



## Insights into genes involved in electricity generation in *Geobacter sulfurreducens* via whole genome microarray analysis of the OmcF-deficient mutant

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### ARTICLE INFO

#### Article history:

Received 1 March 2008  
Received in revised form 15 April 2008  
Accepted 22 April 2008  
Available online 29 April 2008

#### Keywords:

*Geobacter sulfurreducens*  
Cytochrome  
Microbial fuel cells  
Microarray

### ABSTRACT

*Geobacter sulfurreducens* effectively produces electricity in microbial fuel cells by oxidizing acetate with an electrode serving as the sole electron acceptor. Deletion of the gene encoding OmcF, a monoheme outer membrane *c*-type cytochrome, substantially decreased current production. Previous studies demonstrated that inhibition of Fe(III) reduction in the OmcF-deficient mutant could be attributed to poor transcription of the gene for OmcB, an outer membrane *c*-type cytochrome that is required for Fe(III) reduction. However, a mutant in which *omcB* was deleted produced electricity as well as wild type. Microarray analysis of the OmcF-deficient mutant versus the wild type revealed that many of the genes with the greatest decreases in transcript levels were genes whose expression was previously reported to be upregulated in cells grown with an electrode as the sole electron acceptor. These included genes with putative functions related to metal efflux and/or type I secretion and two hypothetical proteins. The outer membrane cytochromes, OmcS and OmcE, which previous studies have demonstrated are required for optimal current generation, were not detected on the outer surface of the OmcF-deficient mutant even though the *omcS* and *omcE* genes were still transcribed, suggesting that the putative secretion system could be involved in the export of outer membrane proteins necessary for electron transfer to the fuel cell anode. These results suggest that the requirement for OmcF for optimal current production is not because OmcF is directly involved in extracellular electron transfer but because OmcF is required for the appropriate transcription of other genes either directly or indirectly involved in electricity production.

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### 1. Introduction

Conversion of waste organic matter and renewable biomass to electricity with electricigens, microorganisms that can completely oxidize organic compounds with quantitative electron transfer to electrodes, is a promising novel energy source [1–5]. However, further optimization of electricigen-based microbial fuel cells is necessary before many of the possible practical applications of these systems can be realized. A better understanding of the mechanisms by which electricigens transfer electrons to the anodes of microbial fuel is likely to aid in this optimization by providing information for better engineering of anode materials and/or genetic engineering of the electricigens.

*Geobacter sulfurreducens* is the electricigen that has been most intensively studied to date because: 1) it produces high current densities in microbial fuel cells [K. P. Nevin, H. Richter, S. F. Covalla, J. P. Johnson, T. L. Woodard, H. Jia, M. Zhang, and D. R. Lovley, Environmental

Microbiology. doi:10.1111/j.1462-2920.2008.01675.x]; 2) the complete genome sequence of this organism is available [6]; 3) a genetic system is available [7–9]; and 4) there has been extensive study on extracellular electron transfer to Fe(III) oxide in this organism which might provide insights into extracellular electron transfer to electrodes [10–14]. Similarities between electron transfer to insoluble Fe(III) oxides and electrodes include requirements for the electrically conductive pili [10,15,16] and the outer membrane *c*-type cytochrome, OmcS [11,17].

Several other *c*-type cytochromes are known to be involved in Fe(III) reduction, but their role in electron transfer to electrodes has not been investigated. Some *c*-type cytochromes, most notably the outer membrane cytochrome *OmcB*, are believed to function as electron transfer components in a chain of redox proteins leading to Fe(III) reduction [14,18]. However, other cytochromes have a less direct role [9,19]. For example, the deletion of the genes for putative *c*-type cytochromes OmcG and OmcH inhibited Fe(III) reduction because OmcB was less expressed in the mutants [19].

OmcF is a monoheme outer membrane *c*-type cytochrome of *G. sulfurreducens* and the OmcF-deficient mutant was impaired in Fe(III) reduction because the *omcB* transcript was dramatically decreased in the mutant [9]. Here we report that deletion of the gene for OmcF also inhibits

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electricity production, but that the mechanism for inhibition is likely to be different than that for Fe(III) reduction.

