Geobacter: The Microbe Electric’s Physiology, Ecology, and Practical Applications

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ABSTRACT

Geobacter species specialize in making electrical contacts with extracellular electron acceptors and other organisms. This permits Geobacter species to fill important niches in a diversity of anaerobic environments. Geobacter species appear to be the primary agents for coupling the oxidation of organic compounds to the reduction of insoluble Fe(III) and Mn(IV) oxides in many soils and sediments, a process of global biogeochemical significance. Some Geobacter species can anaerobically oxidize aromatic hydrocarbons and play an important role in aromatic hydrocarbon removal from contaminated aquifers. The ability of Geobacter species to reductively precipitate uranium and related contaminants has led to the development of bioremediation strategies for contaminated environments. Geobacter species produce higher current densities than any other known organism in microbial fuel cells and are common colonizers of electrodes harvesting electricity from organic wastes and aquatic sediments. Direct interspecies electron exchange between Geobacter species and syntrophic partners.
appears to be an important process in anaerobic wastewater digesters. Functional and comparative genomic studies have begun to reveal important aspects of *Geobacter* physiology and regulation, but much remains unexplored. Quantifying key gene transcripts and proteins of subsurface *Geobacter* communities has proven to be a powerful approach to diagnose the *in situ* physiological status of *Geobacter* species during groundwater bioremediation. The growth and activity of *Geobacter* species in the subsurface and their biogeochemical impact under different environmental conditions can be predicted with a systems biology approach in which genome-scale metabolic models are coupled with appropriate physical/chemical models. The proficiency of *Geobacter* species in transferring electrons to insoluble minerals, electrodes, and possibly other microorganisms can be attributed to their unique “microbial nanowires,” pili that conduct electrons along their length with metallic-like conductivity. Surprisingly, the abundant *c*-type cytochromes of *Geobacter* species do not contribute to this long-range electron transport, but cytochromes are important for making the terminal electrical connections with Fe(III) oxides and electrodes and also function as capacitors, storing charge to permit continued respiration when extracellular electron acceptors are temporarily unavailable. The high conductivity of *Geobacter* pili and biofilms and the ability of biofilms to function as supercapacitors are novel properties that might contribute to the field of bioelectronics. The study of *Geobacter* species has revealed a remarkable number of microbial physiological properties that had not previously been described in any microorganism. Further investigation of these environmentally relevant and physiologically unique organisms is warranted.

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1. INTRODUCTION

*Geobacter* species represent a rare example of a genus of microorganisms that are abundant and play an important biogeochemical role in a diversity of natural environments, yet are easily cultured and can be genetically manipulated for physiological studies. Although there are other Fe(III)-reducing microorganisms that have been studied in more detail, it is clear that *Geobacter* species are generally the predominant Fe(III)-reducing microorganisms in many soils and sediments in which Fe(III) reduction is an important process. Physiological studies with *Geobacter* species have revealed a number of novel microbial properties that have an important impact on the geochemistry of some anaerobic soils and sediments and, in some instances, have practical applications.

As detailed in subsequent sections, the following microbial processes were first identified in studies with *Geobacter* species: (1) oxidation of organic compounds to carbon dioxide with Fe(III) or Mn(IV) as the electron acceptor, (2) conservation of energy from organic matter oxidation coupled to Fe(III) or Mn(IV) reduction, (3) production of extracellular...
magnetite from microbial Fe(III) reduction, (4) anaerobic oxidation of an aromatic hydrocarbon in pure culture, (5) microbial reduction of U(VI), (6) microbial reduction of Co(III), (7) utilization of humic substances as an electron acceptor for microbial respiration, (8) oxidation of organic compounds to carbon dioxide with an electrode serving as an electron acceptor, (9) conservation of energy from the oxidation of organic compounds coupled to electron transfer to an electrode, (10) the potential for an electrode to serve as an electron donor to support microbial respiration, (11) use of cytochromes as capacitors to permit respiration in the absence of exogenous electron acceptors, (12) extracellular electron transfer via microbial nanowires, (13) organic metallic-like long-range conduction of electrons along a protein filament, (14) production of conductive biofilms with conductivities comparable to that of synthetic polymers, and (15) the potential for interaction with syntrophic partners via a direct electron transfer (Fig. 1).

The reduction of Fe(III), and to a lesser extent Mn(IV), by Geobacter species can play an important role in carbon cycling in water-saturated soils and aquatic sediments and further influences the geochemistry of these environments through the release of dissolved Fe(II) and Mn(II) as

Figure 1  Time line of important discoveries associated with Geobacter species.
well as trace metals, metalloids, and phosphate that adsorb onto Fe(III) and Mn(IV) oxides. In fact, the studies that led to the discovery of the first Geobacter species were initially designed to better understand the flux of phosphate from aquatic sediments that contributes to algal blooms. Geobacter reduction of U(VI) and radionuclides can have an important influence on the migration of these compounds and is considered to be a potential tool for mitigating environmental contamination. Geobacter species play an important role in degrading a diversity of organic contaminants in groundwater, both under natural attenuation and engineered bioremediation strategies. The ability of Geobacter species to exchange electrons with electrodes has inspired several new strategies for bioenergy and bioremediation. A recent surprise is the realization that Geobacter species are important syntrophic microorganisms, forming partnerships with methanogenic microorganisms, under conditions where they can significantly contribute to the conversion of organic wastes, or hydrocarbon deposits, to methane. The production of Geobacter-based materials with novel electronic properties is a newly emerging field of study.

The number of publications on Geobacter species is relatively small but continues to grow (Fig. 2) as does awareness of the environmental relevance of these organisms and their potential practical applications. The purpose of this review is to provide a broad overview of what has been learned about Geobacter species since they were discovered 25 years ago. Due to time and space constraints, not every publication mentioning Geobacter species could be reviewed.

Figure 2  Publications and citations each year with Geobacter as a topic according to data from the Thomson Reuters ISI Web of Knowledge.
2. DISTRIBUTION AND ABUNDANCE OF **GEOBACTER** SPECIES

The hallmark physiological capability of *Geobacter* species is their ability to couple the oxidation of organic compounds to the reduction of Fe (III), which allows *Geobacter* species to fill key niches in the anaerobic microbial food chain of sedimentary environments such as aquatic sediments, wetlands, rice paddies, and subsurface environments in which Fe(III) reduction is an important terminal electron-accepting process (Lovley, 1987, 1991, 1993, 1995, 2000b). For example, molecular analysis of the metabolically active microorganisms in Fe(III)-reducing rice paddy soils revealed that *Geobacter* species accounted for 85% of the microorganisms consuming acetate, the key intermediate in anaerobic degradation of organic matter (Hori *et al*., 2010). Other factors such as the “remarkably low” maintenance energy requirement of *Geobacter* species may also be an important factor in their success in subsurface environments (Lin *et al*., 2009).

Molecular analyses, which avoid cultivation bias, have generally found that *Geobacter* species are the most abundant Fe(III)-reducing microorganisms in environments in which Fe(III) reduction is actively taking place (see, e.g., Anderson *et al*., 2003; Cummings *et al*., 2003; Holmes *et al*., 2007; Hori *et al*., 2010; Islam *et al*., 2004a; Kerkhof *et al*., 2011; Rooney-Varga *et al*., 1999; Röling *et al*., 2001; Snoeyenbos-West *et al*., 2000; Stein *et al*., 2001; Vrionis *et al*., 2005). Pure culture isolates have been recovered from a diversity of environments (Table 1). Further, molecular (Fig. 3) and/or enrichment studies have detected *Geobacter* in diverse environments such as aquifers contaminated with petroleum (Alfreider and Vogt, 2007; Anderson *et al*., 1998; Bottton *et al*., 2007; Coates *et al*., 1996; Holmes *et al*., 2007; Prakash *et al*., 2010; Rooney-Varga *et al*., 1999; Salminen *et al*., 2006; Snoeyenbos-West *et al*., 2000; Van Stempvoort *et al*., 2009; Winderl *et al*., 2007, 2008); groundwater contaminated with landfill leachate (Kuntze *et al*., 2011; Lin *et al*., 2005, 2007; Röling *et al*., 2001; Staats *et al*., 2011); environments contaminated with organic acids (Azizian *et al*., 2010; Stults *et al*., 2001); contaminated soils and aquatic sediments (Blothe *et al*., 2008; Cummings *et al*., 2000; Haller *et al*., 2011; Halm *et al*., 2009; Manickam *et al*., 2010); uranium-contaminated subsurface sediments amended with organics to promote metal reduction (Akob *et al*., 2008; Amos *et al*., 2007; Anderson *et al*., 2003; Baldwin *et al*., 2008; Brodie *et al*., 2006; Burkhardt *et al*., 2010, 2011; Callister *et al*., 2010; Cardenas *et al*., 2008, 2010; Chandler *et al*., 2010; Chang *et al*., 2005;
Table 1  *Geobacter* species available in pure culture listed in the order in which the species were described.

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<th>Genomic size and information&lt;sup&gt;a&lt;/sup&gt;</th>
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<th>Optimal growth temperature</th>
<th>References&lt;sup&gt;e&lt;/sup&gt;</th>
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<tr>
<td><em>Geobacter metallireducens</em></td>
<td>Aquatic sediments</td>
<td>4,011,182 bp GC%—59.5</td>
<td>Ac, Bz, Bze&lt;sup&gt;f&lt;/sup&gt;, BtOH, Buty, Bzo, BzOH, p-Cr, EtOH, p-HBz, p-HBzOH, IsoB, IsoV, Ph, Prop, PrOH, Pyr, Tol, Val</td>
<td>PCIO, Fe (III)-Cit</td>
<td>Mn(IV), Tc(VII)*, U(VI), AQDS, humics, Nitrate</td>
<td>30</td>
<td>Lovley et al. (1987, 1993), Lovley and Phillips (1988a,b)</td>
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<td><em>Geobacter sulfurreducens</em></td>
<td>Contaminated ditch</td>
<td>3,814,139 bp GC%—60.9</td>
<td>Ac, H&lt;sub&gt;2&lt;/sub&gt;</td>
<td>PCIO, Fe (III)-Cit, Fe(III)-P</td>
<td>Tc(VII)*, Co(III), U(VI), AQDS, S&lt;sup&gt;°&lt;/sup&gt;, Fum, Mal, O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>35</td>
<td>Caccavo et al. (1994), Lin et al. (2004)</td>
</tr>
<tr>
<td>“<em>Geobacter humireducens</em>” (strain JW3)</td>
<td>Contaminated wetland</td>
<td></td>
<td>Ac, EtOH, For, H&lt;sub&gt;2&lt;/sub&gt;, Lac</td>
<td>PCIO, Fe (III)-Cit</td>
<td>Mn(IV), AQDS S&lt;sup&gt;°&lt;/sup&gt;, nitrate, Fum, Mn(IV), AQDS</td>
<td>30</td>
<td>Coates et al. (1998)</td>
</tr>
<tr>
<td><em>Geobacter chapellei</em></td>
<td>Deep subsurface</td>
<td></td>
<td>Ac, EtOH, For, Lac</td>
<td>PCIO, Fe-NTA</td>
<td>Mn(IV), AQDS, Fum</td>
<td>25</td>
<td>Coates et al. (2001)</td>
</tr>
<tr>
<td><em>Geobacter grbiciae</em></td>
<td>Aquatic sediments</td>
<td></td>
<td>Ac, Buty, EtOH, For, Prop, Pyr</td>
<td>PCIO, Fe (III)-Cit</td>
<td>AQDS</td>
<td>30</td>
<td>Coates et al. (2001)</td>
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<tr>
<th>Name</th>
<th>Source</th>
<th>Genome size and information</th>
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<th>Fe forms reduced</th>
<th>Other electron acceptors</th>
<th>Optimal growth temperature</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Geobacter hydrogenophilus</em></td>
<td>Contaminated aquifer</td>
<td></td>
<td>Ac, Buty, Bzo, EtOH, For, H₂, Prop, Pyr, Succ</td>
<td>PCIO, Fe (III)-Cit</td>
<td>AQDS, Fum</td>
<td>30</td>
<td>Coates et al. (2001)</td>
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<td><em>Geobacter breensis</em></td>
<td>Freshwater ditch</td>
<td></td>
<td>Ac, BtOH, Buty, Bzo, EtOH, For, Fum, H₂, Lac, Mal, Prop, PrOH, Pyr, Succ</td>
<td>PCIO</td>
<td>Mn(IV), S₀, Fum, Mal</td>
<td>30</td>
<td>Straub and Buchholz-Cleven (2001)</td>
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<tr>
<td><em>Geobacter pelophilus</em></td>
<td>Freshwater ditch</td>
<td></td>
<td>Ac, EtOH, For, Fum, H₂, Mal, Prop, PrOH, Pyr, Succ</td>
<td>PCIO, Akaganeite</td>
<td>Mn(IV), S₀, Fum, Mal</td>
<td>30</td>
<td>Straub and Buchholz-Cleven (2001)</td>
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<td><em>Geobacter bemidjiensis</em></td>
<td>Fe(III)-reducing subsurface sediment</td>
<td>4,615,150 bp GC% — 60.3</td>
<td>Ac, Bzo, BtOH, Buty, EtOH, Fum, H₂, IsoB, Lac, Mal, Prop, Pyr, Succ, Val</td>
<td>Fe(III)-Cit, Fe(III)-NTA, Fe (III)-P, PCIO</td>
<td>AQDS, Fum, Mal, Mn(IV)</td>
<td>30</td>
<td>Nevin et al. (2005)</td>
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<td><em>Geobacter psychrophilus</em></td>
<td>Acetate-impacted aquifer sediment</td>
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<td>Ac, BtOH, EtOH, For, Lac, Mal, Pyr, Succ</td>
<td>Fe(III)-Cit, Fe(III)-NTA, Fe (III)-P, PCIO</td>
<td>AQDS, Electrode, Fum, Mal, Mn(IV)</td>
<td>17–30</td>
<td>Nevin et al. (2005)</td>
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<td><em>Geobacter lovleyi</em></td>
<td>Freshwater sediment</td>
<td></td>
<td>Ac, Bze, Bzo, Buty, Cit, EtOH, For, Glu, Lac, MeOH, Prop, Succ, Tol, YE, Ac, Buty, BtOH, EtOH, MeOH, Glyc, Lac, Pyr, Succ, Val</td>
<td>Fe(III)-Cit, PCIO</td>
<td>PCE, TCE, nitrate, Fum, Mal, S₀, U(VI), Mn(IV)</td>
<td>35</td>
<td>Sung et al. (2006)</td>
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<td><em>Geobacter pickeringii</em></td>
<td>Sedimentary kaolin strata</td>
<td></td>
<td>Ac, Buty, BtOH, EtOH, MeOH, Glyc, Lac, Pyr, Succ, Val</td>
<td>Fe(III)-Cit, Fe(III)-NTA, Fe (III)-P, PCIO</td>
<td>AQDS, Mal, Fum, Mn(IV), S₀, U(VI)*</td>
<td>30</td>
<td>Shelobolina et al. (2007a,b)</td>
</tr>
<tr>
<td><strong>Geobacter argillaceus</strong></td>
<td>Sedimentary kaolin strata</td>
<td>Ac, Buty, BtOH, EtOH Glyc, Lac, Pyr, Val</td>
<td>Fe(III)-Cit, Fe(III)-NTA, Fe (III)-P, PCIO</td>
<td>Nitrate, Mn(IV), S⁰, U(VI)*</td>
<td>30</td>
<td>Shelobolina et al. (2007b)</td>
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<td><strong>Geobacter thiogenes</strong></td>
<td>Subsurface soil</td>
<td>Ac, Act, H2</td>
<td>Fe(III)-NTA</td>
<td>Mal, Fum, nitrate, S⁰, TCA</td>
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<td>Nevin et al. (2007), De Wever et al. (2000), Shelobolina et al. (2008)</td>
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<td><strong>Geobacter uraniireducens</strong></td>
<td>Uranium-contaminated subsurface sediment</td>
<td>Ac, EtOH, Lac, Pyr</td>
<td>Fe(III)-NTA, Fe (III)-P, PCIO, smectite</td>
<td>AQDS, Fum, Mal, Mn(IV), U(VI)*</td>
<td>32</td>
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<td><strong>Geobacter toluenoxydans</strong></td>
<td>Tar-oil-contaminated sediment</td>
<td>Ac, Buty, Bz, Bzo, BzOH, For, m-Cr, Prop, Pyr, Ph, p-Cr, Tol</td>
<td>Fe(III)-Cit, PCIO</td>
<td>Fum</td>
<td>25–32</td>
<td>Kunapuli et al. (2010)</td>
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<td><strong>Geobacter daltonii</strong></td>
<td>Heavy metal- and hydrocarbon-contaminated shallow subsurface sediment</td>
<td>Ac, Buty, For Bzo⁷, Tol⁷</td>
<td>Fe(III)-Cit, PCIO</td>
<td>Fum, Mal, S⁰, U(VI)</td>
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<td>Prakash et al. (2010)</td>
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<td><strong>“Geobacter andersonii” strain M18</strong></td>
<td>Uranium-contaminated subsurface sediment</td>
<td>Ac, Act, Asp, EtOH, For, Fum, Glt, Lac, Mal, Pyr, Succ, YE</td>
<td>PCIO, subsurface sediment</td>
<td>AQDS</td>
<td>22–25</td>
<td>Holmes et al. (2011c)</td>
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<td><strong>“Geobacter remediiphilus” strain M21</strong></td>
<td>Uranium-contaminated subsurface sediment</td>
<td>Ac, Act, Ala, Bzo, EtOH, For, Glt, Lac, Pyr, Ser, Succ, Xyl, YE</td>
<td>PCIO, subsurface sediment</td>
<td>AQDS, Mn(IV)</td>
<td>22–25</td>
<td>Holmes et al. (2011c)</td>
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<td><strong>“Geobacter aquiferi” strain Ply1</strong></td>
<td>Acetate-impacted aquifer sediment</td>
<td>Ac, Act, Asp, Bzo Cit, Cys, EtOH, For, Fum, Lac, Mal, Pyr, Xyl, YE</td>
<td>PCIO, subsurface sediment</td>
<td>AQDS, Mn(IV)</td>
<td>22–25</td>
<td>Holmes et al. (2011c)</td>
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<th>Other electron acceptors&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Optimal growth temperature</th>
<th>References&lt;sup&gt;e&lt;/sup&gt;</th>
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<td>“Geobacter plymouthensis” strain Ply4</td>
<td>Acetate-impacted aquifer sediment</td>
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<td>PCIO, subsurface sediment</td>
<td>25–30</td>
<td>Holmes &lt;i&gt;et al.&lt;/i&gt; (2011c)</td>
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<sup>b</sup>Abbreviations for electron donors and acceptors: acetate (Ac), acetoin (Act), alanine (Ala), anthraquinone-2,6-disulfonic acid (AQDS), aspartic acid (Asp), benzaldehyde (Bz), benzene (Bze), benzote(Bzo), benzylalcohol (BzOH), butanol (BtOH), butyrate(Buty), citrate (Cit), p-cresol (p-Cr), m-cresol (m-Cr), cysteine (Cys), elemental sulfur (S<sub>e</sub>), ethanol (EtOH), formate (For), fumarate (Fum), glucose (Glu), glutamic acid (Glt), glycerol (Glyc), p-hydroxybenzoate (p-HB), p-hydroxybenzaldehyde (p-HBz), p-hydroxybenzylalcohol (p-HBzOH), hydrogen (H<sub>2</sub>), isobutyrate (IsoB), isovalerate (IsoV), lactate(Lac), malate (Mal), methanol (MeOH), manganese oxide (Mn(IV)), peptone (Pep), phenol (Ph), propanol (PrOH), propionate (Prop), pyruvate (Pyr), serine (Ser), succinate (Succ), tetrachloroethylene (PCE), trichloroethylene (TCE), toluene (Tol), trichloroacetic acid (TCA), valerate (Val), xylose (Xyl), yeast extract (YE).

