TABLE S1. **Primers used in this study.**

Name	Sequence	Enzyme
DNA-binding assays		
flhA-pro1	TTGTCCCTCTGGCACAATTC	
flhA-pro2	AGCTAACTAAAGTAGAAAC	
fgrM-DB1	TCTCATATGGAGACACCGAGGCTGCTCA	NdeI
fgrM-DB2	TCTAAGCTTTGCAAGAGACGTGGAAATTAC	HindIII
Integration of the *fgrM* gene		
fgrM-int1	TCTCATATGGAGACACCGAGGCTGCTCA	NdeI
fgrM-int2	TCTAAGCTTCTATGCAAGAGACGTGGAAATTAC	HindIII
RT-PCR		
fgrM-RTPCR1	GCGGTTGAAGCGGTCGAGGAA	
fgrM-RTPCR2	TCCGACTCCACCGTGCTCA	
flhA-RTPCR1	TTCGTTGCCGTGGAGGAGAA	
flhA-RTPCR2	ATGGCCAACACGGCGGTGGAC	
cheW1-RTPCR1	TTCACCCTCCATGTCCATAACCA	
cheW1-RTPCR2	ATGCAGAATGCACTGCAGACCA	
dcuB-RTPCR1	GGTGCCGACACAGACCGTCA	
dcuB-RTPCR2	ATGATGATGTTCTGGATCCA	
Interruption of the *fgrM* gene		
fgrM-int3	TCTCTCGAGTCCGACTCCACCGTGCTCA	NdeI
fgrM-int4	TCTAAGCTTGCGGTTGAAGCGGTCGAGGAA	HindIII
Construction of histidine kinase mutants		
GHK1-1	TCTCTAGACAGGTGTATGTGACCATC	XbaI
GHK1-2	TCGAATTCAGGAATTCGTTCATGTC	EcoRI
GHK1-3	TCTAAGCTTACCGAGCGGCTCATCCGAC	HindIII
GHK1-4	TCTCTCGAGTGGCTCTCCCTCGTGGATGGGAA	XhoI
GHK2-1	TCTCTAGAGCGGCTACAAGGTATTCA	XbaI
GHK2-2	TCGAATTCTGTTTTCAGGAGTAGCTCCA	EcoRI
GHK2-3	TCTAAGCTTCGAAGACCTGGGACCGCTCA	HindIII
GHK2-4	TCTCTCGAGTCCTCCCGTCGCGGCGTACGC	XhoI
GHK3-1	TCTCTAGAACATCGCCGTGCTCCCCA	XbaI
GHK3-2	TCGAATTCCGGTGGCACTGGATACC	EcoRI
GHK3-3	TCTAAGCTTGCAGCTGGCCAATGTGA	HindIII
GHK3-4	TCTCTCGAGTTGGGTTGTTCCGTCCTCTTTGTC	XhoI
GHK4-1	TCTCTAGACCGGGAAATCCGCGAGGCA	XbaI
GHK4-2	TCGAATTCTCCATGAGATGAATGCA	EcoRI
GHK4-3	TCTAAGCTTGTCAGGAGGTACGGCAGAC	HindIII
GHK4-4	TCTCTCGAGCCTCGCTTCGCCCCGGAGGGGAA	XhoI
GHK5-1	TCTCTAGATATCGTGACCAAGATGCA	XbaI
GHK5-2	TCGAATTCTGGAGAAAGAGCGAGA	EcoRI
GHK5-3	TCTAAGCTTCGGCCAGTCCCGCTACC	HindIII
GHK5-4	TCTCTCGAGGGTGGCTGCGCTGGGGAGGGCAA	XhoI
GHK6-1	TCTCTAGAACAGGAACTCGTCTGAA	XbaI
GHK6-2	TCGAATTCGAACGCCCTGCGCTTGAC	EcoRI
GHK6-3	TCTAAGCTTGGCCGTCAACTGGAGGCACA	HindIII

