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GENOMICS, TRANSCRIPTOMICS, PROTEOMICS

Global transcriptional analysis of *Geobacter sulfurreducens* under palladium reducing conditions reveals new key cytochromes involved

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Abstract

Geobacter sulfurreducens is capable of reducing Pd(II) to Pd(0) using acetate as electron donor; however, the biochemical and genetic mechanisms involved in this process have not been described. In this work, we carried out transcriptome profiling analysis to identify the genes involved in Pd(II) reduction in this bacterium. Our results showed that 252 genes were upregulated while 141 were downregulated during Pd(II) reduction. Among the upregulated genes, 12 were related to energy metabolism and electron transport, 50 were classified as involved in protein synthesis, 42 were associated to regulatory functions and transcription, and 47 have no homologs with known function. RT-qPCR data confirmed upregulation of genes encoding PilA, the structural protein for electrically conductive pili, as well as *c*-type cytochromes GSU1062, GSU2513, GSU2808, GSU2934, GSU3107, OmcH, OmcM, PpcA, and PpcD under Pd(II)-reducing conditions. Δ*pilA* and Δ*pilR* mutant strains showed 20% and 40% decrease in the Pd(II)-reducing capacity, respectively, as compared to the wild type strain, indicating the central role of pili in this process. RT-qPCR data collected during Pd(II) reduction also confirmed downregulation of *omcB*, *omcC*, *omcZ,* and *omcS* genes, which have been shown to be involved in the reduction of Fe(III) and electrodes. The present study contributes to elucidate the mechanisms involved in Pd(II) reduction by *G. sulfurreducens*.

Key points

- *Transcriptome analysis provided evidence on Pd(II) reduction by G. sulfurreducens.*
- *Results indicate that electrically conductive pili is involved in Pd(II) reduction.*
- *G. sulfurreducens was not able to grow under Pd(II)-reducing conditions.*
- *The study contributes to a better understanding of the mechanisms in Pd(II) reduction.*

Keywords Pd(II) reduction · *Geobacter sulfurreducens* · Transcriptome profile · Cytochromes

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Introduction

In the last two decades, the alternative of using bioreductive deposition of precious metals, such as platinum-group metals (PGMs; e.g., Pt, Pd, Rh), for their recovery, has been widely explored. Special interest has been focused on palladium (Pd) due to its high value and extensive use as catalyst. Fe(III) reducing bacteria (IRB), such as *Shewanella oneidensis* (De Windt et al. 2006) and *Geobacter sulfurreducens* (Yates et al. 2013; Pat-Espadas et al. 2013), as well as sulfate reducing bacteria (SRB), such as *Desulfovibrio desulfuricans* (Lloyd et al. 1998), have extensively been explored for this purpose. Despite the great demand for developing efficient microbial processes to recover this valuable element, the mechanisms involved in Pd(II) reduction and subsequent deposition of Pd(0) are poorly understood.

Extracellular formation of Pd(0) nanoparticles (NPs) has been reported in *G. sulfurreducens* (Pat-Espadas et al. 2013). Moreover, differences regarding location of deposited NPs, depending on the strain, have been documented (Lloyd et al. 1998). These findings could be related to specific mechanisms used by each strain, as well as to experimental conditions prevailing. However, further studies are required to fully elucidate the mechanisms involved.

Geobacter species are abundant in nature and have the ability to perform extracellular electron transfer (EET) to reduce a broad array of heavy metals, such as Fe(III), Mn(IV), U(VI), Co(III), and Ag(I), among others (Caccavo et al. 1994; Sanford et al. 2007; Law et al. 2008; Lovley et al. 2011). It has been proposed that the electron transfer mechanisms reported for iron reduction could also be involved in Pd(II) reduction (Pat-Espadas et al. 2014). Nevertheless, the genome of *G. sulfurreducens* has 111 predicted *c*-type cytochromes (Mehta et al. 2005; Ding et al. 2008); hence, it is likely that other cytochromes, as well as conductive pili, could play a role in Pd(II) reduction (Childers et al. 2002; Reguera et al. 2005). This is supported by the observation that some proteins or cytochromes are specifically required to achieve the reduction of certain metals, such as soluble Fe(III), Fe(III) oxides, and U(VI) (Shelobolina et al. 2007; Shi et al. 2007; Ding et al. 2008).

The purpose of this study was to elucidate the mechanisms involved in Pd(II) reduction by *G. sulfurreducens*, based on global transcriptome analysis using RNA sequencing analysis. Quantitative reverse transcription PCR, as well as genetic and physiological tests were also performed to identify the genes involved in Pd(II) reduction.

Materials and methods

Culture procedures

Bacterial strains and oligonucleotides used in this study are listed in Table 1. *G. sulfurreducens* PCA (DSM 12127; ATCC51573) was grown anaerobically at 30 °C in NBAF medium, supplemented with acetate and fumarate; these culture conditions were referred to as "non-Pd(II)-reducing conditions" (Coppi et al. 2001). For experiments conducted under "Pd(II)-reducing" conditions, late logarithmic phase cultures of *G. sulfurreducens* were used. The protocol comprised harvesting cells by centrifugation at 9000*g* for 20 min and washing with sterilized, osmotically balanced buffer. The buffer composition was as follows (in grams per liter): $NaHCO_3$, 2.5; NH_4Cl, 0.25; $NaH_2PO_4{\cdot}H_2O$, 0.006; and KCl, 0.1. Reduction experiments were performed in 120-ml glass serum bottles, including 100 ml of anaerobic basal medium. Anaerobic conditions were established as follows: medium was dispensed into anaerobic pressure bottles, which were sealed with butyl rubber stoppers and flushed with N_2/CO_2 (80:20, v/v) gas mixture to remove dissolved oxygen. The headspace was saturated with the same gas mixture in all bottles, which were subsequently sterilized at 121 °C for 20 min. Cell suspensions, as well as acetate and Na_2PdCl_4 (Sigma-Aldrich) stock solutions were added to yield a concentration of 800 mg l^{-1} cell dry weight (CDW), 5 mM of acetate, and 25 mg Pd(II) l^{-1}, respectively (Pat-Espadas et al. 2013; Pat-Espadas et al. 2014). RNA*Later* stabilization solution (Ambion) was added to cultures for harvesting cells. Bacterial pellets were flash-frozen and stored at − 70 °C.

RNA extraction

G. sulfurreducens cells from both "Pd(II) reduction" and "non-Pd(II) reduction" experimental conditions were used for RNA-Seq and quantitative real-time PCR (RT-qPCR) analyses. All experiments were performed in duplicate by using independent samples. For each biological sample, total RNA samples were extracted using the RNeasy mini kit (Qiagen), then they were examined with an Agilent 2100 Bioanalyzer and quantified using NanoDrop 200c (Thermo Scientific).