## 2. Materials and methods

### 2.1. Bacterial strains and culturing conditions

*G. sulfurreducens* strains DL1 [20], DL6 (*omcB::cam*) [14], DLTM1 (*omcS::spec*) [11], DLMC8 (*omcE::kan*) [11] and DLBK01 (*omcF::kan*) [9] were cultured anaerobically in NBAF (acetate/fumarate) medium at 30 °C as previously described [7]. Steady-state cultures of strain DL1 and DLBK01 were grown in chemostats under strict anaerobic conditions (N<sub>2</sub>–CO<sub>2</sub> [80:20, vol/vol]) in freshwater medium containing fumarate (27.5 mM) as the electron acceptor and acetate (5 mM) as the electron donor, as previously described [21,22]. The dilution rate was 0.05 h<sup>-1</sup>.

### 2.2. Microbial fuel cell system for testing electricity generation

Each *G. sulfurreducens* strain was initially grown in medium with acetate (10 mM) provided as the electron donor and fumarate (40 mM) as the electron acceptor as previously described [17]. The cells were then harvested via centrifugation, washed, and resuspended in anoxic medium [17] lacking electron donor and acceptor. This cell suspension was inoculated anaerobically into the anaerobic anodic chamber (250 ml of medium) of a two-chambered electrode system [23,24] with 10 mM acetate and 20 mM fumarate in order to colonize the graphite electrode surface with *G. sulfurreducens*, as previously described [17]. Once the early stationary phase was reached, the culture medium was completely replaced anaerobically with fresh bicarbonate-buffered growth medium [25] amended with 10 mM acetate as electron donor. All the other conditions for fuel cell operation were the same as previously described [17].

### 2.3. DNA microarray hybridization and statistical analysis

Total RNA was isolated from three different chemostat cultures of both the wild type and the *OmcF*-deficient mutant. Ten micrograms of RNA from wild type and mutant samples were chemically labeled with cyanine 3 or cyanine 5 (Cy3/Cy5) fluorescent dyes respectively, using the MicroMax ASAP RNA Labeling Kit (Perkin Elmer, Wellesley, MA) according to the manufacturer's instructions. Labeled RNA was fragmented in a 20 µl volume at 70 °C for 30 min using Fragmentation Reagent (Ambion Inc., Austin, TX). The resulting products were ethanol precipitated and subsequently resuspended in hybridization buffer. Duplicates of each RNA sample were hybridized to 12 K DNA microarrays (Combinatrix, Mukilteo, WA) as previously described [26]. The arrays were scanned with a GenePix 4000B scanner (Molecular Devices Inc., Sunnyvale, CA), and analyzed using GenePix and Acuity 4.0 software. LIMMA mixed model analysis [R-package LIMMA [27]] was applied to the normalized log<sub>2</sub> ratios to identify differentially expressed genes. The *P*-value was corrected for multiple comparisons according to the Benjamini and Hochberg procedure [28] to control the false discovery rate. Genes demonstrating statistically significant differential expression are listed in Tables 4 and 5 according to the fold changes (<-1.5 for down-regulation and >+1.5 for up-regulation) and the *P*-values. A gene was called differentially expressed if at least half of the corresponding probes for that gene have a *P*-value <0.0001. Detailed information on the design of the arrays including oligonucleotide sequences and raw data are available at the NCBI GEO database ([www.ncbi.nlm.nih.gov/geo](http://www.ncbi.nlm.nih.gov/geo)) under series number GSE7526.

