcH::Kan^r$ mutants also could not reduce Fe(III)-EDTA, consistent with a defect in electron transfer rather than toxicity due to iron or a specific chelator.

Deletion of *imcH* eliminates electron transfer to extracellular Mn(IV) oxides but not Fe(III) oxides. In addition to their inability to transfer electrons to soluble acceptors, $\Delta imcH::Kan^r$ cells also could not reduce laboratory-synthesized Mn(IV) oxides (birnessite) (Fig. 3A). This lack of reduction was observed using Mn(IV) oxide concentrations between 5 and 50 mM, with inoculation sizes ranging from an optical density at 600 nm (OD₆₀₀) of 0.005 to 0.05, suggesting that this was again related to electron transfer and not due to Mn(IV) toxicity or growth inhibition by the metal.

Based upon the general defect with chelated Fe(III) and insoluble Mn(IV), it was expected that the *imcH* mutant also would not reduce insoluble Fe(III) oxides. Unexpectedly, laboratory-synthesized Fe(III) oxides were reduced by the $\Delta imcH::Kan^r$ mutant (Fig. 3B). Using the freshly precipitated Fe(III) oxide ferrihydrite, a minor lag was observed in the first days of Fe(III) reduction by the mutant, but rates and extents converged within 1 week ($n = 4$). The same phenotype of wild-type Fe(III) reduction by $\Delta imcH::Kan^r$ cells was observed using goethite, a more crystalline form of Fe(III) oxide (Fig. 3C). The observation that deletion of a cytochrome prevented reduction of insoluble Mn(IV) oxides but not insoluble Fe(III) oxides contradicted a model where electron acceptors beyond the outer membrane are reduced by similar machinery. Since mutant cultures reduced some forms of Fe(III) (oxides) but not others (chelates), the phenotype also did not support a model where soluble metal reductases handed off electrons to more distant proteins able to interact with all insoluble metals.

In these experiments, a trend did emerge; the *imcH* mutant could not reduce electron acceptors with high redox potentials, such as Fe(III)-EDTA (+0.1 V), Fe(III) citrate (+0.38 V), and Mn(IV) oxides ($> +0.4$ V). The two Fe(III) oxides still reduced by the mutant represented much lower redox potentials, reported to be in the range of -0.2 to 0 V (28, 29, 32). With less energy available per electron to pump protons or drive reduction, we hypothesized that Fe(III) oxides might require a different electron transfer chain and that ImcH was not essential with these low-potential acceptors. The ability to alter electrode potentials in electrochemical reactors while other variables remain constant allowed for the direct testing of this hypothesis.

Mutants lacking *imcH* can transfer electrons only to electrodes poised to mimic low-potential extracellular electron acceptors. To examine the effect of redox potential on the $\Delta imcH::Kan^r$ mutant, poised electrodes were used as electron acceptors. The redox potential typically chosen for bioelectrochemical experiments is relatively high (+0.2 to +0.4 V) (33–35), since electrode-based studies are often aimed at providing an unlimited electron sink for bacteria (34, 36). The $\Delta imcH::Kan^r$ mutant was unable to transfer electrons to an electrode held at this standard potential (+0.24 V), while the wild type grew with a maximum doubling time of ~ 6 h to a value of $400 \mu\text{A}/\text{cm}^2$ within 3 days (Fig. 4A).

In contrast, when electrodes were poised at a lower redox potential (-0.1 V versus SHE), growth of the $\Delta imcH::Kan^r$ mutant occurred. Mutant cells grew at rates identical to that of wild type controls exposed to the same low redox potential. However, at this

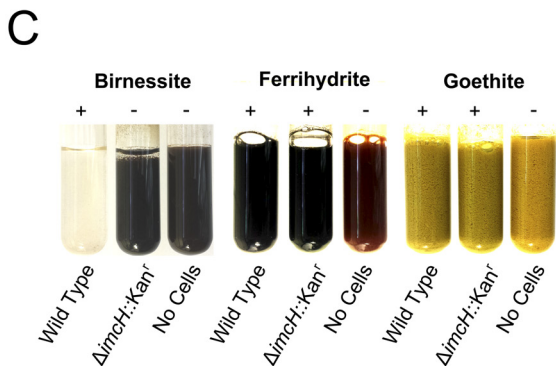
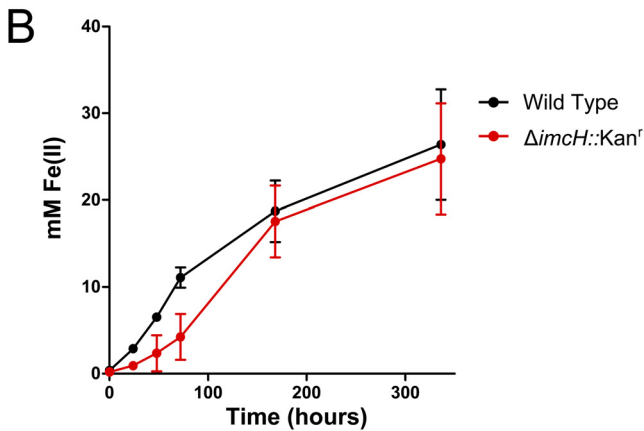
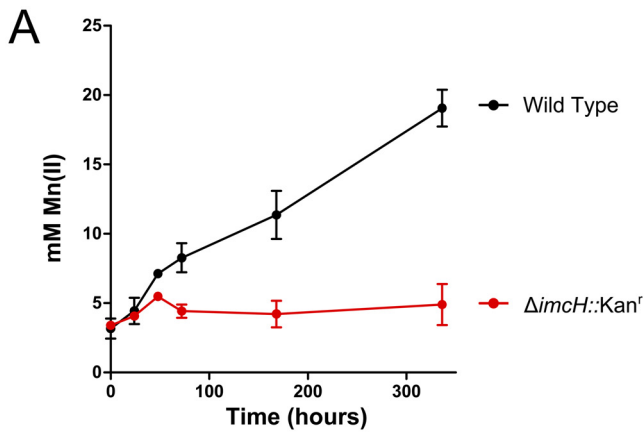