RNA-Seq and data analysis

RNA-Seq was performed using RNA samples extracted from the two experimental conditions tested. Illumina sequencing was performed at USMI (Unidad de Secuenciación Masiva, UNAM, Mexico). Briefly, after removing residual DNA using DNase I (ThermoScientific) and ribosomal RNA with Terminator 5′-Phosphate-dependent exonuclease (Epicentre), the mRNA-enriched RNA was chemically fragmented to 150–200 bp. Based on these cleaved RNA fragments, cDNA was synthesized using a random hexamer primer and reverse transcriptase. After final reparation and ligation of adaptors, obtained products were amplified by PCR, further purified, and used to create the final cDNA library. Libraries were sequenced on an Illumina Genome Analyzer IIx. Differential

Table 1 Bacterial strain and primer sequences used in this work and for RT-qPCR validations

Strain	Description	Reference/source
Geobacter sulfurreducens DSM 12127; ATCC51573		
PCA strain	Wild type	Caccavo et al. (1994)
Δ*pilR* strain	*pilR*::kan, kanamicin resistance	Juárez et al. (2009)
Δ*pilA* strain	*pilA*::kan, kanamicin resistance	Reguera et al. (2005)
Oligonucleotides	Sequence 5′–3′	
qPCRrecCfw	CTGTCGTCACCCTTTGTTCC	This study
qPCRrecCrev	GAAAGGGATAGGAGCCGTTC	This study
qPCRomcMfw	TGGAGACTACCCATGCTGAA	This study
qPCRomcMrev	AGACGTCGAGGTGCTCGTAT	This study
qPCRomcHfw	ATGGACGTGAATGGAAGGAG	This study
qPCRomcHrev	TGGCAGTCAGTACAGGTGGA	This study
qPCRppcDfw	CAGCACTCACCCTGTTCTGT	This study
qPCRppcDrev	TGCTTTTTGTGGTCGAAGGT	This study
qPCRhgtRfw	GAGAGAAAATCCGCGGTACA	This study
qPCRhgtRrev	TAGCCTCCCTCATGATGTCC	This study
qPCRGSU1062fw	TCCAGGATTCCGAAACTCAA	This study
qPCRGSU1062rev	CGGCTCTATTTCGCTCTCAG	This study
qPCRGSU0207fw	AGCAAGGGCTTTGGTTTTCT	This study
qPCRGSU0207rev	TTACCCTATCGCCTTCAGCA	This study
qPCRGSU1650fw	TACCCCGTGTTTCGCTATCT	This study
qPCRGSU1650rev	CCGTGGAAATCGAAGAATTT	This study
qPCRGSU0837fw	GCATTGAACGGATCTTCGAC	This study
qPCRGSU0837rev	GCGGACGTACTGTTCATGTC	This study
qPCRGSU0345fw	CGAGTCACTCTCGTTGCAGA	This study
qPCRGSU0345rev	TGCGAGAGAGCAGATGAAGA	This study
qPCRGSU2813fw	CGGTTCTCAACTCGGTCTTC	This study
qPCRGSU2813rev	GACATAGTCGGGGATGCTGT	This study
qPCRGSU0615fw	ACTTTGCCCTCTGTTTCACG	This study
qPCRGSU0615rev	GAATCCGCTCTTTGACAAGC	This study
qPCRGSU2937fw	GTCACCAGGAAGTCCACGAT	This study
qPCRGSU2937rev	TCAGGTGCTCAACTTTCACG	This study
qPCRGSU1106fw	AGTTCCCCAGTACGTGTTCG	This study
qPCRGSU1106rev	GCGAACTTGGAATCCTTTTG	This study
qPCRomcBfw	GGAGTATGTGGCATCCCTTG	This study
qPCRomcBrev	ACCGTTGGCATTCGTATCTC	This study
qPCRomcCfw	AGAGTACGTGGCATCCCTTG	This study
qPCRomcCrev	CCGTTGGCATTCGTATCTCT	This study
qPCRomcSfw	TCCTACCAGAACAGCAACGA	This study
qPCRomcSrev	ATAGGAACCGCTCAGGGACT	This study
qPCRomcZfw	AAGCCGACTGTCTCGAGTGT	This study
qPCRomcZrev	CGGAGGTATTGATGCAGCTT	This study
qPCRomcEfw	CCAGATCTGCGTGTTCTGTC	This study
qPCRomcErev	CATGCTGCTGGACGAGTAGA	This study
qPCRppcBfw	CCACAAGAAACACCAGACGA	This study
qPCRppcBrev	CACCCCTTGCAGGACTTG	This study
qPCRpilAfw	AATTACCCCCATACCCCAAC	This study
qPCRpilArev	AGCAGCTCGATAAGGGTGAA	This study
qPCRGSU2513Fw	GACCAGGCCCAGTTCAAGTA	This study

Table 1 (continued)

Strain	Description	Reference/source
qPCRGSU2513Rv	GGTGTCGATTTCAATTTCCTG	This study
qPCRGSU2808Fw	CGAAATGCCATACCTCCACT	This study
qPCRGSU2808Rv	TATATTGCCCCGAGTTGTCC	This study
qPCRGSU0592Fw	GGAGTATTCCCCCTGAAGGA	This study
qPCRGSU0592Rv	ATGTTTCTGGTGGCTGAAGG	This study
qPCRGSU2495Fw	ACCAGCTCTGCTTCGACTGT	This study
qPCRGSU2495Rv	GGTTGTGGCAGAGGGTACAT	This study

expression analyses were performed through IDEAmex website (http://zazil.ibt.unam.mx/ideamex/) using three methods: edgeR (Robinson and Oshlack 2010), DESeq (Anders and Huber 2010), and NOISeq (Tarazona et al. 2011). edgeR and NOISeq were performed by applying TMM (Robinson and Oshlack 2010) as the normalization method. To identify differentially expressed genes, we selected those whose p value were < 0.05 and fold change > 2, for each method. Finally, we considered as the best candidates, only genes that appeared differentially expressed in the three methods. The functional annotation of differentially expressed genes, regarding the affected pathways, was obtained from Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto 2000), using our own R's scripts. RNA-Seq transcriptome data were deposited in the NCBI Gene Expression Omnibus database under accession number GSE113152.

