SUPPORTING INFORMATION

Anaerobic Mercury Methylation and Demethylation by *Geobacter bemidjiensis* Bem

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1. Methylmercury species distributions during Hg(II) methylation assay

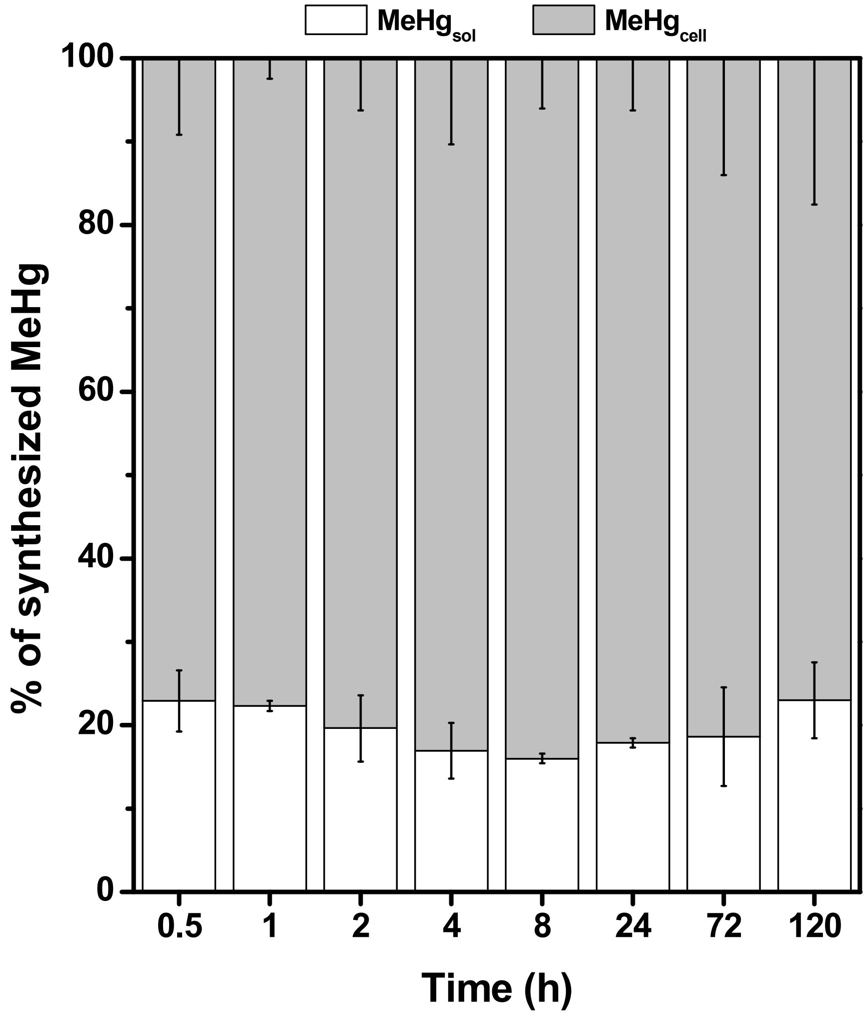


Figure S1. Methylmercury (MeHg) species distribution, expressed as the percentage of the total synthesized MeHg, during Hg(II) methylation assays with washed cells of *G. bemidjiensis* Bem (5×10^8 cell mL^{-1}). Soluble MeHg (MeHg$_{sol}$) were analyzed after removing cells with the 0.2-µm syringe filter, whereas the cell sorbed or associated MeHg (MeHg$_{cell}$) were determined by the difference between total MeHg and the soluble MeHg$_{sol}$ (see Figure 3a for additional details).