FIG 3 *ImcH* is essential for electron transfer to insoluble Mn(IV) oxide but not reduction of insoluble Fe(III) oxide. (A) Reduction of insoluble Mn(IV) oxide (birnessite) to soluble Mn(II) by the wild type versus that by the *imcH* deletion mutant (\pm SD; $n = 3$). (B) Reduction of insoluble Fe(III) oxide (ferrihydrite) to Fe(II) by the wild type versus that by the *imcH* deletion mutant (\pm SD; $n = 3$). (C) Examples of solubility and mineral form changes accompanying incubation with metal oxides by *G. sulfurreducens*. Brightness and contrast of each oxide image were adjusted in an identical manner.

lower potential, the exponential growth rates of both the mutant and the wild type were slower, with current doubling every ~ 9 h, and both cultures achieved a similar current density of $\sim 150 \mu\text{A}/\text{cm}^2$ within 3 days (Fig. 4B). When *imcH* mutants were exposed to low potentials to establish a biofilm capable of the slower growth rate and then switched to high potentials, the slow growth rate persisted (see Fig. S1 in the supplemental material).

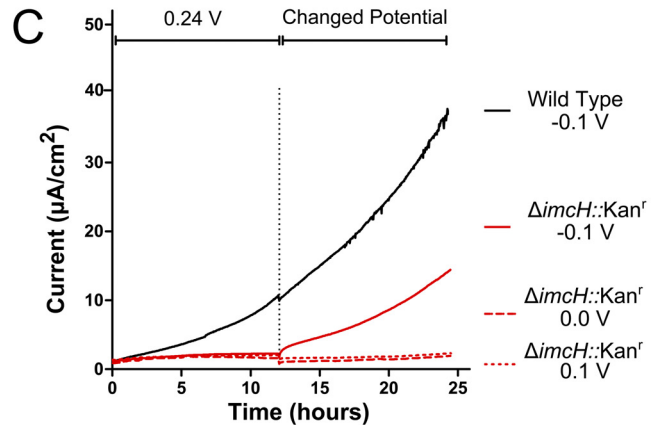
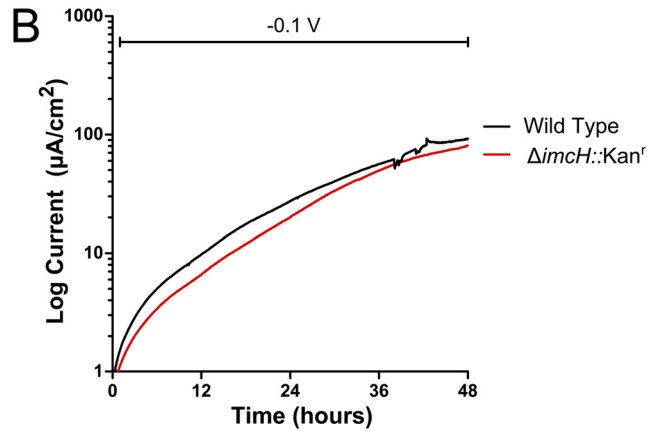
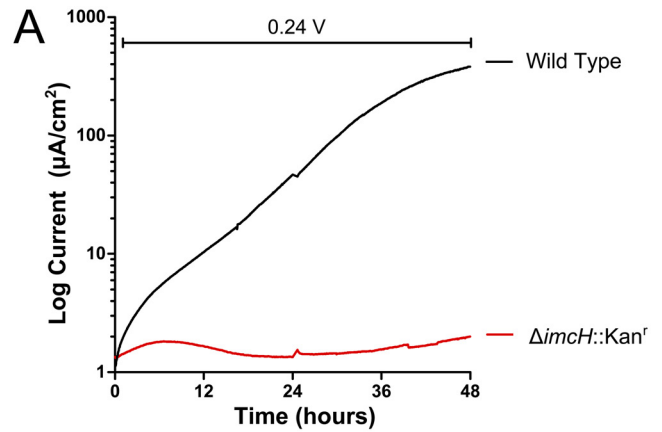


FIG 4 *ImcH* is required for electron transfer to high-potential electrodes but not for low-potential electrodes. Current at a working graphite electrode is positive (anodic) when *Geobacter* oxidizes acetate and transfers electrons to the surface. (A) The wild type versus the *imcH* deletion mutant exposed to a working electrode poised at a potential of $+0.24$ V versus SHE. (B) The wild type versus the *imcH* deletion mutant exposed to a working electrode poised at a potential of -0.1 V versus SHE. (C) The specific effect of electrode potential on growth of the mutant lacking *imcH*. Electrodes were poised at $+0.24$ V versus SHE for 12 h and then changed to the potential indicated. All experiments were performed in triplicate; representative traces are shown.

Additional experiments provided evidence that only specific redox potentials triggered respiration. First, the precision of electrodes allowed testing of whether growth gradually improved as