RT-qPCR

To validate the quality of sequencing data, some differentially expressed genes were selected for RT-qPCR analysis. mRNA was extracted as described in section "RNA extraction" and residual DNA was removed using DNase I (Thermo Scientific). cDNA synthesis was performed using RevertAid H Minus First Strand cDNA Synthesis kit (Thermo Scientific). Subsequently, RT-qPCR was performed using a Maxima SYBR Green/ROXq PCR Master Mix (Thermo Scientific) in a 96-well plate with the Light-Cycler II (Roche). Gene-specific primers used for RT-qPCR are shown in Table 1. *recC* was used as internal gene standard for PCR amplification. Normalized fold changes of the relative expression ratio were quantified by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). All experiments were performed in triplicate, using independent samples, and their average values were calculated.

Cytochrome *c* content

Membrane fractions of *G. sulfurreducens* were isolated as previously described (Kim et al. 2005; Juárez et al. 2009). Outer membrane-enriched fractions were prepared by treating crude membranes with a sarkosyl (sodium N-lauroyl sarcosinate) solution at 1% (wt/vol) to extract inner membrane proteins. Outer membrane proteins were analyzed by Tris-Glycine denaturing polyacrylamide gel electrophoresis, and *c*-type cytochromes were detected by staining with *N*,*N*,*N*,*N*-tetramethylbenzidine, as previously described (Thomas et al. 1976; Francis and Becker 1984). PageRuler pre-stained protein standards were purchased from Thermo Scientific. The Tris-Glycine gel image was digitized using a Gel-doc (Bio Rad).

Immunoblot analysis

Protein extraction from cultures performed under "Pd(II)-reducing" and "non-Pd(II)-reducing" conditions was conducted by western blot as follows: cells pellets were re-suspended in 150 μl of B-PER II Bacterial Protein extraction reagent (Pierce) and incubated for 15 min. Afterwards, 1 mg of total protein per sample was incubated with PAGE-Buffer and boiled for 5 min before separation on a 15% SDS-PAGE. After separation, proteins were transferred to nitrocellulose membranes (Merck-Millipore) for immunoblot analysis using rabbit polyclonal antibodies raised against *G. sulfurreducens* (Yi et al. 2009). Blots were blocked with 3% BSA in PBS overnight at 4 °C and then incubated with a 1/1000 dilution of primary antibody for 4 h at room temperature, washed with PBS, and incubated with a 1/5000 dilution of goat anti-rabbit alkaline phosphatase-conjugated secondary antibody for 3 h at room temperature. After being washed, blots were developed with 5-bromo-4-chloro-3-indolylphosphatase (BCIP)-Nitro Blue Tetrazolium (Pierce) following manufacturer's instructions.

Viability assay

Cell viability assays after exposure to Pd(II) were performed by recovering resting cells in NBAF medium and incubated to measure microbial growth. Prior to inoculation, cell suspensions under Pd(II)-reducing conditions were incubated for 3 h and further transferred to NBAF medium to yield a cellular

density of 0.05 (OD 600_{nm}). Cultures were incubated at 30 °C and growth was periodically monitored as OD 600_{nm}.

Analytical techniques

Reduction of Pd(II) was quantified as follows: 5 ml of samples were filtered using 0.22 μm membrane filters (Millipore, Bedford, USA). Filtered samples were then analyzed by inductively coupled plasma-optic emission spectroscopy (ICP-OES, Varian 730-ES). Cell counts were performed using a fluorescent microscopy and acridine orange to stain cells. Briefly, a 100-μl sample was added to a 900-μl, 2.5% glutaraldehyde solution and mixed thoroughly. Bacterial cell suspensions were stained with an acridine orange solution (final concentration, 0.01%) and incubated at room temperature for 2 min. Samples were then vacuum filtered through a black Isopore membrane filter (pore diameter, 0.2 μm; Millipore).

X-ray diffraction analysis

Analysis of Pd(0) NPs deposited on the different strains of *G. sulfurreducens* was conducted in an X-Ray diffractometer Bruker D8 Advance. Samples were treated as previously described (Pat-Espadas et al. 2013). X-ray diffraction (XRD) patterns were recorded from 20°–90° 2θ with a step time of 2 s and step size of 0.01° 2 .

Results

The main results obtained from the analysis of *c*-type cytochromes, the genes up- and downregulated under Pd(II)-reducing conditions, as well as the contribution of the pili to palladium reduction are described in the following sections.

Differentially expressed genes during Pd(II) reduction

We used high-throughput RNA-Seq approach to profile transcriptional responses during Pd(II) reduction in *G. sulfurreducens*. *p* values and fold changes (FC) were calculated and only genes that showed differential expression by all methods were selected, resulting in 393 differentially expressed genes (Fig. 1a). A cutoff p value < 0.05 and FC > 2 were used.

Out of the 393 genes, 252 displayed statistically significant upregulation (FC > 2), while 141 downregulated under Pd(II)-reducing conditions. Genes showing significant differences in transcript levels were classified into the following functional categories: regulatory functions and transcription, energy metabolism and electron transport, DNA metabolism, transport, carbohydrate metabolism, proteolysis, protein synthesis, amino acids metabolism, mobile and extrachromosomal elements,

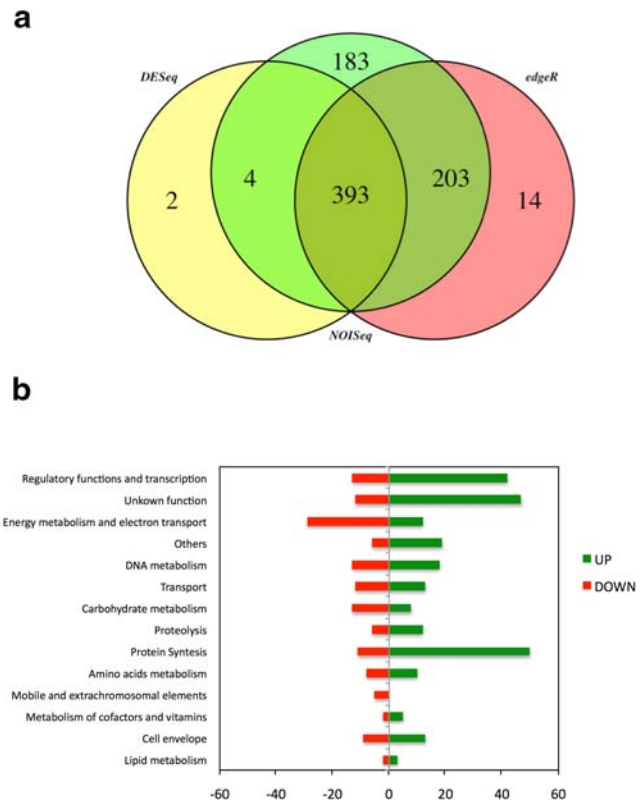

Fig. 1 Transcriptome analysis results from *Geobacter sulfurreducens* under Pd(II)-reducing conditions. **a** Venn diagram representing differential gene expression analysis from Pd(II)-reducing conditions by three statistical methods. **b** Functional overview of the genes that were differentially expressed during Pd(II) reduction

metabolism of cofactors and vitamins, cell envelope, lipid metabolism, unknown function, and others (Fig. 1b).

