LETTERS

Extracellular electron transfer via microbial nanowires

Gemma Reguera[1], Kevin D. McCarthy[2]*, Teena Mehta[1]*, Julie S. Nicoll[1], Mark T. Tuominen[2] & Derek R. Lovley[1]

Microbes that can transfer electrons to extracellular electron acceptors, such as Fe(III) oxides, are important in organic matter degradation and nutrient cycling in soils and sediments[1,2]. Previous investigations on electron transfer to Fe(III) have focused on the role of outer-membrane *c*-type cytochromes[1,3]. However, some Fe(III) reducers lack *c*-cytochromes[4]. *Geobacter* species, which are the predominant Fe(III) reducers in many environments[1], must directly contact Fe(III) oxides to reduce them[5], and produce monolateral pili[6] that were proposed[1,2], on the basis of the role of pili in other organisms[7,8], to aid in establishing contact with the Fe(III) oxides. Here we report that a pilus-deficient mutant of *Geobacter sulfurreducens* could not reduce Fe(III) oxides but could attach to them. Conducting-probe atomic force microscopy revealed that the pili were highly conductive. These results indicate that the pili of *G. sulfurreducens* might serve as biological nanowires, transferring electrons from the cell surface to the surface of Fe(III) oxides. Electron transfer through pili indicates possibilities for other unique cell-surface and cell–cell interactions, and for bioengineering of novel conductive materials.

The role of pili in Fe(III) oxide reduction was studied with *Geobacter sulfurreducens* because a genetic system[9] and the complete genome sequence[10] are available. As expected from previous studies[6], *G. sulfurreducens* produced pili during growth on Fe(III) oxide (Fig. 1a) but not on soluble Fe(III) (Fig. 1b), and the pili were localized to one side of the cell. The formation of pili could also be induced during growth on the alternative electron acceptor fumarate if the cells were grown at the suboptimal temperature of 25 °C (Fig. 2a), indicating that pilin production in *G. sulfurreducens* might be growth-regulated as it is in other bacteria[11].

The genome sequence of *G. sulfurreducens* contained two open reading frames (ORFs), GSU1496 and GSU1776, predicted to code for pilin domain proteins with the conserved amino-terminal amino acid characteristics of type IV pilins[12]. Phylogenetic analyses placed the protein encoded by ORF GSU1776 among bacterial pseudopilins of type II secretion systems, and subsequent studies have confirmed the role of this gene, termed *oxpG*, in protein secretion to the outer membrane[13]. The protein encoded by ORF GSU1496 formed an independent line of descent along with pilin subunits of other members of the *Geobacteraceae* such as *Geobacter metallireducens* and *Pelobacter propionicus* (Fig. 1c). The predicted length of these *Geobacter* pilin proteins was considerably shorter than other bacterial pilins (see Supplementary Fig. S1) and was restricted to the highly conserved N-terminal domain of bacterial type IV pilins, which functions in inner membrane insertion, signal processing and pilin polymerization, and forms the central helical core of the pilus filament[12,14]. The degree of conservation of geopilins at this region was lower than other bacterial pilins and, as a result, geopilins were