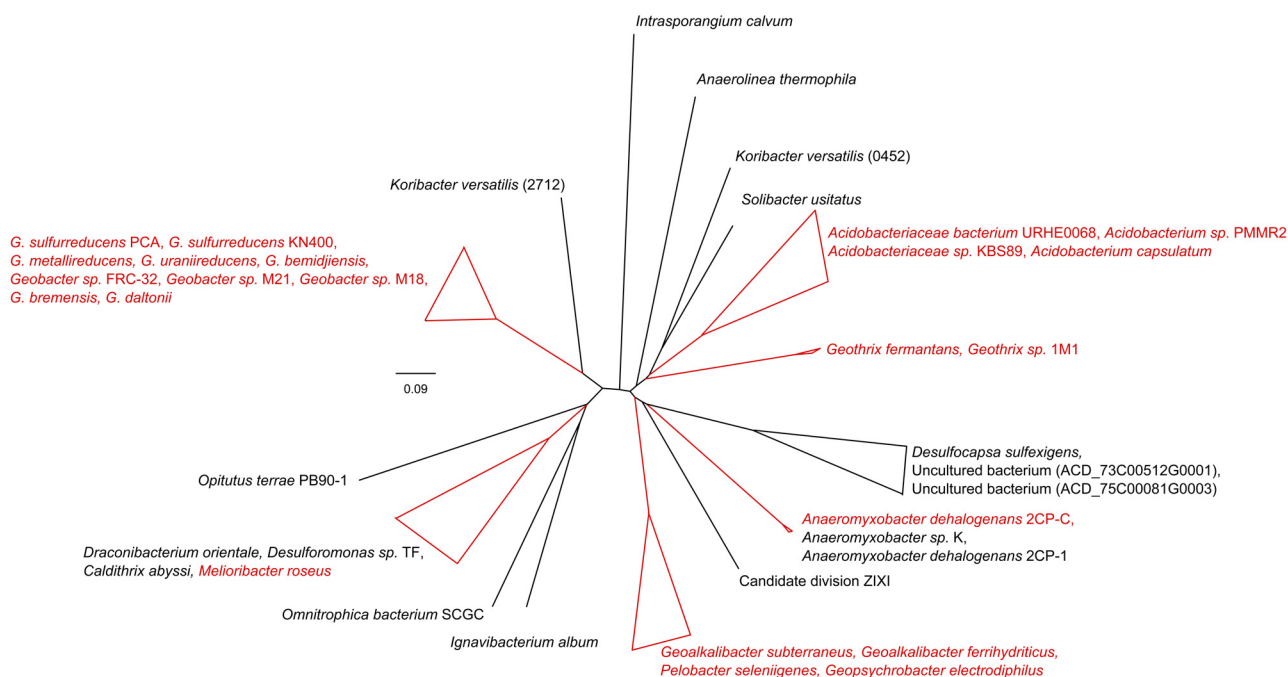


FIG 5 ImcH homologues are widely distributed. Only proteins with at least 75% total of the sequence length of *Geobacter sulfurreducens*' ImcH were included. Alignment was performed using the default settings in the ClustalO program, with FigTree v 1.4.0 used to generate the graphical representation of the alignment. Clusters with 50% or greater identity were collapsed. Clusters in which pure culture-based evidence for extracellular electron transfer has been found are shown in red, with the representative species/strain names also shown in red.

potential was lowered or if there was a threshold required to induce growth of cells lacking *imcH*. External potentials of +0.24 V, +0.1 V, and 0 V did not support growth of *imcH* mutants (Fig. 4A and C). In contrast, lowering the redox potential by only 100 mV further, to -0.1 V, triggered growth (Fig. 4C).

Failure to grow on electrodes could hypothetically be caused by an inability of cells to attach to surfaces (37). To test if attachment played a role, *imcH* mutants were incubated with high-potential (+0.24 V) electrodes, and planktonic cells were washed from the chamber prior to a switch to lower potential (-0.1 V). Growth of *imcH* mutants was again triggered after the potential switch and produced responses similar to data shown in Fig. 4C, showing that cells attached during the high-potential phase. Additionally, electrodes harvested after 12-h incubations had similar levels of attached protein at both permissive (-0.1 V, $18.9 \pm 0.4 \mu\text{g}/\text{electrode}$; $n = 2$) and nonpermissive (0.0 and 0.1 V, 22.40 ± 3.4 and $15.11 \pm 3.9 \mu\text{g}/\text{electrode}$, respectively; $n = 2$) potentials. These data confirmed that the mutation did not affect binding of cells to electrodes and indicated that the redox potential of a solid electron acceptor could be the sole signal causing *G. sulfurreducens* to alter its electron transfer pathway.

DISCUSSION

These results show that *G. sulfurreducens* cannot transfer electrons to high redox potential electron acceptors without the inner membrane multiheme *c*-type cytochrome ImcH. However, after exposure to low redox potentials, either in the form of certain Fe(III) oxides or poised electrodes, *imcH* mutants are able to utilize an alternative pathway that enables respiration and growth. The growth rate of wild-type and mutant cultures at lower redox potentials is slower, supporting the hypothesis that these strategies

are distinct and that cells generate less ATP per electron when using lower-energy strategies. Based on these data, *G. sulfurreducens* has electron transfer pathways which can be differentially utilized in response to the redox potential of an extracellular electron acceptor, such as a metal oxide or an electrode. Since the potentials of many environmentally relevant metals lie on opposite sides of the threshold able to trigger this choice, these findings help explain conflicting *Geobacter* mutant phenotypes, suggest that genetic markers may exist for monitoring subsurface redox conditions, and imply that bacteria can sense the redox potential of terminal electron acceptors.

With its transmembrane helices, N-terminal NapC/NirT homology, and *c*-type hemes, ImcH represents a new family of bacterial redox proteins implicated in extracellular electron transfer. Homologs of ImcH are present in all *Geobacteraceae* isolated for a metal-reducing phenotype, as well as related metal-reducing *Anaeromyxobacter* spp., but are notably absent in fermentative (*Pelobacter* spp.) and chlororespiratory (*Geobacter lovleyi*) members of this cluster (Fig. 5). Homologs of *imcH* are found in the *Acidobacteria* and *Planctomyces-Verrumicrobia-Chlorobium* phyla, which contain Fe(III)-reducing genera (*Geothrix* and *Melioribacter*, respectively) (38, 39), suggesting that other relatives should be reinvestigated for their respiratory abilities (Fig. 5). With the increasing availability of single-cell and metagenomic sequences, *imcH* may serve as an aid for prediction of respiratory strategies, but discovery of what proteins interact with ImcH will greatly improve sequence-based predictions.

In the laboratory, electrochemical systems are routinely used to study electron transfer kinetics (3, 36), biofilm development (21, 37, 40), and long range electron flow between metal-reducing bacteria (40–42), with the justification that these insights help

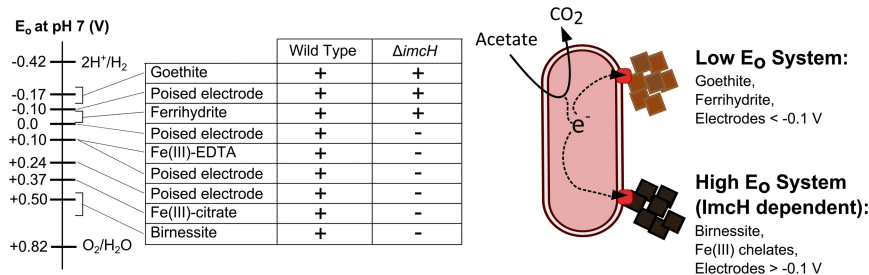


FIG 6 Summary of reduction potentials tested and general model for inner membrane electron transfer pathways. Reduction potentials versus standard hydrogen electrode (SHE) for all metal oxides, chelates, and electrodes tested as electron acceptors for wild-type and $\Delta imcH::Kan^r$ strains in this study. Due to the heterogeneity of metal oxides, a range of potentials from previously reported values are shown. Reduction of the electron acceptor by a given strain is indicated as “+,” whereas lack of reduction is indicated as “-.” According to this model, cells typically route electron flux out of the quinone pool via an ImcH-dependent pathway for reduction of high-potential acceptors, while an as yet undiscovered pathway is used for low-potential acceptors.