### 2.4. Relative quantification of gene expression with quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Quantitative RT-PCR analysis was performed to verify down-regulation of genes in the *OmcF*-deficient mutant observed on whole genome

microarrays. GSU2737 (*omcB*), GSU2731 (*omcC*), GSU2780, GSU3410, GSU1330, GSU1331, GSU1332, GSU1333, GSU1339, GSU1340, and GSU1341 were selected for further analysis. The primers used for qRT-PCR analysis were shown in Table 1 (primers used for qRT-PCR analyses of GSU1331, GSU1332, GSU1339, and GSU1341 are not shown). The Dura-Script enhanced avian RT single-strand synthesis kit (Sigma-Aldrich Co. St Louis, MO) was used to generate cDNA with random primers and cDNA generated by RT-PCR was used for qRT-PCR amplification as previously described [29]. Detection of amplified qRT-PCR products was performed with the GeneAmp 5700 sequence detection system (PE Biosystems, Foster City, CA) according to the manufacturer's instructions. The qRT-PCR was performed as previously described [17]. To verify amplification and correct amplicon size, aliquots from qRT-PCR were examined on an ethidium bromide stained 2% agarose gel. The gene for *recA* (GSU0145), previously shown to be constitutively expressed in *G. sulfurreducens* [29,30], was not identified in this microarray analyses as differentially expressed. Equivalent expression of *recA* was confirmed in both wild type and the *OmcF*-deficient mutant by qRT-PCR analysis (data not shown). As a result, it was used as an endogenous control for relative qRT-PCR analysis. Primers (Table 1) were designed for qRT-PCR analysis according to the manufacturer's instructions (PE Biosystems, Foster City, CA) from the *G. sulfurreducens* genome sequence [6].

### 2.5. DNA and RNA manipulations

PCR product purification was carried out using a QIAGEN Gel Purification kit (QIAGEN Inc., Valencia, CA). Probes for Northern blot analysis were labeled with [ $\alpha$ -<sup>32</sup>P]dATP using a Strip-EZ DNA probe synthesis and removal kit (Ambion Inc., Austin, Texas). [ $\alpha$ -<sup>32</sup>P]dATP was purchased from Perkin Elmer Life and Analytical Sciences, Inc., Boston, MA. All primers used to amplify *G. sulfurreducens* sequences were designed using the *G. sulfurreducens* genome sequence [6]. QIAGEN Taq DNA polymerase (QIAGEN Inc., Valencia, CA) was used for PCR amplifications. Total RNA was purified from steady-state chemostat culture using the RiboPure™-Bacteria Kit (Ambion Inc., Austin, TX) according to the manufacturer's protocol followed by treatment with RNase-free DNase (Ambion Inc., Austin, TX). Northern blot analyses were carried out with the Northern Max-Gly system (Ambion Inc., Austin, TX) according to the manufacturer's instructions. All probes for Northern blot analyses (GSU1330–GSU1333, GSU1339–GSU1341, GSU2731, GSU2737, GSU2780, GSU3409, GSU3410, GSU0618

**Table 1**

Primers used for quantitative reverse transcription polymerase chain reaction (qRT-PCR) for genes whose expression levels were downregulated in the *OmcF*-deficient mutant

Target gene	Primer	Sequences (5'–3')	Length of amplicon (bp)	Reference
GSU0145 (RecA, control)	recA660f	GTGAAGGTGGTCAAGAACAAGGT	78	[17]
GSU2737 ( <i>omcB</i> )	recA737r	GGAAATGCCCTCACCGTAGTAA	212	[22]
	8912	CCCACCTTCGACAACCTATTTCG		
GSU2731 ( <i>omcC</i> )	8908-2	GGTCAGCAGGCCACCGG	232	[22]
	8917	GGTCTTCACCCAGATCTCCG		
GSU2780	8915	GGGTGTGTGGTAGAAGGG	202	This study
	2780QF	TGGTACGGGTTGTGAAAAGG		
GSU3410	2780QR	CAGCATCTCGCACTCTTTGG	112	This study
	3410QF	CAATAGTGGAGTGCTGGTATGG		
GSU1330	3410QR	CAGCATCTCGCACTCTTTGG	191	This study
	1330QF	GCAAGCTCGTTACGGGTCTATAC		
GSU1333	1330QR	CAGGATGCAAGTGATCATTTTCAG	158	This study
	1333QF	GGCCGAGCACATGAAAGC		
GSU1340	1333QR	TGCTCTGGCCTTGTATCC	174	This study
	1340QF	GGCCGAAGATGGACGAATTC		
	1340QR	CAGCCAGGATGTTTTTGTGTTC		

