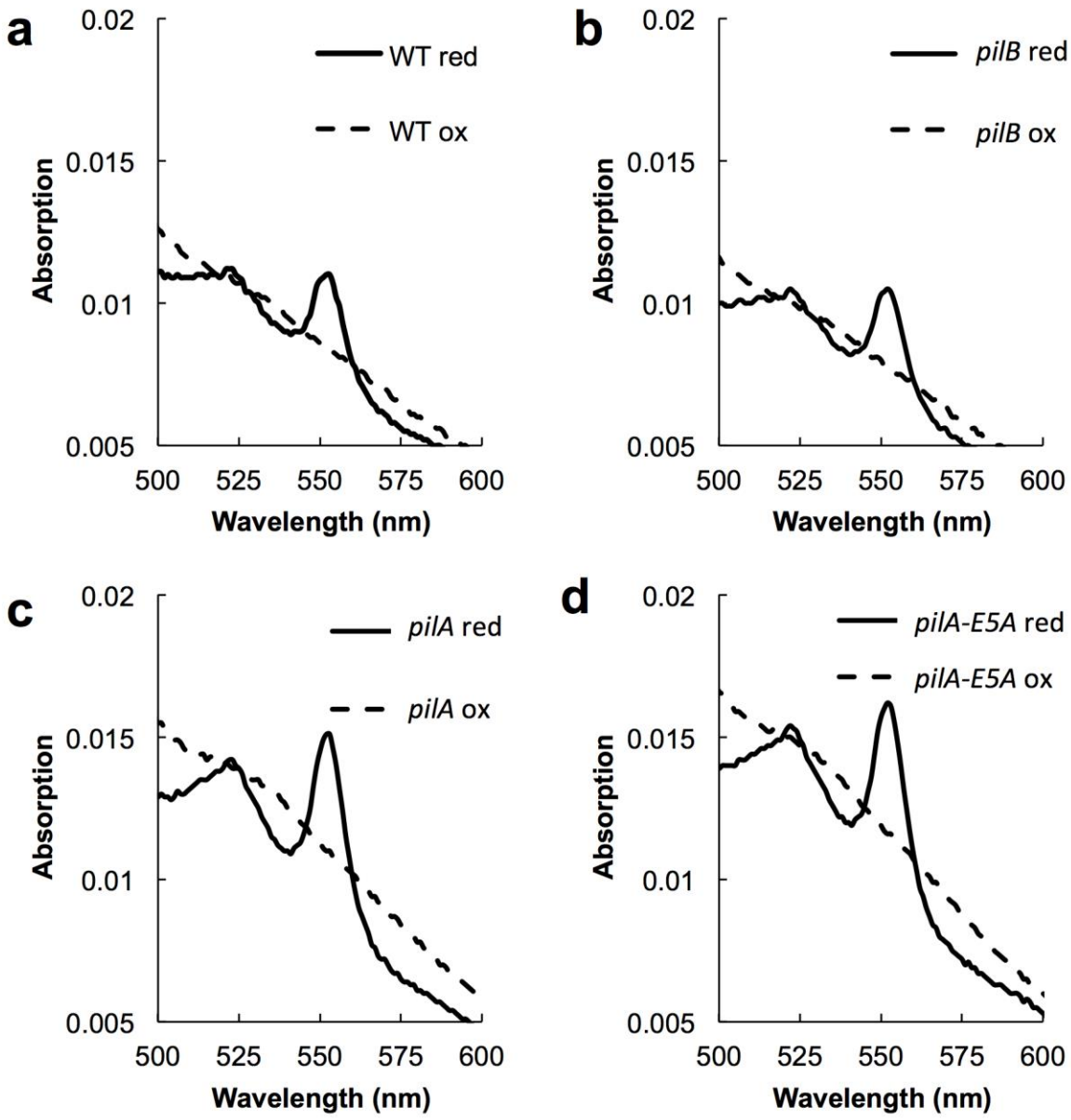
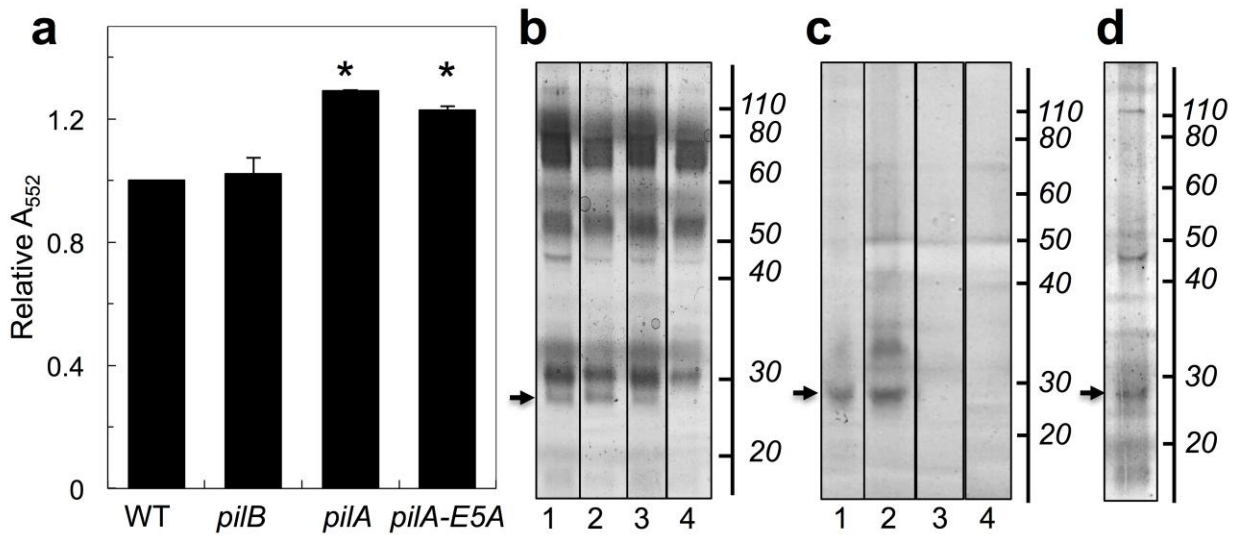