explain reduction of environmentally relevant metal oxides. These data show that most electrodes are not poised at the proper potential to mimic electron transfer to poorly crystalline Fe(III) oxides, such as ferrihydrite (Fig. 6), typically the largest reservoir of reducible Fe(III) in sediments and aquifers (27). Based on recent measurements, the redox potential of laboratory goethite is -0.17 V versus SHE (43), and ferrihydrite/Fe(II) mixtures begin at ~ 0.0 V versus SHE but decrease rapidly to -0.1 V or lower as Fe(II) accumulates in the medium (28, 32, 44). The difference between high-potential laboratory electrode experiments and environmental Fe(III) oxide electron acceptors may explain how outer membrane cytochromes, such as OmcS or OmcB, can appear to be important to reduction of Fe(III) oxides but not electrodes and lead to confusion when proposing electron transfer models (33, 45, 46).

Looking forward, the observation that growth at low redox potential acceptors requires different proteins and induces a characteristically lower growth rate produces a number of hypotheses which can be tested in *Geobacter*. First, multiple inner membrane quinone oxidoreductases must exist in *G. sulfurreducens*, with at least one that is essential for low-potential respiration. These low-potential pathways should demonstrate a lower midpoint potential for electron transfer and result in a lower H^+/e^- stoichiometry than the ImcH-dependent pathway. At least five candidates for alternative quinone oxidoreductases exist in the *G. sulfurreducens* genome, most containing *b*-type cytochromes next to periplasmic *c*-type cytochromes. These “*cbc* family” proteins are among the most conserved within the metal-reducing *Geobacteraceae* (10). Unlike the case of ImcH, which proteomic studies show to be abundant under all conditions (17, 18), *cbc* family protein levels are often altered in the presence of different metals (20), and mutants lacking these proteins yield intermediate phenotypes depending on the metal acceptor (21). It is possible that studies attempting to uncover the role of *cbc* family proteins were confounded by the presence of other electron transfer pathways, such as the ImcH-dependent pathway, and the need to control redox potentials more precisely to observe phenotypes.

A second hypothesis is that each inner membrane cytochrome interacts with separate periplasmic and/or outer membrane redox proteins to create independent conduits out of the cell, based on external redox potential. This could explain why different acceptors induce differential expression of the five periplasmic triheme *c*-type cytochromes (PpcA to -E), why there are many outer membrane cytochromes of *G. sulfurreducens*, and why strains evolved

to overcome some cytochrome deletions regain only 60% of the wild-type growth rate after mutations that lead to expression of repressed or cryptic cytochromes (46). With the identification of ImcH, it may be possible to construct strains lacking one or more routes out of the *Geobacter* cell and study electron transfer pathways independently of each other.

In nearly every microbial respiration, separate strategies are utilized in response to thermodynamic constraints. Oxygen is reduced by multiple terminal oxidases, depending on available concentrations (47). Nitrate reductases exist in the periplasm or cytoplasm, leading to differences in energy conservation (48). Different methanogens compete at distinct thermodynamic thresholds of hydrogen and acetate (49). Since their discovery, metal-reducing bacteria have been outliers, despite the fact that for every 0.5-V difference in redox potential [such as with Mn(IV) oxides versus Fe(III) oxides], enough energy is available to make at least one extra $ATP/2e^-$. These new findings show that *Geobacter* also has a response to this thermodynamic challenge. By sensing and responding to the energy available in external surfaces, *Geobacter*'s respiratory strategy may provide a competitive advantage in subsurface and syntrophic habitats where redox potential, rather than substrate concentration, can be the dominant environmental variable.

MATERIALS AND METHODS

Strains and growth conditions. *Geobacter sulfurreducens* PCA (ATCC 51573) was cultivated for each experiment from colony picks grown from freezer stocks in anaerobic minimal medium (NB salts as described in reference 21) with acetate as the electron donor, pH 6.8. When ferrihydrite (~ 70 mM) or goethite (~ 20 mM) was added as the electron acceptor, 0.69 g/liter $NaH_2PO_4 \cdot H_2O$ stabilized synthesized minerals. Birnessite (20 mM) was added after autoclaving. All medium was buffered with 2 g/liter $NaHCO_3$, made anaerobic by flushing with oxygen-free N_2 - CO_2 (80:20, vol/vol), and sealed with butyl rubber stoppers. All strains used in this study can be found in Table 1.

Deletion of *imcH*. *imcH* (GSU3259) was replaced with a kanamycin resistance cassette (50) using the primers 3259KP1/3259KP2 and 3259KP3/3259KP4 to generate fragments with homology to regions up- and downstream of *imcH*. Primerless fusion with a kanamycin resistance cassette yielded a fragment that was electroporated into wild-type *G. sulfurreducens* (51). Integration was verified by PCR amplification across the region. All primers used in this study can be found in Table 1.

