Supplemental Material

Table S1. Oligonucleotides used in this study. Boldface: annealing nucleotides, italicized: restriction sites, capital letters: Shine-Dalgarno or His-tag sequences.

Primer	Sequence
bamY_Bam+SDGm_for	tcccgg*ggatcc*TAACTAGCTAGCCGGAGGTCCACA**atgcc ctacaatctcaacctgc**
bamY_Hind+cHis_rev	cagtgcc*aagctt*tcaGTGATGGTGATGGTGATG**ggccacgtgc tgcaactc**
Gm_act-pET/D_for	cacc**atgaacaaccaacttgttgagc**
Gm_act-pET/D_rev	**tttgaatcgtgactccattcgg**
bamYDelHind_for	catccc*aagctt***ctgcctcaacaccatgaacc**
bamYDelXba_rev	ggctag*tctaga***atcccgtcgagaatgtccag**
R24	**agcggataacaatttcacacagga**
F24	**cgccagggttttcccagtcacgac**
Sm_for_BglII	ggatca*agatct***ttgaatcgaactaatatttttttgg**
Sm_rev_BglII	gtgatc*agatct***ctagtatgacgtctgtcgcac**

Fig. S1. Growth of *G. metallireducens* on solid medium. Single colonies formed after 4-6 days on agar containing mineral medium under a N_2/CO_2 atmosphere (80:20, by vol.) with benzoate as electron donor and nitrate as electron acceptor.

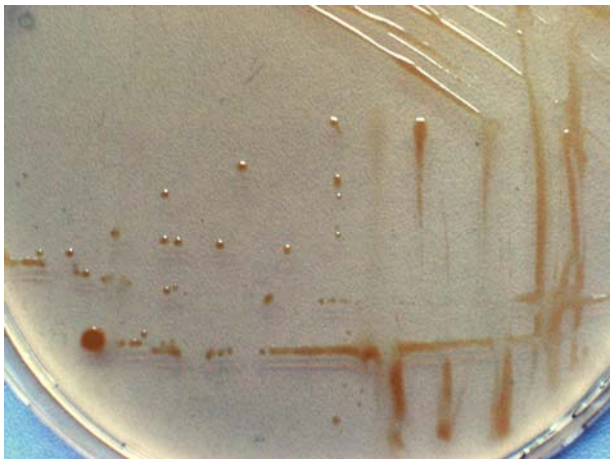


Fig. S2 SDS-PAGE analysis of homologous *bamY* expression. A Cell extracts of *G. metallireducens* grown on acetate/nitrate with (+) and without (-) plasmid pCD*bamY* carrying *bamY* for homologous expression. **B** Purified His-tagged BamY after homologous expression.