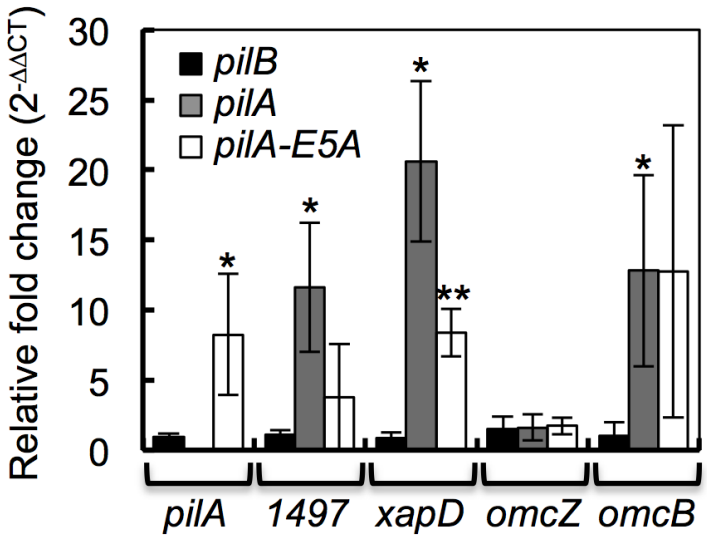