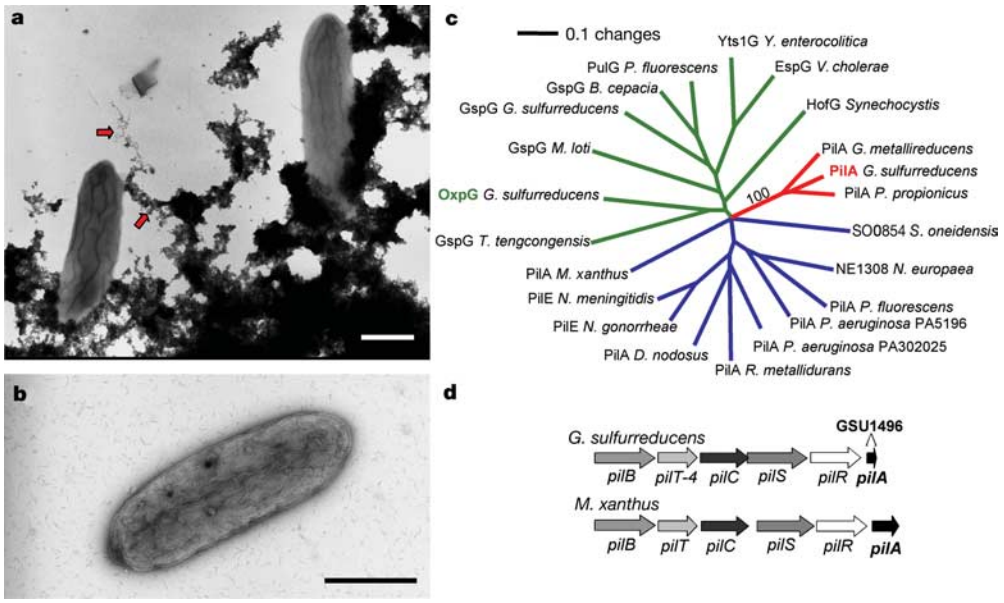

Figure 1 | *Geobacter sulfurreducens* pili. a, **b**, Transmisssion electron micrographs of cells grown with poorly crystalline Fe(III) oxides (**a**) or soluble Fe(III) citrate (**b**). Arrows indicate pili. Scale bars, 0.5 μm. **c**, Phylogenetic distance tree derived from amino-terminal amino acid sequence alignments (see Supplementary Information and Supplementary Fig. S1) showing the relationship of annotated *G. sulfurreducens* pilin-domain proteins, encoded by GSU1496 and GSU1776, and pilins (blue branches) and pseudopilins (green branches) of other bacteria. **d**, Genomic organization of pilus biosynthesis genes surrounding GSU1496 in *G. sulfurreducens* and the *pilA* gene in *M. xanthus*[17].

[1]Department of Microbiology and [2]Department of Physics, University of Massachusetts, Amherst, Massachusetts 01003, USA.
*These authors contributed equally to this work.

phylogenetically distant from other bacterial pilins, including a type IV pilin of another metal reducer, *Shewanella oneidensis* (Fig. 1c). Homologues of genes required for the formation and assembly of pili in other Gram-negative bacteria[15,16] are upstream of the *Geobacter pilA* gene (Fig. 1d), in a genetic arrangement similar to that of the pili genes in *Myxococcus xanthus*[17], a δ-proteobacterium distantly related to *Geobacter*. These results indicate that the GSU1496 gene encodes a pilin subunit, which was designated PilA.

When *pilA* was deleted, *G. sulfurreducens* failed to produce pili (Fig. 2b) and could no longer reduce insoluble electron acceptors such as poorly crystalline Fe(III) oxides (Fig. 3) and Mn(IV) oxides (data not shown). In contrast, the mutant could reduce soluble electron acceptors, such as fumarate and Fe(III) citrate as well as the wild type. The mutant also grew in medium containing Fe(III) oxide if the chelator nitrilotriacetate was added to solubilize some of the Fe(III) or in the presence of anthraquinone-2,6-disulfonate (AQDS) (Supplementary Fig. S2). AQDS serves as a soluble electron shuttle and transfers electrons between the cell surface and the surface of the Fe(III) oxide[18], alleviating the need for direct contact for Fe(III) oxide reduction[5]. Complementation of the *pilA* mutation with a functional copy of the *pilA* gene *in trans* restored the capacity for assembling pili (Fig. 2c) and for Fe(III) oxide reduction (Fig. 3a). These results showed that *G. sulfurreducens* required the assembly of functional pili to reduce insoluble Fe(III) oxides.

One known function of type IV pili in other microorganisms is establishing contact with surfaces[7,8]. Fe(III) oxides are typically smaller than *G. sulfurreducens* cells (Fig. 1a), but it was possible to quantify the potential for attachment of *G. sulfurreducens* to Fe(III) by inoculating fumarate-grown cells into medium in which Fe(III) oxide, attached to glass coverslips, was provided as the sole electron acceptor. Within the first 24 h, the cells of the *pilA*-deficient strain that were added initially attached to Fe(III) oxides as well as the wild type (Fig. 3b), but whereas the wild type grew on the Fe(III) oxide, as indicated by an increase in biomass on the Fe(III) oxide over the next 24 h, the *pilA* mutant could not grow, as shown by a decrease in biomass (Fig. 3b). The *pilA*-deficient mutant did grow on the surface if fumarate was provided as an alternative electron acceptor (data not shown). These results showed that pili are not required for Fe(III) oxides to attach to cells and confirmed the necessity for pili for growth with Fe(III) oxides as the sole electron acceptor. Further evaluation of the nature of the association of the Fe(III) oxides with the cells revealed that, when Fe(III) oxides were added to fumarate-grown cells, the outer surface of the *pilA*-deficient mutant still had the ability to bind Fe(III) oxides (Fig. 3d) but in the wild type there was substantial association of Fe(III) oxides with the pili (Fig. 3c).