GHK6-4	TCTCTCGAGGCAGAGGGTGAAGTAAAAGGTA	XhoI
GHK7-1	TCTCTAGACCAGAGTATCCACCTGATC	XbaI
GHK7-2	TCGAATTCACCATTTTGACGATCAC	EcoRI
GHK7-3	TCTAAGCTTCGTCTCAATGCGCAAGCA	HindIII
GHK7-4	TCTCTCGAGCTCAAGCAGATTGAGGTAATCAA	XhoI
GHK8-1	TCTCTAGACTAGACCTGGCCTCGGGTA	XbaI
GHK8-2	TCGAATTCCTCTTCGCCGGTGTGGCTC	EcoRI
GHK8-3	TCTCTCGAGCCCGGTCAGAGTGAAATAAAACGTC	HindIII
GHK8-4	TCTAAGCTTGGAAGACTGCGTCGAACAA	XhoI
Sp-fwd	TCGAATTCGCACAGGATGACGCCTAAC	EcoRI
Sp-rev	TCTAAGCTTGAAGCGGCGTCGGCTTG	HindIII
In vitro phosphorylation assays		
GHK3-K1	TCTCATATGGCCAGCAACGTAACCGAGCGGA	NdeI
GHK3-K2	TCTCTCGAGTTGGGTTGTTCCGTCCTCTTTGTC	XhoI
GHK4-R1	TCTCATATGACCGAAAAGAAACTGCGAC	NdeI
GHK4-R2	TCTCTCGAGCTCGCGGATTTCCCGGTCCAC	XhoI
fgrM-R1	TCTCATATGGAGACACCGAGGCTGCTCA	NdeI
fgrM-R2	TCTAAGCTTGTGCTCGAGGCCCCGCTTCAC	HindIII

Recognition sequences for restriction enzymes are underlined.

TABLE S2. **Histidine kinases conserved in *Geobacter* species.**

Name	DL-1	Domain organization
GHK1	GSU0007	Unknown-PAS-HK
GHK2	GSU0008	REC-PAS-HK
GHK3	GSU0009	Unknown-PAS-HK
GHK4	GSU0010	REC-PAS-GAF-HK
GHK5	GSU0149	REC-HK
GHK6	GSU1043	Unknown-PAS-HK
GHK7	GSU1630	Unknown-PAS-HK
GHK8	GSU2565	HK

Homology search was conducted by NCBI/BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi/).
REC; receiver, HK; histidine kinase