**Table 2**

Primers used for Northern blot analyses for genes whose expression levels were downregulated in the *OmcF*-deficient mutant

Target gene	Primer	Sequences (5'–3')	Length of amplicon (bp)	Reference
GSU2737 ( <i>omcB</i> )	8916	GGACTGCGCACCATCAAGG	435	[33]
	8908-2	GGTCAGCAGGCCACCGG		[14]
GSU2731 ( <i>omcC</i> )	8914	GCCAGAGTGAGGCCCAGA	543	[33]
	8915	GGGTGTTGTGTAGAAGGG		[22]
GSU2780	2780F	ATGCAATATGCTTTTACGCC	504	This study
	2780R	GCGGTTTTCCATGAGGTCT		
GSU3410	3410F	ATGAAAACACAGCCATCGC	225	This study
	3410R	GTGAGTCGTGGCTCCTTCT		

and GSU2504) were purified from PCR product. Primers for amplifying probes are listed in Table 2 (primers used for GSU1330–GSU1333, GSU1339–GSU1341, and GSU3409 are not shown). Northern blot analysis of GSU0618 (*omcE*) and GSU2504 (*omcS*) were performed by previously reported primers [11]. The PCR amplification condition was as follows: 96 °C for 4 min followed by 30 cycles of 96 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 10 min. RT-PCR analysis was used to determine whether GSU1330, GSU1331, GSU1332, and GSU1333 comprise an operon, and whether GSU1339, GSU1340, and GSU1341 comprise another operon. The primers (Table 3) amplified a segment of the mRNA extended from the 3' end of one gene into the 5' end of the next gene.

### 2.6. Detection of loosely bound outer membrane *c*-type cytochromes

The loosely bound outer membrane-enriched protein fractions (LBOP) of *G. sulfurreducens* DL1 [20], DLMT1 (*omcE::kan*) [11], DLMC8 (*omcS::spec*) [11], and DLBK01 (*omcF::kan*) [9] strains were isolated as previously described [11]. The LBOP were separated by Tris–Tricine denaturing polyacrylamide gel electrophoresis following by staining with *N,N,N',N'*-tetramethylbenzidine as previously described [31,32].

## 3. Results and discussion

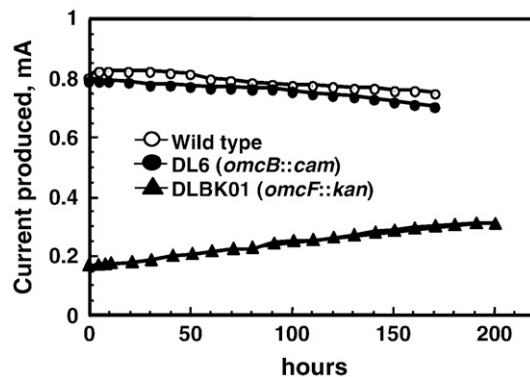
### 3.1. Current generation by the *OmcF*-deficient mutant

A strain of *G. sulfurreducens* in which the gene for *OmcF* was deleted was defective in electricity production in a microbial fuel cell (Fig. 1). The *OmcF*-deficient mutant was previously shown to be defective in the production of *OmcB* [9]. However, a lack of *OmcB*, was not a factor resulting in diminished current production because an *OmcB*-deficient mutant produced current at the same level as wild type (Fig. 1) as previously reported [17].

**Table 3**

Primers used for determining whether GSU1330, GSU1331, GSU1332, and GSU1333 comprise an operon, and whether GSU1339, GSU1340, and GSU1341 comprise another operon

Target genes	Primer	Sequences (5'–3')	Length of amplicon (bp)	Reference
GSU1330–GSU1331	1330OF	GAACAATGCCATTCCTTGG	481	This study
	1331OR	ATCCGGGCTTGCTTGTATG		
GSU1331–GSU1332	1331OF	AAGTGATCGCCTCCTCCG	469	This study
	1332OR	TCACGTAGATCATCGAAAAGCC		
GSU1332–GSU1333	1332OF	CGATCATGTGGTCCAGCG	503	This study
	1333OR	CCTGGGTAATGGCCTTGC		
GSU1339–GSU1340	1339OF	TGCAGGATGCGCGCTTC	494	This study
	1340OR	TGATCCGCCCCAGGTC		
GSU1340–GSU1341	1340OF	TTCATGGCCGAAAAGCAAG	511	This study
	1341OR	AGCATGACGTTCTCCAGGG		