Generation of *imcH* complement. The primers 3259CompF and 3259CompR were used to amplify *imcH* from wild-type DNA. The resulting fragment was digested with XbaI plus BamHI, ligated into pSRKGm (52), and transformed into *Escherichia coli* DH5 α . The purified plasmid

TABLE 1 Strains, plasmids, and primers used in this work

Name	Relevant characteristic or sequence	Source or usage
<i>Geobacter sulfurreducens</i>		
Wild type	ATCC 51573	Caccavo et al. (2)
$\Delta imcH::Kan^r$ strain	Replacement of GSU3259 with a kanamycin resistance cassette	This study
<i>imcH</i> -His \times 6 strain	C-terminally polyhistidine-tagged ImcH	This study
<i>Escherichia coli</i>		
DH5 α	Host for cloning	Invitrogen
WM3064	Donor strain for conjugation: <i>thrB1004 pro thi rpsL hsdS lacZ</i> Δ M15 RP4-1360 Δ (<i>araBAD</i>)567 Δ <i>dapA1341::[erm pir(wt)]</i>	Saltikov and Newman (53)
Plasmids		
pSRKGm	Empty vector for complementation	Khan et al. (52)
<i>pimcH</i>	Complementation vector- <i>imcH</i> in pSRKGm	This study
pBBR1MCS-2	Source of kanamycin resistance cassette	Kovach et al. (50)
Primers ^a		
3259K1	CGTCTGGGGGGTTCGGTTCGC	Upstream fragment for gene replacement
3259K2	AGCTGGCAATTCGGTTCGCTTCTCTCCACTTTGAAACGC	Upstream fragment for gene replacement, homology to Kan ^r in italics
3259K3	GCCTTCTTGACGAGTTCTTCTGAAAGCACTGACACGGCCTGCA	Downstream fragment for gene replacement, homology to Kan ^r in italics
3259K4	GTCCGACACACGGGGGGACT	Downstream fragment for gene replacement
KanF	AAGCGAACCGAATTGCCAGCT	Kanamycin cassette from pBBR1MCS-2
KanR	TCAGAAGAAGCTCGTCAAGAAGGC	Kanamycin cassette from pBBR1MCS-2
3259CompF	CGCGICTAGAGGTAATAAAGCGTTTCAAAGTGGAG	GSU3259 for complementation, restriction site underlined
3259CompR	TAGAGGATCCTCAGTGCTTGCCGGGGC	GSU3259 for complementation, restriction site underlined
3259KHis1	CCCCGATGAAGTCACTG	3' end of GSU3259 for C-terminal polyhistidine tag
3259KHis2	GCTCAGTGATGGTATGGTATGTCCGCCTCCGTGCTTGCCGG	3' end of GSU3259 for C-terminal polyhistidine tag, polyhistidine tag in italics
3259KHis3	CATCACCATCACCATCACTGAGCAAGCGAACCGAATTGCCAGCT	Kanamycin resistance cassette, polyhistidine tag in italics
3259KHis4	CGGGGGGCTTGCTGCTGTCAGGCCGTGTCAGAAGAACTCGTCAAGAAGGC	Kanamycin resistance cassette
3259KHis5	GCCTTCTTGACGAGTTCTTCTGACACGGCCTGCAGCAGCAAGCCCCCG	Downstream region of GSU3259 for C-terminal polyhistidine tag
3259KHis6	CGGAAGATCATCGACACAA	Downstream region of GSU3259 for C-terminal polyhistidine tag

^a Primers were used to amplify the product listed, and were used as described in Materials and Methods.

was used to transform *E. coli* WM3064 (53), which was mated on a 0.2- μ m filter with the $\Delta imcH::Kan^r$ mutant and recovered as previously described (37).

Generation of chromosomal C-terminal polyhistidine-tagged ImcH (ImcH-His \times 6). The primers 3259KHis1 and 3259KHis2 amplified an ~300-bp fragment of the 3' end *imcH*. Primers 3259KHis5 and 3259KHis6 were used to amplify genomic DNA downstream of *imcH*, and 3259KHis3 and 3259KHis4 were used to amplify a kanamycin resistance cassette with ends homologous to the amplified products described above. Gel-purified products were fused using primerless PCR, and the linear fragment was electroporated into *G. sulfurreducens* cells (51). Integration of the fragment was assessed by PCR amplification across the region. Cells

containing the genomic poly-His fusion were verified to retain the ability to reduce Fe(III) citrate using the methods described below.

Sucrose gradient separation of membrane fractions. Late-log-phase cells grown under fumarate-reducing conditions were harvested at 3,700 \times g for 15 min and washed with 50 mM Tris-HCl (pH 8.0) plus 1 mM MgSO₄, 0.2 mM CaCl₂, and 1 mM EDTA. Cell pellets were resuspended in 50 mM Tris-HCl (pH 7.5) plus 1 mM MgSO₄ and 0.2 mM CaCl₂ and subjected to three passes through a French pressure cell. Unlysed cells were removed at 12,000 \times g for 20 min, and the supernatant was centrifuged at 100,000 \times g for 1 h to pellet membranes. Membranes were resuspended in the Tris-Mg-Ca buffer, loaded on a 70/50/30% (wt/vol) sucrose gradient, and centrifuged at 113,000 \times g in a swinging basket rotor for

20 h. Fractions were diluted extensively and filter concentrated through centrifugation.

Protein was quantified using the bicinchoninic acid (BCA) assay (Thermo Scientific Pierce, Waltham, MA). Succinate dehydrogenase activity was assessed anaerobically in the presence of the phenazine methosulfate (PMS) (40 μ M) and 2,6-dichlorophenolindophenol (DCPIP) (50 μ M) by monitoring the absorbance at 600 nm, after addition of succinate. Protein fractions were resolved on 12% polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes at 100 V for 90 min. Membranes were allowed to incubate overnight at 4°C with a mouse anti-His primary antibody (Thermo Scientific Pierce, Waltham, MA) in 2% casein, followed by goat anti-mouse secondary antibody conjugated to alkaline phosphatase (Sigma-Aldrich, St. Louis MO) and visualization with nitroblue tetrazolium (NBT)-5-bromo-4-chloro-3-indolylphosphate (BCIP) (Thermo Scientific Pierce, Waltham, MA).

Reduction of Fe(III) and Mn(IV). For soluble Fe(III) growth assays, late-log-phase cells were inoculated to an OD₆₀₀ of 0.005 into medium containing 55 mM Fe(III) citrate and 20 mM acetate. Fe(II) was measured in samples kept in 0.5 N HCl using a modified FerroZine assay (54). For washed-cell assays, cells were washed 3× via centrifugation at 3,700 × g for 10 min using an anaerobic minimal medium lacking ammonium, phosphate, and sulfate, with 100 mM NaCl to maintain osmotic pressure. Wild-type and mutant cells were incubated at an OD₆₀₀ of 0.05 with Fe(III) citrate (50 mM) at 30°C using 10 mM acetate as the electron donor and monitored using a modified FerroZine assay (54).