FIGURE S1

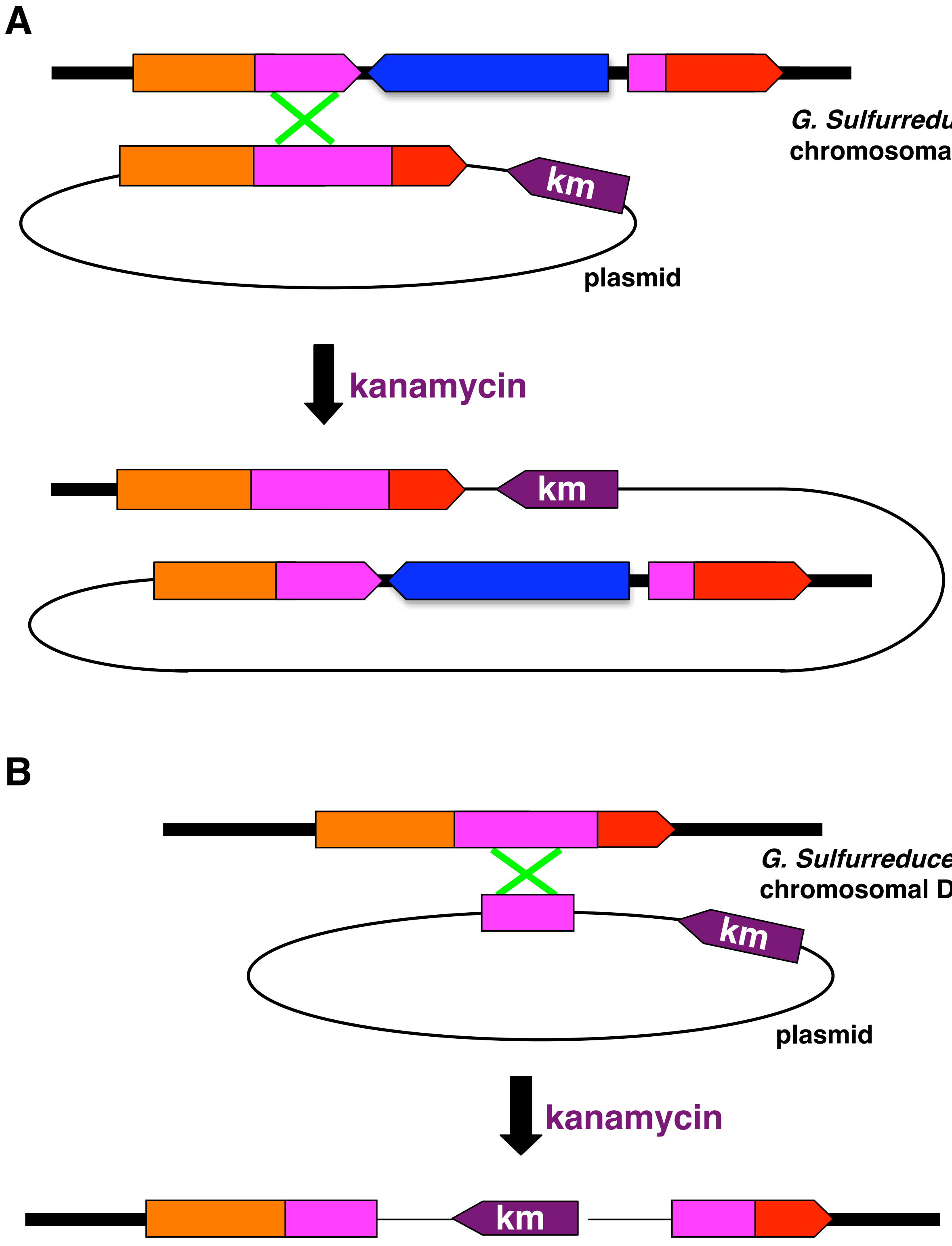

FIGURE S1. **Schemes for genetic manipulation.** A, Integration of the *fgrM* gene into *G. sulfurreducens* DL-1. B, Interruption of the *fgrM* gene in *G. sulfurreducens* KN400.

FIGURE S2

A

flagella	
flhA	C**TGGCAC**AACGG**TTGC**A
flgB	C**TGG**T**ACG**GCTT**TTGC**T
fliL	C**TGGCA**TTTCGG**TTGC**A
flgJ	T**TGGCAC**ATAACA**TGC**T
fliD	G**TGG**AG**C**CGGTT**TTGC**A
chemotaxis	
cheY1	C**TGG**A**AC**AAAGTA**TGC**A
mcp2	T**TGGC**TGAACAA**TTGC**A
others	
dcuB	T**TGG**T**A**TCCCCC**TTGC**T
pilA	T**TGG**TT**CG**GCTT**TTGC**T
fdnG	T**TGGCAC**AATGC**TTGC**C
nifH	T**TGGCA**T**G**GACGG**TGC**T
gnfK	C**TGGCA**T**G**CCTCG**TGC**C
CONSENSUS	**TGGCACG**NNNN**TTGC**
	-24 **-12**