**Fig. 1.** Current generated by microbial fuel cells inoculated with *G. sulfurreducens* wild type strain (Wild type), the *OmcB*-deficient mutant (DL6), and the *OmcF*-deficient mutant (DLBK01). The data shown are from a representative fuel cell of triplicate fuel cells for each treatment.

### 3.2. Genes with lower transcript levels in the *OmcF*-deficient mutant versus wild type *G. sulfurreducens*

In order to evaluate other mechanisms by which deletion of the gene for *OmcF* might negatively impact current production, gene expression in the *OmcF*-deficient mutant was compared to gene expression in wild type with a whole genome DNA microarray. This comparison was carried out in chemostat cultures with fumarate as the electron acceptor, because it was not possible to grow sufficient biomass of the mutant on electrodes for microarray analysis. There were 12 genes with lower transcript levels in the mutant versus the wild type and 13 genes with higher transcript levels in the mutant when changes of more than 1.5 fold and *P*-values less than 0.0001 were chosen as the criteria for significant differences. Analysis of transcript levels with qRT-PCR and Northern blot analyses (Table 4, Fig. 2) confirmed the results from the microarray analysis in each case investigated. The genes on either side of *omcF* are GSU2431, a putative

**Table 4**

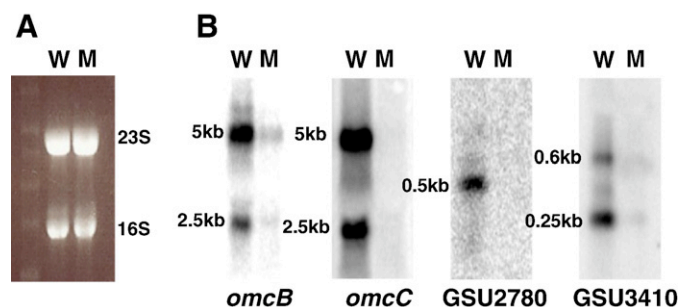
Genes that were significantly downregulated in the *OmcF*-deficient mutant compared to the wild type in acetate-limiting, fumarate-grown continuous culture<sup>a</sup>

Locus ID	Annotation	Fold change		
		DNA microarray		qRT-PCR
		<sup>b</sup> <i>omcF</i>	<sup>c</sup> Electrode	<i>omcF</i>
GSU1333	Hypothetical protein	–3.25	3.75	–4.17
GSU2780	Hypothetical protein	–2.66	5.49	–8.91
GSU2732	Cytochrome <i>c</i> family protein ( <i>orf2-2</i> : second gene of <i>omcC</i> operon)	–2.40	NDE	ND
GSU2738	Cytochrome <i>c</i> family protein ( <i>orf2-1</i> : second gene of <i>omcB</i> operon)	–2.40	NDE	ND
GSU2739	Hypothetical protein ( <i>orf1-1</i> : first gene of <i>omcB</i> operon)	–2.26	NDE	ND
GSU2733	Hypothetical protein ( <i>orf1-2</i> : first gene of <i>omcC</i> operon)	–2.23	NDE	ND
GSU2731	Polyheme membrane-associated cytochrome <i>c</i> ( <i>omcC</i> )	–2.01	NDE	–17.4
GSU1340	ABC transporter, permease protein	–1.88	2.59	–2.14
GSU2737	Polyheme membrane-associated cytochrome <i>c</i> ( <i>omcB</i> )	–1.78	NDE	–3.30
GSU1330	Metal ion efflux outer membrane protein, putative	–1.68	3.33	–3.30
GSU3410	Hypothetical protein	–1.67	3.21	–8.51
GSU2741	Transcriptional regulator, TetR family (regulator of <i>omcB</i> operon)	–1.53	NDE	ND

<sup>a</sup> Abbreviations: NDE, not differentially expressed; ND, not determined.