The main differentially expressed genes were those involved in protein synthesis, where 50 genes were upregulated, while 11 were downregulated. The second group corresponds to genes involved in regulatory functions and transcription with 55 genes (42 upregulated and 13 downregulated). The third group of genes is involved in energy metabolism and electron transport (12 upregulated and 29 downregulated), from which 20 code for *c*-type cytochromes.

Expression of *c*-type cytochromes genes during Pd(II) reduction

Approximately half of the differentially expressed genes involved in energy metabolism and electron transport were related to *c*-type cytochromes (9 upregulated and 11 downregulated); 8 are located in the outer membrane, 8 in the periplasmic, 1 in the cytoplasm, and 1 attached to the inner membrane, while the location of the remaining 2 is unknown (Table 2).

Among the most highly upregulated *c*-type cytochromes under Pd(II)-reducing conditions were GSU1062, GSU2808 and PpcA. Additionally, the outer membrane cytochromes OmcH and OmcM were also overexpressed under Pd(II)-

Table 2 *c*-Type cytochrome and putative cytochrome genes differentially expressed during Pd(II) reduction

Locus ID	Gene annotation	Gene name	Fold change (FC)	Log2 FC	*p* value	Signal peptide	Cellular location
Cytochromes genes that were upregulated in RNA-Seq experiments							
GSU0612	Cytochromec3, 3 heme-binding sites	*ppcA*	0.381	1.390	1.4E−06	Yes	Periplasmic
GSU1024	Cytochromec3, 3 heme-binding sites	*ppcD*	0.440	1.186	2.4E−03	Yes	Periplasmic
GSU1062	Cytochrome c putative, 1 heme-binding site	–	0.281	1.830	4.1E−05	Yes	Periplasmic
GSU2513	Cytochrome c family protein, 1 heme-binding site	–	0.423	1.243	2.4E−02	Yes	Unknown
GSU2808	Cytochrome c family protein, 5 heme-binding sites	–	0.338	1.563	3.0E−05	Yes	Outer membrane
GSU2934	Cytochrome c family protein, 9 heme-binding sites	*cbcN*	0.476	1.071	2.6E−04	Yes	Periplasmic
GSU3107	Ribosomal protein L31, 1 heme-binding site	*rpmE*	0.404	1.307	5.5E−03	NO	Cytoplasmic
GSU2883	Cytochrome c family protein, 18 heme-binding sites	*omcH*[a]	0.789	0.342	1.0E+00	Yes	Outer membrane
GSU2294	Cytochrome c family protein, 4 heme-binding sites	*omcM*[a]	0.530	0.917	5.8E−02	Yes	Outer membrane
Cytochromes genes that were downregulated in RNA-Seq experiments							
GSU0283	Sensor histidine kinase, 1 heme-binding site	–	2.442	−1.288	7.9E−05	NO	Cytoplasmic membrane
GSU0592	Cytochrome c family protein, 11 heme-binding sites	*omcQ*	2.491	−1.317	1.6E−05	Yes	Outer membrane
GSU0615	Cytochrome c family protein, 5 heme-binding sites	–	4.283	−2.099	1.9E−03	Yes	Periplasmic
GSU2076	Cytochrome c family protein, 5 heme-binding sites	*omcZ*	2.181	−1.125	4.8E−06	Yes	Outer membrane
GSU2495	Cytochrome c family protein, 22 heme-binding sites	–	3.333	−1.737	2.5E−03	NO	Unknown
GSU2731	Polyheme membrane-associated cytochrome c, 8 heme-binding sites	*omcC*	2.125	−1.088	2.3E−03	Yes	Outer membrane
GSU2737	Polyheme membrane-associated cytochrome c, 8 heme-binding sites	*omcB*	4.047	−2.017	4.6E−02	Yes	Outer membrane
GSU2811	Cytochrome c Hsc, 1 heme-binding site	*cccA*	2.657	−1.410	6.8E−16	Yes	Periplasmic
GSU2813	Cytochrome c551 peroxidase, 2 heme-binding sites	*ccpA*	3.285	−1.716	4.5E−13	Yes	Periplasmic
GSU2937	Cytochrome c family protein, 5 heme-binding sites	–	3.523	−1.817	9.9E−03	Yes	Periplasmic
GSU2504	Cytochrome c family protein, 1 heme-binding site	*omcS*[a]	1.829	−0.871	1.0E+00	Yes	Outer membrane

Protein cellular location was predicted with PSORT-B (Gardy et al. 2003) and SignalP 3.0 Server (Emanuelsson et al. 2007)

[a] Values are out of cutoff for fold change but validated by RT-qPCR

reducing conditions. Other *c*-type cytochromes upregulated during Pd(II) reduction were PpcD, GSU2513, GSU2934, and GSU3107. PpcD was also overexpressed in the reduction of Mn(IV) oxides, while the *gsu2934* gene was overexpressed during the reduction of Fe(III) oxides (Aklujkar et al. 2013). The putative cytochrome GSU2513 has not been reported previously and its function needs to be elucidated. Additional genes expressed under Pd(II)-reducing conditions by *G. sulfurreducens* are presented and discussed in supplementary material (SM, Tables S1 and S2).

Cytochrome *c* and PilA proteins content during palladium reduction

In order to assess if mRNA expression correlates with protein content of some *c*-type cytochromes differentially expressed under Pd(II)-reducing conditions, we evaluated their content by heme-staining of SDS-PAGE gels. As shown in Fig. 2a, inner membrane and outer membrane proteins extracted from cultures incubated under Pd(II)-reducing and non-P(II)-reducing conditions revealed differences in abundance of *c*-type cytochromes. This was particularly evident for OmcB and OmcC outer membrane multiheme *c*-type cytochromes, which are required for Fe(III) reduction in *G. sulfurreducens* (Leang et al. 2005; Liu et al. 2015). OmcS, which is also required for Fe(III) oxide reduction (Qian et al. 2011), as well as the outer membrane multiheme *c*-type cytochrome, OmcZ, which is essential for optimal current production in microbial fuel cells (Inoue et al. 2011), were more abundant in non-Pd(II)-reducing conditions (acetate/fumarate) as compared to the level observed under Pd(II)-reducing conditions.