B

fliC	TT**CTAAAG**C**TTT**TCCGGCTGCC**GCCGATA**C**GT**G
fliD	CA**CTAAAGTTTT**TCCCAGTCAG**G**T**CGATA**CA**T**A
flgJ	G**GCTAAAGTTTT**TCAGAAGTTG**GCCGATA**G**GT**G
flaG	T**GCTAAAGTTT**CCCTGCAGTCAT**CCGATA**T**GA**G
flbD	A**GCTAAAGTTTT**CCGGTCCGCG**GCCGA**A**A**C**GA**T
cheW1	TA**CTAAA**A**TT**A**T**GGACTCTTGC**GCCGAT**GA**GA**A
cheY4	T**GCTAAA**CGAAACGGGCAACCT**GCCGATA**T**GA**A
mcp1	A**GCTAAAGT**CACAGCCATTTTG**G**T**CGATA**CT**A**A
mcp3	T**G**T**TAAAG**G**T**C**T**GGGGAGGCCT**GCCGATA**CA**A**T
mcp6	CC**CTAAAGT**A**T**CGCCCTGATTGC**GCCGATA**ACT
mcp7	TA**CTAAAG**AAA**T**ATACATCTCTCT**CGATA**A**GT**A
mcp9	G**GCTAAA**C**T**A**T**CCGCCTTCCCC**GCCGATA**CA**T**T
mcp10	TA**CTAAA**CA**TTT**TCTTACGACA**GCCGATA**AA**T**T
mcp11	TC**CTAAAGTTT**AGTCGAACCGT**GCCGATA**G**G**GT
mcp13	GAT**TAAAGTTTT**TCGGGATTGT**GCCGA**A**A**TT**A**C
mcp15	AC**CTAAAGT**CAATCAGGAAAATC**CCGATA**T**G**GA
mcp18	G**GCTAAA**TA**T**A**T**TCACGTCTCA**G**T**CGAT**GA**GA**T
mcp20	GAT**TAAAGT**AA**T**CCGCGAATTT**G**T**CGATA**C**GA**T
mcp24	C**G**T**TAAAG**A**T**GGGTGGCGGGCC**G**T**CGA**A**A**GA**T**A
mcp32	CCT**TAAAGTT**A**T**TCGGGTATGCT**CCGATA**C**G**CA
mcp33	TCA**TAAAG**AA**T**CTGCTTTGGTT**GCCGAT**GTAGA
hrfP	T**GCTAAAGTT**TCCAACGGTTGT**GCCGATA**AC**T**T
Consensus	**GCTAAAGTTTT** **GCCGATA GA/T**

FIGURE S2. **Promoter elements in flagella-related genes in *G. sulfurreducens* DL-1.** A, RpoN-dependent promoter elements. Highly conserved nucleotides are indicated in bold letters. RpoN-dependent promoter elements of *flgB*, *fliL*, *dcuB*, *fdnG*, *nifH*, *gnfK*, and *pilA* in *G. sulfurreducens* DL-1 were previously reported (Juáez *et al*, 2009; Leang *et al*, 2009; Ueki and Lovley, 2010), while those of *flhA*, *flgJ*, *fliD*, *cheY1*, and *mcp2* were predicted on the basis of the conservation of sequences in *Geobacter* species. Most of the flagella-related genes shown here appear to be the first gene of the operon. B, FliA-dependent promoter elements. FliA-dependent promoter elements were predicted on the basis of the conservation of sequences in *Geobacter* species. Some of them were previously predicted (Tran *et al*, 2008).