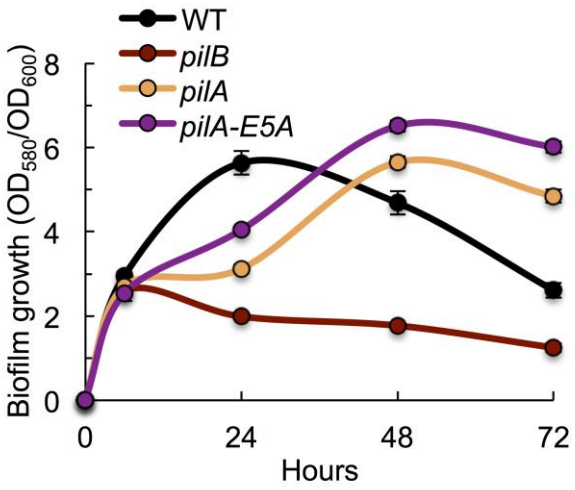
Supplementary Fig. 1: Representative absorption spectra of WT (**a**), *pilB* (**b**), *pilA* (**c**), and *pilA-E5A* (**d**) oxidized (dashed) and dithionite-reduced (solid) biofilm cell extracts, which were used to estimate their cytochrome content.



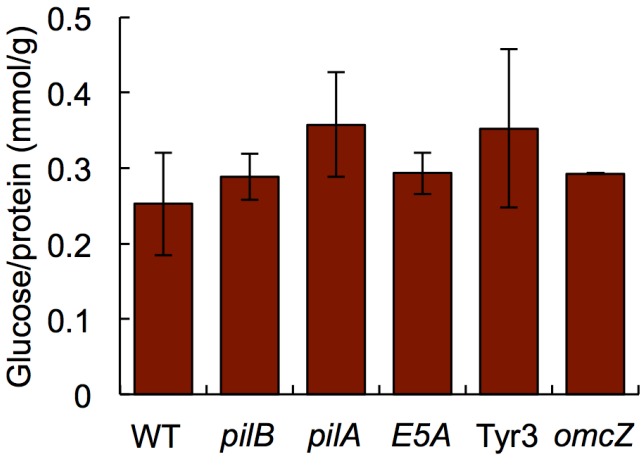
Supplementary Fig. 2: Cytochrome expression in planktonic cells (a-b) and biofilms (c-d) of the WT and pili-deficient mutants. (a) Cytochrome content (total cellular heme, measured with the pyridine hemochrome method) of planktonic cells of the pili-deficient strains *pilB*, *pilA*, and *pilA-E5A* relative to the WT. Shown are average and standard error of duplicate samples for each strain. Significant changes relative to the WT values were identified in *t*-test pairwise comparisons and are indicated with a star (* p <0.05) (**b**) Heme-stained proteins in whole-cell extracts of planktonic cells of WT (lane 1), *pilB* (lane 2), *pilA*, (lane 3), and *omcZ* (lane 4), which was used as negative control lane for the ~ 30-kDa $OmcZ_S$ band (arrow). (**c-d**) Heme-stained proteins in the biofilm matrix of the WT, *pilB*, *pilA*, and *pilA-E5A* (lanes 1 to 4, respectively, in panel **c**), and Tyr3 (panel **d**). The arrows point at the migration of the $OmcZ_S$ band.



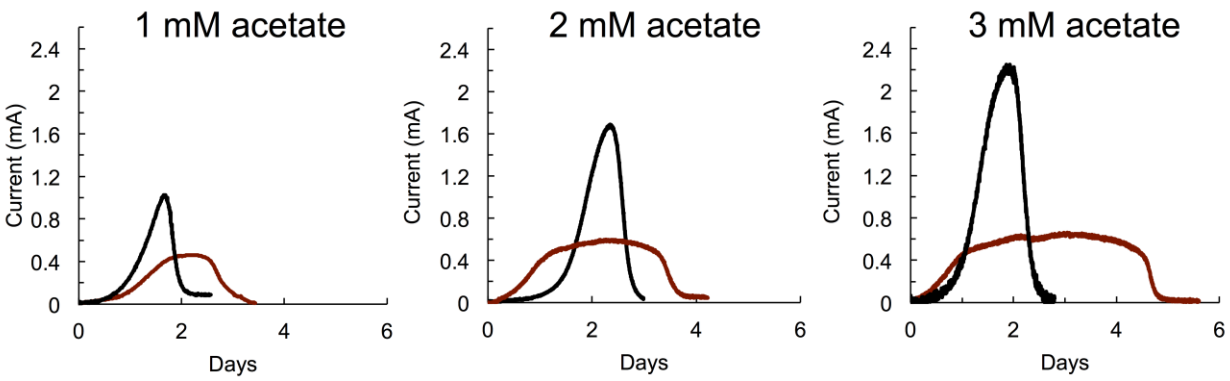
Supplementary Fig. 3: Expression of genes in the pilin operon (*pilA* and GSU1497), EPS synthesis (*xapD*), and outer membrane *c*-type cytochromes (*omcZ* and *omcB*) in 48-h old biofilms formed by the pili-deficient strains *pilB* (black), *pilA* (gray) and *pilA-E5A* (white) compared to WT. The constitutive gene *rpoD* was used as internal control. Data shown are average and standard deviation of four replicates. Statistically significant changes relative to the *pilB* values were identified in *t*-test pairwise comparisons and are indicated with one or two stars (* p <0.05; ** p <0.005)



Supplementary Fig. 4: Time course biofilm formation assay showing biofilm growth (OD_{580} of solubilized crystal violet associated with the biofilm normalized by OD_{600} of the culture) of the WT, *pilB*, *pilA*, and *pilA-E5A*. Each time point shows the average and standard deviation of data collected for six replicate biofilm samples per strain.