We also examined the expression of *pilA* gene (GSU1496), which was upregulated under Pd(II)-reducing conditions. To verify the PilA protein content under these conditions, Immunobloting analysis was performed using anti-PilA antibodies. As shown in Fig. 2b, PilA was overproduced under these conditions.

Contribution of pili on Pd(II) reduction

In order to assess the contribution of pili to the reduction of Pd(II), experiments were performed with *ΔpilA* (pilin-deficient mutant) and *ΔpilR* mutant strains. Strain *ΔpilR* does not produce PilR, which is the main transcriptional activator of *pilA* gene, encoding for pilin, the structural protein of pili (Reguera et al. 2005). Therefore, in this mutant, PilA is severely decreased (Juárez et al. 2009). Results showed differences in Pd(II)-reducing capacity for the mutant strains as compared to the wild type (WT) strain during the same incubation period (Fig. 3a). It was quantified 98%, 81%, and 61% of Pd(II) reduction for WT, *ΔpilA* and *ΔpilR* mutant strains, respectively. The nature of produced NPs was analyzed by XRD (Fig. 3b), which confirmed the formation of Pd(0). The pattern of XRD in all samples showed five strong Bragg reflections at 2 < theta > values around 40.11, 46.66, 68.13, and 82.11, which correspond to planes (111), (200), (220), and (311) of a face-centered cubic lattice (fcc) (XRD pattern was indexed to ICDD card 89–4897 (fcc palladium syn)). XRD pattern showed that Pd NP's were crystalline in nature.

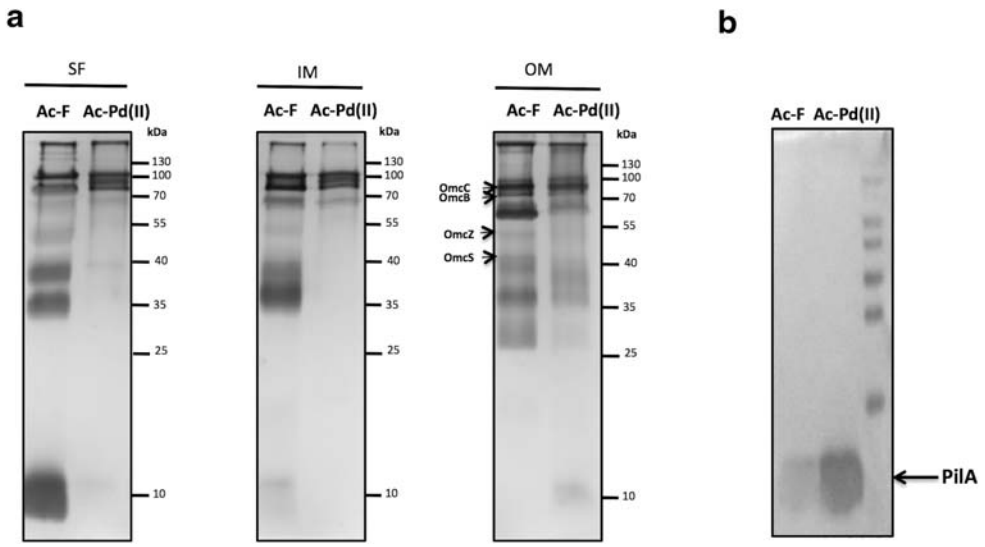

Fig. 2 *c*-Type cytochrome and PilA protein content found under Pd(II)-reducing conditions. **a** SDS-PAGE heme stained. Outer membrane (OM), inner membrane (IM) and soluble fraction (SF) were prepared from PCA strain with acetate-fumarate (Ac-F) or acetate-Pd(II) (Ac-Pd(II)). The localization of OmcC, OmcB, OmcS, OmcZ, and PpcA were labeled based on expected molecular weight (78.96, 74.89, 42.94, 47.09, and 7.72 kDa, respectively). **b** Immunoblot analysis for PilA. The PageRuler Pre-stained Protein Ladder standard (ThermoScientific) was used as a molecular weight

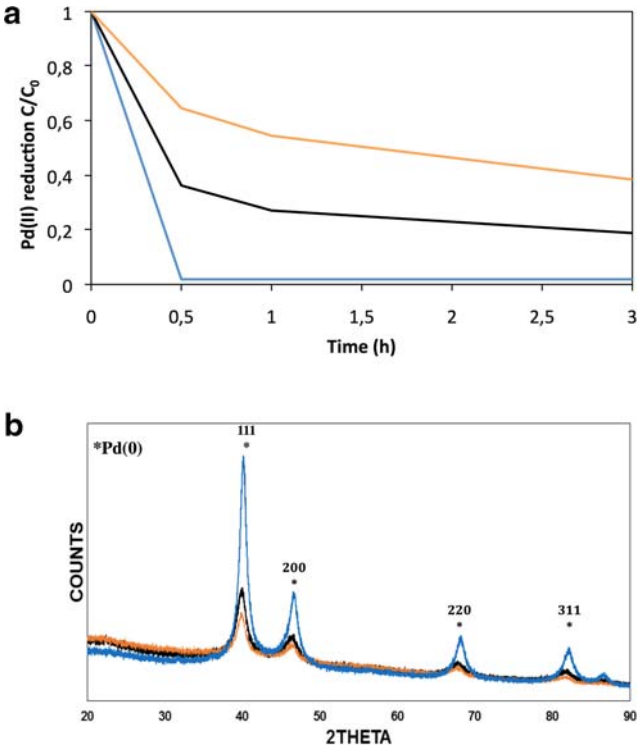

Fig. 3 Palladium reduction by different strains of *G. sulfurreducens*. **a** Kinetics of Pd(II) reduction. **b** Comparison of XRD patters corresponding to cells and black precipitates obtained from cultures of wild type (WT), Δ*pilA* and Δ*pilR* strains under Pd(II)-reducing conditions. WT strain, blue line; Δ*pilA* strain, black line; Δ*pilR* strain, orange line

Viability of *G. sulfurreducens* after exposure to Pd(II)

In order to verify if *G. sulfurreducens* is able to grow after exposure to Pd(II), cells were harvested from Pd(II)-reducing incubations and subsequently cultured in NBAF medium. Results showed that *G. sulfurreducens* could recover its viability after exposure to Pd(II), as shown in SM (Fig. S1). However, *ΔpilA* and *ΔpilR* mutant strains spent slightly more time than the WT strain to recover viability after exposure to Pd(II) (SM, Fig. S1). While our results confirmed that Pd(II) can be used as electron acceptor by *G. sulfurreducens*, no evidence demonstrating microbial growth was obtained under P(II)-reducing conditions. To corroborate if the lack of growth was not related to electron acceptor limitation, subsequent additions of Pd(II) were done and cells were able to reduce Pd(II) in several consecutive cycles, but growth was not observed (SM, Fig. S2).