It has previously been proposed that *Geobacter*'s pili might mediate surface motility, which might aid *G. sulfurreducens* in locating Fe(III) or Mn(VI) oxides[1,2,6], but no twitching motility of the wild-type cells was observed on glass surfaces coated with Fe(III) oxide. Furthermore, deleting a putative *pilT* gene (Supplementary Information), which is required for twitching motility in other organisms[19], had no effect on Fe(III) oxide reduction.

These results indicated that the pili might have a more direct role in electron transfer to Fe(III) oxides. To evaluate this we measured the electrical conductivity through the pili. Pili and other proteins released from the outer surface of *G. sulfurreducens* grown with fumarate (Supplementary Fig. S3) were immobilized on a graphite surface and analysed with an atomic force microscope (AFM) equipped with a conductive tip and electronics that permitted mapping of the local conductance from the tip to the substrate (Fig. 4). Topographic analysis revealed pili as well as other, unidentified, more globular, proteins which were also sheared off the outer cell surface (Fig. 4a). When a voltage was applied to the tip there was a strong current response along the pilus filament, which was positive when a positive voltage was applied and negative with a negative voltage (Fig. 4b, c). In contrast, the non-pilin proteins had no detectable conductivity and in instances in which the non-pilin proteins covered the pili filaments they insulated the pili from the conductive tip. This general response, initially observed in relatively large-scale scans (Fig. 4a–c), was even clearer in cross-sections in which high current was associated with the slight increase in topography associated with the pilus filament, but the higher topography, associated with non-pilin material, had no detectable current (Fig. 4d, middle panel). A scan across a portion of the pilin filament overlain by other material also yielded no detectable current (Fig. 4d, bottom panel). Current line scans generated after applying different voltages while scanning the same region of a pilus demonstrated a linear, ohmic, correspondence between current and voltage applied (Fig. 4e, f). When similar studies were performed with pili from the metal reducer *Shewanella oneidensis* or the non-metal reducer *Pseudomonas aeruginosa* (Supplementary Fig. S4 and S5), no conductance was detected.

These results show that the pili of *G. sulfurreducens* are highly conductive. This indicates that *G. sulfurreducens* requires pili in order to reduce Fe(III) oxides because pili are the electrical connection between the cell and the surface of the Fe(III) oxides. This contrasts with the nearly universal concept that outer-membrane cytochromes are the proteins that transfer electrons to Fe(III) oxide in Fe(III)