For Fe(III) oxide reduction, late-log-growth-phase cells were inoculated to an OD₆₀₀ of 0.005 into medium containing 70 mM ferrihydrite (55) as the electron acceptor and 20 mM acetate as the electron donor. Samples were dissolved in 0.5 N HCl overnight, and Fe(II) concentrations were measured via a modified FerroZine assay (54).

Birnessite [Mn(IV) oxide] (55) reduction was monitored by a modified FerroZine assay based on Fe(II) reduction of residual Mn(IV). Late-log-growth-phase cells were inoculated to an OD₆₀₀ of 0.005 into medium containing 10 mM acetate and ~20 mM birnessite. Samples were diluted into 2N HCl containing 4 mM FeSO₄ and allowed to incubate overnight in the dark. The quantity of Fe(II) remaining in solution was determined using the FerroZine assay (54).

Electrochemical analysis. Three-electrode bioreactors were prepared using working graphite electrodes polished with 1,500-grit wet/dry sandpaper (Ali Industries Inc., Fairborn, OH) and sonicated for 1 h in deionized (DI) water with multiple water exchanges to remove particulates. Reactors contained Pt counterelectrodes and calibrated Calomel reference electrodes (34). Sterile anaerobic reactors were poised at the shown potentials using a VMP3 multichannel potentiostat, and a 50% (vol/vol) volume of OD₆₀₀ 0.50 cells entering acceptor limitation was used to initiate experiments with 20 mM acetate as the electron donor. Reactors were purged with humidified 80%:20% N₂-CO₂ passed over a heated copper column to remove trace oxygen.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <http://mbio.asm.org/lookup/suppl/doi:10.1128/mbio.02034-14/-DCSupplemental>.

Figure S1, TIF file, 1.7 MB.

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REFERENCES

- Lovley DR, Holmes DE, Nevin KP. 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Adv. Microb. Physiol.* 49:219286. [http://dx.doi.org/10.1016/S0065-2911\(04\)49005-5](http://dx.doi.org/10.1016/S0065-2911(04)49005-5).
- Caccavo F, Lonergan DJ, Lovley DR, Davis M, Stolz JF, McInerney MJ. 1994. *Geobacter sulfurreducens* sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. *Appl. Environ. Microbiol.* 60:3752–3759.
- Bond DR, Lovley DR. 2003. Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl. Environ. Microbiol.* 69:1548–1555. <http://dx.doi.org/10.1128/AEM.69.3.1548-1555.2003>.
- Snoeyenbos-West OL, Nevin KP, Anderson RT, Lovley DR. 2000. Enrichment of *Geobacter* species in response to stimulation of Fe(III) reduction in sandy aquifer sediments. *Microb. Ecol.* 39:153–167. <http://dx.doi.org/10.1007/s002480000018>.
- Anderson RT, Vrionis HA, Ortiz-Bernad I, Resch CT, Long PE, Dayvault R, Karp K, Marutzky S, Metzler DR, Peacock A, White DC, Lowe M, Lovley DR. 2003. Stimulating the in situ activity of *Geobacter* species to remove uranium from the groundwater of a uranium-contaminated aquifer. *Appl. Environ. Microbiol.* 69:5884–5891. <http://dx.doi.org/10.1128/AEM.69.10.5884-5891.2003>.
- Tadanier CJ, Schreiber ME, Roller JW. 2005. Arsenic mobilization through microbially mediated deflocculation of ferrihydrite. *Environ. Sci. Technol.* 39:3061–3068. <http://dx.doi.org/10.1021/es048206d>.
- Islam FS, Gault AG, Boothman C, Polya DA, Charnock JM, Chatterjee D, Lloyd JR. 2004. Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. *Nature* 430:68–71. <http://dx.doi.org/10.1038/nature02638>.
- Bond DR, Holmes DE, Tender LM, Lovley DR. 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. *Science* 295:483–485. <http://dx.doi.org/10.1126/science.1066771>.
- Holmes DE, Bond DR, O'Neil RA, Reimers CE, Tender LR, Lovley DR. 2004. Microbial communities associated with electrodes harvesting electricity from a variety of aquatic sediments. *Microb. Ecol.* 48:178–190. <http://dx.doi.org/10.1007/s00248-003-0004-4>.
- Butler JE, Young ND, Lovley DR. 2010. Evolution of electron transfer out of the cell: comparative genomics of six *Geobacter* genomes. *BMC Genomics* 11:40. <http://dx.doi.org/10.1186/1471-2164-11-40>.
- Myers JM, Myers CR. 2000. Role of the tetraheme cytochrome CymA in anaerobic electron transport in cells of *Shewanella putrefaciens* MR-1 with normal levels of menaquinone. *J. Bacteriol.* 182:67–75. <http://dx.doi.org/10.1128/JB.182.1.67-75.2000>.
- Coursolle D, Baron DB, Bond DR, Gralnick JA. 2010. The Mtr respiratory pathway is essential for reducing flavins and electrodes in *Shewanella oneidensis*. *J. Bacteriol.* 192:467–474. <http://dx.doi.org/10.1128/JB.00925-09>.
- 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. <http://dx.doi.org/10.1128/AEM.00146-07>.
- Clarke TA, Edwards MJ, Gates AJ, Hall A, White GF, Bradley J, Reardon CL, Shi L, Beliaev AS, Marshall MJ, Wang Z, Watmough NJ, Fredrickson JK, Zachara JM, Butt JN, Richardson DJ. 2011. Structure of a bacterial cell surface decaheme electron conduit. *Proc. Natl. Acad. Sci. U. S. A.* 108:9384–9389. <http://dx.doi.org/10.1073/pnas.1017200108>.
- Saffarini DA, Schultz R, Beliaev A. 2003. Involvement of cyclic AMP (cAMP) and cAMP receptor protein in anaerobic respiration of *Shewanella oneidensis*. *J. Bacteriol.* 185:3668–3671. <http://dx.doi.org/10.1128/JB.185.12.3668-3671.2003>.
- Nevin KP, Kim BC, Glaven RH, Johnson JP, Woodard TL, Methé BA, Didonato RJ, Covalla SF, Franks AE, Liu A, Lovley DR. 2009. Anode biofilm transcriptomics reveals outer surface components essential for high density current production in *Geobacter sulfurreducens* fuel cells. *PLoS One* 4:e5628. <http://dx.doi.org/10.1371/journal.pone.0005628>.
- Ding YH, Hixson KK, Aklujkar MA, Lipton MS, Smith RD, Lovley DR, Mester T. 2008. Proteome of *Geobacter sulfurreducens* grown with Fe(III) oxide or Fe(III) citrate as the electron acceptor. *Biochim. Biophys. Acta* 1784:1935–1941. <http://dx.doi.org/10.1016/j.bbapap.2008.06.011>.
- Ding YH, Hixson KK, Giometti CS, Stanley A, Esteve-Núñez A, Khare T, Tollaksen SL, Zhu W, Adkins JN, Lipton MS, Smith RD, Mester T, Lovley DR. 2006. The proteome of dissimilatory metal-reducing microorganism *Geobacter sulfurreducens* under various growth conditions. *Biochim. Biophys. Acta* 1764:1198–1206. <http://dx.doi.org/10.1016/j.bbapap.2006.04.017>.
- Holmes DE, Chaudhuri SK, Nevin KP, Mehta T, Methé BA, Liu A, Ward JE, Woodard TL, Webster J, Lovley DR. 2006. Microarray and