Validation of selected differentially expressed genes using RT-qPCR

To verify the results obtained from RNA-Seq experiments and to get quantitative data to compare the transcript abundances under Pd(II)-reducing conditions, RT-qPCR analyses from 24 selected genes encoding proteins involved in electron transfer, transcriptional regulators and central metabolism (Table 3) were performed. These genes include the *c*-type cytochromes genes (*omcH*, *omcM*, *omcB*, *omcC*, *omcS*, *omcZ*, *ppcD*, *gsu1062*, *ppcB*, *omcE*, *ccpA*, *gsu0615*, *gsu2937*, *gsu2513*, *gsu2808*, *omcQ*, and *gsu2495*), as well as the cold shock DNA/RNA-binding protein, *gsu0207*, the transcriptional regulators, *hgtR* and *gsu0837*, the menaquinol oxidoreductase complex Cbc3, *gsu1650*, the pilin protein, *pilA*, the NADH dehydrogenase I, *nuoH*-1, and the citrate synthase I, *gltA*.

Upregulation of *omcH*, *omcM*, *gsu1062*, *gsu2513*, *gsu2808*, and *ppcD* genes that encoded for *c*-type cytochromes under Pd(II)-reducing conditions was confirmed by RT-qPCR. Similarly, the expression of *pilA*, *gsu0207*, *hgtR*, and *gsu1650* was high under these conditions according to RT-qPCR results. On the other hand, the low transcription of *omcB*, *omcC*, *omcS*, *omcZ*, *gsu0837*, *gsu0345*, *gsu2813*, *gsu0615*, *gsu2937*, *omcQ*, *gsu2495*, and *gsu1106* observed in RNA-Seq analyses was confirmed by RT-qPCR. Furthermore, the low transcription of *nuoH*-1 and *gltA* genes was also observed, in agreement with the high expression of *hgtR*, which is a negative regulator of these genes (Ueki and Lovley 2010).

Discussion

The results obtained from the analysis of the genes differentially expressed under Pd(II)-reducing conditions accounted for approximately 11% of the genes in *G. sulfurreducens* genome, indicating that Pd(II) reduction triggered significant global gene expression changes. The group of genes with the main notable change under these conditions was that involved in tRNA synthesis and ribosomal proteins, such as *rpsU*-1, *rpsB*, *rpsB*, *rpsT*, *rpmB*, *rplU*, and *rpsL*. The results also revealed a high number of upregulated transcriptional regulators, which points out the response of this bacterium to use Pd(II) as electron acceptor. Significant number of differentially expressed genes was associated to *c*-type cytochromes under Pd(II)-reducing conditions as it is shown in Table 2. For instance, the highly upregulated *c*-type cytochrome GSU1062 is a putative *c*-type cytochrome, which is abundant under ferric-citrate-reducing conditions (Ding et al. 2006). Similarly, *gsu2808* encodes for an outer membrane cytochrome, and also reported overexpressed under Fe(III)-reducing conditions, while its expression decreases in OmcB-deficient mutant (Leang et al. 2005; Methé et al. 2005). On the other hand, cytochrome PpcA, upregulated during Pd(II) reduction, participates in electron transfer in the periplasm. A *ppcA* mutant showed a decrease in Fe(III) and U(VI) reduction capacities (Lloyd 2003; Mehta et al. 2005).

Overexpression of cytochromes *omcH* and *omcM* under Pd(II)-reducing conditions suggests that they could be involved in the extracellular reduction of this electron acceptor

Table 3 Expression of genes with relevant phenotype observed in RNA-Seq analysis validated by RT-qPCR

Locus ID	Common name	Avg +Pd/Avg −Pd
GSU2294	OmcH, cytochrome c	2.87
GSU2883	OmcM, cytochrome c	1.29
GSU1024	PpcD, cytochrome c	5.80
GSU1062	Cytochrome c, 1 heme-binding site	5.63
GSU0207	Cold shock DNA/RNA-binding protein	12.91
GSU1650	Cytochrome b/b6 complex	2.14
GSU3364	HgtR, hydrogen-dependent growth transcriptional regulator	9.42
GSU1496	PilA, pilin protein	1.50
GSU2513	Cytochrome c, 1 heme-binding site	1.91
GSU2808	Cytochrome c, 5 heme-binding site	9.44
GSU2737	OmcB, lipoprotein cytochrome c	0.28
GSU3731	OmcC, lipoprotein cytochrome c	0.33
GSU2076	OmcZ, cytochrome c	0.28
GSU0618	OmcE, cytochrome c	0.88
GSU2504	OmcS, cytochrome c	0.56
GSU0364	PpcB, cytochrome c	0.98
GSU0592	OmcQ, cytochrome c	0.40
GSU2495	Cytochrome c, 22 heme-binding site	0.20
GSU0837	Response regulator	0.04
GSU0345	nuoH-1, NADH dehydrogenase I	0.74
GSU2813	CcpA, cytochrome c peroxidase	0.35
GSU0615	Cytochrome c, 5 heme-binding site	0.28
GSU2937	Cytochrome c, 5 heme-binding site	0.49
GSU1106	GltA, type I citrate synthase	0.48

n-fold changes were calculated based on the 2^{-DDCT} method (Livak and Schmittgen 2001)

Avg average, *−Pd* = without palladium, *+Pd* with palladium

since previous work has shown that *omcH* was overexpressed under growing conditions with insoluble Fe(III) oxides, while mutations in the *omcH* and *omcM* genes affect the reduction of Fe(III) oxides (Aklujkar et al. 2013).