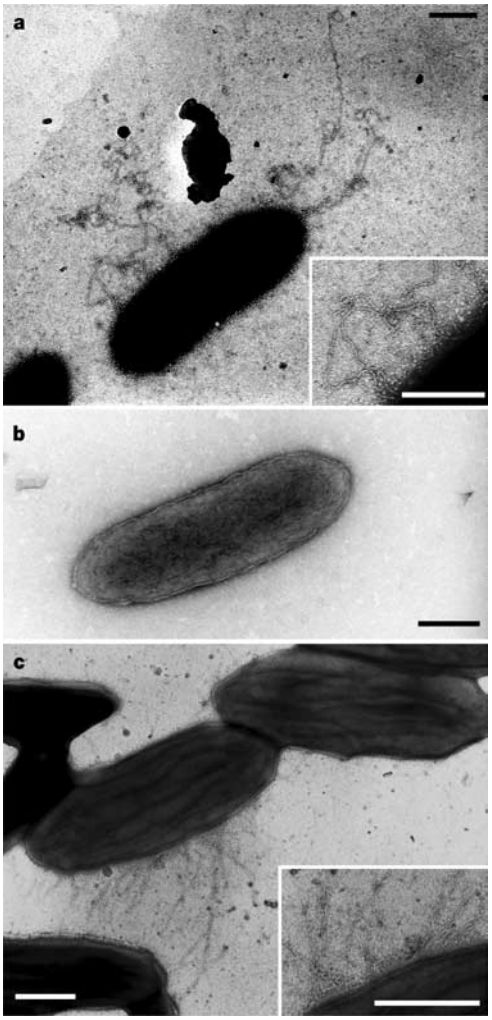

Figure 2 | Transmission electron microscopy analyses. Shown are cells of a wild-type strain (**a**), a *pilA*-deficient mutant strain (**b**) and a complemented mutant strain (**c**) of *G. sulfurreducens*. Cells were grown in medium with acetate and fumarate at 25 °C to induce the formation of pili, then negatively stained. Insets in **a** and **c** show details of pili produced by the wild-type and complemented mutant strains, respectively. Scale bars, 0.2 μm.

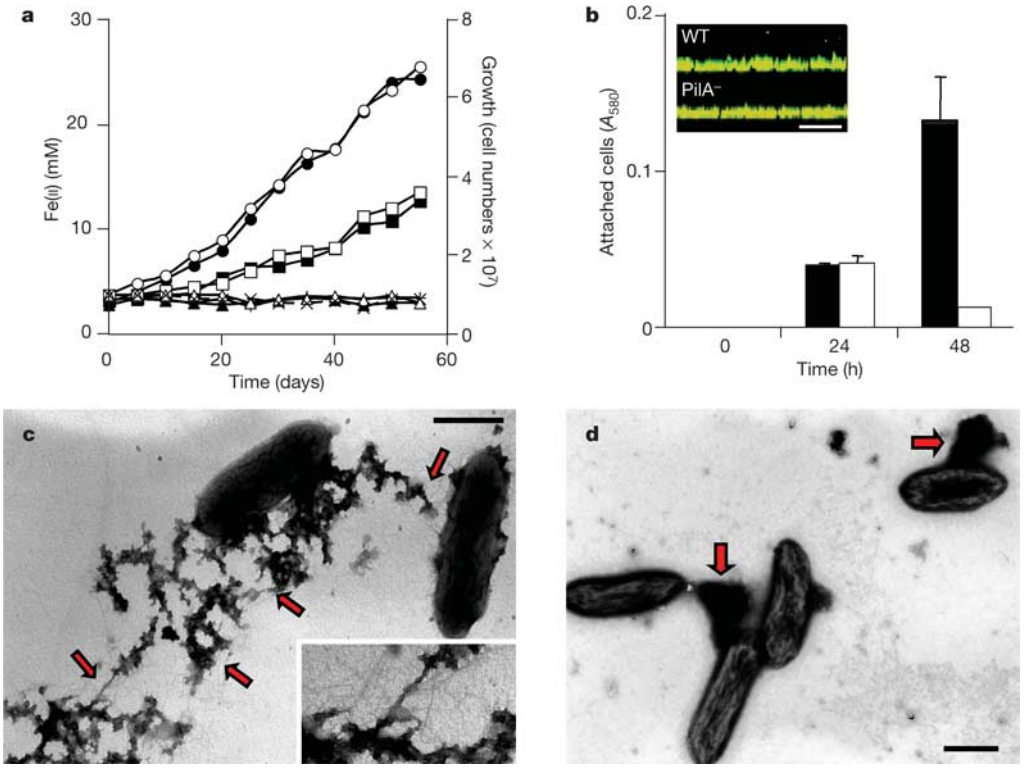

Figure 3 | Effect of a mutation in pilin production on reduction of Fe(III) oxide and attachment. **a**, Cells (open symbols) of the wild-type (circles), Δ*pilA* mutant (triangles) and complemented Δ*pilA* mutant (squares) strains and Fe(II) produced from Fe(III) reduction (filled symbols). Plus signs, Fe(II) in uninoculated medium; crosses, cells in uninoculated medium. **b**, Biomass of cells attached to Fe(III) oxide-coated coverslips over time; inset, confocal scanning laser microscopy of biomass attached in the first 24 h to the Fe(III) oxide, which is at the bottom of each image. Scale bar, 20 μm. Solid bars, wild type (WT); open bars, Δ*pilA* mutant (PilA$^-$). Error bars show s.d. **c**, **d**, Transmission electron micrographs of fumarate-grown WT (**c**) and PilA$^-$ (**d**) cells amended with Fe(III) oxides (indicated by arrows). Scale bars, 0.5 μm. Inset in (**c**) shows WT pili intertwined with Fe(III) oxides.