FIGURE S3

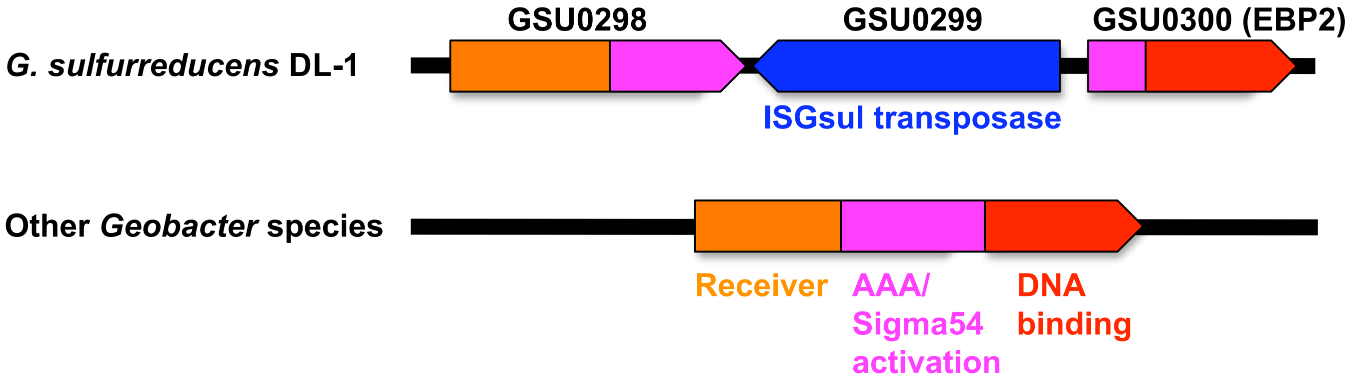

FIGURE S3. **Gene organization.** The gene encoding EBP2 in *G. sulfurreducens* DL-1 lacks a regulatory domain and most of a AAA/Sigma54 activation domain, while its homologues in other *Geobacter* species encode a typical enhancer-binding protein consisting of an N-terminal regulatory (receiver) domain, a central AAA/Sigma54 activation domain, and a C-terminal DNA-binding domain. In *G. sulfurreducens* DL-1, a gene encoding a regulatory domain and most of a AAA/Sigma54 activation domain that are homologous to those of the EBP2 homologues in other *Geobacter* species is located upstream of the gene encoding a transposase that is located upstream of the EBP2 gene.