<sup>c</sup>Fe(III) forms: Poorly crystalline iron oxide (PCIO), ferric citrate (Fe(III)-cit), ferric nitrilotriacetic acid (Fe(III)-NTA), ferric pyrophosphate (Fe(III)-P).

<sup>d</sup>Organism has the ability to reduce the metal but not determined whether energy to support growth is conserved from reduction of this metal.

<sup>e</sup>Reference in which the capacity to grow via Fe(III) reduction is described, followed by references with other physiological traits.

<sup>f</sup>Zhang <i>et al.</i> (2011).

<sup>g</sup>Electron donors utilized with fumarate as electron acceptor only.
Figure 3  Neighbor-joining tree showing the phylogenetic relationship within the genus Geobacter based on 16S rRNA gene sequences. The clone sequences having >98% 16S rRNA gene sequence identities were grouped into a single cluster. Cultured representatives (black), including isolates whose genomes are fully sequenced (red) are shown in the figure. Isolation source and the reference for both pure culture isolates (blue) and representatives environmental clone sequences (black) are also shown at the right side of the tree. The sequences assigned as unpublished in the NCBI and SILVA databases are presented with their accession number. All sequences (>1300 bases) were obtained from the SILVA SSU_106 Ref database (Pruesse et al., 2007) and manually aligned in ARB program (Ludwig et al., 2004) before the phylogenetic tree construction. The scale bar represents 10% sequence divergence.
Holmes et al., 2002; Hwang et al., 2009; Istok et al., 2004; Kerkhof et al., 2011; Michalsen et al., 2007; Mohanty et al., 2008; North et al., 2004; Peacock et al., 2004; Scala et al., 2006; Wan et al., 2005; Wilkins et al., 2007, 2009; Williams et al., 2011; Xu et al., 2010); subsurface environments with high arsenic concentrations (Héry et al., 2008; Islam et al., 2004a; Lear et al., 2007; Weldon and MacRae, 2006); environments contaminated with chlorinated compounds or dechlorinating enrichment cultures (Amos et al., 2007; Bedard et al., 2007; Imfeld et al., 2010; Kim et al., 2010; Macbeth et al., 2004; Sorensen et al., 2010; Sung et al., 2006; Yoshida et al., 2005); wetland and aquatic sediments (Brofft et al., 2002; Cifuentes et al., 2000; Coates et al., 1998; Costello and Schmidt, 2006; Costello et al., 2009; Martins et al., 2011; Musat et al., 2010; Roden et al., 2006, 2008; Stein et al., 2001; Straub et al., 1998); freshwater seeps (Blothe and Roden, 2009; Bruun et al., 2010); acidic springs, peat, or sediments (Adams et al., 2007; Kusel et al., 2008, 2010; Percent et al., 2008); pristine aquifers (Flynn et al., 2008; Holmes et al., 2007); rice paddy or other soils (Cahyani et al., 2008; Conrad et al., 2007; Friedrich et al., 2004; Hansel et al., 2008; Hiraishi et al., 2005; Ishii et al., 2010; Ishii et al., 2009; Noll et al., 2005; Scheid et al., 2004; Zhu et al., 2009); soil rhizosphere (Fernando et al., 2008); mangrove sediments (Zhang et al., 2008); a 1700-year-old wooden spear shaft (Helms et al., 2004); iron-rich snow (Kojima et al., 2009); clay wall material (Kitajima et al., 2008); dental unit water supply systems (Singh et al., 2003); methanogenic digesters (Cervantes et al., 2003, 2004; Morita et al., 2011; Riviere et al., 2009; Tsushima et al., 2010; Werner et al., 2011); and the deep subsurface (Coates et al., 1996, 2001; Kovacik et al., 2006; Shimizu et al., 2006). In many of these studies, it was concluded that Geobacter species had an important role in influencing the soil/sediment/groundwater biogeochemistry and/or promoting bioremediation.

Another environment in which Geobacter species or closely related Desulfiuromonas, Geopsychrobacter, and Pelobacter species are often abundant is on the surface of electrodes harvesting electricity from organic matter in wastewater or systems initiated with wastewater inocula (Aelterman et al., 2008; Borole et al., 2009; Butler et al., 2010a; Call et al., 2009; Chang et al., 2008; Choo et al., 2006; Cusick et al., 2010; Freguia et al., 2010; Ishii et al., 2008; Jung and Regan, 2007, 2011; Kiely et al., 2011a,b; Kim et al., 2007, 2008b; Lee et al., 2003, 2008, 2009; Li et al., 2010; Liu et al., 2008; Luo et al., 2010; Parameswaran et al., 2010; Shimoyama et al., 2009; Torres et al., 2009a; Xing et al., 2009), as well as sediments (Bond et al., 2002; De Schamphelaire et al., 2010; Holmes et al., 2004a,d; Kato et al., 2010; Liu et al., 2007; Reimers et al., 2006; Tender et al., 2002; White et al., 2009; Williams et al., 2010).
3. BRIEF DESCRIPTION OF GEOBACTER SPECIES

A significant number of pure culture isolates of Geobacter species are available (Table 1; Fig. 3). All Geobacter isolates are Gram-negative rods that are capable of oxidizing acetate with the reduction of Fe(III). Other commonly conserved features include the ability to reduce Mn(IV), U(VI), elemental sulfur, and humic substances or the humic substance analog anthraquinone-2,6-disulfonate (AQDS). Many isolates have the ability to use other small molecular weight organic acids, ethanol, or hydrogen as an electron donor (Table 1).

The two most heavily studied Geobacter species have been G. metallireducens and G. sulfurreducens. G. metallireducens was the first Geobacter species recovered in pure culture (Lovley and Phillips, 1988a; Lovley et al., 1987, 1993a). It was with this isolate that many of the novel physiological attributes listed in Section 1 were discovered. The recent development of a genetic system for G. metallireducens (Tremblay et al., 2011a) is likely to refocus attention on this organism to elucidate the physiology of important novel properties, such as anaerobic benzene degradation.

Geobacter sulfurreducens was the first Geobacter species for which methods for genetic manipulation were developed (Aklujkar and Lovley, 2010; Coppi et al., 2001; Kim et al., 2005; Lloyd et al., 2003; Park and Kim, 2011; Rollefson et al., 2009; Ueki and Lovley, 2010a), and therefore it has served as the Geobacter of choice for functional genomic studies designed to understand Geobacter metabolism, gene regulation, and extracellular electron transfer. It was the first Geobacter species found to use hydrogen as an electron donor, or to grow with elemental sulfur as an electron acceptor. The originally isolated strain was referred to as strain PCA (Caccavo et al., 1994). A commonly used strain of G. sulfurreducens derived from strain PCA is frequently referred to as strain DL-1 (Coppi et al., 2001) because this culture was maintained for many transfers in the laboratory and may have accumulated a significant number of mutations that were not present in the originally isolated PCA strain. For example, the DL-1 strain only poorly reduces Fe(III) oxide unless it is adapted for growth on Fe(III) oxide for long periods of time. The capacity for effective Fe (III) oxide reduction was recovered via adaptive evolution (Tremblay et al., 2011b).

Another valuable strain of G. sulfurreducens is strain KN400, which was recovered in a study designed to adaptively evolve G. sulfurreducens for growth on electrodes (Yi et al., 2009). Although the KN400 and DL-1 strains have an identical 16S rRNA gene sequence, they have some
important physiological differences. In addition to producing more current than DL-1 (Yi et al., 2009), KN400 also reduces Fe(III) oxides much faster (Flannagan et al., 2011). One reason for this may be greater expression of pili in KN400, which, as discussed below, is thought to be a major conduit for electron transfer to Fe(III) oxide. Further, strain KN400 is motile, whereas strain DL-1 is not. This can be attributed to interruption of the gene for the master regulator for flagella gene expression, FrgM, in DL-1 (Ueki et al., 2011). Motility is important in Fe(III) oxide reduction, as described below, and flagella could play a role in biofilm formation on electrodes.

Some Geobacter isolates have been isolated in studies focused on novel physiological properties such as the ability to use aromatic compounds (G. toluenoxydans; Kunapuli et al., 2010) or reduction of Fe(III) in clays (G. pickeringii, G. argillaceus; Shelobolina et al., 2007b). G. lovleyi (Sung et al., 2006) is the only Geobacter species that has been shown to reductively dechlorinate the chlorinated solvents tetrachloroethylene (PCE) and trichloroethylene (TCE) that are common groundwater contaminants and 16S rRNA gene sequences closely related to the pure culture have been recovered in dechlorinating enrichment cultures (Daprato et al., 2007; Dennis et al., 2003; Duhamel and Edwards, 2006, 2007; Duhamel et al., 2004) as well as subsurface environments contaminated with chlorinated solvents (Amos et al., 2007; Kim et al., 2010; Macbeth et al., 2004; Sorensen et al., 2010; Sung et al., 2006). G. (formerly Trichlorobacter) thiogenes is the only other known dechlorinating Geobacter, reducing trichloroacetic acid (De Wever et al., 2000; Nevin et al., 2007).

One of the goals of isolating pure cultures of Geobacter species is to obtain isolates that are representative of the Geobacter species that predominate in environments of interest. Therefore, the isolates Geobacter uraniireducens (Shelobolina et al., 2008), Geobacter andersonii (Holmes et al., 2011c), and Geobacter remediiphilus (Holmes et al., 2011c) are of special interest because their 16S rRNA gene sequences match 16S rRNA gene sequences that were found to predominate during active Fe(III) reduction in the uranium-contaminated aquifer in Rifle, CO when it is amended with acetate. These isolates may be particularly useful for elucidating the physiology of Geobacter species in such systems.

The isolates from the Rifle, CO site are in a phylogenetic clade, known as subsurface clade I, which as discussed in the next section, is a phylogenetically coherent group of Geobacter species that have been found to predominate in a diversity of aquifers in which Fe(III) reduction is important (Holmes et al., 2007). Other isolates that are also in the clade include G. bemidjiensis, G. humireducens, G. bremensis, G. daltonii, and
G. plymouthensis. Some of these isolates were recovered from surface sediments (Table 1).

Subsurface clade II of the Geobacter species includes some subsurface isolates such as G. chapellei, which was isolated from a deep subsurface aquifer in which Fe(III) reduction was important (Lovley et al., 1990). G. psychrophilus and Geobacter aquiferi were isolated from the same acetate-impacted aquifer from which the subsurface clade I isolate G. plymouthensis was derived (Table 1).

4. PHYLOGENY AND GENOMIC RESOURCES

Geobacter species are in the family Geobacteraceae, which is within the domain Bacteria, phylum Proteobacteria, class Deltaproteobacteria, and order Desulfuromonadales. The order Desulfuromonadales branches phylogenetically between the orders Syntrophobacterales and Desulfarculales. The Geobacteraceae family can be further divided into three distinct clusters: Geobacter, Desulfuromonas, and Desulfuromusa (Holmes et al., 2004b). The genera Malonomonas and Geopsychrobacter fall within the Desulfuromusa cluster, Geothermobacter and Geoalkalibacter fall within the Desulfuromonas cluster, and Pelobacter species are scattered throughout all three clusters (Fig. 4).

Comparative genomics suggest that the last common ancestor of the Geobacteraceae was an acetate-oxidizing, respiratory species capable of extracellular electron transfer, and that specialization for fermentative/syntrophic growth in Pelobacter species evolved at least twice (Butler et al., 2009). Pelobacter species have lost numerous genes, including most of the c-type cytochromes, important in extracellular electron transfer, while gaining unique genes for fermentative and syntrophic growth (Butler et al., 2009; Haveman et al., 2006). P. carbinolicus may have lost multiheme c-type cytochrome genes and other genes with multiple closely spaced histidine codons, including the 14-subunit NADH dehydrogenase complex, due to an autoimmune response of its CRISPR locus against the histidyl-tRNA synthetase gene (Aklujkar and Lovley, 2010). Initial studies suggested that Pelobacter species could reduce Fe(III) (Lovley et al., 1995); further investigation with P. carbinolicus revealed that Fe(III) reduction was indirect through a sulfur shuttle (Haveman et al., 2008). P. carbinolicus can grow as a syntroph (Schink, 1992) but does this via interspecies hydrogen transfer rather than the direct electron transfer that has been documented with Geobacter species (Summers and Lovley, 2011). Early on it was proposed that P. propionicus should be
Figure 4 Maximum-likelihood tree showing the phylogenetic relationship between the members of the family Geobacteraceae within the class Deltaproteobacteria using 16S rRNA gene (>1300 bp). The numbers at the branch points are tree puzzle support values. Only values greater than 50 are shown. For further methodological details, see the legend to Fig. 3.
placed in the genus Geobacter (Lonergan et al., 1996), but its significantly different evolutionary trajectory and physiology might warrant a separate genus designation.

Geobacter species can be grouped (Fig. 3) into three distinct clades: “subsurface clade 1,” “subsurface clade 2,” and the “G. metallireducens clade” (Holmes et al., 2007). Molecular studies have shown that most of the Geobacter species that predominate in Fe(III)-reducing subsurface environments fall into “subsurface clade 1” or “subsurface clade 2” (Holmes et al., 2007).

Nine Geobacter genomes and two Pelobacter genomes have been completely sequenced, including two strains of G. sulfurreducens (Table 1). Descriptions and comparisons of these genomes are available (Aklujkar et al., 2009, 2010; Butler et al., 2009, 2010b) as are in silico metabolic models based on these genomes (Mahadevan and Lovley, 2008; Mahadevan et al., 2006, 2011; Scheibe et al., 2009; Segura et al., 2008; Sun et al., 2009, 2010; Yang et al., 2010) and other computational analyses (Krushkal et al., 2007, 2011; Mahadevan et al., 2006; Qu et al., 2009; Tran et al., 2008; Yan et al., 2007). Also datasets of numerous genome-scale transcriptional and proteomic analyses that may be a useful resource are available (Ahrendt et al., 2007; Butler et al., 2007; Conlon et al., 2009; Ding et al., 2008; Franks et al., 2010b; Holmes et al., 2006, 2009; Khare et al., 2006; Kim et al., 2008a; Krushkal et al., 2007; Leang et al., 2009; Methe et al., 2005; Nevin et al., 2009; Nunez et al., 2006; Postier et al., 2008; Qiu et al., 2010; Strycharz et al., 2011a; Yan et al., 2006).

5. ELECTRON ACCEPTORS

Geobacter species can use a diversity of electron acceptors to support anaerobic growth (Table 1), and there is evidence that G. sulfurreducens can grow via oxygen reduction at low oxygen tensions (Lin et al., 2004). Soluble electron acceptors that can be reduced intracellularly include nitrate, fumarate, and chlorinated compounds (Table 1). Biochemical studies have identified protein fractions with nitrate- and nitrite-reductase activity (Murillo et al., 1999; Naik et al., 1993; Senko and Stolz, 2001) in G. metallireducens, and the fumarate reductase of G. sulfurreducens, which has the dual role of acting as succinate dehydrogenase, was identified with a gene deletion approach (Butler et al., 2006). Separate subsections discussing major extracellular electron acceptors follow.
5.1. Fe(III)

At the circumneutral pH in most environments in which *Geobacter* thrive, Fe (III) is highly insoluble. In subsurface sediments in which *Geobacter* species were active, poorly crystalline Fe(III) hydroxides and structural Fe(III) of phyllosilicates were the Fe(III) sources in the clay fraction that were reduced (Shelobolina *et al.*, 2004). Fe(III) forms that mimic these are the best insoluble Fe(III) sources for cultivating *Geobacter* species. Crystalline Fe(III) oxides, which microorganisms do not appear to significantly reduce in natural sediments (Phillips *et al.*, 1993), are poor electron acceptors for the cultivation of *Geobacter* species. Initial attempts to enrich for acetate-oxidizing, Fe(III)-reducing microorganisms with crystalline Fe(III) forms only yielded methane-producing enrichment cultures. It was the adoption of poorly crystalline Fe(III) oxide, synthesized by neutralizing Fe(III) chloride solutions, that led to successful enrichment and isolation of *G. metallireducens*. As outlined in the references in Table 1, subsequent *Geobacter* species have been enriched and isolated on a diversity of electron acceptors. However, Fe(III) that either comes from the environment of interest (Shelobolina *et al.*, 2007b), or closely resembles that Fe(III), may be most likely to lead to the isolation of the most environmentally relevant strains.

