Genome of *Geobacter sulfurreducens:* Metal Reduction in Subsurface Environments

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The complete genome sequence of *Geobacter sulfurreducens*, a δ-proteobacterium, reveals unsuspected capabilities, including evidence of aerobic metabolism, one-carbon and complex carbon metabolism, motility, and chemotactic behavior. These characteristics, coupled with the possession of many two-component sensors and many c-type cytochromes, reveal an ability to create alternative, redundant, electron transport networks and offer insights into the process of metal ion reduction in subsurface environments. As well as playing roles in the global cycling of metals and carbon, this organism clearly has the potential for use in bioremediation of radioactive metals and in the generation of electricity.

G. sulfurreducens, a member of the δ-Proteobacteria and of the family Geobacteraceae, is an important component of subsurface biota. *Geobacter* spp. generate energy as adenosine triphosphate by using metal ion–mediated electron transport to oxidize organic compounds to CO_2. For instance, Fe(III) oxides are abundant in the subsurface environment and are commonly used as terminal electron acceptors. However, the considerable interest in using *Geobacter* spp. for bioremediation stems from their ability to precipitate soluble metals, such as uranium, as a product of electron transport. Preferential stimulation of native populations of *Geobacter* spp. to promote metal precipitation from groundwater is readily achieved in situ by the addition of acetate (*1*–*3*). Beyond the opportunities for bioremediation, interest in *Geobacter* spp. lies in biotechnological efforts to capture energy from the catabolism of organic waste with energy-harvesting electrodes (*4*). Not forgetting, of course, the critical roles that *Geobacter* spp. play in the global cycling of metals and carbon.

The *G. sulfurreducens* genome is a single circular chromosome of 3,814,139 base pairs (bp) with a total of 3466 predicted protein-encoding open reading frames [coding sequences (CDSs)] (Table 1 and fig. S1) (*5*). This genome offers a phylogenetic framework for evolutionary studies on metal ion reduction. Analysis of gene distribution patterns across lineages (phylogenetic profiling) revealed that *G. sulfurreducens* and the metal ion–reducing γ-Proteobacterium *Shewanella oneidensis* (*6*) shared only two genes not found in any other species: both encoding c-type cytochromes. Hence, the metal ion–reducing capabilities of these species are not simply related to their sharing an exclusive set of genes; the expansion of specific gene families and the presence of novel genes are also involved. Similar analysis revealed many global similarities in gene content across a wider range of taxa (table S1). One example is a cluster of 20 genes found in organisms including *Desulfovibrio vulgaris*, low-G+C firmicutes, and Archaea. Most of the genes with known functions in this cluster are involved in energy metabolism (such as those encoding heterodisulfide reductases). The presence of four additional conserved hypothetical CDSs in this cluster suggests that their functional roles may be related to energy metabolism as well.

G. sulfurreducens encodes genes for glycolysis, the tricarboxylic acid (TCA) cycle, and the pentose phosphate pathway. With one notable exception, the TCA genes appear to be bacterial forms; however, both *G. sulfurreducens* and *G. metallireducens* (*7*) encode a form of citrate synthase previously reported only in eukaryotes (*8*) (fig. S2).

Central to the metabolism of *G. sulfurreducens* is the ability to anaerobically oxidize acetate (an abundant electron donor and carbon source in subsurface zones) completely to CO_2 and water using a variety of electron acceptors including metal ions, elemental sulfur, and fumarate. The lack of identifiable transporters for sugar uptake highlights the central importance of acetate metabolism to this organism (fig. S3). Based on its predicted membrane transporter complement, amino acids and carboxylates appear to be the predominant organic substrates for *G. sulfurreducens*. However, *G. sulfurreducens* does possess a complex set of phosphotransferase enzymes that presumably serve solely regulatory purposes.

G. sulfurreducens encodes enzymes that might participate in the acetyl–coenzyme A (acetyl-CoA) pathway. This versatile pathway can use acetate and one-carbon (C_1) compounds as substrates for energy generation, and can also be used to assimilate carbon by CO_2 reduction (*9*–*11*). However, *G. sulfurreducens* is missing a key enzyme of this pathway: formyl tetrahydrofolate synthetase (FTS). Instead, it may use reverse electron transport (coupling energetically unfavorable redox reactions to the expenditure of a membrane ion gradient), analogously to some methanogens, hence circumventing the need for the missing FTS (*12*) (fig. S3).