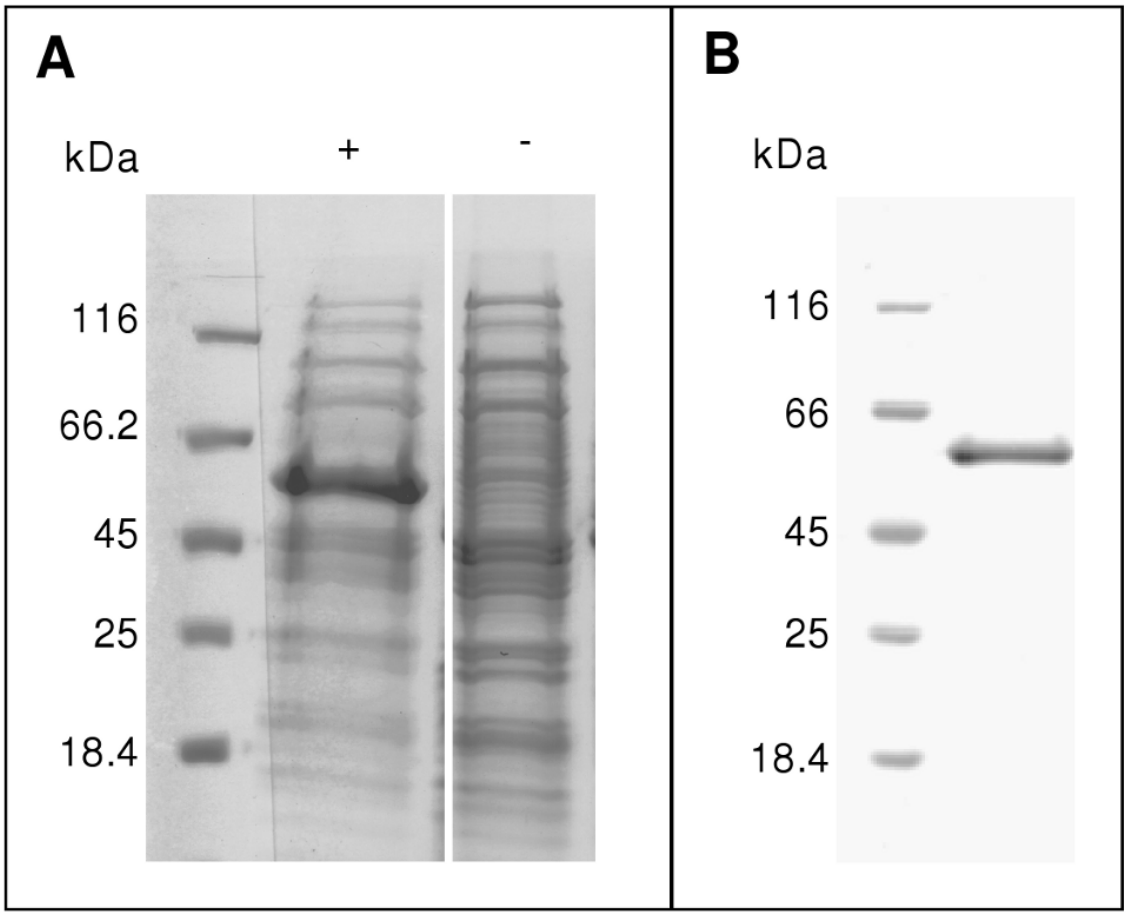
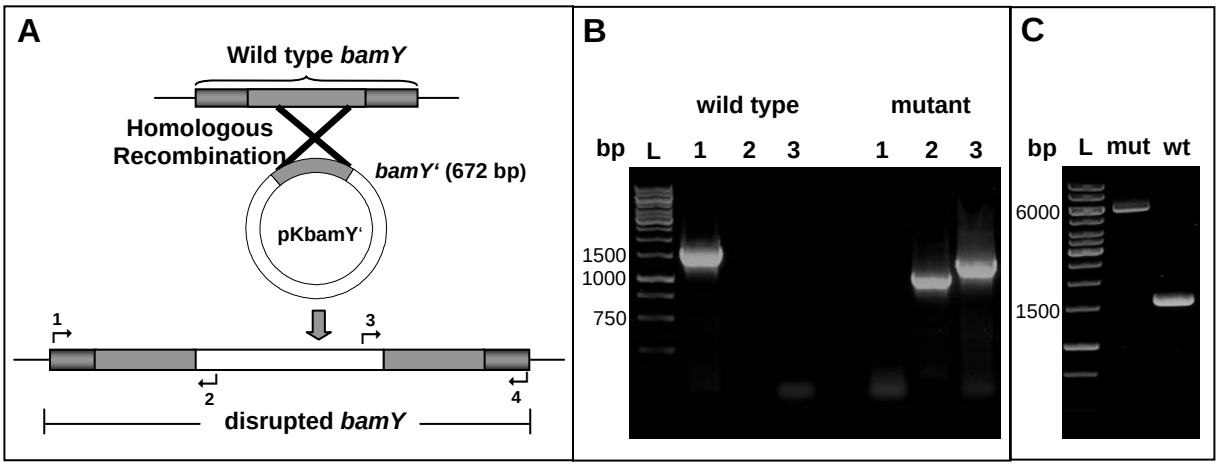


Fig. S3 Disruption of *bamY* via homologous recombination and PCR analysis of the resulting *bamY::kan* mutant. A A central 672 bp fragment of *bamY* (*bamY'*) was inserted into plasmid pK18*mob* which subsequently recombined with the *G. metallireducens* chromosome resulting in the insertion of the plasmid into *bamY*. Arrows and numbers indicate primers used for control PCRs for verifying correct gene disruption. **B** Agarose gels of control PCR products: L, DNA ladder; **1**, full-length wild type *bamY* (1.5 kb) using primers P1 and P4; **2**, PCR-product obtained with primers P1 and P2, and, **3**, P3 and P4. **C** Agarose gel of Phusion™-PCRs amplifying full length *bamY* using genomic DNA from the mutant strain (mut) and the wild type (wt). The sizes of the PCR products differed by the size of the plasmid integrated in the mutant strain. For primers used see Table S1 in the supplemental data.



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