- genetic analysis of electron transfer to electrodes in *Geobacter sulfurreducens*. *Environ. Microbiol.* 8:1805–1815. <http://dx.doi.org/10.1111/j.1462-2920.2006.01065.x>.
20. Aklujkar M, Coppi MV, Leang C, Kim BC, Chavan MA, Perpetua LA, Giloteaux L, Liu A, Holmes DE. 2013. Proteins involved in electron transfer to Fe(III) and Mn(IV) oxides by *Geobacter sulfurreducens* and *Geobacter uraniireducens*. *Microbiology* 159:515–535. <http://dx.doi.org/10.1099/mic.0.064089-0>.
 21. Rollefson JB, Levar CE, Bond DR. 2009. Identification of genes involved in biofilm formation and respiration via mini-Himar transposon mutagenesis of *Geobacter sulfurreducens*. *J. Bacteriol.* 191:4207–4217. <http://dx.doi.org/10.1128/JB.00057-09>.
 22. Mahadevan R, Palsson BØ, Lovley DR. 2011. In situ to in silico and back: elucidating the physiology and ecology of *Geobacter* spp. using genome-scale modelling. *Nat. Rev. Microbiol.* 9:39–50. <http://dx.doi.org/10.1038/nrmicro2456>.
 23. Lovley DR. 2006. Bug juice: harvesting electricity with microorganisms. *Nat. Rev. Microbiol.* 4:497–508. <http://dx.doi.org/10.1038/nrmicro1442>.
 24. Shi L, Squier TC, Zachara JM, Fredrickson JK. 2007. Respiration of metal (hydr)oxides by *Shewanella* and *Geobacter*: a key role for multiheme c-type cytochromes. *Mol. Microbiol.* 65:12–20. <http://dx.doi.org/10.1111/j.1365-2958.2007.05783.x>.
 25. Liu Y, Wang Z, Liu J, Levar CE, Edwards MJ, Babauta JT, Kennedy DW, Shi Z, Beyenal H, Bond DR, Clarke TA, Butt JN, Richardson DJ, Rosso KM, Zachara JM, Fredrickson JK, Shi L. 2014. A trans-outer membrane porin-cytochrome protein complex for extracellular electron transfer by *Geobacter sulfurreducens* PCA. *Environ. Microbiol. Rep.*, in press. <http://dx.doi.org/10.1111/1758-2229.12204>.
 26. Yoho RA, Papat SC, Torres CI. 2014. Dynamic potential-dependent electron transport pathway shifts in anode biofilms of *Geobacter sulfurreducens*. *ChemSusChem*, in press. <http://dx.doi.org/10.1002/cssc.201402589>.
 27. Cornell RM, Schwertmann U. 2003. The iron oxides: structure, properties, reactions, occurrences and uses, 2nd ed. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
 28. Majzlan J. 2012. Minerals and aqueous species of iron and manganese as reactants and products of microbial metal respiration, p 112–28. In Giescher J, Kappler A (ed), *Microbial metal respiration; from geochemistry to potential applications*, 1st ed. Springer Verlag, New York, NY.
 29. Thamdrup B. 2000. Bacterial manganese and iron reduction in aquatic sediments, p 4112–84. In Schink B (ed), *Advances in Microbial Ecology*, 16th ed. Springer Verlag, New York, NY.
 30. Simon J, Gross R, Einsle O, Kroneck PM, Kröger A, Klimmek O. 2000. A NapC/NirT-type cytochrome *c* (NrfH) is the mediator between the quinone pool and the cytochrome *c* nitrite reductase of *Wolinella succinogenes*. *Mol. Microbiol.* 35:686–696. <http://dx.doi.org/10.1046/j.1365-2958.2000.01742.x>.
 31. Yu NY, Wagner JR, Laird MR, Melli G, Rey S, Lo R, Dao P, Sahinalp SC, Ester M, Foster LJ, Brinkman FSL. 2010. PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. *Bioinformatics* 26:1608–1615. <http://dx.doi.org/10.1093/bioinformatics/btq249>.
 32. Boland DD, Collins RN, Glover CJ, Waite TD. 2013. An in situ quick-EXAFS and redox potential study of the Fe(II)-catalysed transformation of ferrihydrite. *Colloids Surf. Physicochem. Eng. Aspects* 435:2–8. <http://dx.doi.org/10.1016/j.colsurfa.2013.02.009>.
 33. Richter H, Nevin KP, Jia H, Lowy DA, Lovley DR, Tender LM. 2009. Cyclic voltammetry of biofilms of wild type and mutant *Geobacter sulfurreducens* on fuel cell anodes indicates possible roles of OmcB, OmcZ, type IV pili, and protons in extracellular electron transfer. *Energy Environ. Sci.* 2:506–516. <http://dx.doi.org/10.1039/b816647a>.
 34. Marsili E, Rollefson JB, Baron DB, Hozalski RM, Bond DR. 2008. Microbial biofilm voltammetry: direct electrochemical characterization of catalytic electrode-attached biofilms. *Appl. Environ. Microbiol.* 74:7329–7337. <http://dx.doi.org/10.1128/AEM.00177-08>.
 35. Speers AM, Reguera G. 2012. Electron donors supporting growth and electroactivity of *Geobacter sulfurreducens* anode biofilms. *Appl. Environ. Microbiol.* 78:437–444. <http://dx.doi.org/10.1128/AEM.06782-11>.
 36. Marsili E, Sun J, Bond DR. 2010. Voltammetry and growth physiology of *Geobacter sulfurreducens* biofilms as a function of growth stage and imposed electrode potential. *Electroanalysis* 22:865–874. <http://dx.doi.org/10.1002/elan.200800007>.