Additional evidence of C_1 metabolism is supplied by the presence of the anaerobic form of carbon monoxide dehydrogenase (CODH), which catalyzes the oxidation of carbon monoxide to CO_2 and hydrogen. In addition to participation in the acetyl-CoA

Table 1. General features of the *G. sulfurreducens* genome.

Size (bp)	3,814,139
G+C percentage	60.9
Number of predicted CDSs	3466
Average size of CDS (bp)	989
Percentage coding	90
Number of ribosomal RNA operons (16*S*-23*S*-5*S*)	2
Number of transfer RNAs	49
Number of structural RNAs	2
Number of CDSs similar to known protein	2011
Number of CDSs similar to proteins of unknown function	445
Number of conserved hypothetical proteins	384
Number of hypothetical proteins	633
Number of Rho-independent terminators	376

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pathway, CODH can be used chemolithoautotrophically in reactions distinct from this pathway (*12*).

G. sulfurreducens appears to have a versatile approach to capturing energy and carbon, having three enzyme systems, each of which is capable of converting pyruvate to acetyl-CoA. These include pyruvate-ferredoxin oxidoreductase and pyruvate-formate lyase, used by anaerobes, and a putative pyruvate dehydrogenase complex found largely in aerobic organisms (*13*).

Alternate electron transport pathways in *G. sulfurreducens* take electrons generated from central metabolism in the cytoplasm and transfer them by direct contact to extracellular electron acceptors, such as Fe(III) oxides (*2*). In contrast, other metal ion reducers, including *Shewanella* spp., also use soluble electron shuttling compounds in addition to direct contact (*14*). There were an unprecedented number of putative c-type cytochromes found in *G. sulfurreducens*, with 111 CDSs containing at least one match to the c-type cytochrome motif that identifies heme groups (*15*) (table S2). Seventy-three c-type cytochromes contain two or more heme groups, including one that possesses 27. The abundance of cytochromes highlights the importance of electron transport to this organism and suggests that flexibility and redundancy in the electron transfer networks it can create are important for the reduction of diverse metal ions in natural environments.

Of the c-type cytochromes in *G. sulfurreducens*, many are more similar to those from *S. oneidensis* than from the metal ion–reducing δ-Proteobacterium *D. vulgaris* (*16*). For instance, 23 are best matches to CDSs in *S. oneidensis*, as compared with 10 in *D. vulgaris*. Grouping c-type cytochromes into families revealed that some are shared across all three genomes (tables S3 and S4), although 43 candidates were unique to *G. sulfurreducens* (table S6).

Other well-recognized electron transport components, including dehydrogenases, quinones, iron-sulfur proteins, and b-type cytochromes, are present. Overall, however, the *G. sulfurreducens* genome has a markedly different set of electron transport components as compared with those of other metal ion reducers (*12*) (figs. S4 and S5); for instance, 51% of the electron transport proteins in *G. sulfurreducens* have no homolog in *S. oneidensis* (table S5).

G. sulfurreducens can couple the oxidation of hydrogen to the reduction of Fe(III). Besides using environmental hydrogen sources to fuel this reaction, molecular hydrogen can also be formed as a byproduct of the organism's own metabolism (for example, from nitrogen fixation) and subsequently cycled back into the electron transport network to yield energy. *G. sulfurreducens* has at least three NiFe-hydrogenases (large- and small-subunit) (*12*) that could be involved, as well as two multisubunit nicotinamide adenine dinucleotide (NAD^+)–reducing hydrogenases. The latter are similar to the NADH: quinone oxidoreductases (proton pumps that establish membrane ion gradients) and may also be involved in reverse electron transport or hydrogen cycling (*12*).