reducers[1,3]. However, the outer-membrane cytochrome model for Fe(III) reduction has serious limitations. For example, whereas *Geobacter* and *Desulfuromonas* species in the *Geobacteraceae* family of Fe(III)-reducing microorganisms contain abundant *c*-type cytochromes, no *c*-type cytochromes could be detected in *Pelobacter* species[4], which are phylogenetically intertwined with *Geobacter* and *Desulfuromonas* species[20,21]. Yet *Pelobacter* species—which, like *Geobacter* species, contain pili localized on one side of the cell—also are capable of reducing Fe(III) oxides[4].

The conductive pili provide the opportunity to extend electron transfer capabilities well beyond the outer surface of the cells, which might be especially important in soils in which Fe(III) oxides exist as

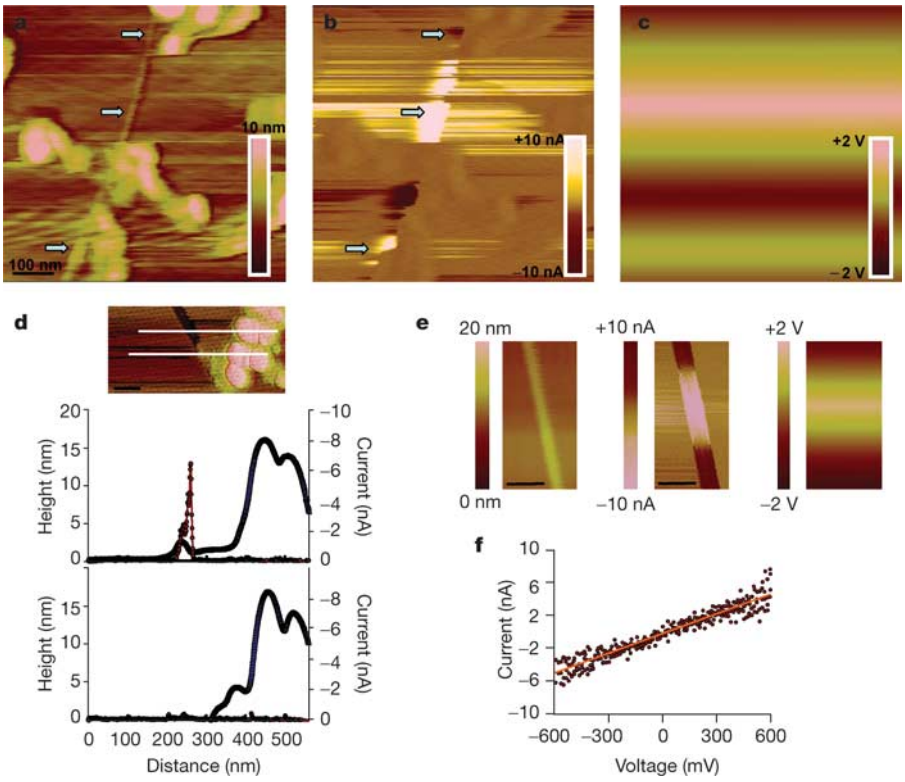

Figure 4 | Conducting-probe atomic force microscopy. **a**, Topography of a pilus (indicated by arrows) and non-pilin globular proteins. **b**, **c**, Current image (**b**) of the same field when a slow, triangular sweep bias voltage (**c**) was applied to the tip. **d**, Image showing both the topography and current of a pilus (top); the top and bottom white lines show the locations of the scans in the middle and bottom panels, respectively. Thick line (blue open circles where visible), height; red line, current. **e**, Results from disabling the slow axis to repeatedly scan horizontally across the same portion of a pilus filament. The apparent increased width of the pilus is an artefact of this form of scanning. **f**, Correspondence between current and applied voltage. Scale bars, 100 nm.

heterogeneously dispersed coatings on clays and other particulate matter. The pilus apparatus is anchored in the periplasm and outer membrane of Gram-negative cells, thus offering the possibility that pili accept electrons from periplasmic and/or outer membrane electron transfer proteins. These intermediary electron transfer proteins need not be the same in all organisms, which is consistent with the differences in cytochrome content and/or composition in different Fe(III) reducers[1]. The likely function of the pili is to complete the circuit between these various intermediary electron carriers and the Fe(III) oxide.