Reducing the particle size of Fe(III) oxides may accelerate rates of Fe(III) reduction (Bosch *et al.*, 2010), but whether *Geobacter* species can be conveniently cultivated on nanoscale Fe(III) oxides has not yet been determined. *G. metallireducens* could use the insoluble Fe(III) complex Prussian Blue (Fe₄[Fe(CN)₆]₃), an environmental contaminant, as an electron acceptor to support growth (Jahn *et al.*, 2006).

*Geobacter* species can be conveniently grown with soluble chelated, Fe(III) forms (Lovley, 2000b), but whole genome gene expression (Holmes *et al.*, 2011b) and proteomic (Ding *et al.*, 2008) studies have suggested that cells grown with soluble Fe(III) have significantly different physiologies than cells grown on Fe(III) oxide. Soluble Fe(III) appears to be reduced at the outer cell surface (Coppi *et al.*, 2007), but as discussed below, the number of proteins that *G. sulfurreducens* requires for reducing chelated Fe(III) is significantly less than for Fe(III) oxide reduction.

5.2. Electrodes

After Fe(III) reduction, electron transfer to electrodes is probably the most studied form of respiration in *Geobacter* species (Lovley, 2006a,b, 2008b, 2011a; Lovley and Nevin, 2011). All *Geobacter* species that have
been evaluated have the capacity for electron transfer to electrodes, which, as discussed in subsequent sections, may have several practical applications. *Geobacter* and closely related *Desulfuromonas* and *Geopsychrobacter* species were the first microorganisms found to conserve energy to support growth by coupling the oxidation of organic matter with electron transfer to electrodes and appear to be most effective microorganisms in carrying out this form of respiration (Bond et al., 2002; Holmes et al., 2004d; Ieropoulos et al., 2005; Ren et al., 2007). *Geobacter* or closely related species are frequently the most abundant microorganisms that colonize electrodes from mixed species inocula or natural communities, especially under strict anaerobic conditions (see many references in Section 2). Frequently, the *Geobacter* species enriched are most closely related to *G. sulfurreducens*, which is consistent with the finding that *G. sulfurreducens* strains produce the highest current densities of any known pure cultures and accomplish this with very high (>90%) coulombic efficiencies (Nevin et al., 2008; Yi et al., 2009). This is expected to give them a competitive advantage in colonizing electrodes.

A wide diversity of conductive materials are appropriate electrode surfaces for *Geobacter* species. Solid graphite is the most commonly employed (Bond and Lovley, 2003), but other graphite/carbon materials (Adachi et al., 2008; Srikanth et al., 2008; Ieropoulos et al., 2010; Selimbo et al., 2010), carbon cloth (Nevin et al., 2008), gold (Richter et al., 2008), steel (Dumas et al., 2008a), platinum (Marsili et al., 2008; Yi et al., 2009), and a diversity of conductive polymers (K.P. Nevin, unpublished data) are also effective.

### 5.3. Other Extracellular Electron Acceptors

Mn(IV) oxides are typically much less abundant than Fe(III) oxides in soils and sediments and are more susceptible to abiotic reduction (Lovley and Phillips, 1988b). There has been much less focus on Mn(IV) reduction than Fe(III) in *Geobacter* studies, but many of the aspects of mechanisms for Fe(III) oxide reduction described below are likely to apply to Mn(IV) reduction.

*Geobacter* species can reduce a wide diversity of metal ions. It is possible to grow *Geobacter* species on some of these electron acceptors when they are provided in high concentrations in laboratory cultures, but all are generally found in low abundance in natural environments and would not support significant amounts of growth. Therefore, it is unlikely that *Geobacter* species have evolved specific mechanisms to conserve energy to support growth with these metal ions as electron acceptors. It seems more likely
that the ability of *Geobacter* species to reduce metal ions results from the generalized ability to move electrons to the outer cell surface and onto redox-carriers that can nonspecifically transfer electrons to a wide variety of redox-active species, as described in subsequent sections. The environmental significance of the reduction of these metals is that, in general, reduction decreases solubility, and hence mobility. This can have important geochemical consequences in natural environments, such as aiding in ore deposit formation and, as described in subsequent sections, can be an effective bioremediation tool.

For example, the discovery that *Geobacter* species can reduce soluble U(VI) to the less soluble U(IV) provided a microbial model for U(VI) reduction in sedimentary environments where U(VI) reduction had previously been considered to be an abiotic phenomenon and suggested a strategy for removing uranium from contaminated waters (Lovley *et al.*, 1991). Some *Geobacter* species can grow with U(VI) as the sole electron acceptor (Lovley *et al.*, 1991; Sanford *et al.*, 2007), but even in contaminated environments, U(VI) availability is much less than that of Fe(III), which supports most of the *Geobacter* growth (Finneran *et al.*, 2002a).

*G. sulfurreducens* can use Co(III)-EDTA as an electron acceptor to support growth (Caccavo *et al.*, 1994). 60Co(III) chelated with EDTA is a contaminant of nuclear operations and is highly mobile, whereas the Co(II) produced from microbial reduction is much less mobile (Gorby *et al.*, 1998).

*G. metallireducens* conserved energy to support growth from the reduction of soluble V(V) to less soluble V(IV) with acetate as the electron donor (Ortiz-Bernad *et al.*, 2004b). In groundwater in which V(V) was a co-contaminant with U(VI), stimulating the growth of *Geobacter* effectively removed V(V). However, abiotic reduction of V(V) with Fe(II) produced from Fe(III) oxide reduction may have also contributed to V(V) removal (Ortiz-Bernad *et al.*, 2004a).

In a similar manner, *Geobacter* species may enzymatically reduce Tc(VII), but abiotic reduction by Fe(II), produced by *Geobacter* species or other organisms, is a more likely reaction in soils and sediments (Lloyd and Macaskie, 1996; Lloyd *et al.*, 2000). *G. metallireducens* appeared to reduce Cr(VI) (Lovley *et al.*, 1993a), but this is another species that Fe(II) can readily reduce abiotically. Other contaminant radionuclides that *Geobacter* species can reduce include Np(V) (Lloyd *et al.*, 2000) and Pu (IV) (Boukhalfa *et al.*, 2007; Rusin *et al.*, 1994).

*Geobacter* species can reduce Ag(I) precipitating Ag(0) (Law *et al.*, 2008; Lovley *et al.*, 1993a) and Hg(II) reduction has also been reported (Lovley *et al.*, 1993a; Wiatrowski *et al.*, 2006). Although a diversity of other
Fe(III)-reducing microorganisms reduced Au(III), the *Geobacter* species tested did not (Kashefi et al., 2001).

Some *Geobacter* species have the ability to reduce elemental sulfur (Table 1). Elemental sulfur is expected to be abundant in sediments near the interface of the zones of Fe(III) reduction and sulfate reduction because sulfide produced in the sulfate reduction zone and entering a zone containing Fe(III) will abiotically reduce the Fe(III) with the formation of elemental sulfur. *G. metallireducens* (Kaden et al., 2002) and possibly other *Geobacter* species may be sensitive to sulfide, therefore the reported inability of some *Geobacter* species to grow with elemental sulfur as the electron acceptor may be related to sulfide sensitivity or inappropriate culture conditions. As discussed below, the mechanisms for elemental sulfur reduction in *G. sulfurreducens* are expected to be rather nonspecific, and it is expected that all *Geobacter* species should be capable of transferring electrons to sulfur in a similar manner.

Humic substances are a heterogeneous class of complex organic compounds that can be the most abundant form of organic matter in some soils and sediments. The structure of humic substances is poorly understood, but it is known that they contain quinone moieties that are potential electron acceptors for *Geobacter* species and some other microorganisms (Coates et al., 1998; Jiang and Kappler, 2008; Klapper et al., 2002; Lovley and Blunt-Harris, 1999; Lovley et al., 1996a, 1998; Roden et al., 2010; Scott et al., 1998; Wolf et al., 2009). If Fe(III) is also available, the hydroquinone moieties produced when the quinones are reduced can abiotically reduce Fe(III) to Fe(II), regenerating the quinone state. In this manner, the humic substances or humic substance analogs, such as AQDS, function as electron shuttles between the *Geobacter* species and the Fe(III). Providing humic electron shuttles can accelerate the reduction of Fe(III) oxide and make it feasible for *Geobacter* species to reduce some Fe(III) forms, such as crystalline Fe(III) oxides, that are otherwise only poorly reduced (Lovley et al., 1996a, 1998).

5.4. Other Microorganisms—Syntrophy

In the absence of alternative electron acceptors, *Geobacter* species can transfer electrons to syntrophic partners. For example, early studies demonstrated that most *Geobacter* species could produce hydrogen from a variety of organic electron donors (Cord-Ruwisch et al., 1998), suggesting that these *Geobacter* species might form syntrophic associations with the well-established principle of interspecies hydrogen transfer (McInerney...
et al., 2009; Stams and Plugge, 2009) in which one microorganism disposes of electrons via the production of hydrogen gas and the partner organism consumes the hydrogen with electron transfer to an electron acceptor the other partner cannot utilize.

Acetate-oxidizing cocultures could be established with *G. sulfurreducens* and either *Wolinella succinogenes* or *Desulfovibrio vulgaris* the first time that rapid anaerobic syntrophic oxidation of acetate at moderate temperatures was demonstrated (Cord-Ruwisch et al., 1998). However, growth yields of *G. sulfurreducens* in the coculture with *W. succinogenes* were higher than expected if *G. sulfurreducens* was disposing of electrons via hydrogen production. This initially led to the suggestion that a soluble c-type cytochrome released in the medium served as an electron shuttle between the two species (Cord-Ruwisch et al., 1998), followed by the suggestion that cysteine added to the medium was the electron shuttle (Kaden et al., 2002). A third alternative, currently under investigation, is direct interspecies electron transfer as described below.

Cell suspensions of *G. metallireducens* and *W. succinogenes* oxidized toluene with the reduction of fumarate (Meckenstock, 1999). The electron carrier between the two organisms was not investigated, but the poor capacity for *G. metallireducens* to produce hydrogen would suggest that an alternative to interspecies hydrogen transfer might have been necessary.

The ability of *Geobacter* species to form syntrophic interactions was further investigated in cocultures of *G. metallireducens* and *G. sulfurreducens* (Summers et al., 2010). Multiple lines of evidence suggested that as the coculture adapted for rapid syntrophic growth, electrons were directly exchanged between the two species via electrically conductive connections that will be described later. As detailed later, there is increasing evidence that direct interspecies electron transfer may be an important feature of *Geobacter* in anaerobic environments (Lovley, 2011a,c, Morita et al., 2011).

Another possibility in natural environments is that environmental components may aid in interspecies electron transfer. For example, in the reduced state, humic substances and other organics that have quinone/hydroquinone moieties can serve as electron donors to support anaerobic respiration (Lovley et al., 1999). *G. metallireducens* was able to transfer electrons derived from acetate oxidation much more rapidly to *W. succinogenes* in the presence of the humics analog AQDS, which served as an electron acceptor for acetate oxidation by *G. metallireducens*, and the reduced AQDS served as an electron donor for *W. succinogenes* (Lovley et al., 1999).
6. ELECTRON DONORS

6.1. Acetate, Other Fatty Acids, Hydrogen, Electrodes, Humics, Fe(II), U(IV)

The universal ability of all *Geobacter* species to oxidize acetate with Fe (III) serving as the sole electron-acceptor points to their key ecological/biogeochemical role in soils and sediments. Acetate is the key extracellular intermediate in the anaerobic degradation of organic matter (Lovley and Chapelle, 1995). Although there are some Fe(III)-reducing microorganisms that can completely oxidize fermentable organic compounds, such as sugars and amino acids (Lovley *et al.*, 2004), they do not appear to be competitive with fermentative microorganisms. For example, in sediments in which Fe(III) reduction was the predominant terminal electron-accepting process, glucose was metabolized to acetate and other minor fermentation acids (Lovley and Phillips, 1989). Therefore, mineralization of organic matter can only take place if there are Fe(III) reducers capable of coupling the oxidation of acetate to the reduction of Fe(III). Further, acetate is a common additive to stimulate the activity of *Geobacter* species for in situ bioremediation of uranium-contaminated groundwater. Therefore, understanding acetate metabolism is key for understanding the ecology of *Geobacter* species.

Acetate is oxidized via the TCA cycle in *Geobacter* species (Champine and Goodwin, 1991; Champine *et al.*, 2000; Mikoulinskaia *et al.*, 1999). As recently reviewed in detail (Mahadevan *et al.*, 2011), studies on acetate transporters (Risso *et al.*, 2008a) as well as iterative genome-scale metabolic modeling and experimental studies (Mahadevan *et al.*, 2006; Risso *et al.*, 2008b; Segura *et al.*, 2008; Tang *et al.*, 2007) have identified additional features of the pathways for acetate metabolism that will not be repeated here.

Even though the *Geobacter* species whose genomes have been sequenced were isolated from geographically diverse environments, there is high conservation of acetate metabolism genes including the genes encoding acetate transporters and the eight enzymes of acetate oxidation via the TCA cycle (citrate synthase, aconitase, isocitrate dehydrogenase, 2-oxoglutarate:ferredoxin oxidoreductase, succinyl:acetate CoA-transferase, succinate dehydrogenase/fumarate reductase, fumarase, and malate dehydrogenase) (Butler *et al.*, 2010b). All the genomes encode a 14-subunit NADH dehydrogenase complex, except *G. lovleyi* encodes a 12-subunit NADH dehydrogenase complex. All except *G. metallireducens* encode the
NAD-dependent ferredoxin:NADP oxidoreductase (Nfn) complex, and all encode a putative NADPH:menaquinone oxidoreductase (Sfr) complex (Coppi et al., 2007). The genes that encode ATP synthase are divided into two conserved operons in every Geobacter genome (Butler et al., 2010b).

In addition to acetate, other short-chain fatty acids, alcohols, and hydrogen serve as electron donors for some Geobacter species (Table 1). In G. sulfurreducens, the enzyme required for hydrogen oxidation has been identified as a four-subunit NiFe hydrogenase (Coppi et al., 2004). However, this enzyme is only conserved in G. sulfurreducens, G. uranireducens, G. bemidjiensis, “G. andersonii,” “G. remediiphilus,” and G. lovleyi (Butler et al., 2010b). In addition to the respiratory hydrogenase, there are multiple other cytoplasmic and membrane-bound hydrogenases found in Geobacter species, all quite distinct from the well-studied hydrogenases in related Desulfovibrio species (Coppi, 2005). There is a hydrogenase specific to Geobacter that predominate in the subsurface (Butler et al., 2010b).

Genome analysis suggests that G. metallireducens metabolizes propionate via the 2-methylcitrate pathway (Aklujkar et al., 2009; Bond et al., 2005), whereas G. bemidjiensis is predicted to metabolize propionate via the methylmalonyl-CoA pathway (Aklujkar et al., 2010). Activation of butyrate and valerate with a kinase and a transferase to form butyryl-CoA and valeryl-CoA, respectively, has been predicted from genome sequences (Aklujkar et al., 2009, 2010), but the expression of butyrate kinase did not increase in G. metallireducens fed butyrate (Peters et al., 2007a). G. bemidjiensis also possesses a long-chain fatty acyl-CoA dehydrogenase, fadE, which is absent from the genome of G. metallireducens, suggesting that G. bemidjiensis has the potential for long-chain fatty acid metabolism. Long-chain fatty acid degradation was documented in the closely related Desulfuromonas palmitatis (Coates et al., 1995).

G. metallireducens and G. sulfurreducens could reduce nitrate and fumarate, respectively, with the reduced humic substance analog AQDS (Lovley et al., 1998). G. metallireducens can oxidize Fe(II) and U(IV) with nitrate as an electron acceptor (Finneran et al., 2002b; Weber et al., 2006). However, the capacity to conserve energy to support growth with these electron donors has not been demonstrated.

Electrodes poised at a low potential can serve as an electron donor for Geobacter species. Electrode-dependent reduction of nitrate (Gregory and Lovley, 2005), fumarate (Gregory et al., 2004), U(VI) (Gregory and Lovley, 2005), and chlorinated compounds (Strycharz et al., 2008) has been documented, as has the reduction of protons to produce hydrogen gas (Geelhoed and Stams, 2011). Initial studies have suggested that the route
for electron transfer from electrodes to Geobacter species may be different from electron flow in the opposite direction for current production (Dumas et al., 2008b; Strycharz et al., 2011a).

6.2. Aromatic Compounds

As detailed later, Geobacter species are often abundant within zones of petroleum-contaminated aquifers in which aromatic hydrocarbons are being removed, and it has been suggested that they can play an important role in converting hydrocarbons in systems designed for recovery of hydrocarbon deposits as methane. G. metallireducens was the first Geobacter species found to degrade aromatic compounds and the first microorganism of any kind in pure culture found to degrade an aromatic hydrocarbon (Lovley and Lonergan, 1990; Lovley et al., 1989). Of particular interest is the ability of G. metallireducens to anaerobically degrade benzene (Zhang et al., 2011). Other than the hyperthermophile Ferroglobus placidus (Holmes et al., 2011d), G. metallireducens is the only organism in pure culture that has been reported to be capable of degrading benzene through defined anaerobic pathways.

A number of other Geobacter species, isolated from a diversity of contaminated environments, are capable of degrading aromatic compounds (Table 1). Geopsychrobacter electrodiphilus, which is closely related to Geobacter species, also metabolizes aromatic compounds (Holmes et al., 2004d). All Geobacter species that are capable of degrading aromatic compounds degrade benzoate. Only four (G. metallireducens, G. grbiciae, G. toluenoxydans, and G. daltonii) are capable of metabolizing toluene.