Many of the electron transport proteins were predicted to reside in the periplasm or outer membrane of *G. sulfurreducens*, making export through the plasma membrane important to energy metabolism. Supporting evidence included sequences encoding machinery for twin arginine transport and a type II secretian-dependent pathway for the translocation of proteins from the periplasmic space to the outer membrane. Several sequences representing components of the type II secretion system in *G. sulfurreducens* were divergent as compared to other type II secretion protein sequences, suggesting distinctions in its transport mechanisms.

Geobacter spp. have previously been characterized as strict anaerobes (*17*). However, there was considerable evidence in the genome of an oxidative capacity, which could be used to exploit or provide protection from oxic episodes. Homologs have been found for the high-oxygen-affinity cytochrome d–ubiquinol terminal oxidase and rubredoxin-oxygen oxidoreductase, as well as for the low-oxygen-affinity cytochrome c oxidase from the heme-copper oxidase superfamily (*18, 19*). The occurrence of homologs for catalase, superoxide dismutase, ruberythrin, and other peroxidases suggests an ability to scavenge oxygen radicals. *G. sulfurreducens* also possesses a CDS related to the oxygen-dependent form of the enzyme protoporphyrinogen oxidase, which catalyzes the penultimate step in the porphyrin biosynthetic pathway. This is unexpected, because most facultative or anaerobic bacteria capable of this reaction use a multienzyme complex linked to the respiratory chain. It is more usual for strict aerobes to use this single protein, which is dependent on oxygen as a terminal electron acceptor (*20*).

It is possible that *G. sulfurreducens* metabolizes complex carbon compounds through aerobic metabolism. A putative dioxygenase could break aromatic rings, and the presence of isoquinoline-oxidoreductase indicates complex heterocyclic ring catabolism (*21*). The presence of indolepyruvate oxidoreductases suggests that aryl pyruvates can be catabolized anaerobically. Hence, *G. sulfurreducens* seems to have a choice of carbon catabolism pathways it can use, permitting versatility under changing conditions.

The large number of regulatory genes found probably reflects the need to adapt to rapidly changing conditions (*6*). Four percent of the CDSs were two-component regulators (histidine kinases and response regulators); of these, 43% have PAS (PER-ARNT-SIM) domains or sensory modules that detect oxygen tension, redox state, or light or energy levels (*22*). Transcriptional regulators in the *G. sulfurreducens* genome included multiple members of DNA binding protein families that regulate metal-responsive genes, such as the Fur and ArsR families (*23, 24*).

Chemotactic behavior requires complex regulatory networks and a mechanism for motility. *G. sulfurreducens* possesses multiple copies of methyl-accepting chemotaxis proteins (MCPs), including at least two previously undescribed and a homolog of DifA, which is crucial to social gliding motility (*25*). Essential Che proteins are also found in multiple copies, including the sensor kinase CheA and response regulator CheY. Although *G. sulfurreducens* was previously thought to be nonmotile, our analysis has revealed CDSs for both flagella and pili production.

The *G. sulfurreducens* genome has not only provided remarkable new insights into its unique metabolic capabilities and strategies for environmental survival, but has also made us rethink *Geobacter* physiology. This species may be neither immobile nor a strict anaerobe. It also possesses extraordinary electron transport capability and sensory potential, highlighted by the unprecedented collection of newly reported c-type cytochromes, the range and depth of which will best be appreciated in the light of comparative studies. Genomic analysis continues to further our understanding of the role of *Geobacter* spp. in the environment, as well as the evolution of metal ion reduction and how these processes relate to bioremediation and energy generation.

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Materials and Methods
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Crystal Structure of the RC-LH1 Core Complex from *Rhodopseudomonas palustris*

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The crystal structure at 4.8 angstrom resolution of the reaction center–light harvesting 1 (RC–LH1) core complex from *Rhodopseudomonas palustris* shows the reaction center surrounded by an oval LH1 complex that consists of 15 pairs of transmembrane helical α- and β-apoproteins and their coordinated bacteriochlorophylls. Complete closure of the RC by the LH1 is prevented by a single transmembrane helix, out of register with the array of inner LH1 α-apoproteins. This break, located next to the binding site in the reaction center for the secondary electron acceptor ubiquinone (UQ_B), may provide a portal through which UQ_B can transfer electrons to cytochrome b/c_1.