 37. Rollefson JB, Stephen CS, Tien M, Bond DR. 2011. Identification of an extracellular polysaccharide network essential for cytochrome anchoring and biofilm formation in *Geobacter sulfurreducens*. *J. Bacteriol.* 193:1023–1033. <http://dx.doi.org/10.1128/JB.01092-10>.
 38. Coates JD, Ellis DJ, Gaw CV, Lovley DR. 1999. *Geothrix fermentans* gen. nov., sp. nov., a novel Fe(III)-reducing bacterium from a hydrocarbon-contaminated aquifer. *Int. J. Syst. Bacteriol.* 49:1615–1622. <http://dx.doi.org/10.1099/00207713-49-4-1615>.
 39. Podosokorskaya OA, Kadnikov VV, Gavrilo SN, Mardanov AV, Merkel AY, Karnachuk OV, Ravin NV, Bonch-Osmolovskaya EA, Kublanov IV. 2013. Characterization of *Meliobacter roseus* gen. nov., sp. nov., a novel facultatively anaerobic thermophilic cellulolytic bacterium from the class *Ignavibacteria*, and a proposal of a novel bacterial phylum *Ignavibacteriae*. *Environ. Microbiol.* 15:1759–1771. <http://dx.doi.org/10.1111/1462-2920.12067>.
 40. Renslow R, Babauta J, Dohnalkova A, Boyanov M, Kemner K, Majors P, Fredrickson J, Beyenal H. 2013. Metabolic spatial variability in electrode-respiring *Geobacter sulfurreducens* biofilms. *Energy Environ. Sci.* 6:1827–1836. <http://dx.doi.org/10.1039/c3ee40203g>.
 41. Bond DR, Strycharz-Glaven SM, Tender LM, Torres CI. 2012. On electron transport through *Geobacter* biofilms. *ChemSusChem* 5:1099–1105. <http://dx.doi.org/10.1002/cssc.201100748>.
 42. Snider RM, Strycharz-Glaven SM, Tsoi SD, Erickson JS, Tender LM. 2012. Long-range electron transport in *Geobacter sulfurreducens* biofilms is redox gradient-driven. *Proc. Natl. Acad. Sci. U. S. A.* 109:15467–15472. <http://dx.doi.org/10.1073/pnas.1209829109>.
 43. Orsetti S, Laskov C, Haderlein SB. 2013. Electron transfer between iron minerals and quinones: estimating the reduction potential of the Fe(II)-goethite surface from AQDS speciation. *Environ. Sci. Technol.* 47:14161–14168. <http://dx.doi.org/10.1021/es403658g>.
 44. Schwertmann U, Rochelle CM. 2000. Iron oxides in the laboratory: preparation and characterization. Wiley-VCH Verlag, New York, NY.
 45. Mehta T, Coppi MV, Childers SE, Lovley DR. 2005. Outer membrane c-type cytochromes required for Fe(III) and Mn(IV) oxide reduction in *Geobacter sulfurreducens*. *Appl. Environ. Microbiol.* 71:8634–8641. <http://dx.doi.org/10.1128/AEM.71.12.8634-8641.2005>.
 46. Leang C, Coppi MV, Lovley DR. 2003. OmcB, a c-type polyheme cytochrome, involved in Fe(III) reduction in *Geobacter sulfurreducens*. *J. Bacteriol.* 185:2096–2103. <http://dx.doi.org/10.1128/JB.185.7.2096-2103.2003>.
 47. Green J, Paget MS. 2004. Bacterial redox sensors. *Nat. Rev. Microbiol.* 2:954–966. <http://dx.doi.org/10.1038/nrmicro1022>.
 48. Simon J, van Spanning RJM, Richardson DJ. 2008. The organisation of proton motive and non-proton motive redox loops in prokaryotic respiratory systems. *Biochim. Biophys. Acta* 1777:1480–1490. <http://dx.doi.org/10.1016/j.bbabi.2008.09.008>.
 49. Thauer RK, Kaster AK, Seedorf H, Buckel W, Hedderich R. 2008. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nat. Rev. Microbiol.* 6:579–591. <http://dx.doi.org/10.1038/nrmicro1931>.
 50. 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. [http://dx.doi.org/10.1016/0378-1119\(95\)00584-1](http://dx.doi.org/10.1016/0378-1119(95)00584-1).
 51. Lloyd JR, Leang C, Hodges Myerson AL, Coppi MV, Cuifio S, Methe B, Sandler SJ, Lovley DR. 2003. Biochemical and genetic characterization of PpcA, a periplasmic c-type cytochrome in *Geobacter sulfurreducens*. *Biochem. J.* 369:153–161. <http://dx.doi.org/10.1042/BJ20020597>.
 52. Khan SR, Gaines J, Roop RM, Farrand SK. 2008. Broad-host-range expression vectors with tightly regulated promoters and their use to examine the influence of TraR and TraM expression on Ti plasmid quorum sensing. *Appl. Environ. Microbiol.* 74:5053–5062. <http://dx.doi.org/10.1128/AEM.01098-08>.
 53. Saltikov CW, Newman DK. 2003. Genetic identification of a respiratory arsenate reductase. *Proc. Natl. Acad. Sci. U. S. A.* 100:10983–10988. <http://dx.doi.org/10.1073/pnas.183403100>.
 54. Lovley DR, Phillips EJ. 1987. Rapid assay for microbially reducible ferric iron in aquatic sediments. *Appl. Environ. Microbiol.* 53:1536–1540.
 55. Lovley DR, Phillips EJ. 1986. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl. Environ. Microbiol.* 51:683–689.