FIGURE S4

```
GDL1(EBP2) --------------------------------------------------------------------------------
GDL1(0298) METPRLLIADDDKKTRDFVTAFLKYKGYDVVQAFDGQDALEKLEVEDVHLVITDLMMPRVNGLEFVKKLKAMRPGTVVIA
GKN400     METPRLLIADDDKKTRDFVAAFLKYKGYDVVQAFDGQDALEKLEVEDVHLVITDLMMPRVNGLEFVKKLKAMRPGTVVIA
Gmet       MDTPRLLIADDDKKTRDFVAAFLKYKGYDVVQACDGQDALDKLETEEVHLVITDLMMPRVNGLEFVKKLKTMRPGTVIIA
Gura       MDSARILIADDDKKTRDFVAAFLSYKGYQVFQASDGQDALEKIELNDVHMVITDIMMPRVNGLEFIKKLKAMRPEIVTIA
GFRC32     MESARILIADDDKKTRDFVAAFLSYKGYQVFQAGDGQDALEKIELNDVQMVITDIMMPRINGLEFIKKLKAMRPEIVTIA
Gbem       METPKILIADDDKKTRDFVAAFLSYKGYQVYQAFDGQDALQKIELHEVQMVITDIMMPRVNGLEFIKQLKSIRPDIVTIA
GM21       METPKILIADDDKKTRDFVAAFLSYKGYQVYQAFDGQDALQKIELHEVQMVITDIMMPRVNGLEFIKQLKSIRPDIVTIA
                                                                 *

GDL1(EBP2) --------------------------------------------------------------------------------
GDL1(0298) YSAFGNYEMTSNLLKAGVFFYLEKPFNLDELETHVKRGLEHQSLQSQTYRSKPALKSRSLIPNIIGESPRMLSLFELIEK
GKN400     YSAFGNYEMTSNLLKAGVFFYLEKPFNLDELETHVKRGLEHQSLQSQTYRSKPALKSRSLIPNIIGESPKMLSLFELIEK
Gmet       YSAFGNYEMASNLLKAGVFFYLEKPFNLDELETHVKRGFEHQSLQSQTYRAKPALKNRALMPNIIGESSKMLSLFELIEK
Gura       YSAFANFEMTANLLKAGAFFYLEKPFNLEELETHVKRGLEHQALQRQSFKSKPCIKNRSLLNNIIGESDRMLSLFEMIEK
GFRC32     YSAFANYEMTANLLKAGAFFFLEKPFNLEELETHVKRGLEHQTLQRQSFKAKPCIKNRSLLNHIIGESEKMLSLFEMIEK
Gbem       YSAFANFEMTANLLKAGAFFYLEKPFNLEELETHVKRGLEHQALQSKSFRSKPCIKNRALLNNIIGESEKMLSLFEMIEK
GM21       YSAFANFEMTANLLKAGAFFYLEKPFNLEELETHVKRGLEHQALQSKSFRSKPCIKNRALLNNIIGESEKMLSLFEMIEK

GDL1(EBP2) --------------------------------------------------------------------------------
GDL1(0298) VAESDSTVLIQGESGTGKELVARAVHDLSNRKNRNFVPVNCAAIPDELLESELFGHVKGSFTGAIATRIGRFEMADKGTL
GKN400     VAESDSTVLIQGESGTGKELVARAVHDLSNRKNRNFVPVNCAAIPDELLESELFGHVKGSFTGAIATRIGRFEMADKGTL
Gmet       VAESDSTVLIQGESGTGKELVARAVHDLSNRKTRNFVPVNCAAIPDELLESELFGHVKGSFTGAVANRIGRFEMADRGTL
Gura       VAGSDSTVLIQGESGTGKELVARAIHDLSNRATKNFVPVNCAAIPDDLLESELFGHVKGSFTGAVATRIGRFEMADRGTL
GFRC32     VAGSDSTVLIQGESGTGKELVAKAIHDLSNRATKNFVPVNCAAIPDDLLESELFGHVKGSFTGAVATRIGRFEMADRGTL
Gbem       VATSDSTVLIQGESGTGKELVARAIHDLSNRSDKNFVALNCAAIPDELLESELFGHVKGSFTGAVASRIGRFEMADKGTL