Supplementary Fig. 5: EPS content (as glucose equivalents, in mmol, per mg of cell protein) of biofilms of the WT, *pilB*, *pilA*, *pilA-E5A* (*E5A*), Tyr3, and *omcZ* strains. Shown are average and standard error of two independent experiments, each containing the matrix extracted from 48-h biofilm biomass collected from the wells of two 48-well plates.



Supplementary Fig. 6. Representative plots of current generation by WT (black) and *pilB* (maroon) in MECs operated in batch and fed an initial concentration of 1, 2, or 3 mM acetate.

Supplementary Table 1. Strains and plasmids used in this study.

Bacterial strain or plasmid	Relevant genotype and properties[a]	Source or reference(s)
Geobacter sulfurreducens		
WT	Wild type strain PCA	[1]
pilA	Δ*pilA*::*aaaC1*, Gm^r	This study
pilB	Δ*pilB*::*aaaC1*, Gm^r	This study
omcZ	Δ*omcZ*::*aaaC1*, Gm^r	This study
pilA-E5A	$pilA^{E5A}$::*aadA*, $Spec^r$	This study
Δ*pilB*	Δ*pilB*::*loxP*	This study
pilB+	Δ*pilB* complemented with pRG5-*pilB*	This study
pilB gspE	Δ*pilB* strain carrying the Δ*gspE*::*aaaC1* mutation, Gm^r	This study
pilB mshE	Δ*pilB* strain carrying the Δ*mshE*::*aaaC1* mutation, Gm^r	This study
Tyr3	*pilA*Y27,32,57A::*aadA*, $Spec^r$	This study
Plasmids		
pCM351	Amp^r, Tet^r, Gm^r, ColE1 *ori*, *oriT*	[2]
pCM158	Km^r, *trfA*, *oriT*, *oriV*, ColE1 *ori*, *cre*	[2]
pCR2.1-TOPO	Amp^r, Km^r, ColE1 *ori*	Invitrogen
pRG5	Shuttle vector for *G. sulfurreducens* Spc^r, P_{taclac}	[3]
pRG5-*pilB*	pRG5 with *G. sulfurreducens pilB*	This study

Supplementary Table 2. Primers used in this study.