The mechanisms for the degradation of aromatic compounds in Geobacter species have primarily been investigated in G. metallireducens. Benzoyl-CoA is a central intermediate in the metabolism of all monoaromatic substrates (Fig. 5). Benzoyl-CoA reductase catalyzes the dearomatization of benzoyl-CoA to cyclohexan-1,5-diene-1-carboxyl-CoA via a Birch-type reduction (Boll, 2005b; Boll and Fuchs, 1995; Boll et al., 2000; Kung et al., 2009; Peters et al., 2007b). G. metallireducens has a class II benzoyl-CoA reductase, which until recently (Holmes et al., 2011d) was thought to be found in all anaerobes degrading benzoate (Löffler et al., 2011). Whereas class I benzoyl-CoA reductases couple the hydrolysis of two ATP with the transfer of two electrons from reduced ferredoxin to benzoyl-CoA (Boll, 2005a), the class II enzymes, which were initially discovered in G. metallireducens (Kung et al., 2009), do not have an ATP requirement (Löffler et al., 2011). In order to overcome the high redox
barrier associated with ring reduction, class II benzoyl-CoA reductases may be driven by either membrane potential or electron bifurcation (Kung et al., 2010).

A cluster of eight benzoate-induced genes (Bam BCDEFGHI) is thought to code for the class II benzoyl-CoA reductase (Kung et al., 2009). BamBC subunits are sufficient to catalyze the reductive dearomatization of benzoyl-CoA in vitro (Kung et al., 2010). BamB contains the tungstopterin active site and an Fe–S cluster, whereas BamC contains three more Fe–S clusters (Kung et al., 2009). BamDEFGHI are thought to participate in an uncharacterized electron activation process. Proteome analysis revealed that BamFG was found exclusively in the membrane fraction (Heintz et al., 2009). Therefore, it was proposed that the benzoyl-CoA reductase complex is membrane associated. Activity of class II benzoyl-CoA reductase was also detected in extracts of G. bemidjiensis and several other obligate anaerobes (Löffler et al., 2011).

Figure 5 Aromatic hydrocarbon oxidation pathways in *Geobacter* species. Enzymes of the aromatics degradation pathways indicated: benzylsuccinate synthase (BssABCD), benzylsuccinate CoA-transferase (BbsEF), benzylsuccinyl-CoA dehydrogenase (BbsG), phenylitaconyl-CoA hydratase (BbsH), 2-[hydroxy(phenyl) methyl]-succinyl-CoA dehydrogenase (BbsCD), benzoylsuccinyl-CoA thiolase (BbsAB), alcohol dehydrogenase (adh), 4-hydroxybenzaldehyde dehydrogenase (PcmO), benzoate-CoA ligase (BamY), phenylphosphate synthase (PpsABC), phenylphosphate carboxylase (PpcBD—these are enzymes distinct from the PpcB and PpcD cytochromes), p-cresol methylhydroxylase (PcmIJ), 4-hydroxybenzoyl-CoA reductase (PcmRST), benzoyl-CoA reductase (BAmBCDEFGI), cyclohexadienyl-CoA hydratase (BamR), hydroxyenoyl-CoA dehydrogenase (BamQ), and oxoenoyl-CoA hydrolase (BamA).
Additional reactions (Fig. 5) complete the conversion of benzoyl-CoA to 3-hydroxypimelyl-CoA (Wischgoll et al., 2005), which is then oxidized to acetyl-CoA and CO₂ via beta-oxidation. This process includes the oxidative decarboxylation of glutaryl-CoA to crotonyl-CoA by the decarboxylating glutaryl-CoA dehydrogenase of *G. metallireducens* (Wischgoll et al., 2009).

The toluene degradation pathway of *G. metallireducens* (Fig. 5) appears to be similar to the one found in the denitrifier *Aromatoleum aromaticum* (Butler et al., 2007; Rabus, 2005; Rabus et al., 2005). The benzylsuccinate synthase adds toluene as a radical to fumarate to form benzylsuccinate. Benzylsuccinate is then activated to benzylsuccinyl-CoA, which is metabolized to benzoyl-CoA (Rabus, 2005; Rabus et al., 2005). Proteomic and functional genomics studies have suggested that the *p*-cresol degradation pathway in *G. metallireducens* is similar to the one found in *Pseudomonas putida* (Butler et al., 2007; Cronin et al., 1999; Peters et al., 2007a). PcmO, a 4-hydroxybenzaldehyde dehydrogenase, could be responsible for the conversion of 4-hydroxybenzaldehyde to 4-hydroxybenzoate in the *p*-cresol pathway and for the conversion of benzaldehyde to benzoate in the benzyl alcohol pathway (Butler et al., 2007). *G. metallireducens* expressed more PcmO when grown on *p*-cresol compared to acetate or benzoate (Peters et al., 2007a). No experimental evidence suggests that PcmO is involved in benzyl alcohol oxidation.

Phenol degradation in *Geobacteraceae* is believed to be similar to that in the facultative anaerobic denitrifier *Thaurea aromatica*, which was initially used to describe the phenol pathway (Butler et al., 2007). Genes coding for phenylphosphate synthase and carboxylase were upregulated when *G. metallireducens* was grown on phenol compared to acetate and benzoate (Schleinitz et al., 2009). Out of four phenylphosphate carboxylase subunits of *T. aromatica*, only homolog genes coding for two subunits were found in the *G. metallireducens* and *Geobacter daltonii* genomes. Despite the absence of the other genes, phenylphosphate carboxylase activity was still detected (Schleinitz et al., 2009). Further studies are necessary to understand the biochemistry of phenylphosphate carboxylation in *Geobacteraceae*. It was suggested that the benzoate-CoA ligase might also catalyze the conversion of 4-hydroxybenzoate to 4-hydroxybenzoyl-CoA (Butler et al., 2007). However, biochemical studies demonstrated that the 4-hydroxybenzoate-CoA ligase activity in *G. metallireducens* is found in another enzyme (Peters et al., 2007a; Wischgoll et al., 2005).

Most of the aromatic degradation genes are found on a 300-kb genomic island in *G. metallireducens* (Butler et al., 2007). This region contains 244 genes, which code for, among other things, the benzoyl-CoA reductase, the benzoate-CoA ligase, phenol and *p*-cresol degradation pathways,
cyclohexadienoyl-CoA hydratase, the hydroxyenoyl-CoA dehydrogenase, the oxoenoyl-CoA hydrolase, and fatty acid oxidation enzymes (Butler et al., 2007). Identical transposons and repetitive sequences found in this region indicate that possible horizontal transfer events might be responsible for the acquisition of those genes (Butler et al., 2007). Most of those 244 genes have no ortholog in Geobacteraceae species that are unable to degrade aromatic compounds (Butler et al., 2007). Genes coding for the toluene degradation pathway are found on two operons located in another genomic island of 167 kb (Butler et al., 2007).

Little is known about the regulatory network associated with aromatic degradation in Geobacteraceae. Recently a G. metallireducens two-component system formed by the histidine kinase BamV and the response regulator BamW was found to regulate the transcription of bamY, the gene coding for the benzoate-CoA ligase (Juárez et al., 2010). BamVW caused an increase in the transcription of bamY after exposure to benzoate, 4-hydroxybenzoate, or p-cresol. A transcriptional regulator, which in the presence of acetate represses the expression of bamA, and possibly other genes whose expression is induced during growth on benzoate, has been identified in G. bemidjiensis (Ueki, 2011). Genes for phenol or p-cresol degradation are also transcribed when benzoate is the sole electron source (Peters et al., 2007a; Schleinitz et al., 2009). However, accumulation of the corresponding proteins was observed only during growth on phenol or p-cresol, but not with benzoate (Peters et al., 2007a; Schleinitz et al., 2009). Therefore, the existence of posttranscriptional regulation mechanism(s) was proposed to explain these observations.

Many aspects of aromatic degradation pathways are still unknown and need to be characterized in order to further comprehend the role of Geobacter species in contaminated environments. These studies should be accelerated by the recent development of methods for genetic manipulation of G. metallireducens.

7. EXTRACELLULAR ELECTRON TRANSFER

Effective extracellular electron transfer is one of the hallmark physiological features of Geobacter species. The capacity to exchange electrons with its extracellular environment defines the unique ecological niche of Geobacter species and is an important feature of the many practical applications of this genus. Extracellular electron transfer in Geobacter...
species is accomplished through unique mechanisms that have yet to be described in any other organism.

7.1. Microbial Nanowires

One of the most surprising discoveries in the study of extracellular electron transfer in *Geobacter* species has been the finding that *G. sulfurreducens*, and presumably other *Geobacter* species, produces pili that are electrically conductive (Malvankar *et al.*, 2011b; Reguera *et al.*, 2005). Initial indications that pili were important in extracellular electron transfer came from the observation that *G. metallireducens* expressed pili when grown on Fe(III) or Mn(IV) oxides, but not when grown with soluble, chelated Fe(III) as the electron acceptor (Childers *et al.*, 2002). Studies on pili in *G. sulfurreducens* have demonstrated that this organism can produce pilin-like filaments from several different proteins, but the most abundant filaments are those comprising PilA (Klimes *et al.*, 2010).

Deletion of the gene for PilA, the structural pilin protein, inhibited Fe(III) oxide reduction (Reguera *et al.*, 2005). Conducting atomic force microscopy demonstrated that the pili were conductive across their diameter (Reguera *et al.*, 2005). The atomic force microscopy revealed that there were other proteins associated with the pili, but they acted as insulators. Therefore, it was proposed that a method for electron transfer to Fe(III) oxide was long-range electron transport along the pilin filaments. Further, although electron hopping between cytochromes is the accepted method for biological electron transfer over distance, it was suggested that cytochromes did not mediate the electron transport along the pili (Reguera *et al.*, 2005). This concept was seriously questioned (Shi *et al.*, 2007) because there was no known mechanism for electron transfer along protein filaments.

However, subsequent studies have provided a mechanism. The pili of *G. sulfurreducens* possess metallic-like conductivity comparable to synthetic conducting polymers, such as the organic metal polyaniline (Malvankar *et al.*, 2011b). When pilin preparations were spotted on a two-electrode system, they formed a network that conducted electrons between the two electrodes. Preparations from a *pilA* deletion mutant had conductivities comparable to the buffer control. Treating the pilin preparation to denature any cytochromes that might have remained associated with the pili had no impact on conductivity. Upon cooling from room temperature, the pilin conductivity increased exponentially, a hallmark of quasi-one-dimensional organic metals. The temperature response would not have been observed if electron hopping between cytochromes
was responsible for the electron transfer. Studies on the impact of pH changes on conductivity and X-ray diffraction analysis of purified pilin preparations suggested that π–π interchain stacking between aromatic moieties of pilin amino acids may confer the metallic-like conductivity. This hypothesis is currently under investigation.

The possibility of electron transport along a protein filament without the involvement of cytochromes is a paradigm shift in biology. The metallic-like mechanism for electron transport along the pili of *G. sulfurreducens* under *in vivo* conditions is fundamentally different than the conductivity proposed for filaments of other microorganism such as *Shewanella oneidensis*, which was only demonstrated in fixed preparations and was reported to be dependent on the presence of cytochromes (Gorby et al., 2006).

### 7.2. Cytochromes and Multicopper Proteins

One of the most striking features of *Geobacter* species is their abundant *c*-type cytochromes and the large diversity of cytochromes encoded in *Geobacter* genomes (Butler et al., 2010b; Ding et al., 2006; Méthé et al., 2003). With the exception of *G. lovleyi*, *Geobacter* species possess ca. 100 *c*-type cytochrome genes per genome (Butler et al., 2010b). There are nine families of well-conserved *c*-type cytochromes, four of which are encoded together and may constitute a quinone:ferricytochrome *c* oxidoreductase. However, most of the cytochromes are poorly conserved among the *Geobacter* species and some cytochrome families have only been found in a single species of *Geobacter* (Butler et al., 2010b). This, coupled with the fact that the function of *c*-type cytochromes has only been significantly studied in *G. sulfurreducens*, makes it difficult to make broad generalizations about cytochrome function in *Geobacter* species.

One family of *c*-type cytochromes that is well conserved is the PpcA family of triheme periplasmic cytochromes. These are among the most abundant *c*-type cytochromes in *Geobacter* species and were first studied biochemically in the closely related *Desulfuromonas acetoxidans* (Banci et al., 1996; Bruschi et al., 1997; Czjzek et al., 2001) and *G. metallireducens* (Afkar and Fukumori, 1999; Champine et al., 2000) and then with more detailed functional studies in *G. sulfurreducens*.

PpcA purified from *G. sulfurreducens* contained the expected three hemes with a molecular weight of 9.6 kDa and a midpoint potential of −169.5 mV (Lloyd et al., 2003). Although PpcA is related to the earlier studied cytochrome in *D. acetoxidans*, its redox properties are distinct (Pessanha et al., 2006). Purified PpcA reduced Fe(III) and other metals,
but its periplasmic location makes direct reduction of Fe(III) unlikely (Lloyd et al., 2003). The heme groups of PpcA are oriented in parallel or perpendicular to each other (Morgado et al., 2010b), an arrangement expected to facilitate rapid electron transfer within and between proteins (Mowat and Chapman, 2005). Deletion of ppcA did not impact fumarate reduction but did impact reduction of the extracellular electron-acceptors Fe(III), AQDS, and U(VI) with acetate as the electron donor. However, with hydrogen as the electron donor, reduction of extracellular electron acceptors in the mutant and wild type were comparable.

There are four homologs of PpcA in G. sulfurreducens, designated PpcB-PpcE. The function of these homologs appears to be different. Surprisingly, deletions of ppcB, ppcC, or ppcE increased rates of Fe(III) reduction (Shelobolina et al., 2007a). Whereas PpcA appears to be expressed constitutively, PpcD expression is enhanced during growth on Fe(III) oxide (Ding et al., 2008). Only PpcB is downregulated in cultures grown on soluble iron (Ding et al., 2008). Structural and thermodynamic characterizations such as the organization, redox potential, and oxidation of hemes further suggest different functions of the homologues (Morgado et al., 2008, 2010a; Pokkuluri et al., 2010). Only PpcA and PpcD can couple e\(^{-}\)/H\(^{+}\) translocation across the inner membrane (Morgado et al., 2010a), and only PpcC displays polymerization and redox-dependent conformational changes (Morgado et al., 2007). Much more research into the function of these periplasmic cytochromes and their interaction with inner and outer membrane components is required.

Early studies on G. sulfurreducens found significant Fe(III) reductase activity in membrane fractions, which involved cytochromes (Gaspard et al., 1998; Magnuson et al., 2000). One of these cytochromes was purified (Magnuson et al., 2001) and was most likely the subsequently described OmcB (Leang et al., 2003). This cytochrome has a molecular weight of 89 kDa, 12 hemes, and gross midpoint potential of \(-190\) mV with some hemes appearing to have much more negative potentials (Magnuson et al., 2001). The purified protein was capable of reducing Fe(III) oxide and chelated Fe(III). OmcB is embedded in the outer membrane, with a portion of the molecule exposed to the outer surface (Qian et al., 2007). Deleting the gene for OmcB inhibited reduction of Fe(III) citrate and Fe(III) oxide (Leang et al., 2003). Deletion mutants adapted to growth on Fe(III) citrate, but not Fe(III) oxide (Leang et al., 2005). The presence of multiple RpoS-dependent promoters upstream of upregulated cytochromes in the Fe(III) citrate-adapted mutant suggests that an activated RpoS response permitted G. sulfurreducens to compensate for the loss of OmcB (Krushkal et al., 2009).
OmcB is encoded downstream from another cytochrome, designated Orf2, in an operon with a third protein of unknown function (Leang and Lovley, 2005). This operon has been duplicated, and the other copy is immediately downstream. In all the other Geobacter species genomes, there is at least one operon with similarity to this one, and in several, there are tandem repeats of the operon as well (Butler et al., 2010b). While the Orf2 cytochrome is well conserved across all species, the sequence of the gene in the omcB position in the operon varies substantially. In all cases, this gene encodes a multiheme cytochrome, but some of the sequences are very divergent and cannot be called omcB homologs. Thus, while the operon is conserved and even duplicated, and the sequence of the Orf2 cytochromes is well conserved, there may be less pressure for the large outer membrane cytochrome to maintain a specific sequence.

Whereas OmcB is embedded in the outer membrane, several of the G. sulfurreducens c-type cytochromes are fully exposed on the outer cell surface. OmcS is a six-heme c-type cytochrome with a molecular weight of 47 kDa (Qian et al., 2011). Its midpoint redox potential is −212 mV, more negative than that of the periplasmic c-type cytochromes. However, the available evidence suggests that individual hemes span a wide range of potentials. The gene for OmcS is the most upregulated gene during growth on Fe(III) oxide versus growth on Fe(III) citrate (Holmes et al., 2011b) and this is reflected in the proteome (Ding et al., 2008) and in initial studies that detected omcS transcripts in cells grown on Fe(III) oxide, but not Fe(III) citrate (Mehta et al., 2005). It is also highly expressed under some conditions during growth on electrodes (Holmes et al., 2006) and in cocultures of G. sulfurreducens and G. metallireducens (Summers et al., 2010). Purified OmcS reduced a diversity of potential extracellular electron acceptors for G. sulfurreducens, including Fe(III) oxide, U(VI), and humics, and also bound Fe(III) oxide (Qian et al., 2011). OmcS is specifically associated with the pili of G. sulfurreducens (Leang et al., 2010) and is required for growth on Fe(III) oxide, but not Fe(III) citrate (Mehta et al., 2005).

OmcE is another c-type cytochrome found on the outer cell surface, but its specific localization has yet to be pinpointed. It also has not been purified but is predicted to have a molecular weight of 32 kDa and four hemes (Mehta et al., 2005). Expression patterns of OmcE (Ding et al., 2008; Holmes et al., 2006; Kim et al., 2008a; Nevin et al., 2009), as well as gene deletions studies (Mehta et al., 2005), suggest that OmcE plays a role in extracellular electron transfer in wild-type cells, but cells can adapt to the loss of OmcE.