GM21       VATSDSTVLIQGESGTGKELVARAIHDLSNRSDKNFVALNCAAIPDELLESELFGHVKGSFTGAVASRIGRFEMADKGTL

GDL1(EBP2) --------------------------------------------------------------------------------
GDL1(0298) FLDEIGDMKPNLQVKLLRVLQNRELEPVGATRSKKVDVRIIAATNQNLEKLVASKQFREDLFYRISVIPIFIPPLRDRRE
GKN400     FLDEIGDMKPNLQVKLLRVLQNRELEPVGATRSKKVDVRIIAATNQNLEKLVASKQFREDLFYRISVIPIFIPPLRDRRE
Gmet       FLDEIGDMKPNLQVKLLRVLQNRELEPVGATRSKKIDVRIIAATNQNLEKLVATKQFREDLYYRLSVIPIFIPPLRERKE
Gura       FLDEIGDMKPNLQVKLLRVLQNKELEPVGAARTKKVDVRIIAATNQNLEHLVASKIFREDLYYRLSVIPITLPPLRERKT
GFRC32     FLDEIGDMKPNLQVKLLRVLQNKELEPVGAARTKKVDVRIIAATNQNLEHLVASKIFREDLYYRLSVIPIMLPPLRERKS
Gbem       FLDEIGDMKANLQVKLLRVLQSRELEPVGSTRSKKVDVRIIAATNQNLDHMVASKEFREDLYYRLSVIPIMLPPLRERGS
GM21       FLDEIGDMKANLQVKLLRVLQSRELEPVGSTRSKKVDVRIIAATNQNLDHMVASKEFREDLYYRLSVIPIMLPPLRERGS

GDL1(EBP2) MGQQWVNTFLDRFNRNKKSKVKGIDADAMEILCGYDWPGNVRELENLVERLVILKGFGTLGVKDLPEKYTGVSLSA--PS
GDL1(0298) DIPLLVNTTAVP--------------------------------------------------------------------
GKN400     DIPLLVNTFLDRFNRNKKSKVKGIDADAMELLCGYDWPGNVRELENLVERLVILKGFGTLGVKDLPEKYTGVSLSA--PS
Gmet       DIPLLVNSFLERFNRNKKSKVKGIDSDAMDLLVHYDWPGNVRELENLVERLVILKGFGAIGVNDLPEKFSGVTISP--PS
Gura       DIPLLLNNFLEKFNRNKQRKVQSFDKNTMDILCNFDWPGNVRELENLVERLVILKGSGTITFQDLPEKYRGIKSAP--KS
GFRC32     DVPLLINNFLEKFNKNKQRKVQSFDKSTMDILCNYDWPGNVRELENLVERMVIIKGSGTILFNDLPEKYRGIKSAG--KS
Gbem       DVPLLLNSFLEKFNRSKQRKVQGFDKQVMEILSNYDWPGNVRELENLVERLVIIKTTGVISIHDLPEKYRGSRTTTVLHG
GM21       DVPLLLNSFLEKFNRSKQRKVQGFDKQVMEILSNYDWPGNVRELENLVERLVIIKTTGVISIHDLPEKYRGGRTTTVLHG

GDL1(EBP2) ETLALPDAGICLNTVVEEFENNLILQALKKTGGNKKEAALLLNLKRTTLIEKLKKRKLDVISTSLA
GDL1(0298) --------------------------------------------------------------------
GKN400     ETLALPDAGICLNTVVEEFENNLILQALKKTGGNKKEAALLLNLKRTTLIEKLKKRKLDVISTSLA
Gmet       ESMTLPDSGICLNTVVEEFENNLILQALKKTGGNKKEAALLLNLKRTTLIEKLKKRRLDTIATSFA
Gura       ERLTLPDTGFCLNSAVEDFENQLILQALEKTGGNKKEAATLLNLKRTTLIEKLKKKKLVFNDVSLSGS
GFRC32     EQLTLPDSGFCLNSAVEDFENRLILQALEKTGGNKKEAANLLNLKRTTLIEKLKKKKLLFNDASFSSS
Gbem       EPMTLPDDGFCLNSAVEEFENRLILQALEKSGGNKKEAAELLNLKRTTLIEKLKKKKLVYGEGSATP
GM21       EPMTLPDDGFCLNSAVEEFENRLILQALEKSGGNKKEAAELLNLKRTTLIEKLKKKKLVYGEGSATP
```