<sup>b</sup> Fold changes in the *OmcF*-deficient mutant compared to the wild type.

<sup>c</sup> The fold changes were previously reported from whole genome microarray analysis of *G. sulfurreducens* during growth with an electrode as the sole electron acceptor versus growth on Fe(III) citrate [17].



**Fig. 2.** Northern blot analyses of downregulated genes. Total RNA was isolated from steady-state cultures from chemostats of the wild type *G. sulfurreducens* (W) and the OmcF-deficient mutant (M). (A) A gel was run and stained with ethidium bromide, revealing the 16S and 23S rRNA, as a loading control. (B) *omcB* (GSU2737), *omcC* (GSU2731), GSU2780, and GSU3410 that were identified to be downregulated in the OmcF-deficient mutant.

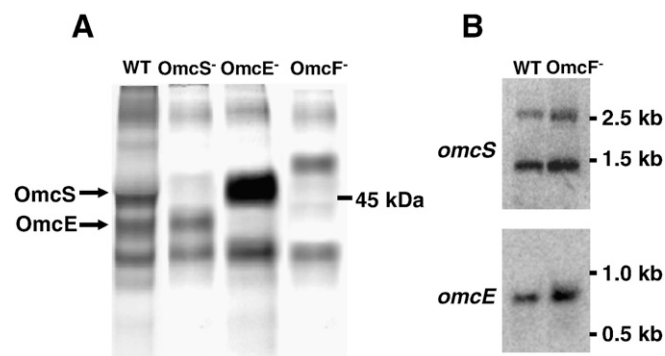
membrane protein similar to *Escherichia coli* ClpB, and GSU2433, a putative ATP-dependent protease similar to *E. coli* Lon. Transcript levels of both genes were not significantly different in the mutant (fold changes of 1.000465 and  $-1.129$  for GSU2431 and GSU2433, respectively), consistent with a lack of polar effects in the OmcF-deficient mutant [9].

The microarray analysis, as well as analysis with qRT-PCR and Northern blots, confirmed the previously described [9] down-regulation of the *omcB* and *omcC* operons (Table 4, Fig. 2). All the components of these operons had significantly lower transcript levels in microarray analysis (Table 4). The gene for a transcriptional regulator of the *omcB* operon, GSU2741 [33], also had lower transcript levels in the OmcF-deficient mutant.

The GSU1330–GSU1333 and GSU1339–GSU1340 gene clusters, which were the most upregulated genes in wild type *G. sulfurreducens* grown on the anode surface of a microbial fuel cell [17] were among the genes with the greatest decrease in transcript levels in the OmcF-deficient mutant compared to the wild type (Table 4). The GSU1330–GSU1333 cluster was predicted to be an operon [34] and RT-PCR analysis with primers designed to amplify transgene fragments (Table 3) produced the expected products in each instance (data not shown), suggesting that this operon prediction was correct. Analysis with qRT-PCR (Table 4) and Northern blots (data not shown) demonstrated that transcript levels for all genes in the GSU1330–GSU1333 cluster were much lower (ca.  $-2.1$  to  $-4.2$  fold) in the OmcF-deficient mutant. These genes are annotated [6] as follows: putative metal ion efflux outer membrane protein (GSU1330), efflux transporter, RND family, MFP subunit (GSU1331), heavy metal efflux pump, Czca family (GSU1332), and hypothetical protein (GSU1333).

Another gene, GSU1340, located near the GSU1330–GSU1333 gene cluster appeared to be downregulated in the OmcF-deficient mutant (Table 4). GSU1339, GSU1340, and GSU1341 were predicted to be components of one operon [34] and the appropriate primers (Table 3) amplified all of the expected transgene fragments (data not shown) confirming these genes could be co-transcribed. Analysis with qRT-PCR (Table 4) and Northern blots (data not shown) demonstrated that transcript levels for all genes in the GSU1339–GSU1341 cluster were much lower (ca.  $-2.1$  to  $-5.0$  fold) in the OmcF-deficient mutant. The genes are annotated as: hypothetical protein (GSU1339), ABC transporter, permease protein (GSU1340), ABC transporter, ATP-binding protein (GSU1341).