In contrast to OmcE and OmcS, OmcZ is not required for the reduction of insoluble Fe(III). However, of all G. sulfurreducens cytochromes studied
to date, only OmcZ is absolutely necessary for high-density current production (Nevin et al., 2009). In its mature extracellular form, OmcZ has a molecular weight of 30 kDa, with eight hemes, including an unusual CX₁₄CH motif (Inoue et al., 2010). Its midpoint potential is $-220 \text{ mV}$, but as with other multiheme cytochromes individual hemes cover a wide range of potentials. The purified protein can reduce a range of typical soluble extracellular electron acceptors, and Mn(IV) oxides, but only poorly reduced Fe(III) oxide. This corresponds with increased expression of OmcZ during growth on Mn(IV) oxide, but not Fe(III) oxide, versus growth on Fe(III) citrate (Holmes et al., 2011b). The poor solubility of OmcZ in water might help maintain it within the extracellular matrix (Inoue et al., 2010). OmcZ is specifically localized at the biofilm–anode interface in high-current density biofilms (Inoue et al., 2011). It does not associate with filaments and its expression patterns suggest that its natural function may be to promote the reduction of extracellular soluble electron acceptors.

The cytochrome encoded by gene GSU1334 is homologous to OmcZ and a deletion mutant exhibited defects in Fe(III) oxide and U(VI) reduction (Shelobolina et al., 2007a). However, caution in interpreting such phenotypes is warranted without additional study. For example, deleting genes for several cytochromes predicted to be on the outer surface inhibited Fe(III) reduction, but this could be attributed to the lack of proper expression or localization of OmcB or other outer-surface cytochromes in these mutants (Kim et al., 2005, 2006, 2008a). In a similar manner, deletion of the gene for MacA, a cytochrome of interest because it is more highly expressed during growth on Fe(III), inhibited Fe(III) reduction (Butler et al., 2004), but deletion of macA was also associated with a lack of OmcB (Kim and Lovley, 2008).

Another cytochrome of interest is PgcA, which is upregulated during growth on Fe(III) oxide (Ding et al., 2008; Holmes et al., 2011b). Selection for enhanced growth of G. sulfurreducens on Fe(III) oxide selected for mutations that increased expression of PgcA (Tremblay et al., 2011b). Further, PgcA is a member of one of the cytochrome families conserved across several Geobacter species (Butler et al., 2010b). Further study of this cytochrome is underway.

The putative multicopper protein, OmpB, which is localized to the outer surface of G. sulfurreducens (Qian et al., 2007), also appears to be involved in the reduction of Fe(III) oxide but is not required for the reduction of soluble Fe(III) (Mehta et al., 2006). The pseudo-pilin OxpG is required for OmpB export. The OmpB homolog, OmpC, is also important for optimal Fe(III) oxide reduction but has not been experimentally localized and
has different expression patterns than OmpB (Holmes et al., 2008). Homologs with four copper-binding sites, two at the N-terminus and two at the C-terminus, are found in all of the Geobacter genomes, though the protein size ranges from ca. 800 to 1700 aa (Butler et al., 2010b). Phylogenetically, the omp genes form two distinct clades, the B-type and the C-type, and not all genomes contain both types (Holmes et al., 2008). No homologs were found in the two Pelobacter genomes. Various potential roles for OmpB and OmpC have been suggested (Holmes et al., 2008; Mehta et al., 2006), but purification and characterization of the proteins are required to better evaluate these possibilities.

The many other underexplored cytochromes and other putative redox-active proteins in G. sulfurreducens warrant further study, as do proteins likely to be involved in cytochrome export (Afkar et al., 2005), and the cytochromes in other Geobacter species. For example, G. uraniireducens increases the transcriptional expression of several cytochromes when cultured in anoxic sediments versus growth on soluble electron acceptors (Holmes et al., 2009). Additional structural studies will provide important insights into their function (Londer et al., 2002, 2006a,b; Morgado et al., 2007, 2009; Pessanha et al., 2004, 2006; Pokkuluri et al., 2004, 2008, 2009, 2011).

Development of genetic systems for Geobacter species other than G. sulfurreducens will also aid in functional analysis, as will the approach of determining which cytochrome functions can be completed in mutants of G. sulfurreducens with cytochrome gene sequences from other Geobacter species (Yun et al., 2011a).

### 7.3. Model for Extracellular Electron Transfer to Fe(III) Oxide

Several models have been advanced for how Geobacter species transfer electrons to insoluble Fe(III) oxides. A miscalibrated spectrophotometer in initial studies with G. metallireducens (Gorby and Lovley, 1991) resulted in the mistaken suggestion that \(b\)-type cytochrome(s) were important in extracellular electron transfer, but subsequent studies demonstrated a role for \(c\)-type cytochromes in the reduction of Fe(III) and other metals (Lovley et al., 1993a). An early model for Fe(III) oxide reduction by Geobacter sulfurreducens suggested that it released a low-molecular-weight \(c\)-type cytochrome, which acted as an electron shuttle between cells and Fe(III) oxide (Seeliger et al., 1998). However, this concept was refuted in a number of studies, including studies in the laboratory, which initially developed the electron shuttling concept (Lloyd et al., 1999; Nevin and Lovley, 2000; Straub and Schink, 2003).
Evidence consistent with the need for direct contact is the lack of Fe(III) reduction when cells are separated from Fe(III) oxide contained within microporous alginate beads (Nevin and Lovley, 2000) or agar (Straub and Schink, 2003). This was observed with G. metallireducens (Nevin and Lovley, 2000) as well as G. sulfurreducens, G. brementis, and G. pelophilus (Straub and Schink, 2003). In contrast, Shewanella (Nevin and Lovley, 2002b) and Geothrix (Nevin and Lovley, 2002a) species, and Fe(III)-reducing enrichment cultures (Straub and Schink, 2003), produced shuttles that permitted reduction of Fe(III) oxide at a distance. Further, G. metallireducens also did not appear to produce chelators that could solubilize Fe(III), whereas Shewanella (Nevin and Lovley, 2002b) and Geothrix (Nevin and Lovley, 2002a) species did solubilize Fe(III) under similar conditions.

Although some of the components that appear to be involved in electron transfer to Fe(III) oxides have been identified, the understanding of how these, and potentially other components, fit together is far from complete. As noted above, OmcS is likely to have an important role in Fe(III) oxide reduction because (1) OmcS expression is highly upregulated during growth on Fe(III) oxide (Ding et al., 2008; Holmes et al., 2011b; Mehta et al., 2005); (2) gene deletion studies indicate that the OmcS is required for Fe(III) oxide reduction (Mehta et al., 2005); (3) OmcS is specially associated with pili (Leang et al., 2010), which, as described above, are electrically conductive and are required for Fe(III) oxide reduction; and (4) purified OmcS can transfer electrons to Fe(III) oxide and may bind Fe(III) (Qian et al., 2011). The simplest explanation for these observations is that electrons that are transported along the pili are transferred to Fe(III) oxide via OmcS. There is no obvious route for electrons to get to OmcS other than the pili and the lack of Fe(III) reduction in the absence of OmcS suggests that electrons cannot be directly transferred from the pili to Fe(III) oxide.

There is little information on how electrons are transferred to the pili. This could conceivably take place in the periplasm, or even the inner membrane, but the requirement for OmcB, which is located in the outer membrane, suggests that electron transfer near the outer surface of the cell is more likely. The fact that OmcB is embedded in the outer membrane suggests that it might be difficult for OmcB and pili to associate closely enough for electron transfer between the two. The need to mediate electron transfer from OmcB to the pili at the outer cell surface may explain why other potentially redox-active outer-surface components, such as other c-type cytochromes and the putative multicopper proteins OmpB and OmpC, are important in Fe(III) oxide reduction.
The role of other outer-surface cytochromes in Fe(III) oxide reduction is also not completely understood. OmcE can be an abundant c-type cytochrome under some growth conditions, but cells can eventually overcome deletion of omcE and reduce Fe(III) oxide (Mehta et al., 2005). It has been proposed that OmcZ localized in an extracellular matrix could be important in Fe(III) oxide reduction (Rollefson et al., 2011), but this is not consistent with several observations including (1) OmcZ is not required for Fe(III) oxide reduction (Nevin et al., 2009), (2) low expression of omcZ in cells growing on Fe(III) oxide (Holmes et al., 2011b), and (3) purified OmcZ only poorly reduces Fe(III) oxide (Inoue et al., 2010).

If OmcB is the conduit for electrons out of the cell and toward pili, then the next question is what is the electron donor for OmcB? Periplasmic cytochromes are potential sources, ferrying electrons from the inner membrane to the outer membrane. As noted above, a number of periplasmic c-type cytochromes have been identified in G. sulfurreducens, but no electron transfer link between these cytochromes and OmcB, or any other electron acceptor, has been documented.

Diagrams for how the electrons may flow to Fe(III) oxide from G. sulfurreducens are available (Lovley, 2011c), but clearly we are still at the hypothesis stage and more research on electron transfer out of the cell is warranted. Novel strategies for elucidating important components are likely to be helpful. For example, adaptive evolution for improved Fe(III) oxide reduction in G. sulfurreducens provided further evidence for the importance of pili in Fe(III) oxide reduction as well as identifying an additional c-type cytochrome that may be involved (Tremblay et al., 2011b).

Studies on species other than G. sulfurreducens are also warranted to look for commonalities that are general features of electron transfer to Fe(III) oxides in all Geobacter species. For example, unique PilA sequences are conserved in Geobacter species (Reguera et al., 2005) and recent gene deletion studies have demonstrated that PilA is required for Fe(III) oxide reduction in G. metallireducens (Tremblay et al., 2011a).

In contrast, outer-surface cytochromes’ sequences are poorly conserved in Geobacter species (Butler et al., 2010b), suggesting that there is less specificity in cytochrome requirements. However, there is still an opportunity to look for commonality in mechanisms. For example, if electrons cannot be directly transferred from pili to Fe(III) oxides, then it would be expected that G. metallireducens, which does not have an OmcS homolog (Butler et al., 2010b), would possess another cytochrome, which like OmcS, is associated with pili and necessary for Fe(III) oxide reduction.

Additional research is also required on the early steps of electron transfer across the inner membrane and to the electron carriers responsible for
the terminal steps in electron transfer to Fe(III) and other extracellular electron acceptors. Although possible electron carriers can be identified from genome sequences, experimental studies are required before definitive models can be developed. One of the key features of extracellular electron transfer in Geobacter species is the poor energy yields available from this mode of respiration in comparison with the reduction of soluble electron acceptors within the cell (Esteve-Nunez et al., 2004, 2005; Mahadevan et al., 2006). This can be attributed, at least in part, to the fact that intracellular reduction of electron acceptors consumes protons along with electrons, but when electrons are transferred out of the cell, this proton sink is lost, requiring export of protons that does not contribute to the development of a proton-motive force across the inner membrane (Mahadevan et al., 2006, 2011).

7.4. Model for Extracellular Electron Transfer to Electrodes

Like Fe(III) oxide, electrodes represent an insoluble, extracellular electron acceptor. Initial studies with G. sulfurreducens suggested that it did not produce electron shuttles in order to promote electron transfer to electrodes (Bond and Lovley, 2003) and electrochemical studies supported this conclusion (Busalmen et al., 2008, 2010; Marsili et al., 2008, 2010; Marsili et al.; Richter et al., 2009). This is consistent with the similar concept of direct electron transfer to Fe(III) oxide.

However, there are major differences between the electrodes and Fe(III) oxide because electrodes function as stable long-term electron acceptors, whereas once Fe(III) is reduced in one location cells need to find additional sources of Fe(III). The stability of the electrode as an electron acceptor makes it possible for Geobacter to produce thick (>50 μm) biofilms on electrodes (Franks, 2010; Franks et al., 2009; Nevin et al., 2009; Reguera et al., 2006), which are not formed during growth on Fe(III) oxide. Thus, the necessity to transfer electrons through a biofilm may require different electron transport strategies and may place different selective pressures on cells.

Fashioning one coherent model for electron transfer from G. sulfurreducens to electrodes that can accommodate all the data available in the literature is difficult. There is substantial confusion in the literature because models generated from preliminary data are often ruled out as more data becomes available. For example, early studies in our laboratory investigated electron transfer in systems producing relatively low amounts of current in which most of the cells were closely associated with
the anode surface. Under those conditions, OmcS was highly expressed and was essential for current production (Holmes et al., 2006). In contrast, in subsequent studies with systems producing much more current, OmcS was not highly expressed and cells adapted to produce current comparable to that of wild type when OmcS was deleted (Nevin et al., 2009). Rather, OmcZ was highly expressed in the high-current density biofilms. OmcZ and OmcS do not appear to have equivalent functions, based on their different localization and other factors, and it is generally the case that when OmcS is highly expressed OmcZ expression is low and vice versa. The geometry of the electrode material may also influence gene expression patterns, with expression patterns on graphite fiber electrodes resembling more closely the expression in the early low-current density biofilms on planar graphite surfaces (K.P. Nevin et al., unpublished data). Therefore, instead of attempting to develop one universal model for electron transfer to electrodes, we have focused on electron transfer in thick (>50 μm) electrode biofilms, which produce high-current densities, because a major goal is to understand the production of high-current densities in order to further optimize current output.

An initial observation in the development of higher current densities was that the increase in current was proportional to the increase in biomass on the anode, suggesting that cells at great distance from the anode were contributing to current production (Reguera et al., 2006). Subsequent studies have confirmed the high metabolic activity of such cells (Franks et al., 2010b). The finding that deleting pilA prevented high-current densities led to the hypothesis that networks of pili in the G. sulfurreducens biofilms conferred conductivity on the biofilm and a route for electrons released from cells at distance to be transported to the electrode (Reguera et al., 2006). Consistent with this concept, modeling studies indicated that the high-current density in microbial fuel cells would be feasible only if Geobacter biofilms were assumed to be electrically conductive (Marcus et al., 2007; Torres et al., 2008, 2009b). However, the suggestion that biofilms of Geobacter species could be conductive contrasted with previous studies, which had demonstrated that the biofilms of bacteria act as insulators (Dheilly et al., 2008; Herbert-Guillou et al., 1999; Muñoz-Berbel et al., 2006).

Measurement of the conductance of viable G. sulfurreducens biofilms with a novel two-electrode system revealed that the biofilms that had been grown with an electrode as the electron acceptor had remarkable conductivity, comparable to that of synthetic organic conducting polymers, such as polyaniline and polyacetylene (Malvankar et al., 2011b). In contrast, biofilms grown in the same system, but with fumarate as the electron acceptor, had low conductivity. The biofilms of Escherichia coli and Pseudomonas
*G. sulfurreducens* were not conductive. Evaluation of strains of *G. sulfurreducens* with different biofilm conductivities demonstrated a strong correlation between the abundance of PilA in the biofilm and conductivity, suggesting that the conductivity was related to the extent of pilin production.

The temperature dependence of biofilm conductivity was similar to that of pilin preparations, demonstrating a metallic-like conduction mechanism, which was further confirmed with electrochemical gating studies (Malvankar et al., 2011b). These results suggested that the biofilm conductivity was related to the metallic-like conductivity of the pilin network. None of these results support the concept of electron hopping through biofilms via c-type cytochromes. Further, denaturing the c-type cytochromes in the biofilms had no impact on conductance and there was no correlation between conductance and cytochrome content of the biofilms. These results suggest that the novel metallic-like conductivity in *G. sulfurreducens* can be attributed to the surprising metallic-like conductivity of its pilin networks.

Consistent with the apparent importance of pili in conduction of electrons through *G. sulfurreducens* biofilms, the gene for PilA is among the most highly upregulated genes in current-producing biofilms (Nevin et al., 2009). Selective pressure for enhanced current production yielded a strain of *G. sulfurreducens* that produced more pili (Yi et al., 2009). Deletion of pilA significantly inhibited current production, with only cells near the electrode surface remaining metabolically active (Reguera et al., 2006). Although the pilin constructed of PilA may have a structural role in biofilm formation under some conditions (Reguera et al., 2007), the pilA deletion mutant readily formed thick biofilms on the graphite electrode material if fumarate was provided as an alternative electron acceptor (Nevin et al., 2009).

The concept of electron transport through *G. sulfurreducens* biofilms via conductive pilin networks contrasts with many studies that have suggested that more traditional electron transfer via cytochromes moves electrons through the biofilms. Biofilms of wild-type *G. sulfurreducens* growing on electrodes are visibly red, due to the cytochrome abundance. Many studies have provided evidence that cytochromes are oxidized and reduced in *G. sulfurreducens* biofilms in electrical contact with electrodes (Esteve-Núñez et al., 2011; Fricke et al., 2008; Jain et al., 2011; Liu et al., 2010b, 2011; Marsili et al., 2008, 2010; Millo et al., 2011; Richter et al., 2009; Srikanth et al., 2008; Strycharz et al., 2011b), but the interpretation that this represents electron transfer through the biofilm by electron hopping via c-type cytochromes in analogy with redox hydrogels (Heller, 2006; Richter et al., 2009) is not consistent with the studies (Malvankar et al., 2011b) on biofilm conductance.
The likely explanation for this apparent discrepancy is that the electrochemical analyses only probed the biofilm-electrode interface and not the entire biofilm (Dumas et al., 2008a; Franks et al., 2010a). The cytochromes at the interface may function as an electrochemical gate, promoting electron transfer to the electrode surface (Dumas et al., 2008a).

A likely candidate for a cytochrome functioning as an electrochemical gate is the outer-surface c-type cytochrome OmcZ. The OmcZ gene is one of the most highly upregulated genes in current-producing cells, and if omcZ is deleted, the cells produce low levels of current (Nevin et al., 2009). There is much higher resistance for electron transfer to electrodes in cells lacking OmcZ, which was originally interpreted as OmcZ conferring conductivity throughout the biofilm (Richter et al., 2009). However, this cannot be correct as the conductance of biofilms of a strain with lower abundance of OmcZ was higher than those of wild type (Malvankar et al., 2011b). Further, cells throughout the biofilm express omcZ (Franks et al., 2011). OmcZ accumulates at the biofilm-electrode interface, consistent with the electrochemical gate hypothesis (Inoue et al., 2011).

The reason that OmcZ or other cytochromes might be required to facilitate current production is that a significant energy barrier might exist across the biofilm-electrode interface similar to a semiconductor–metal interface (Lange and Mirsky, 2008). The wide range of reduction potentials (−420 to −60 mV) of the multiple hemes in OmcZ (Inoue et al., 2010) might help overcome this energy barrier in a manner similar to electrochemical gating in molecular electronics (Vanmaekelbergh et al., 2007).