FIGURE S4. **Alignment of EBP2 homologues.** The putative phosphorylation site (D, Asp) is indicated in red with an asterisk. GDL1; *G. sulfurreducens* DL-1, GKN400; *G. sulfurreducens* KN400, Gmet; *G. metallireducens*, Gura; *G. uraniireducens*, GFRC; *Geobacter* sp. FRC-32, Gbem; *G. bemidjiensis*, GM21; *Geobacter* sp. M21.

FIGURE S5

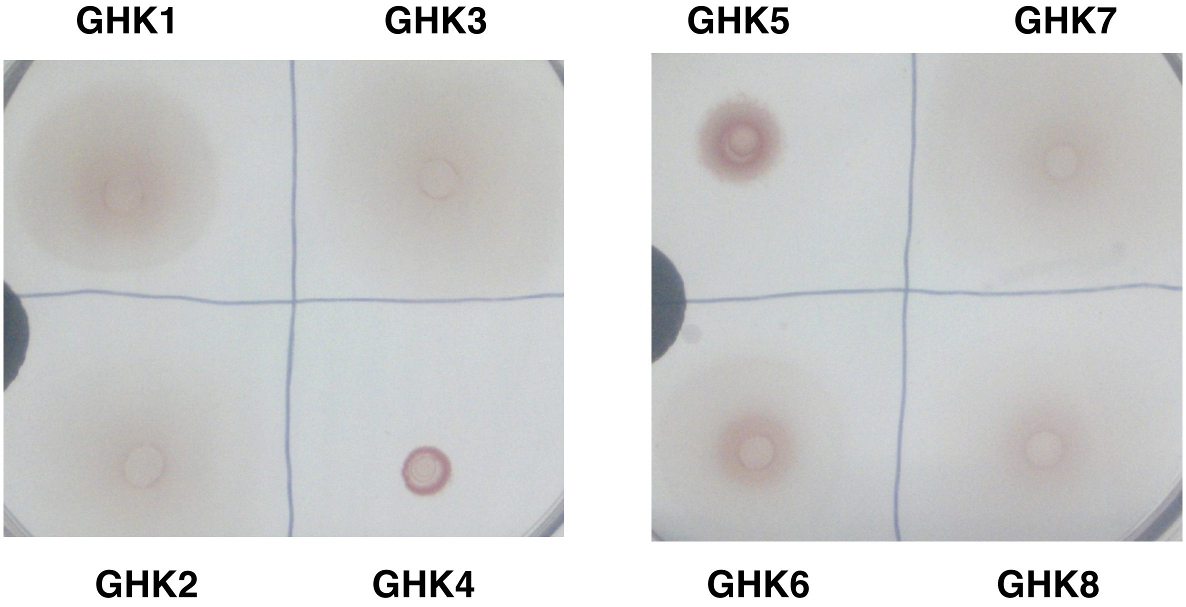

FIGURE S5. **Motility of mutants of histidine kinases conserved in *Geobacter* species.** Mutants were spotted on agar (0.3%) plates containing the NBAF medium and incubated for an extended period (8days).

FIGURE S6

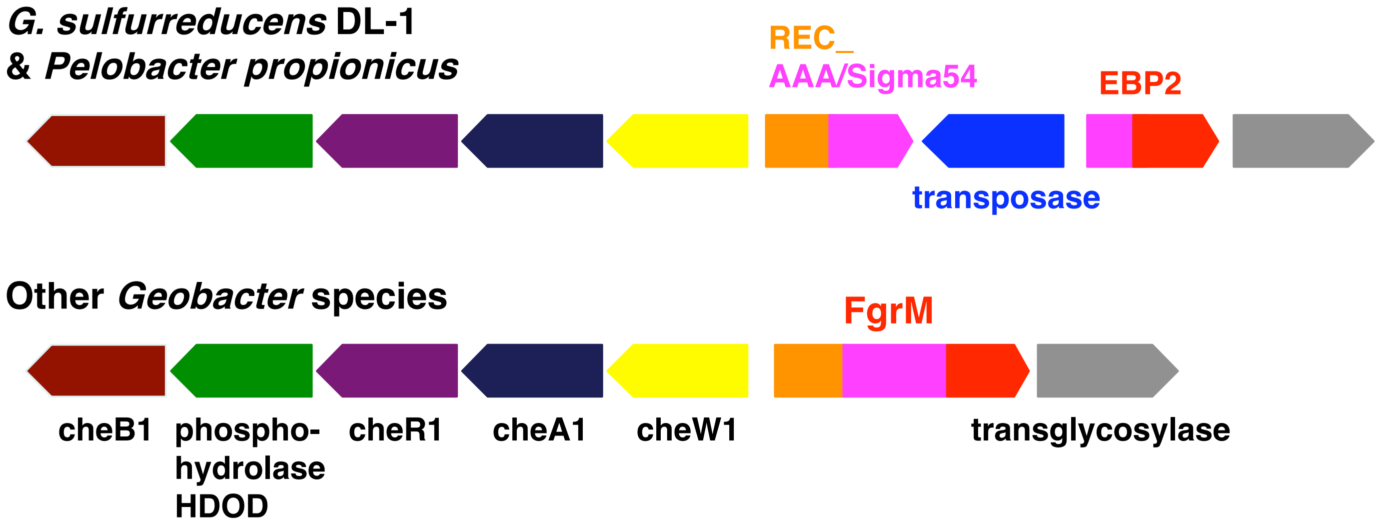

FIGURE S6. **Arrangement of genes surrounding the *fgrM* gene.** Genes surrounding the *fgrM* gene are conserved in *Geobacter* species. The same arrangement with that in *G. sulfurreducens* DL-1 is found in *Pelobacter propionicus*.

Supplementary References

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