2. Elemental Hg(0) production during methylmercury degradation assays

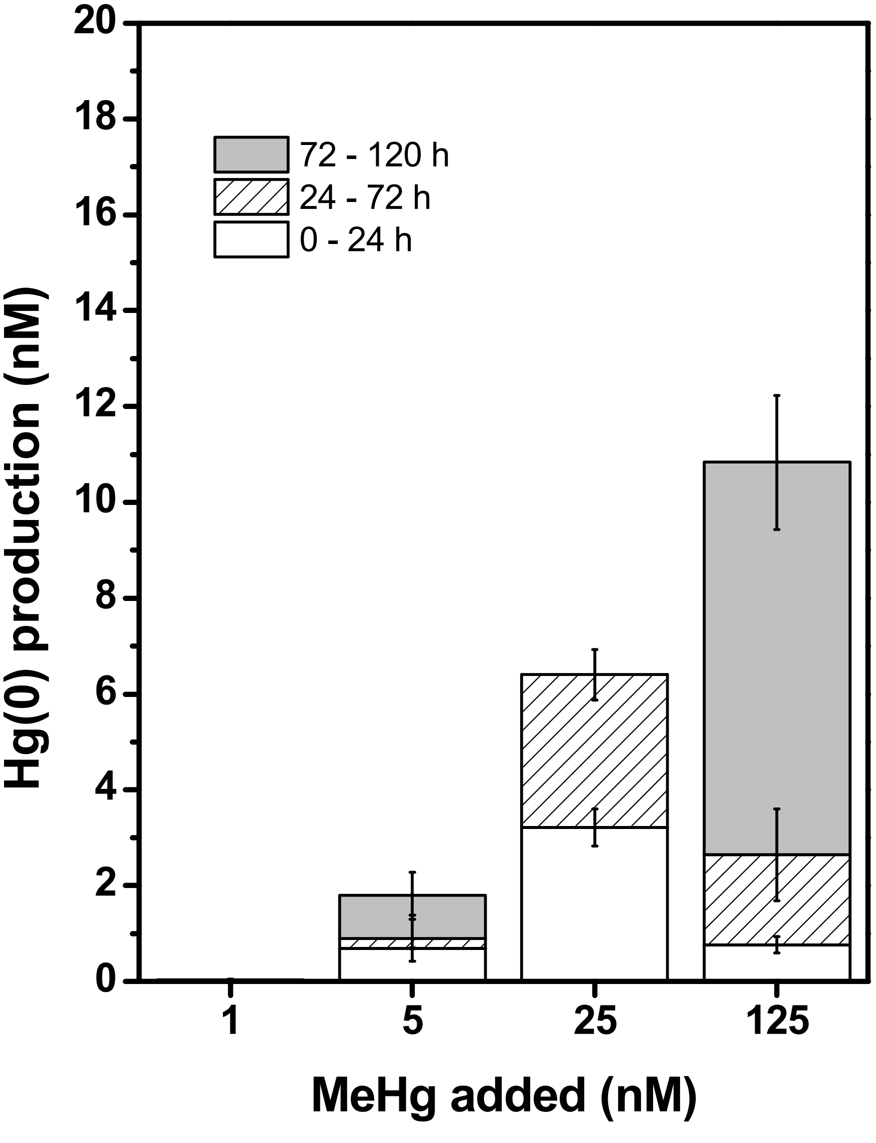


Figure S2. Cumulative production of Hg(0) (at 24, 72, and 120 h) during methylmercury (MeHg) degradation assays at varying MeHg concentrations (5, 25, and 125 nM) with washed cells of *G. bemidjiensis* Bem (5×10^{8} cell mL^{-1}) in MOPS buffer (pH 6.8) (see Figure 4b and text for additional details). Data points represent an average of two independent batch experiments, and error bars represent one standard deviation from 2–3 replicate samples.

Table S1: Template sequences used in BLAST searches. A. Organomercurial lyase MerB from plasmid pDU1358, Genbank accession number AAA88369; B. Mercuric reductase MerA from plasmid pDU1358, Genbank accession number ADM52740.

A.

MKLAPYILERLTSVNRTNGTADLLVPLLRELAKGRPVSRTTLAGILDWPAERVAAVLEQ
ATSTEYDKDGNIIGYGLTLRETSYVFEIDDRRLYAWCALDTLIFPALIGRTARVSSHCAAT
GAPVSLTVSPSEIQAVEPAGMAVSLVLPQEAADVRQSFCCHVHFFASVPTAEDWASKHQ
GLEGLAIVSVHEAFGLGQEFNRHLLQTMSSRTP

B.

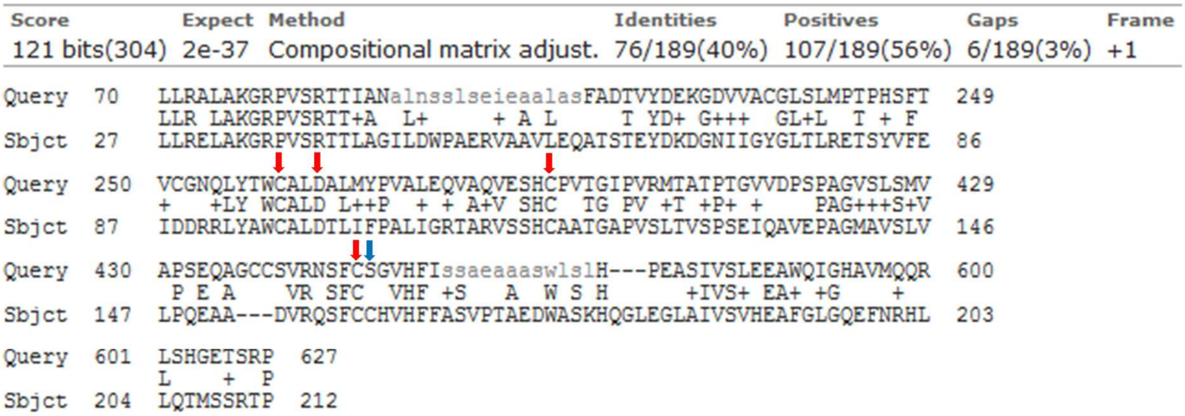
MTHLKITGMTCDSCAAHVKEALEKVPGVQSAIVSYAKGAAQLALDPGTAPDALTAAVA
GLGYKAMLADAPPTDNRTGLFDKVRGWMGAADKGSGGERPLQVAVIGSGGAAMAAA
LKAVEQGAQVTLIERGTIGGTCVNVGCVPSKIMIRAAHIAHLRRESPFDGGMPPTPPTILR
ERLLAQQQARVEELRHAKYEGILDGNSAITVLHGEARFKDDQSLIVSLNEGGERVVMFD
RCLVATGASPAMPPIPGLKESPYWTSTEALVSDTIPERLAVIGSSVVALELAQAFARLGS
QVTILARNTLFFRDDPSIGEAVTAAFRAEGIKVLEHTQASQVAHVNGEFVLTTGHGEVR
ADKLLVATGRTPNTRSLALDAAGVTVNAQGAIVIDKGMRTSTPHIYAAGDCTDQPQFV
YVAAAAGTRAAINMTGGDAAINLTAMPAVVFTDPQVATVGYSEAEAHHDGIETDSRTL
TLDNVPRALANFDTRGFIKLVIEEGSGRLIGVQVVAPEAGELIQTAVLAIRNRMTVQELA
DQLFPYLTMVEGLKLAAQTFTKDVKQLSCCAG