Primer	Sequence (5'-3')	Amplification	Use
RS1	GATCTGGTCGGATACAACACC	*pilB* upstream	*pilB*
RS2	TTATGCGGCCGCCATATGCATCTGCTAGCCTGCATAGACTCTCC	*pilB* upstream	*pilB*
RS3	GTGTTAACCGGTCATATGCAGTGGCTGACGACTAAACAAAATGCC	*pilB* downstream	*pilB*
RS4	CTCTTGTGAGGATGCAGGTAC	*pilB* downstream	*pilB*
RS5	ATGGACCTCGAAGCCTACCT	*pilA* upstream	*pilA*
RS6	TTATGCGGCCGCCATATGCATCTGAAGCATAAGTG	*pilA* upstream	*pilA*
RS7	GTGTTAACCGGTCATATGCACGCCCGAAAGTTAA	*pilA* downstream	*pilA*
RS8	TCCATGCATCATTTTCGATG	*pilA* downstream	*pilA*
RS9	ATATGGCGTTTACCGCAGAG	*omcZ* upstream	*omcZ*
RS10	TTATGCGGCCGCCATATGCAGCTCCGAAGAAAGTCAAACG	*omcZ* upstream	*omcZ*
RS11	GTGTTAACCGGTCATATGCAGATGCGCCAATCAGTACCTT	*omcZ* downstream	*omcZ*
RS12	CACAGCCAGGTACCATCTGA	*omcZ* downstream	*omcZ*
RS13	TTTCTCAGCAATCCATCGAG	*gspE* upstream	*pilB gspE*
RS14	TTATGCGGCCGCCATATGCAATCTGTTCCATGTCGTGTGC	*gspE* upstream	*pilB gspE*
RS15	GTGTTAACCGGTCATATGCAGTATGCCGACCTTCCGGTAT	*gspE* downstream	*pilB gspE*
RS16	AGTGATTTTGCTCCGAATGG	*gspE* downstream	*pilB gspE*
RS17	TTTCGGCCATGTACTCCTTT	*mshE* upstream	*pilB mshE*
RS18	TTATGCGGCCGCCATATGCATCCTTGACGATGCTTTCCAT	*mshE* upstream	*pilB mshE*
RS19	GTGTTAACCGGTCATATGCACTCCTCCCTGCACGGTTGA	*mshE* downstream	*pilB mshE*
RS20	CGACATCTTGTTCTCGTGGA	*mshE* downstream	*pilB mshE*
RS21	TGCATATGGCGGCCGCATAA	*aaaC1 loxP*	Excible Gm marker
RS22	TGCATATGACCGGTTAACAC	*aaaC1 loxP*	Excible Gm marker
RS23	GTGGTGAAGGGGTAGGTTGA	*pilA* upstream	*pilA::aadA*
RS24	GTTAGGCGTCATCCTGTGCTTAACTTTCGGGCGGATAGG	*pilA* upstream	*pilA::aadA*
RS25	CAAGCCGACGCCGCTTCTTGATTAAATACATACTGGAGG	*pilA* downstream	*pilA*-Sp
RS26	GCGACTTCCACTCGGTACC	*pilA* downstream	*pilA*-Sp
RS27	GCACAGGATGACGCCTAAC	*aadA*	Sp marker
RS28	GAAGCGGCGTCGGCTTG	*aadA*	Sp marker
RS29	GGTTTCACCCTTATC**GCG**CTGCTGATCGTCGTT	*pilAE5A::aadA*	*pilA-E5A*
RS30	AACGACGATCAGCAG**CGC**GATAAGGGTGAAACC	*pilAE5A::aadA*	*pilA-E5A*

RS31	TCCGCAGTTCTCGGCG**GCT**CGTGTCAA GGCGTAC	*pilAY27A::aadA*	Tyr3
RS32	GTACGCCTTGACACG**AGC**CGCCGAGA ACTGCGGA	*pilAY27A::aadA*	Tyr3
RS33	GTATCGTGTCAAGGCG**GCC**AACAGCG CGGCGTC	*pilAY32A::aadA*	Tyr3
RS34	GACGCCGCGCTGTT**GGC**CGCCTTGAC ACGATAC	*pilAY32A::aadA*	Tyr3
RS35	CCGCATTTGCTGATGATCAAACC**GCT**C CGCCCGAAAGTTAA	*pilAY57A::aadA*	Tyr3
RS36	TTAACTTTCGGGCGG**AGC**GGTTTGATC ATCAGCAAATGCGG	*pilAY57A::aadA*	Tyr3
RS37	CCAACACAAGCAGCAAAAAG	*pilA*	qPCR
RS38	GCAGCGAGAATACCGATGAT	*pilA*	qPCR
RS39	ATGGGTGGCAAGGACTTTAC	GSU1497	qPCR
RS40	ACACCCGGTTACCAGAAGAG	GSU1497	qPCR
RS41	GTCCAACAAAGGGAACTGCT	*xapD*	qPCR
RS42	CCTCCGCAGAGAGGTAATCA	*xapD*	qPCR
RS43	CACGAGCCTGACACTCACTC	*omcZ*	qPCR
RS44	AAGGTTGCTGACCTTGTTGG	*omcZ*	qPCR
RS45	GACACGGTCAACCAGAACAA	*omcB*	qPCR
RS46	GGTCCCAGTTTACGACAGGA	*omcB*	qPCR
RS47	AGTTCTCGACGTACGCCACT	*rpoD*	qPCR
RS48	TCAGCTTGTTGATGGTCTCG	*rpoD*	qPCR

Supplementary References

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2 Marx, C. J. & Lidstrom, M. E. Broad-host-range *cre-lox* system for antibiotic marker recycling in gram-negative bacteria. *Biotechniques* **33**, 1062-1067 (2002).

3 Kim, B. C. *et al.* OmcF, a putative *c*-type monoheme outer membrane cytochrome required for the expression of other outer membrane cytochromes in *Geobacter sulfurreducens*. *J. Bacteriol.* **187**, 4505-4513 (2005).