Based on homology to genes in *E. coli*, the proteins encoded by the genes in clusters GSU1330–GSU1333 and GSU1339–GSU1341 could contribute to a system that exports proteins to the outer cell surface in *G. sulfurreducens*. GSU1330 has homology with the gene for TolC in *E. coli* K12 (32% similarity, NCBI accession number NP417507). When TolC assembles with a protein providing energy from proton antiport (corresponding to GSU1331 and GSU1332), this complex pumps out



**Fig. 3.** Expression of outer-surface c-type cytochromes. (A) Tris-Tricine polyacrylamide gel electrophoresis and heme staining of loosely bound outer membrane-enriched protein fractions prepared from wild type (WT), OmcS-deficient (OmcS<sup>-</sup>, *omcS::spec*) [11], OmcE-deficient (OmcE<sup>-</sup>, *omcE::kan*) [11], and OmcF-deficient (OmcF<sup>-</sup>, *omcF::kan*) [9] strains. OmcS and OmcE heme staining bands were not detected in the OmcF-deficient mutant. (B) Northern analysis of *omcS* and *omcE* expression in the wild type (WT) and the OmcF-deficient mutant (OmcF<sup>-</sup>). Equal amounts of total RNA (5  $\mu$ g) were loaded for each strain. Ethidium bromide staining of 16S and 23S rRNA is shown in Fig. 2A as confirmation of RNA quantification.

small compounds such as detergents, organic solvents, and anti-bacterial drugs for bacterial survival [35,36]. However, if TolC cooperates with inner membrane complexes providing energy from ATP hydrolysis (corresponding to GSU1340 and GSU1341), this complex (Type I secretion system) exports large proteins such as hemolysin and proteases [36–38]. Thus, the function of the putative type I secretion by *G. sulfurreducens* might include exporting some outer membrane proteins which are important in cell–anode interaction.

In order to determine whether deletion of *omcF* impacted on the export of proteins to the outer surface, loosely bound outer membrane-enriched protein fractions were separated from the OmcF-deficient and wild type strains and examined for cytochrome content (Fig. 3A). OmcS and OmcE, outer membrane c-type cytochromes that are required for optimal current production [11,17], were not detected in the loosely bound outer membrane proteins of the OmcF-deficient mutant (Fig. 3A). The microarray analysis described above, as well as Northern blot analysis (Fig. 3B), indicated that transcription of *omcS* and *omcE* was comparable in the OmcF-deficient mutant and in wild type cells. These results are consistent with the concept that decreased expression of the putative secretion system (GSU1330–GSU1333 and GSU1339–GSU1341) in the OmcF-deficient mutant

**Table 5**

Genes that were significantly upregulated in the OmcF-deficient mutant compared to the wild type in acetate-limiting, fumarate-grown continuous culture

Locus ID	Annotation	Fold change	Role category
GSU2407	Hypothetical protein	2.81	Function unknown
GSU2408	Heat shock protein, Hsp20 family	2.41	Protein folding and stabilization
GSU3406	Amino acid ABC transporter, periplasmic amino acid-binding protein	2.32	Transport and binding proteins
GSU1947	Hypothetical protein	2.14	Function unknown
GSU2404	Pentapeptide repeat domain protein	2.06	Function unknown
GSU2390	Heat shock protein HtpG	1.89	Protein folding and stabilization
GSU2406	dnaJ domain protein	1.89	Protein turnover, chaperones
GSU1945	Fibronectin type III domain protein	1.76	Function unknown
GSU0655	RNA polymerase sigma-32 factor ( <i>rpoH</i> )	1.67	Transcription
GSU2355	Hypothetical protein	1.66	Function unknown
GSU0658	ClpB protein	1.64	Protein turnover, chaperones
GSU1072	Transcriptional regulator, IclR family	1.60	Regulatory functions
GSU1752	Translation elongation factor P	1.56	Protein synthesis

could prevent proper localization of outer membrane proteins required for electron transfer to fuel cell anodes.