7.5. Extracellular Electron Transfer in Syntrophy

The finding that key elements of extracellular electron exchange in G. sulfurreducens were required for effective electron exchange between G. metallireducens and G. sulfurreducens and the finding that the aggregates were electrically conductive (Summers et al., 2010) suggest that components of these cells can form an electrically conductive matrix which permits direct electron exchange between the partners (Lovley, 2011a,c). OmcS was very abundant in the aggregates, which was attributed to a mutation in a regulatory gene of G. sulfurreducens that was selected for during adaption for ethanol metabolism. Introducing a strain of G. sulfurreducens with the regulator inactivated hastened adaption for ethanol metabolism, whereas deleting the gene for OmcS or PilA prevented aggregate formation and ethanol metabolism. These results suggest that
electron transfer through OmcS and the pili of *G. sulfurreducens* is an important part of the interspecies electron exchange.

Which components of *G. metallireducens* are important for the electron exchange are not known, but this question should now be addressable because of the recent development of methods for genetically manipulating *G. metallireducens* (Tremblay *et al.*, 2011b). The study of direct electron transfer from electrodes to cells (Strycharz *et al.*, 2011a) may help elucidate the electron-receiving component of the model.

It is important to recognize that aggregation of the two syntrophic partners may not be a prerequisite for direct interspecies electron transfer. In a manner similar to the mechanisms proposed for Fe(III) oxide reduction, *Geobacter* species could establish temporary contact with a recipient cell, offload electrons, and then move on.

### 7.6. Model for Extracellular Electron Transfer to Other Extracellular Electron Acceptors

The display of multiple low-potential *c*-type cytochromes on the outer surface of *Geobacter* species confers the capacity to reduce a wide diversity of soluble electron acceptors at the outer cell surface. Reduction of these electron acceptors may be rather nonspecific. For example, deleting the genes for individual outer-surface cytochromes only partially inhibited the ability of *G. sulfurreducens* to reduce humic substances and AQDS. Only when the genes for OmcB, OmcE, OmcS, OmcT, and OmcZ were deleted in the same strain was humic substance and AQDS reduction eliminated (Voordeckers *et al.*, 2010).

Although the final product of U(VI) reduction is U(IV), the initial reduction of U(VI) may be a one electron transfer followed by disproportionation of U(V) to U(VI) and U(IV) (Renshaw *et al.*, 2005). Initially it was considered that U(VI) might be reduced in the periplasm (Lloyd *et al.*, 2002), but the accumulation of uranium in the periplasm that was a main line of evidence for periplasmic reduction was later found to be an artifact (Shelobolina *et al.*, 2007a). Systematic deletion of the genes for the most abundant outer-surface *c*-type cytochromes in a study comparable to one on reduction of humic substances has indicated that the site of reduction is the outer surface of the cell (R. Orellana, unpublished data). Purified OmcZ (Inoue *et al.*, 2010) and OmcS (Qian *et al.*, 2011) reduce U(VI), and it is likely that many low-potential *c*-type cytochromes will be capable of U(VI) reduction (Lovley *et al.*, 1993b). It seems likely that
the other metallic ions that *Geobacter* species can reduce may also be reduced in a similar nonspecific manner.

In *vitro* studies with the abundant periplasmic *c*-type cytochrome of the closely related *Desulfuromonas acetoxidans* demonstrated that this cytochrome could reduce elemental sulfur *in vitro* (Pereira et al., 1997) and periplasmic reduction of sulfur has been a model. However, systematic reduction of the outer-surface *c*-type cytochromes of *G. sulfurreducens* has suggested that elemental sulfur is also reduced at the outer cell surface (S. Dar, unpublished results).

### 7.7. Capacitor Role of Cytochromes

In addition to their role in extracellular electron transfer discussed above, and possible environmental sensing discussed later, the abundant *c*-type cytochromes of *Geobacter* species may have the additional role of functioning as capacitors for electron storage under some environmental conditions (Esteve-Nunez et al., 2008; Lovley, 2008a). The sheer abundance of *c*-type cytochromes in cells growing under electron-acceptor-limiting conditions or in biofilms producing electrical current makes the cultures/biofilms visibly red. The capacity to store electrons in periplasmic and outer-surface cytochromes may be beneficial for *Geobacter* species because Fe(III) sources are heterogeneously dispersed and there are likely to be periods when *Geobacter* species are not in direct contact with Fe(III) oxides. In fact, when *Geobacter* species are most actively growing during *in situ* uranium bioremediation they are highly planktonic and thus periodically out of contact with Fe(III) (Anderson et al., 2003; Dar et al., 2011; Kerkhof et al., 2011). Cytochromes positioned beyond the inner membrane, in the oxidized state, can accept electrons from inner membrane electron carriers, permitting continued respiration even when Fe(III) is not available. Energy conservation under such conditions is expected to be similar to when Fe(III) is available because conservation of energy results from electron transfer components in the inner membrane. Subsequent steps in electron transport to extracellular electron acceptors do not conserve additional energy for the microorganism; they are just necessary in order to provide electron acceptors for electron transfer across the inner membrane. Once cells contact Fe(III), the electrons stored in the cytochromes can be discharged.

Another observation consistent with this concept is that *G. metallireducens* specifically expresses flagella when grown on insoluble Fe(III) or Mn(IV) oxides, but not when grown on soluble Fe(III) citrate (Childers...
et al., 2002). Deleting a gene for flagella production inhibited Fe(III) oxide reduction in G. metallireducens, but not the reduction of Fe(III) citrate (Tremblay et al., 2011a). Changing the sequence of a gene for a master regulator of flagella to confer motility in the otherwise nonmotile G. sulfurreducens enhanced Fe(III) oxide reduction (Ueki et al., 2011). The negative impact on Fe(III) reduction of making G. metallireducens nonmotile was stronger when the cells were grown with sediment Fe(III) oxides as the electron acceptor than in cultures with synthetic Fe(III) oxides, consistent with the greater dispersal of Fe(III) oxides in sediments (Tremblay et al., 2011a). Chemotaxis might guide Geobacter species to Fe(III) and Mn(IV) oxide sources (Childers et al., 2002).

Studies with current-producing biofilms of G. sulfurreducens have confirmed this inference of c-type cytochromes conferring capacitance (Malvankar et al., 2011a). The biofilms had capacitance comparable to that of synthetic supercapacitors. Multiple lines of evidence demonstrated that the biofilm capacitance could be attributed to the c-type cytochromes. As discussed below, this novel form of capacitance may be a useful contribution to the field of bioelectronics.

8. REGULATION OF METABOLISM

In order to understand how Geobacter species function in diverse environments, and how they are likely to change their metabolism in response to changes in environmental conditions, it is important to understand how gene expression is regulated. The elucidation of regulatory networks in Geobacter species is in its infancy, but some progress has been made.

8.1. Sigma Factors

Sigma factors play a key role in the regulation of gene expression in response to changing environments, and bacteria typically employ multiple sigma factors to optimize their responses. Each species of sigma factors recognizes specific promoter elements of a certain set of genes and initiates their transcription. The genome of G. sulfurreducens encodes homologs of RpoD (σ^70), RpoS (σ^32), RpoH (σ^32), RpoN (σ^54), RpoE (σ^24), and FliA (RpoF, σ^28) found in E. coli and many other bacteria (Méthé et al., 2003). In other bacteria, RpoD is generally the major sigma factor for most housekeeping genes (Ishihama, 2000). Alternative sigma
factors are required for stress response genes. For example, RpoS is required for general stress response genes including stationary-phase genes. RpoH is necessary for heat-shock genes. RpoN is involved in transcription of genes for nitrogen deficiency and some other stresses. FliA participates in regulation of flagella and chemotaxis genes. RpoE represents extracytoplasmic function (ECF) sigma factors, which are involved in regulation of genes for outer membrane or periplasmic proteins.

The RpoD homolog appears to be the major sigma factor in *G. sulfurreducens* as transcriptomic analysis has demonstrated that a large number of genes were transcribed by RNA polymerase containing RpoD (Qiu et al., 2010). It is likely that *G. sulfurreducens* RpoD recognizes promoter elements similar to those of other bacterial RpoD homologs (Qiu et al., 2010; Yan et al., 2006). However, genetic and biochemical studies for RpoD have not been conducted.

The RpoS homolog is the stationary-phase sigma factor in *G. sulfurreducens* (Nunez et al., 2004). It is also involved in response to oxygen exposure and in growth with oxygen as the electron acceptor. An *rpoS*-deletion mutant exhibited less viability at the stationary phase than the wild-type strain and slower recovery after exposure to oxygen. The mutant was also defective in reduction of insoluble Fe(III) oxide but not of soluble Fe(III) citrate. Transcriptome and proteome analyses revealed genes in a variety of cellular functions under the control of *G. sulfurreducens* RpoS, which include oxidative stress and nutrient limitation response genes and genes for *c*-type cytochromes (Nunez et al., 2006). Among the *c*-type cytochromes is MacA, which is known to be critical for Fe(III) reduction (Butler et al., 2004). It appears that promoters recognized by RpoS in *G. sulfurreducens* are similar to those recognized by RpoD, as found in other bacteria (Yan et al., 2006). However, genes regulated by RpoS in *G. sulfurreducens* are diversified from those in other bacteria (Santos-Zavaleta et al., 2011). These studies suggest that the RpoS homolog plays important roles in *G. sulfurreducens* under conditions that *Geobacter* species typically encounter in subsurface environments.

RpoH is the heat-shock sigma factor in *G. sulfurreducens* (Ueki and Lovley, 2007). Expression of the *rpoH* gene was induced by heat shock from 30 to 42 °C and appears to be controlled by RpoH itself as well as the HrcA/CIRCE system. HrcA is known to be a repressor for heat-shock genes by binding the CIRCE (Controlling Inverted Repeat of Chaperon Expression) element in other bacteria (Schulz and Schumann, 1996; Zuber and Schumann, 1994). An *rpoH*-deletion mutant of *G. sulfurreducens* was unable to grow at 42 °C, whereas the wild-type strain could. The expression of heat-shock response genes decreased dramatically in the *rpoH*-deletion mutant.
In contrast to most other bacteria for which RpoN is dispensable under some conditions, the RpoN homolog appears to be a vital sigma factor in *G. sulfurreducens* (Leang et al., 2009). Transcriptome analysis demonstrated that RpoN regulates a large number of genes involved in a wide range of cellular functions including those encoding enzymes for ammonia assimilation, which are predicted to be essential under all growth conditions in *G. sulfurreducens*. RpoN also regulates genes that were shown to be important for growth in subsurface environments or electricity production in microbial fuel cells, such as flagella biosynthesis, pili biosynthesis, and c-type cytochromes. Promoter elements recognized by the *G. sulfurreducens* RpoN are highly similar to those recognized by other bacterial RpoN homologs. Transcription initiation by RNA polymerase containing RpoN requires an enhancer-binding protein, and the *G. sulfurreducens* genome encodes more putative transcription factors in the enhancer-binding protein family than most bacteria (Karlin et al., 2006; Méthé et al., 2003). Thus, the RpoN homolog is a global regulator controlling a complex transcriptional network modulating physiological responses in *G. sulfurreducens*.

The FliA homolog appears to regulate flagella and chemotaxis genes in *G. sulfurreducens* as analysis of the *G. sulfurreducens* genome identified sequences similar to those recognized by other bacterial FliA homologs (Leang et al., 2009; Tran et al., 2008). As noted elsewhere, flagellar motility and chemotaxis are likely to be important for growth in subsurface environments. However, the function of the FliA homolog has not been experimentally studied in *Geobacter* species.

The RpoE homolog has not been characterized in *Geobacter* species. RpoE homologs or ECF sigma factors have been shown to control a variety of cellular functions in other bacteria. Amino acid sequences of ECF sigma factors appear to be more diverse than those of other families of sigma factors (e.g., RpoD, RpoS, RpoH, RpoN, and FliA). Therefore, it is difficult to predict the function of the RpoE homologs in *Geobacter* species solely by their sequences. Whereas homologs of RpoD, RpoS, RpoH, RpoN, and FliA are highly conserved among *Geobacter* species whose genome sequences are available, RpoE homologs are not, suggesting that RpoE might be involved in regulation of species-specific features.

### 8.2. Transcription Factors

Transcription factors generally regulate genes for more specific cellular functions than sigma factors and further fine-tune gene regulation in response to environmental and physiological changes. Transcription
Factors include an activator and a repressor, which promote or inhibit transcription by RNA polymerase, respectively. Some transcription factors can function as both an activator and a repressor. The *G. sulfurreducens* genome encodes 151 putative transcription factors (Méthé *et al.*, 2003). Transcription factors classified as a response regulator on the basis of sequence similarity are described in the section on two-component systems below.

The novel transcriptional repressor, HgtR, is induced in *G. sulfurreducens* when hydrogen is available as an electron donor and represses expression of citrate synthase and other genes encoding enzymes involved in central metabolism (Ueki and Lovley, 2010a). HgtR also regulates a gene encoding a putative transcription factor in the GntR family. Target genes of this GntR homolog in *Geobacter* species have not been identified. A transcription factor in the RpoN-dependent enhancer-binding protein family appears to regulate expression of *hgtR*, which has sequences highly similar to the RpoN recognition consensus sequences in its promoter region. Further, a gene encoding an enhancer-binding protein is located upstream of the *hgtR* homologs in *Geobacter* species. These enhancer-binding protein homologs contain a domain similar to the C-terminal domain of the iron-only hydrogenase large subunit at the N-terminus. It is possible that these enhancer-binding protein homologs sense environmental and/or intracellular hydrogen and activate the *hgtR* homologs. Therefore, it is likely that a novel regulatory cascade mediated by multiple transcription factors’ genes controls expression of central metabolism genes in *Geobacter* species.

Studies on the adaption of *G. sulfurreducens* to grow on lactate revealed a transcriptional regulator that regulates expression of the genes for succinyl-CoA synthetase (Summers *et al.*, 2011), a TCA cycle enzyme that is required for growth on lactate, but not acetate (Galushko and Schink, 2000; Segura *et al.*, 2008). The *G. sulfurreducens* gene GSU0514, which is a homolog of the IclR transcription factor, encodes a transcriptional repressor for the succinyl-CoA synthetase subunit genes *sucC* and *sucD* (Summers *et al.*, 2011). Mutations in GSU0514 were selected for during adaption for enhanced growth on lactate, which enhanced expression of *sucC* and *sucD* and promoted lactate metabolism (Summers *et al.*, 2011).

Another adaptive evolution study identified the transcription factor, GSU1771, which controls the expression of genes that are important for Fe(III) oxide reduction (Tremblay *et al.*, 2011b). GSU1771 is a homolog of *Streptomyces* antibiotic regulatory protein (SARP) (Wietzorrek and Bibb, 1997). Adaption of *G. sulfurreducens* for more rapid growth on Fe(III) oxide yielded strains that accumulated a mutation that interrupted the GSU1771 gene. Inactivation of GSU1771 in the wild-type strain
enhanced the ability to reduce Fe(III) oxide and increased the expression of the gene for PilA, the structural protein for the electrically conductive pili.

Although dissimilatory metal reduction by Geobacter species has been extensively studied, effects of the availability of metals for assimilatory purposes on growth and activity of Geobacter species have gained less attention. For instance, Fe(II) appears to play a critical physiological role in Geobacter species as they contain an unusually large number of iron-sulfur proteins such as c-type cytochromes, which have been shown to be essential for dissimilatory metal reduction. The Fe(II)-dependent transcription factor, Fur, is an important regulator for Fe(II) influx in other bacteria (Escolar et al., 1999) and all available Geobacter genomes contain a cluster consisting of homologs of fur, as well as feoB, which encodes an iron uptake protein and ideR, another Fe(II)-dependent transcription factor (O’Neil et al., 2008). In chemostat cultures, the expression of the fur-feoB-ideR cluster decreased as Fe(II) concentrations increased, suggesting that transcript abundance could serve as an indication of limitation of iron for assimilation. Monitoring transcript abundance of the Geobacter species in groundwater surprisingly revealed that iron availability might be limiting under some bioremediation conditions (O’Neil et al., 2008). Analyses of Geobacter genomes identified sequences in feoB and other genes that are similar to other bacterial Fur-binding sites, suggesting that Fur controls feoB in Geobacter species.

A number of biological processes in energy generation, nitrogen assimilation, and detoxification require nickel-dependent enzymes such as hydrogenase, carbon monoxide dehydrogenase, and urease (Mulrooney and Hausinger, 2003; Zhang et al., 2009). The Ni(II)-dependent transcription factor NikR is known to regulate nickel transporters in other bacteria (Chivers and Sauer, 1999; Wang et al., 2009). The G. uranireducens NikR homolog was shown to bind the promoter regions of two different genes, nik(MN)1 and nik(MN)2, which encode ABC-type transporters (Benanti and Chivers, 2010). The DNA-binding mode of the G. uranireducens NikR homolog was distinct from other members of the NikR family.

Geobacter species are likely to encounter oxygen intrusions in subsurface environments, particularly at the oxic/anoxic interface where Fe(III) sources are abundant. Thus, the ability of Geobacter species to tolerate exposure to low concentrations of oxygen and even grow with oxygen as the terminal electron acceptor may be a critical factor to survival in subsurface environments (Lin et al., 2004; Mouser et al., 2009a). Many bacterial cells are equipped with oxidative responsive systems, which are regulated by RecA and LexA (Butala et al., 2009; Cox, 2007). LexA is a transcription factor controlling genes in the SOS system. The G. sulfurreducens genome
encodes two independent LexA homologs, which appear to be autoregulated (Jara et al., 2003). Unlike other bacterial LexA, *G. sulfurreducens* LexA homologs may not control *recA* and other genes known to be involved in the SOS system because sequences similar to *G. sulfurreducens* LexA-binding sites located in the *G. sulfurreducens* *lexA* genes are absent from these SOS system genes in *G. sulfurreducens*. Thus, *G. sulfurreducens* may employ unique regulatory mechanisms in oxidative responsive systems.