Photosynthesis is one of the most important biological reactions on Earth. It provides all of the oxygen we breathe and, ultimately, all the food we eat. Purple photosynthetic bacteria have proved to be excellent model systems in which to study the light reactions of photosynthesis. Their light reactions usually begin with the absorption of a photon by the light harvesting (LH) or antenna system (*1*). This absorbed energy is then rapidly and efficiently transferred to the reaction center (RC), where it is used to initiate cyclic electron transport between the RC, cytochrome b/c_1, and cytochrome c (Fig. 1), producing a proton gradient that drives adenosine triphosphate (ATP) synthase and ultimately converts solar energy into useful chemical energy. These reactions take place in the photosynthetic membrane, where the RC is surrounded by two types of antenna complexes, called LH1 and LH2 (*2*). The LH1 antenna forms a stoichiometric complex with the RC, called the RC-LH1 core complex. Both types of LH complexes are constructed on the same modular principle. Bacteriochlorophyll *a* (Bchl*a*) and carotenoids are noncovalently bound to two types of low molecular weight (5 to 7 kD), hydrophobic apoproteins, called α and β, each of which has a single membrane-spanning α helix. The functioning LH complexes are oligomers of these αβ pairs, together with their associated pigments (*2*).

In the past few years, structural analysis of some of these pigment-protein complexes has substantially advanced the field. The structure of the purple bacterial RC was determined in 1985 (*3*) and is now known to a resolution of better than 2 Å (*4*). The structure of the LH2 complex from the purple bacterium *Rhodopseudomonas acidophila* was determined in 1995 (*5*), and it is now also at 2 Å resolution (*6*). LH2 has a nonameric ring structure. Nine α-apoproteins form an inner ring of α helices, while nine β-apoproteins form an outer ring of α helices. The Bchl*a* and carotenoid molecules are located between these two cylinders of α helices. The ATP synthase (*7*) and cytochrome b/c_1 (*8*) complex structures from bovine heart mitochondria have also been determined, to resolutions of 2.8 and 3.0 Å, respectively.

The structure of the LH1 complex has been investigated previously by electron cryomicroscopy on two-dimensional (2D) crystals. An 8.5 Å resolution projection map of a reconstituted LH1 complex from *Rhodospirillum rubrum* (*9*) revealed a large $(\alpha\beta)_{16}$ cyclic structure with features similar to LH2 and in which the "hole" in the middle was sufficient to accommodate an RC. Similar projection maps of RC-LH1 core complexes from several species of purple bacteria (*R. rubrum*, *Rhodobacter sphaeroides*, and *Rps. acidophila*) have confirmed that the RC is located within the LH1 ring (*10–13*). However, some studies have questioned whether, when the RC is present, the LH1 ring is actually complete (*14*).

Figure 1 shows a flow of electrons from the RC to the cytochrome b/c_1 complex through the fully reduced ubiquinone UQ_BH_2 (ubiquinol), which is generally thought to migrate from the RC into the membrane (*15*). Models in which the RC is completely surrounded by a double palisade of LH1 α helices raise the question of how UQ_BH_2 could escape from the core complex into the membrane (*16*). Two species of purple bacteria, *Rb. sphaeroides* and *Rb. capsulatus*, have a gene called *pufX* that appears to play a role in this process (*17*, *18*). When the PufX protein is present, reduced and oxidized ubiquinones can rapidly shuttle between the RC and the cytochrome b/c_1 complex (*19*, *20*). However, if this gene is deleted, the bacteria will grow photosynthetically only when LH1 is also deleted or modified so that full-size complexes are not formed (*21–23*). It has been suggested therefore that the PufX protein forms a gate in the LH1 ring through which the UQ can exchange (*16*).

We determined the crystal structure of the RC-LH1 core complex from the purple bacterium *Rhodopseudomonas palustris* at 4.8 Å resolution (*24*). Initial phases at 4.8 Å were calculated by the molecular replacement (MR) method with the RC from *Rb. spha-*

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