GSU2780 and GSU3410, which are annotated as encoding hypothetical proteins, were also downregulated in the OmcF-deficient mutant (Table 4). Gene expression analysis during growth on electrodes indicated that GSU2780 had the greatest increase in transcript levels of any hypothetical protein [17]. It has 5 other homologues (GSU0834, GSU2131, GSU3111, GSU3305, and GSU3402) in *G. sulfurreducens* genome, but none of these had increased transcript levels during growth on electrode. Transcript levels of GSU3410 are also higher during growth on electrode [17]. GSU3409 is a homologue of GSU3410 and Northern blot showed that GSU3409 co-transcribed with GSU3410 (data not shown) and is downregulated in the OmcF-deficient mutant, indicating GSU3409 and GSU3410 are in an operon. This is consistent with operon predictions [34].

### 3.3. Genes with higher transcript levels in the OmcF-deficient mutant

The OmcF-deficient mutant had increased transcript levels in genes related to heat shock response, such as the RpoH heat shock sigma factor [39], as well as several heat shock proteins and chaperones (GSU2408, GSU2390, GSU2406, and GSU0658) (Table 5). The heat shock protein encoded by GSU2408 is in a family of low-molecular-mass chaperone,  $\alpha$ -crystallin-type proteins [40], that have been proposed to function in membrane stabilization [41] or to prevent irreversible aggregation of unfolding proteins [42]. GSU2390 encodes heat shock protein HtpG which possess ATPase for its chaperone activity in vivo and has been proposed to prevent aggregation of unfolded proteins [43] or to promote optimal folding of proteins in stressed cells [44]. GSU2406, which also appeared to be upregulated, encodes DnaJ, which is generally believed to be more important for protein folding than  $\alpha$ -crystallin-type heat shock proteins. These results suggest that the absence of OmcF may have triggered a stress response to potential mislocalization or misfolding of proteins. For example, OmcB, which is constitutively expressed in wild type cells, is likely to be a component of a multi-protein complex (Tunde Mester, unpublished data). When OmcB is not expressed in the OmcF-deficient mutant, this could cause misassembly of the complex.

## 4. Conclusion

The results demonstrate that OmcF, which was previously shown to be necessary for optimum Fe(III) reduction by *G. sulfurreducens*, is also necessary for optimum current production in *G. sulfurreducens* fuel cells, but that the mechanisms by which OmcF influences Fe(III) reduction and electricity generation are different. In Fe(III) reduction OmcF is considered to play a role in regulating the transcription of *omcB*, a gene encoding an outer membrane c-type cytochrome required for Fe(III) reduction [14] rather than participating directly in an electron transfer chain to Fe(III) [9]. The requirement for OmcF for proper *omcB* transcription does not appear to be important in current production because, unlike Fe(III) reduction, OmcB is not required for electricity production. Rather, OmcF is required for proper levels of transcription of other genes that previous studies have shown are among the genes most highly upregulated when *G. sulfurreducens* grows on the anode surface of a microbial fuel cell versus growth on a soluble electron acceptor [17]. In turn, some of the proteins encoded by these genes may play an important role in the proper localization of proteins, such as OmcS and OmcE, which are known to be important in electron transport to anodes [11,17].

A common approach to evaluating the function of outer-surface proteins in extracellular electron transfer to insoluble electron acceptors, such as Fe(III) oxides or electrodes, is to assume that if deletion of the gene for the outer-surface protein inhibits electron transfer then that outer-surface protein is a likely candidate as a conduit for electron transfer to the extracellular electron acceptor.

However, as the studies reported here demonstrate, it is important to evaluate potential indirect consequences of such gene deletions.

## Acknowledgements

This research was supported by the Office of Science (BER), U.S. Department of Energy, Cooperative Agreement No. DE-FC02-02ER63446 and Office of Naval Research Award No. N00014-07-1-0966.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioelechem.2008.04.023.

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