Transcriptional regulators control the expression of genes involved in the degradation of aromatic compounds. BgeR is a transcriptional repressor that regulates genes for the metabolism of aromatic compounds as well as another transcription factor involved in aromatic metabolism in *G. bemidjiensis* (Ueki, 2011). BgeR belongs to the Rrf2 family, but the similarity is limited to the N-terminal region, which is likely a DNA-binding domain. Its C-terminal region does not show similarity to known proteins except for BgeR homologs in other *Geobacter* species. It is likely that genes for aromatic compound degradation are controlled by regulatory cascades consisting of multiple transcription factors in *Geobacter* species. This is a topic that warrants further study because of its potentially important role in bioremediation.

### 8.3. Two-Component Systems

*Geobacter* species have one of the highest IQs of bacteria, which is a measure of the adaptive potential of an organism on the basis of the total number of signaling proteins including the two-component system encoded in a given genome (Galperin, 2005). The genomes of *Geobacter* species encode an unusually large number of genes for the two-component signaling proteins (Aklujkar et al., 2009, 2010; Méthé et al., 2003). The two-component system typically consists of a sensor histidine kinase, which senses environmental signals, and a response regulator, which generally influences the gene expression necessary for the adaptation (Egger et al., 1997). The two components are often colocalized in the same operon in other bacteria (Mizuno, 1997), but this is often not the case in *Geobacter* species. Most of the putative sensor domains of the two-component systems in *Geobacter* species are unique or show similarity to uncharacterized systems in other bacteria. Some of the sensor domains have a c-type heme-binding motif (Londer et al., 2006b; Pokkuluri et al., 2008) and may participate in redox control of complex biological processes in *Geobacter* species.
An important response regulator is PilR, which is an RpoN-dependent enhancer-binding protein that regulates expression of pilA, the gene for the structural pilin protein (Juarez et al., 2009). The histidine kinase PilS appears to regulate PilR because pilS is immediately upstream of pilR. Transcriptomic and bioinformatic approaches identified a number of genes including those for c-type cytochromes, such as OmcB and OmcS, under the control of PilR (Juarez et al., 2009; Krushkal et al., 2010). Adaptive evolution for syntrophic growth of G. metallireducens and G. sulfurreducens selected for a strain of G. sulfurreducens with a mutation in pilR, which enhanced production of the c-type cytochrome OmcS (Summers et al., 2010).

Novel regulatory cascades consisting of two two-component systems regulate nitrogen-fixation gene expression in G. sulfurreducens (Ueki and Lovley, 2010b). GnfM, a member of the enhancer-binding protein family, contains a receiver domain at the N-terminus and thus is also a response regulator of a two-component system in which the activity of GnfM appears to be regulated by the histidine kinase GnfL. The GnfM gene seems to be essential for growth, probably because it regulates the expression of genes involved in nitrogen metabolism that are important even when ammonium is present (Leang et al., 2009). In addition to the nitrogen metabolism genes, the GnfL/GnfM system activates genes encoding regulatory proteins of a two-component system during nitrogen fixation that comprises the histidine kinase GnfK and the response regulator GnfR, which has an RNA-binding domain. GnfK modulates the activity of GnfR by phosphorylation. Phosphorylated GnfR exhibits RNA-binding activity. The GnfK/GnfR system regulates by transcription antitermination the expression of a subset of the nitrogen-fixation genes, such as nifH, nifEN, nifX, glkN, and amtB, whose transcription is activated by the GnfL/GnfM system and whose promoter region contains transcription termination signals. The GnfK/GnfR system plays a critical role in the nitrogen-fixation gene regulation as deletion mutants of gnfK or gnfR are defective in growth dependent on nitrogen fixation.

Expression of the gene bamY, which encodes benzoate-CoA ligase, is induced during growth of G. metallireducens on benzoate (Butler et al., 2007; Wischgoll et al., 2005). The bamV and bamW genes encoding a putative histidine kinase and a putative response regulator, respectively, are located in the vicinity of the bamY gene (Wischgoll et al., 2005). Aromatic compounds induced the expression of the bamV gene (Butler et al., 2007; Wischgoll et al., 2005). The bamY gene contains an RpoN-dependent promoter and the response regulator BamW is also an enhancer-binding protein (Juárez et al., 2010). Addition of benzoate, p-cresol, or p-hydroxybenzoate to cultures of E. coli heterologously expressing bamV
and bamW induced expression of a β-galactosidase gene fused to the bamY promoter, demonstrating the role of this two-component system in controlling aromatics metabolism (Juárez et al., 2010).

FgrM, which is a member of the enhancer-binding protein family as well as a response regulator, is the master transcriptional regulator for flagellar gene expression in Geobacter species (Ueki et al., 2011). FgrM interacts with RpoN to control transcription of a number of flagella genes, including the gene for FliA, which controls expression of some flagella-related genes, including chemotaxis genes. Thus, it appears likely that the expression of flagella-related genes is controlled in a cascade manner.

8.4. Chemotaxis

Unlike well-characterized motility systems such as those found in E. coli and Bacillus subtilis, both of which have a single chemotaxis system, Geobacter species contain multiple chemotaxis systems or homologs of the chemotaxis system (Tran et al., 2008). One of the chemotaxis(-like) systems, designated the α-group, is unique to Geobacter species and is predicted to be involved in flagellar motility (Ueki et al., 2011). A second chemotaxis-like system, designated the β-group and found in δ-proteobacteria, is involved in the regulation of the expression of extracellular proteins, such as the c-type cytochromes, OmcS and OmcZ (Tran et al., 2011). Proteins of both the α and β-groups were abundant in groundwater during acetate-stimulated in situ uranium bioremediation (Wilkins et al., 2009).

Geobacter species appear to have an unusually large number of chemoreceptor (MCP) genes (Aklujkar et al., 2009, 2010; Méthé et al., 2003; Tran et al., 2008). Several MCP genes are located in proximity to other chemotaxis genes on the genome in Geobacter species but most are scattered on the genome. Some are predicted to play a role in chemotaxis (Ueki et al., 2011). It is possible that other MCP genes are involved in signal transduction pathways mediated by chemotaxis-like systems.

8.5. Nucleotide-Based Second Messenger

Stringent response, originally observed during amino acid starvation in E. coli, is affected by (p)ppGpp, which is known to act as a global regulator in physiological adaptation to a variety of environmental changes (Braeken et al., 2006; Potrykus and Cashel, 2008). In G. sulfurreducens, ppGpp and ppGp were produced in response to nutrient limitations and
ppGpp accumulated as the result of oxygen exposure (DiDonato et al., 2006). The production of ppGpp in *G. sulfurreducens* was dependent on the *rel* gene encoding a homolog of the bifunctional RelA/SpoT protein, which has both (p)ppGpp synthetase and hydrolase activity. Deleting *rel* affected expression of genes involved in protein synthesis, stress responses, and electron transport systems, and enhanced growth with fumarate as the electron acceptor, but increased oxygen sensitivity and diminished the capacity for Fe(III) reduction. Bioinformatic analysis suggested that genes influenced by the Rel/ppGpp signaling system are also controlled by Fur and RpoS (Krushkal et al., 2007).

Riboswitches, noncoding RNA elements found in the untranslated region of mRNA, are known to sense and bind cellular metabolites to control gene expression (Lioliou et al., 2010; Waters and Storz, 2009). *Geobacter* species possess riboswitches termed GEMM (genes related to the environment, membranes and motility) (Weinberg et al., 2007), which have been shown to sense c-di-GMP in other bacteria (Sudarsan et al., 2008). *G. uraniireducens* has the largest number of c-di-GMP riboswitch homologs among bacteria whose genomes have been sequenced (Weinberg et al., 2007). In *G. sulfurreducens*, genes known to be differentially regulated during metal reduction and electricity production, such as *omcS* and *omcT*, were found to contain a c-di-GMP riboswitch signature in their noncoding region of mRNA (Weinberg et al., 2007). The 5′ untranslated region of *omcS* mRNA is critical for *omcS* expression (B-C. Kim et al., unpublished data).

Another *c*-type cytochrome whose expression appears to be controlled with a GEMM riboswitch is PgcA, which contains a GEMM riboswitch sequence between the predicted RpoD-dependent promoter and the start codon (Tremblay et al., 2011b). An increase in c-di-GMP in *E. coli* results in the upregulation of *lacZ* under the control of the *pgcA*-associated GEMM riboswitch (B-C. Kim et al., unpublished data). Adaptive evolution of *G. sulfurreducens* for improved growth on Fe(III) oxide selected for strains that had either a single base-pair change or a one-nucleotide insertion in the GEMM riboswitch of the *pgcA* gene. Introduction of either of the GEMM riboswitch mutations in the *pgcA* gene into the wild-type strain increased the abundance of *pgcA* transcripts, consistent with increased expression of *pgcA* in the adapted strains.

### 8.6. Summary Statement on Regulation

The abundance of regulatory genes and novel sensing capabilities found in *Geobacter* species suggest that they are highly attuned to their...
environment and have evolved to be able to sense a wide diversity of environmental cues. For the most part, even the regulatory systems that have already been studied so far have only been examined in a rather preliminary manner and there are many other regulatory systems that have yet to be investigated. A better understanding of these systems will greatly aid the development of models to predict the activity of Geobacter species under different environment conditions.

9. ENVIRONMENTAL SYSTEMS BIOLOGY OF GEOBACTER

The availability of pure cultures of Geobacter species closely related to those that are abundant in Fe(III)-reducing environments has made it possible to take a systems approach to the study of Geobacter ecology in subsurface environments. For example, quantifying key gene transcripts or proteins can provide a diagnosis of the in situ physiological status of Geobacter species, providing insights into metabolic patterns that are likely to be much different than when the microorganisms were grown under nutritionally replete conditions in the laboratory. Even estimating how fast microorganisms are metabolizing in natural environments can be difficult, especially in subsurface environments which are difficult to sample. Understanding in situ physiological status is key for bioremediation, making it possible to rationally design strategies to modify the in situ activity of Geobacter species (Lovley, 2003; Lovley et al., 2008).

9.1. Environmental Transcriptomics and Proteomics

Several strategies were investigated to elucidate the rate of activity of Geobacter species in the subsurface. One successful approach was based on monitoring gene transcript abundance (Holmes et al., 2005; Williams et al., 2011) or protein abundance (Wilkins et al., 2011; Yun et al., 2011b) of the key TCA cycle enzyme citrate synthase. The citrate synthase sequences of Geobacter species are more closely aligned with those of eukaryotes, rather than other prokaryotes (Bond et al., 2005; Méthé et al., 2003), simplifying the task of designing PCR primers to specifically amplify citrate synthase sequences of Geobacter species. Chemostat studies with G. sulfurreducens demonstrated a direct correlation between rates of acetate metabolism and transcript abundance for citrate synthase (Holmes et al., 2005). Subsequent field studies in which the in situ levels of
Geobacter citrate synthase gene transcripts were monitored demonstrated that as acetate availability in the groundwater was artificially manipulated, the metabolism of the in situ Geobacter community responded accordingly, increasing expression of citrate synthase when acetate concentrations were elevated and repressing expression when acetate levels dropped (Holmes et al., 2005; Williams et al., 2011). A similar metabolic response was noted when citrate synthase protein levels were quantified with an antibody-based approach (Yun et al., 2011b).

Attempts to monitor bulk rates of respiration by quantifying the transcript abundance of genes specifically involved in electron transfer processes was less successful (Chin et al., 2004), but recent studies with sulfate reducers have demonstrated that important insights into per-cell rates of metabolism might be obtained with such an approach (Miletto et al., 2011; Villanueva et al., 2008), suggesting that it might be productive to revisit this approach in Geobacter species.

Recent studies demonstrated that the abundance of a predominant c-type cytochrome in groundwater correlated well with the activity of Geobacter species and the effectiveness of uranium bioremediation (Yun et al., 2011b). Subsequent functional analysis suggested that this cytochrome might function similarly to the OmcS of G. sulfurreducens.

As important as it is to understand rates of metabolism, it is equally important to understand the factors that control those rates. Quantifying key gene transcripts or proteins has been shown to be a useful tool for diagnosing which nutrients or stresses might be limiting the growth of the subsurface Geobacter community. For example, measuring transcript abundance (Holmes et al., 2004c; Mouser et al., 2009b) or protein abundance (Yun et al., 2011b) of the nitrogen-fixation protein NifD can indicate whether Geobacter species in the subsurface are limited for ammonium and need to fix atmospheric nitrogen. This information can guide bioremediation because it may be beneficial for cells to be ammonium-limited during uranium bioremediation, but for bioremediation of hydrocarbon-contaminated groundwater ammonium limitation is likely to slow contaminant removal.

In a similar manner, molecular analysis of the in situ physiological status of the subsurface Geobacter community has provided insights into phosphate limitation (N’Guessan et al., 2009), acetate availability (Elifantz et al., 2011), iron limitation (O’Neil et al., 2008), and oxidative stress during uranium bioremediation (Mouser et al., 2009a). Monitoring transcript abundance for a ribosomal protein made it feasible to estimate growth rates of Geobacter species during uranium bioremediation and is expected to be an important tool in evaluating the proposed slow growth of
microorganisms in undisturbed subsurface environments (Holmes et al., 2011a). The increased expression of a key *Geobacter* enzyme in the degradation of aromatic hydrocarbons in response to hydrocarbon contamination in groundwater was documented with an antibody-based approach (Yun et al., 2011b). Continued analysis of *Geobacter* communities with high-throughput proteomics (Callister et al., 2010; Wilkins et al., 2009) and broad transcriptomic approaches (Holmes et al., 2009) is expected to identify other key gene transcripts and proteins that will serve as diagnostic tools for better understanding the ecology of *Geobacter* species during bioremediation and in undisturbed soils and sediments.

### 9.2. BUGS (Bottom-Up Genome-Scale) Modeling

A major goal in microbial ecology is to be able to not only describe the distribution of microorganisms and their activity, but to predict microbial distributions and interactions with other microorganisms and the environment under a diversity of environmental conditions. One strategy for this is to couple genome-scale metabolic models with the appropriate models that can describe physical/chemical conditions and their changes in response to predicted microbial activity (Lovley, 2003; Lovley et al., 2008; Mahadevan et al., 2011; Zhao et al., 2010).

We have termed this modeling approach bottom-up genome-scale modeling, abbreviated BUGS modeling, to differentiate it from the increasingly popular top-down approach of beginning with a global analysis of genes, gene transcripts, and proteins in environments of interest. The two approaches are complementary. An advantage of the top-down community wide approach is that it can rapidly provide a “parts list,” an accounting of the diversity of genes and proteins and their relative abundance. However, this is a highly descriptive approach and it is difficult to make predictions about the response of microorganisms to changes in environmental conditions from such lists. In BUGS modeling, genome-scale metabolic models are made for the microorganisms that predominate in an environment of interest and their interaction with each other and their environment is modeled. BUGS modeling is a slower, iterative process, but in the end provides a knowledge base, based on first principles of microbial physiology, that should have broad applicability and predictive power.

Subsurface environments in which *Geobacter* species predominate have proven to be good test cases for the BUGS modeling concept. The addition of acetate to groundwater to stimulate dissimilatory metal reduction results in a bloom of *Geobacter* species, which are the primary microorganisms
influencing subsurface biogeochemistry during this period. Further, Geo-
bacter is one of the rare examples where isolates of the species that pre-
dominate in the environment of interest are available in pure culture. In
these still early days of the annotation of microbial genomes, the study of
relevant pure cultures is necessary because many important physiological
features, as well as the function of many genes, cannot be ascertained
solely from genomic sequences.

The details of the generation of genome-scale models for environmental
studies have recently been reviewed (Mahadevan et al., 2011) and will not
be repeated here. The initial genome-scale modeling of G. sulfurreducens
proved to be an important driver for hypothesis-driven research and rev-
ealed important metabolic features (Mahadevan et al., 2006, 2011; Yang
et al., 2010). For example, the mechanisms for acetate uptake, catabolic
and anabolic utilization, as well as energy conservation during reduction
of internal (fumarate) or external (Fe(III)) electron acceptors were
elucidated in an iterative process of laboratory experimentation and in sil-
ico modeling. The genome-scale model of G. sulfurreducens was an impor-
tant tool for analyzing the incorporation of carbon into amino acids, and
revealed that isoleucine was synthesized via the citramalate pathway
(Risso et al., 2008b). Continued development of genome-scale metabolic
models in other Geobacter species (Sun et al., 2009) and closely related
organisms (Sun et al., 2010) is identifying commonalities in metabolic
strategies as well as adding new metabolic modules, such as the pathways
for the degradation of aromatic compounds found in G. metallireducens
and other Geobacter species.

To date, BUGS modeling of the biogeochemical impacts of Geobacter
species has been applied to relatively simple subsurface environments.
In initial studies, the genome-scale metabolic model of G. sulfurreducens
was coupled with reactive transport models to determine geochemical
changes when acetate was added to groundwater to stimulate in situ ura-
nium bioremediation (Scheibe et al., 2009). Initial results were encourag-
ing.

The predominance of Geobacter species during acetate-amended ura-
nium bioremediation is rare and some of the most important ecological
interactions in soils and sediments are those between different
microorganisms. Therefore, in order to expand the BUGS modeling con-
cept, the interactions between Rhodoferax and Geobacter species in the
subsurface were modeled. Like Geobacter species, Rhodoferax fer-
rireducens is an acetate-oxidizing Fe(III) reducer (Finneran et al., 2003).
Rhodoferax has a higher growth yield from acetate, but slower growth rate
than Geobacter and Rhodoferax cannot fix nitrogen whereas Geobacter
can. First, a genome-scale model of R. ferrireducens was generated (Risso
et al., 2009). Then the genome-scale models of *G. sulfurreducens* and *R. ferrireducens* were used to model their growth under different environmental conditions (Zhuang et al., 2010). The modeling predicted that, as has been observed experimentally (Mouser et al., 2009b), *Geobacter* and *Rhodoferax* species are likely to coexist in subsurface environments in which the slow degradation of organic matter deposited with the sediments drives microbial metabolism. Where ammonium concentrations are relatively high, *Rhodoferax* will predominate. However, the modeling predicted that when acetate is added to the groundwater to promote the growth of Fe(III) reducers, *Geobacter* outgrows *Rhodoferax* because of its much faster growth rate. This prediction is consistent with what is observed during bioremediation. The BUGS modeling approach is now being expanded to evaluate the competition between *Geobacter* species and sulfate-reducing *Desulfobacter* species, which compete with *Geobacter* species for acetate. Over time more complex communities, potentially first grown in the laboratory (Miller et al., 2010) and then studied in the field, can be modeled.