In addition to serving as a conduit for electron transfer to Fe(III) oxides, pili could conceivably be involved in other electron transfer reactions. For example, pili of individual *Geobacter* cells are often intertwined, raising the possibility of cell-to-cell electron transfer through pili. These biologically produced nanowires might be useful in nanoelectronic applications[22,23], with the possibility of genetically modifying pilin structure and/or composition to generate nanowires with different functionalities.

METHODS

Bacterial strains and culture conditions. All *G. sulfurreducens* strains were isogenic with the wild-type strain PCA (ATCC 51573). A PilA$^-$ mutant strain was generated by replacement of the +61 to +159 coding region of the *pilA* gene (GSU1496) with a chloramphenicol cassette, as described previously[9]. The *pilA* mutation was complemented *in trans* by introducing plasmid pRG5-*pilA*, a pRG5 derivative[24] carrying a wild-type copy of the coding region of *pilA*.

Cells were routinely cultured at 30 or 25 °C under strictly anaerobic conditions in freshwater medium supplemented with acetate as electron donor, with fumarate, Fe(III)-citrate or poorly crystalline Fe(III) oxides (100 mM) as the electron acceptor[25], and in the presence of chloramphenicol (15 $\mu g\,ml^{-1}$) or spectinomycin (150–300 $\mu g\,ml^{-1}$) for cultures of the PilA$^-$ and pRG5-*pilA* strains, respectively. Rates of Fe(III) oxide reduction were determined by measuring the production of Fe(II), and growth was determined as cell counts of acridine-orange-stained cells. For attachment assays, cells were grown in the presence of coverslips coated with Fe(III) oxide; the attached biomass was determined with crystal violet (see Supplementary Information).

Conducting-probe atomic force microscopy analyses. Pili and other outer-surface proteins that were sheared from the cell surface (see Supplementary Information) were left to adsorb for 20 min on the surface of freshly cleaved, highly oriented pyrolytic graphite, fixed with 1% glutaraldehyde for 5 min, washed twice with double deionized water and blotted dry. Samples were examined with a Veeco Dimension 3100 AFM equipped with a Nanoscope IV controller and a SAM III signal access module to enable electrical interfacing with the tip. A gold-coated AFM tip (nominal spring constant 0.06 $N\,m^{-1}$; Veeco Inc.) was used for the imaging. The AFM was operated in contact mode with simultaneous tip–substrate conductivity mapping. While imaging, a slow 'triangle-sweep' bias voltage was applied to the tip in reference to the graphite surface by using a low-noise battery-powered ramping circuit. Current was measured with a DL Instruments 1211 current preamplifier.

Received 31 October 2004; accepted 21 April 2005.