Surprisingly, during Pd(II) reduction, cytochromes OmcB, OmcC, OmcS, and OmcZ, which are involved in the reduction of Fe(III), Mn(IV), and U(VI) (Aklujkar et al. 2013), were downregulated. A previous model of microbial reduction of Pd(II) by *G. sulfurreducens* suggested that cytochromes OmcB and OmcS, which are important in the reduction of Fe(III), Mn(IV) or U(VI), could be involved in the reduction process (Pat-Espadas et al. 2014). However, our data suggest that Pd(II) reduction does not involve those common cytochromes (Fig. 2a), but others with different biochemical characteristics. Moreover, the overproduction of PilA was surprising since the pili is required for extracellular electron transfer to insoluble electron acceptors, such as metal oxides and electrodes (Reguera et al. 2005), but not for reducing soluble metals. In *G. sulfurreducens*, the pili is an important structure participating in long-distance extracellular electron transfer towards Fe(III) oxides, syntrophic partners, as well as electrodes to generate bioelectricity (Reguera et al. 2005; Summers et al. 2010; Smith et al. 2013). It has been observed that the production of some *c*-type cytochromes decreased under U(VI)-reducing conditions, when a Δ*pilA* mutant strain was used, which resulted in a slight decrease in the reduction of U(VI) to U(IV) (Cologgi et al. 2011). It has been suggested that these effects on the reduction of U(VI) in the pili-deficient strain is due to the decrease in outer membrane *c*-type cytochromes and not to pili deficiency (Orellana et al. 2013). Therefore, the negative effect obtained on the reduction of Pd(II) by the Δ*pilA* mutant strain, in our experiments, could be due to a decrease in the production of *c*-type cytochromes of the outer membrane instead of a negative effect related to the pili. Since PilR is a transcriptional regulator that controls the expression of at least 44 genes, among which are several *c*-type cytochromes, we suggest that the decreased in Pd(II) reduction observed in the Δ*pilR* mutant strain could be related to *c*-type cytochrome content rather than to the absence of pili.

The study revealed an important aspect related to *G. sulfurreducens* and the use of Pd(II) since no microbial growth could be verified, though this metal could be used as electron acceptor. Palladium is a toxic element for many microorganisms, which may inhibit the activity of creatine kinase, aldolase, succinate dehydrogenase, carbonic anhydrase, alkaline phosphatase, and prolyl hydroxylase (Liu et al. 1979). Moreover, it is important to mention that it has been reported that chemically synthesized Pd NP's inhibit growth in *Staphylococcus aureus* and *E. coli* (Wilkins et al. 2013). Thus, deposition of Pd(0) NP's on cells surface, which has been observed in *G. sulfurreducens* under these conditions (Pat-Espadas et al. 2013), may be a limiting factor affecting growth during the reduction of Pd(II).

Biological reduction of Pd(II) has been poorly studied. To date, several bacteria have been reported with the ability to reduce Pd(II) to Pd(0) NPs (De Corte et al. 2012). In

Desulfovibrio fructosivorans and *Escherichia coli*, the biological reduction of Pd(II) to Pd(0) is linked to the activity of a hydrogenase (Mikheenko et al. 2008; Deplanche et al. 2010). *Shewanella oneidensis* and *D. desulfuricans* present a similar Pd(II) reduction mechanism, suggesting that hydrogenase and cytochrome c3 are involved in the reduction process (Lloyd et al. 1998; De Corte et al. 2012). Moreover, *Enterobacter cloacae* SgZ-5 T reduces Pd(II) to Pd(0) nanorods in the extracellular space with hydroquinone and riboflavin as redox mediators (Gardy et al. 2003). The present study contributes to elucidate the mechanisms involved in Pd(II) reduction by *G. sulfurreducens*. However, further studies are required to clarify if Pd(II) reduction proceeds in the periplasm.

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Author's contribution FJC, KJ, AHE, and AMPE conceived the study and designed the research. AHE and MHA conducted the experiments. LVA analyzed the data. AHE, AMPE, KJ, and FJC wrote the manuscript with input from all the authors. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

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*. Microbiol Read Engl 159:515–535

Anders S, Huber W (2010) Differential expression analysis for sequence count data. Genome Biol 11:R106

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

Childers SE, Ciufo S, Lovley DR (2002) *Geobacter metallireducens* accesses insoluble Fe(III) oxide by chemotaxis. Nature 416:767–769

Cologgi DL, Lampa-Pastirk S, Speers AM, Kelly SD, Reguera G (2011) Extracellular reduction of uranium via *Geobacter* conductive pili as a protective cellular mechanism. Proc Natl Acad Sci U S A 108:15248–15252

Coppi MV, Leang C, Sandler SJ, Lovley DR (2001) Development of a genetic system for *Geobacter sulfurreducens*. Appl Environ Microbiol 67:3180–3187

De Corte S, Sabbe T, Hennebel T, Vanhaecke L, De Gusseme B, Verstraete W, Boon N (2012) Doping of biogenic Pd catalysts with Au enables dechlorination of diclofenac at environmental conditions. Water Res 46:2718–2726

De Windt W, Boon N, Van den Bulcke J, Rubberecht L, Prata F, Mast J, Hennebel T, Verstraete W (2006) Biological control of the size and reactivity of catalytic Pd(0) produced by *Shewanella oneidensis*. Antonie Van Leeuwenhoek 90:377–389

Deplanche K, Caldelari I, Mikheenko IP, Sargent F, Macaskie LE (2010) Involvement of hydrogenases in the formation of highly catalytic Pd(0) nanoparticles by bioreduction of Pd(II) using *Escherichia coli* mutant strains. Microbiology 156:2630–2640

Ding Y-HR, 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 BBA - Proteins Proteomics 1764:1198–1206

Ding Y-HR, 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 BBA - Proteins Proteomics 1784:1935–1941

Emanuelsson O, Brunak S, von Heijne G, Nielsen H (2007) Locating proteins in the cell using targetP, SignalP, and related tools. Nat Protoc 2(4):953–971

Francis RT, Becker RR (1984) Specific indication of hemoproteins in polyacrylamide gels using a double-staining process. Anal Biochem 136:509–514

Gardy JL, Spencer C, Wang K, Ester M, Tusnády GE, Simon I, Hua S, deFays K, Lambert C, Nakai K, Brinkman FSL (2003) PSORT-B: improving protein subcellular localization prediction for gram-negative bacteria. Nucleic Acids Res 31:3613–3617

Inoue K, Leang C, Franks AE, Woodard TL, Nevin KP, Lovley DR (2011) Specific localization of the c-type cytochrome OmcZ at the anode surface in current-producing biofilms of *Geobacter sulfurreducens*. Environ Microbiol Rep 3:211–217

Juárez K, Kim B-C, Nevin K, Olvera L, Reguera G, Lovley DR, Methé BA (2009) PilR, a transcriptional regulator for pilin and other genes required for Fe(III) reduction in *Geobacter sulfurreducens*. J Mol Microbiol Biotechnol 16:146–158

Kanehisa M, Goto S (2000) KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 28:27–30