At some point, it may be possible to greatly accelerate the BUGS modeling process by building models from genomes sequenced directly from the environment. However, as of now, gene annotation and the ability to predict even simple physiological properties, such as growth rate, from the genome sequence are not sufficiently advanced.

10. BIOGEOCHEMICAL IMPACTS OF *GEOBACTER* SPECIES

Previous reviews have detailed many of the substantial geochemical impacts that *Geobacter* species can have on anaerobic soils and sediments (Lovley, 1991, 1993, 1995, 2000a,b), and these topics will not be covered in detail here. Important geochemical changes that take place in Fe(III)- and Mn(IV)-reducing environments in which *Geobacter* species are abundant can include the production of magnetite, siderite, and other Fe(II) and Mn(II) minerals; the release of iron, trace metals, metalloids, and phosphate into pore waters; other changes that influence the pH and ionic strength of pore waters; and changes in soil porosity as the result of reduction of Fe(III) in clays. The degradation of organic carbon in soils and sediments coupled to the reduction of Fe(III) and Mn(IV) can contribute significantly to anaerobic organic matter degradation with the release of carbon dioxide. Any organic matter degraded in this manner results in less reduction of sulfate and less production of methane. Ions of metals and
metalloids are natural constituents of soils and sediments, and as noted in other sections, the ability of *Geobacter* species to reduce these can influence their geochemical fate.

In some instances, the activity of *Geobacter* species in subsurface environments can have deleterious impacts on groundwater quality. For example, undesirably high concentrations of Fe(II) and Mn(II) as the result of microbial reduction of Fe(III) and Mn(IV) are common (Anderson and Lovley, 1997; Chapelle and Lovley, 1992; Lovley, 1997).

*Geobacteraceae* are abundant in groundwaters with high arsenic concentrations in which Fe(III) reduction has been implicated in the release of arsenic (Héry *et al.*, 2010; Islam *et al.*, 2004a; Smedley and Kinniburgh, 2002; Weldon and MacRae, 2006). Possibilities for arsenic fluxes from sediments to groundwater include release of arsenic adsorbed onto Fe(III) oxide and/or the reduction of As(V) to As(III), which is more soluble. For example, *Geobacter* species are thought to play a key role in the mobilization of arsenic from West Bengal sediments (Héry *et al.*, 2008; Islam *et al.*, 2004a,b, 2005a,b) where arsenic release takes place after Fe(III) reduction, rather than occurring simultaneously (Islam *et al.*, 2004b). It has been proposed that *Geobacter* species related to *G. uraniireducens* and *G. lovleyi* may be the primary catalysts for As(V) reduction (Héry *et al.*, 2010) but As(V) reduction in these species has not yet been documented.

Some *Geobacter* species can methylate mercury and their activity may be an important source of this environmental toxin in iron-rich freshwater sediments (Fleming *et al.*, 2006). Pure cultures capable of mercury methylation include *Geobacter* strain CLFeRB (Fleming *et al.*, 2006) as well as *G. hydrogenophilus*, *G. metallireducens*, and *G. sulfurreducens* and the closely related *Desulfuromonas palmitatis* (Kerin *et al.*, 2006). Environmental conditions that control the extent to which *Geobacter* species methylate mercury are beginning to be examined (Schaefer and Morel, 2009; Schaefer *et al.*, 2011) and warrant further study.

### 11. PRACTICAL APPLICATIONS OF *GEOBACTER* SPECIES

#### 11.1. Bioremediation: Natural Attenuation and Engineered

##### 11.1.1. Aromatic Hydrocarbons

*Geobacter* species are often important components of the microbial community in aquifers polluted with petroleum or landfill leachate (Alfreider and...
Vogt, 2007; Botton et al., 2007; Holmes et al., 2007; Lin et al., 2005, 2007; Röling et al., 2001; Rooney-Varga et al., 1999; Staats et al., 2011; Van Stempvoort et al., 2009; Winderl et al., 2007, 2008) which can be attributed, at least in part, to the ability described above of some Geobacter species to degrade aromatic compounds. Prior to contamination most shallow aquifers are aerobic, but anaerobic conditions rapidly develop once organic contaminants are introduced. Fe(III) is generally an abundant electron acceptor for anaerobic degradation and early studies demonstrated a removal of aromatic hydrocarbon contaminants from petroleum-contaminated groundwater associated with geochemical signatures for Fe(III) reduction (Lovley et al., 1989). Subsequent analysis demonstrated an abundance of Geobacter species in the Fe(III) reduction zone (Anderson et al., 1998; Holmes et al., 2004c, 2007; Lovley et al., 1989; Nevin et al., 2005; Rooney-Varga et al., 1999), accounting for 41% of the active microbial community in the groundwater (Holmes et al., 2007). In a similar manner, 25% of the microbial community comprised Geobacter species in a landfill leachate-contaminated aquifer (Röling et al., 2001). Quantifying specific genes or proteins known to be involved in the degradation of aromatic compounds has further demonstrated the importance of Geobacter species in naturally removing aromatic contaminants (Hosoda et al., 2005; Kane et al., 2002; Kuntze et al., 2008, 2011; Winderl et al., 2007, 2008; Yun et al., 2011b).

The finding that Geobacter species could reduce chelated Fe(III) faster than Fe(III) oxides (Lovley and Phillips, 1988a; Lovley and Woodward, 1996) and that electron shuttles promoted Geobacter reduction of Fe(III) oxide (Lovley et al., 1996a) led to studies evaluating whether the addition of Fe(III) chelators could stimulate the degradation of aromatic hydrocarbons (Lovley et al., 1994, 1996b). In the presence of these stimulants, even benzene could be degraded as rapidly with Fe(III) as the electron acceptor as it could with the introduction of oxygen.

The most practical method for enhancing electron-acceptor availability to Geobacter species involved in the degradation of organic contaminants in contaminated groundwater or aquatic sediments may be the concept of “subsurface snorkels” (Lovley, 2011c). Studies with G. metallireducens, as well as natural communities, demonstrated that providing an electrode as an electron acceptor may be a good strategy for stimulating the degradation of aromatic hydrocarbon contaminants (Lovley et al., 2010). Graphite electrodes may be the best option as they are inexpensive and durable and have the added advantage of adsorbing aromatic contaminants on their surface. This colocalizes the contaminant, the electron acceptor, and the Geobacter species at the electrode surface. Initial studies focused on the use of relatively complex systems in which the potential of the
The ability of *Geobacter* to reduce soluble ions of metals to less soluble forms shows promise as a bioremediation tool. Metals may be removed from water in this manner in reactors, or stimulating the activity of *Geobacter* species for *in situ* immobilization is an option. In some instances, *Geobacter* species might naturally attenuate the movement of metals via reduction.

Uranium has been the contaminant metal of greatest focus because the rapid kinetics of bacterial U(VI) reduction and low solubility of U(IV) make this process an attractive option for removing uranium from groundwaters below drinking water standards (Williams *et al.*, 2011, and references therein). The rather nonspecific nature in which *Geobacter* species reduce U(VI) (see above) and the fact that even in uranium-contaminated environments U(VI) is likely to be a minor electron acceptor (Finneran *et al.*, 2002a) make it difficult to definitely determine if *Geobacter* species are the agents for U(VI) reduction in studies in which dissimilatory metal reduction has been stimulated to promote uranium bioremediation. However, the consistent pattern of effective U(VI) removal being associated with increased growth and activity of *Geobacter* species at least at some sites (Williams *et al.*, 2011, and references therein) suggests that *Geobacter* species play a role.

Stimulating the activity of *Geobacter* species may also remove a variety of other toxic metals that *Geobacter* species have the potential to reduce in pure culture, but the reduction of these contaminants may be indirect in subsurface environments, because as noted above in Section 5, these electron acceptors can also be reduced by Fe(II) that *Geobacter* species generate during Fe(III) oxide reduction.

Although the commonly considered approach to stimulating the activity of *Geobacter* species for bioremediation of uranium and related contaminants is to add organic electron donors, a more effective approach might be to provide *Geobacter* species electrons with electrodes (Gregory and Lovley, 2005). Long-term stimulation of anaerobic respiration has
several potential negative impacts (Williams et al., 2011). These include (1) release of trace metals and arsenic that were associated with Fe(III) oxides into the groundwater (Burkhardt et al., 2010), (2) deterioration of the groundwater quality from accumulations of dissolved Fe(II) or sulfide, and (3) aquifer plugging due to biomass or mineral accumulations (Williams et al., 2011). Further, reductive immobilization of uranium in this manner leaves the uranium contamination in the subsurface.

Therefore, a better alternative may be to feed *Geobacter* species electrons with electrodes (Gregory and Lovley, 2005). Maintenance of the electron addition to the subsurface with electrodes is much simpler than complex pumping strategies for the controlled introduction of organic electron donors and the electrode strategy is sustainable, easily powered with solar panels. Further, this strategy specifically provides electrons for the reduction of the soluble contaminant of interest and the U(IV) produced precipitates on electrodes. It would be a simple matter to periodically remove the electrodes, extract the U(IV) under aerobic conditions in bicarbonate (Phillips et al., 1995), and return the electrodes to the subsurface. This approach would alleviate all the negative side effects of adding the organic electron donors listed above as well as remove the uranium from the subsurface.

### 11.1.3. Chlorinated Contaminants

In a similar manner, providing electrons to *Geobacter* species in the subsurface with electrodes may be an effective strategy for stimulating reductive dechlorination (Strycharz et al., 2008, 2010). Although no *Geobacter* species are known to completely dechlorinate chlorinated solvents, electrodes have the potential to specifically localize the electron donor and the dechlorinating organisms. Therefore, cathodes colonized with *Geobacter* species and positioned near source zones of chlorinated solvents could convert the solvents to the much more soluble products, susceptible to aerobic degradation, that could be degraded downgradient at the anode which produces oxygen (Lovley and Nevin, 2011). As noted above, *Geobacter* species have frequently been detected in subsurface environments contaminated with chlorinated solvents and in dechlorinating enrichments.

The formation of reactive Fe(II) minerals by *Geobacter* species during Fe(III) oxide reduction may accelerate the removal of carbon tetrachloride (McCormick et al., 2002). In a similar manner, humic substances may provide an electron shuttle to promote carbon tetrachloride reduction (Cervantes et al., 2004).
11.2. Producing Methane from Organic Wastes and Hydrocarbon Deposits

Conversion of organic wastes and biomass to methane has been a long-standing bioenergy strategy whose use could be expanded if the process could be accelerated and made more consistently stable. *Geobacter* species may have a role in this process development because the ability of *Geobacter* species to function as syntrophs may permit them to significantly contribute to rapid conversion of organic matter to methane.

Molecular analyses have demonstrated that *Geobacter* and closely related species can account for over 20% of the microbial community in the methanogenic aggregates that form in anaerobic digesters treating brewery wastes (Morita et al., 2011; Werner et al., 2011). Detailed analysis of aggregates from one of these digesters demonstrated that the aggregates had a conductivity similar to that of *Geobacter* co-culture aggregates (Morita et al., 2011). The temperature dependence of the conductance suggested an organic metallic-like conductivity, similar to that observed (Malvankar et al., 2011b) in pilin preparations of *G. sulfurreducens* and *G. sulfurreducens* biofilms. Several lines of evidence suggest that methanogenic microorganisms might be able to directly accept electrons and that direct electron transfer, rather than interspecies hydrogen or formate transfer, was the primary mechanism for electron exchange with the aggregates (Morita et al., 2011). The understanding that *Geobacter* species, and possibly other microorganisms, may be directly transferring electrons to methanogens, via direct interspecies electron transfer, may lead to new reactor designs to better promote this interaction and accelerate the process (Lovley, 2011a,c).

It has also been proposed that the capacity of *Geobacter* species to function in methanogenic syntrophic interactions can be used to enhance the recovery of hydrocarbons from coal and hydrocarbon deposits (Jones et al., 2010; Siegert et al., 2010). In fact, in an enrichment culture converting coal to methane, the most important microorganisms appeared to be *Geobacter* and *Methanosaeta* species (Jones et al., 2010), which is similar to what was found in wastewater methanogenic aggregates (Morita et al., 2011). This suggests that principles for *Geobacter* contributions to methanogenic wastewater treatment may apply to hydrocarbon recovery from the subsurface.

11.3. Microbial Fuel Cells, Electrosynthesis, and Bioelectronics

There is significant interest in the development of large-scale microbial fuel cell systems for wastewater treatment. Given the consistent
enrichment of *Geobacteraceae* on anodes of effectively operating microbial fuel cells, pre-enrichment of anodes with *Geobacter* species may be an important step in scale-up (Cusick *et al.*, 2011).

There may be significant potential for increasing the current output of microbial fuel cells via strain selection/design (Izallalen *et al.*, 2008; Yi *et al.*, 2009). The anode of a microbial fuel cell is not a natural electron acceptor, and thus it is unlikely that there has been significant selective pressure on *Geobacter* species to optimize current production under the conditions found in microbial fuel cells (Lovley, 2006a). For example, increasing pilin expression of *G. sulfurreducens*, via strain selection or genetic engineering, increased biofilm conductivity and current production (Malvankar *et al.*, 2011b). As more is learned about the mechanisms for electron transfer to electrodes in *Geobacter* species, it may be possible to further enhance power output.

Even without strain improvement there may be some short-term practical applications for microbial fuel cells, such as powering electrical devices in remote locations, such as at the bottom of the ocean (Tender *et al.*, 2008). The fact that *Geobacter* species are often the primary microorganisms colonizing electrodes harvesting current from a diversity of environments suggests that they are likely to play an important role in any applications of microbial fuel cells in which current is harvested in open environments in which there will be competition for anode colonization. *Geobacter*-based sensors may also be practical (Davila *et al.*, 2010). Novel system designs make it feasible to consider producing current with *Geobacter* species, even in completely aerobic environments (Nevin *et al.*, 2011b). Electrodes deployed in subsurface environments are naturally colonized by *Geobacter* species (Williams *et al.*, 2010) and may function as sensors of subsurface microbial activity (Tront *et al.*, 2008; Williams *et al.*, 2010).

Microbial electrosynthesis is a process in which electrons are provided to microorganisms colonizing an electrode to support the reduction of carbon dioxide to organic compounds that are excreted from the cells (Lovley, 2011b; Lovley and Nevin, 2011; Nevin *et al.*, 2010, 2011a). When powered with solar technology, microbial electrosynthesis is an artificial form of photosynthesis in which sunlight drives the conversion of carbon dioxide and water to organic compounds and oxygen. Proof-of-concept studies have demonstrated acetate production with acetogenic microorganisms as the catalysts (Nevin *et al.*, 2010, 2011a).

Genome annotation led to the surprising discovery of enzymes for carbon dioxide fixation in some *Geobacteraceae* (Aklujkar *et al.*, 2010). Within the *G. metallireducens* genome, a pair of genes is predicted to encode an ATP-dependent citrate lyase, which would allow the reverse TCA cycle
to produce acetyl-CoA. Further, genes for all of the identified enzymes of the dicarboxylate/4-hydroxybutyrate cycle of carbon dioxide fixation are predicted in the *G. metallireducens* genome. *G. metallireducens* is also capable of electrosynthesis, and investigations with genetically modified strains of other *Geobacter* species are ongoing because of the ability of *Geobacter* species to interact so effectively with electrodes. *G. sulfurreducens* can also use electrons derived from an electrode to reduce protons to hydrogen (Geelhoed and Stams, 2011), potentially providing a renewable catalyst that is much less expensive than the metal catalysts typically employed for hydrogen production.

One of the most exciting practical applications for *Geobacter* species could be bioelectronics. Electronically functional biomaterials are very attractive because they can be synthesized from relatively inexpensive feedstocks and do not contain toxic components (Hauser and Zhang, 2010). Further, conductive materials comprising living bacteria are self-renewing because bacteria can self-repair and replicate. Initial studies have already demonstrated the possibility of tuning the electronic properties of *Geobacter* biofilms via simple genetic engineering and more sophisticated modifications are feasible. Further elucidation of the mechanisms for electron transport along pili and ability of cytochromes to function as capacitors could aid in the biomimetic design of new materials. Therefore, it is expected that microbiobased electronically functional materials will have significant potential for next-generation biotechnological applications.

### 12. CONCLUSIONS

Studies to date have demonstrated the importance of *Geobacter* species to the anaerobic degradation of organic matter in sedimentary environments and its importance in iron, manganese, and trace-metal biogeochemistry. *Geobacter* species can naturally attenuate the migration of organic and metal contaminants, and strategies for artificially stimulating contaminant removal by *Geobacter* species are being developed.

The novel electrical properties of *Geobacter* species, and their pili and cytochromes, coupled with their ability to form direct electrical connections with man-made electronics, are amazing and provide new paradigms for the function of microbial communities and the development of next-generation bioelectronics. It has been suggested that “if it were not for the bacterium GS-15, we would not have radio and television today” because one of the first discovered properties of *G. metallireducens* was its ability to make
magnetite and it was the study of magnetite lodestone that contributed to the early understanding of electricity (Verschuur, 1993). Therefore, it may be fitting that one of the most recently discovered properties of Geobacter species, metallic-like conductivity along pili, has the possibility to make a more direct contribution to further the development of electronics.

Our understanding of the ecology, physiology, biochemistry, and bioelectronics of Geobacter is very rudimentary. Rapid advancements in omic technologies greatly facilitated a substantial increase in the understanding of Geobacter over the past decade. Continued functional genomic analyses of many aspects of Geobacter metabolism and genetic regulation will be essential for continued progress. Further, contributions from other fields, such as physics, materials science, and engineering, will be important not only to increase basic understanding of Geobacter species but also for the development of many promising, novel applications.

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