1. Lovley, D. R., Holmes, D. E. & Nevin, K. P. in *Advances in Microbial Physiology* Vol. 49 (ed. Poole, R. K.) 219–286 (Elsevier Academic, London, 2004).
2. Lovley, D. R. Cleaning up with genomics: applying molecular biology to bioremediation. *Nature Rev. Microbiol.* **1,** 35–44 (2003).
3. Richardson, D. J. Bacterial respiration: a flexible process for a changing environment. *Microbiology* **146,** 551–571 (2000).
4. Lovley, D. R., Phillips, E. J., Lonergan, D. J. & Widman, P. K. Fe(III) and S0 reduction by *Pelobacter carbinolicus*. *Appl. Environ. Microbiol.* **61,** 2132–2138 (1995).
5. Nevin, K. P. & Lovley, D. R. Lack of production of electron-shuttling compounds or solubilization of Fe(III) during reduction of insoluble Fe(III) oxide by *Geobacter metallireducens*. *Appl. Environ. Microbiol.* **66,** 2248–2251 (2000).
6. Childers, S. E., Ciufo, S. & Lovley, D. R. *Geobacter metallireducens* accesses insoluble Fe(III) oxide by chemotaxis. *Nature* **416,** 767–769 (2002).
7. Nassif, X. *et al.* Type-4 pili and meningococcal adhesiveness. *Gene* **192,** 149–153 (1997).
8. Doig, P. *et al.* Role of pili in adhesion of *Pseudomonas aeruginosa* to human respiratory epithelial cells. *Infect. Immun.* **56,** 1641–1646 (1988).
9. Coppi, M. V., Leang, C., Sandler, S. J. & Lovley, D. R. Development of a genetic system for *Geobacter sulfurreducens*. *Appl. Environ. Microbiol.* **67,** 3180–3187 (2001).
10. Methé, B. A. *et al.* Genome of *Geobacter sulfurreducens*: metal reduction in subsurface environments. *Science* **302,** 1967–1969 (2003).
11. Sahu, S. N. *et al.* The bacterial adaptive response gene, *barA*, encodes a novel conserved histidine kinase regulatory switch for adaptation and modulation of metabolism in *Escherichia coli*. *Mol. Cell. Biochem.* **253,** 167–177 (2003).
12. Strom, M. S. & Lory, S. Structure-function and biogenesis of the type IV pili. *Annu. Rev. Microbiol.* **47,** 565–596 (1993).
13. Childers, S. E., Mehta, T., Ciufo, S. & Lovley, D. R. *Abstracts 103rd General Meeting* 361 (American Society for Microbiology, Washington DC, 2003).
14. Parge, H. E. *et al.* Structure of the fibre-forming protein pilin at 2.6 Å resolution. *Nature* **378,** 32–38 (1995).
15. Alm, R. A. & Mattick, J. S. Genes involved in the biogenesis and function of type-4 fimbriae in *Pseudomonas aeruginosa*. *Gene* **192,** 89–98 (1997).
16. Aho, E. L., Murphy, G. L. & Cannon, J. G. Distribution of specific DNA sequences among pathogenic and commensal *Neisseria* species. *Infect. Immun.* **55,** 1009–1013 (1987).
17. Wall, D. & Kaiser, D. Type IV pili and cell motility. *Mol. Microbiol.* **32,** 1–10 (1999).
18. Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P. & Woodward, J. C. Humic substances as electron acceptors for microbial respiration. *Nature* **382,** 445–448 (1996).
19. Merz, A. J., So, M. & Sheetz, M. P. Pilus retraction powers bacterial twitching motility. *Nature* **407,** 98–102 (2000).
20. Holmes, D. E., Nevin, K. P. & Lovley, D. R. Comparison of 16S rRNA, *nifD*, *recA*, *gyrB*, *rpoB* and *fusA* genes within the family *Geobacteraceae* fam. nov. *Int. J. Syst. Evol. Microbiol.* **54,** 1591–1599 (2004).
21. Lonergan, D. J. *et al.* Phylogenetic analysis of dissimilatory Fe(III)-reducing bacteria. *J. Bacteriol.* **178,** 2402–2408 (1996).
22. Davis, J. J. Molecular bioelectronics. *Phil. Trans. R. Soc. Lond. A* **361,** 2807–2825 (2003).
23. Sayler, G. S., Simpson, M. L. & Cox, C. D. Emerging foundations: nano-engineering and bio-microelectronics for environmental biotechnology. *Curr. Opin. Microbiol.* **7,** 267–273 (2004).
24. Butler, J. E. *et al.* A single bifunctional enzyme for fumarate reduction and succinate oxidation in *Geobacter sulfurreducens* and *Geobacter metallireducens*. *J. Bacteriol.* (submitted).
25. Lovley, D. R. & Phillips, E. J. P. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl. Environ. Microbiol.* **54,** 1472–1480 (1988).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank T. Russell and D. Bryant for helpful suggestions. This research was supported by grants to D.R.L. from the Department of Energy's Genomics:GTL and NABIR programmes and DARPA, by a grant to M.T.T. from the National Science Foundation, and by a postdoctoral fellowship to G.R. from the Ministerio de Educación y Ciencia of Spain.

Author Information Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to D.R.L. (dlovley@microbio.umass.edu).