Kim B-C, Leang C, Ding Y-HR, Glaven RH, Coppi MV, Lovley DR (2005) OmcF, a putative c-type monoheme outer membrane cytochrome required for the expression of other outer membrane cytochromes in *Geobacter sulfurreducens*. J Bacteriol 187:4505–4513

Law N, Ansari S, Livens FR, Renshaw JC, Lloyd JR (2008) Formation of nanoscale elemental silver particles via enzymatic reduction by *Geobacter sulfurreducens*. Appl Environ Microbiol 74:7090–7093

Leang C, Adams LA, Chin K-J, Nevin KP, Methé BA, Webster J, Sharma ML, Lovley DR (2005) Adaptation to disruption of the electron transfer pathway for Fe (III) reduction in *Geobacter sulfurreducens*. J Bacteriol 187:5918–5926

Liu TZ, Lee SD, Bhatnagar RS (1979) Toxicity of palladium. Toxicol Lett 4:469–473

Liu Y, Fredrickson JK, Zachara JM, Shi L (2015) Direct involvement of ombB, omaB, and omcB genes in extracellular reduction of Fe(III) by *Geobacter sulfurreducens* PCA. Front Microbiol 6:1075

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods San Diego Calif 25:402–408

Lloyd JR (2003) Microbial reduction of metals and radionuclides. FEMS Microbiol Rev 27:411–425

Lloyd JR, Yong P, Macaskie LE (1998) Enzymatic recovery of elemental palladium by using sulfate-reducing bacteria. Appl Environ Microbiol 64:4607–4609

Lovley DR, Ueki T, Zhang T, Malvankar NS, Shrestha PM, Flanagan KA, Aklujkar M, Butler JE, Giloteaux L, Rotaru A-E, Holmes DE, Franks AE, Orellana R, Risso C, Nevin KP (2011) Geobacter: the microbe electric's physiology, ecology, and practical applications. In: Pole RK (ed) Advances in microbial physiology. Academic Press 59:1–100

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

Methé BA, Webster J, Nevin K, Butler J, Lovley DR (2005) DNA microarray analysis of nitrogen fixation and Fe(III) reduction in *Geobacter sulfurreducens*. Appl Environ Microbiol 71:2530–2538

Mikheenko IP, Rousset M, Dementin S, Macaskie LE (2008) Bioaccumulation of palladium by *Desulfovibrio fructosivorans* wild-type and hydrogenase-deficient strains. Appl Environ Microbiol 74:6144–6146

Orellana R, Leavitt JJ, Comolli LR, Csencsits R, Janot N, Flanagan KA, Gray AS, Leang C, Izallalen M, Mester T, Lovley DR (2013) U(VI) reduction by diverse outer surface c-type cytochromes of *Geobacter sulfurreducens*. Appl Environ Microbiol 79:6369–6374

Pat-Espadas AM, Razo-Flores E, Rangel-Mendez JR, Cervantes FJ (2013) Reduction of palladium and production of nano-catalyst by *Geobacter sulfurreducens*. Appl Microbiol Biotechnol 97:9553–9560

Pat-Espadas AM, Razo-Flores E, Rangel-Mendez JR, Cervantes FJ (2014) Direct and quinone-mediated palladium reduction by *Geobacter sulfurreducens:* mechanisms and modeling. Environ Sci Technol 48:2910–2919

Qian X, Mester T, Morgado L, Arakawa T, Sharma ML, Inoue K, Joseph C, Salgueiro CA, Maroney MJ, Lovley DR (2011) Biochemical characterization of purified OmcS, a c-type cytochrome required for insoluble Fe(III) reduction in *Geobacter sulfurreducens*. Biochim Biophys Acta BBA - Bioenerg 1807:404–412

Reguera G, McCarthy KD, Mehta T, Nicoll JS, Tuominen MT, Lovley DR (2005) Extracellular electron transfer via microbial nanowires. Nature 435:1098–1101

Robinson MD, Oshlack A (2010) A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol 11:R25

Sanford RA, Wu Q, Sung Y, Thomas SH, Amos BK, Prince EK, Löffler FE (2007) Hexavalent uranium supports growth of *Anaeromyxobacter dehalogenans* and Geobacter spp. with lower than predicted biomass yields. Environ Microbiol 9:2885–2893

Shelobolina ES, Coppi MV, Korenevsky AA, DiDonato LN, Sullivan SA, Konishi H, Xu H, Leang C, Butler JE, Kim B-C, Lovley DR (2007) Importance of c-type cytochromes for U(VI) reduction by *Geobacter sulfurreducens*. BMC Microbiol 7:16

Shi L, Squier TC, Zachara JM, Fredrickson JK (2007) Respiration of metal (hydr)oxides by *Shewanella* and *Geobacter*: a key role for multihaem *c* -type cytochromes. Mol Microbiol 65:12–20

Smith JA, Lovley DR, Tremblay P-L (2013) Outer cell surface components essential for Fe(III) oxide reduction by *Geobacter metallireducens*. Appl Environ Microbiol 79:901–907

Summers ZM, Fogarty HE, Leang C, Franks AE, Malvankar NS, Lovley DR (2010) Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. Science 330: 1413–1415. 526

Tarazona S, García-Alcalde F, Dopazo J, Ferrer A, Conesa A (2011) Differential expression in RNA-seq: a matter of depth. Genome Res 21:2213–2223

Thomas PE, Ryan D, Levin W (1976) An improved staining procedure for the detection of the peroxidase activity of cytochrome P-450 on sodium dodecyl sulfate polyacrylamide gels. Anal Biochem 75: 168–176

Ueki T, Lovley DR (2010) Genome-wide gene regulation of biosynthesis and energy generation by a novel transcriptional repressor in *Geobacter* species. Nucleic Acids Res 38:810–821

Wilkins MJ, Wrighton KC, Nicora CD, Williams KH, McCue LA, Handley KM, Miller CS, Giloteaux L, Montgomery AP, Lovley DR, Banfield JF, Long PE, Lipton MS (2013) Fluctuations in species-level protein expression occur during element and nutrient cycling in the subsurface. PLoS One 8:e57819

Yates MD, Cusick RD, Logan BE (2013) Extracellular palladium nanoparticle production using *Geobacter sulfurreducens*. ACS Sustain Chem Eng 1:1165–1171

Yi H, Nevin KP, Kim BC, Franks AE, Klimes A, Tender LM, Lovley D (2009) Selection of variant of Geobacter sulfurreducens with enhanced capacity for current production in microbial fuel cells. Biosens Bioelectron 24(12):3498–3503