Table S2. Nucleotide sequence of *merB* in *G. bemijiensis* Bem strain used in the present study. NCBI reference sequence WP_012528757, Genbank accession number CP001124.

ATGGCAGGAGGCACTTTACACGATGTACTGGCTGGCTTGCACTGTGACCACCCACGG
GTCTGGTTGTTCCTCCTCCGTGCTCTGGCGAAGGGGCGGCCTGTTTCCCGCACCACT
ATAGCGAACGCTCTTAACAGTTCTCTCTCCGAGATTGAGGCGGCGTTGGCGTCCTTT
GCCGACACGGTGTACGACGAGAAAGGCGACGTGGTGGCCTGCGGGTTGTCGCTCAT
GCCTACGCCGCATTCCTTTACGGTCTGCGGCAATCAGCTTTACACCTGGTGTGCCCT
GGATGCCCTCATGTATCCCGTGGCGCTGGAACAGGTCGCACAGGTGGAATCGCATTG
CCCGGTTACGGGCATCCCGGTTCGTATGACGGCAACTCCGACGGGGGTCGTCGATCC
GTCTCCGGCTGGAGTTAGCCTTTCGATGGTTGCACCTTCAGAGCAAGCCGGCTGCTG
CAGCGTCCGTAACAGCTTTTGCAGTGGGGTGCACTTCATAAGTTCCGCTGAAGCTGC
AGCTTCGTGGCTGTCTCTGCATCCCGAAGCAAGCATAGTGTCGTTGGAAGAAGCGTG
GCAGATCGGACATGCAGTAATGCAGCAACGACTGTCGCATGGAGAGACGTCACGCC
CTTGA

METHODS: Genomic DNA of *Geobacter bemidjiensis* Bem was extracted using Ultra-clean™ microbial DNA Isolation Kits (MoBio Laboratory, USA) according to the manufacturer's protocol.[1] The *merB* gene was amplified using forward primer (5’-ATGGCAGGAGGCACTTTACACG-3’) and reverse primer (5’- TCAAGGGCGTGACGTCT CTCCATGCGACAGTCGTTGC-3’). The PCR reaction mixture (25 µl) consisted of 12.5 µl premix (Takara Bio Inc., Japan), 0.5 µl each of 10 µM primer pair, 2 µl DNA template (1-10 ng), and 9.5 µl PCR grade water. Thermal-cycling conditions were set as follows: 5 min initial denaturation at 95°C, 35 cycles at 95°C (for 15 s) and 59°C (for 20 s), followed by a final elongation at 72°C for 10 min. PCR products were purified with PCR Clean-Up System (Promega, USA)[2] and subsequently sequenced using an ABI 3700 sequencer (Applied Biosystems, USA).

Table S3: BLASTX output showing sequence identity between the translated *G. bemidjiensis* Bem sequence (Query, WP_012528757.1) and the organomercurial lyase MerB encoded by pDU1358 (Sbjct, AAA88369.1). Arrows indicate amino acid residues required for activity are Cys96, Asp99 and Cys159 and. Cys117 is a highly conserved cysteine and serves a structural rather than a catalytic role.[3,4] A mutation of Cys160 to a serine (blue arrow) was found to retain 37% of wild-type activity in a mutant of MerB from the broad spectrum resistance plasmid R831b.[3]



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Score           Expect  Method                         Identities     Positives       Gaps         Frame
121 bits(304)   2e-37   Compositional matrix adjust.   76/189(40%)    107/189(56%)    6/189(3%)    +1

Query  70   LLRALAKGRPVSRTTIANalnsslseieaalasFADTVYDEKGDVVACGLSLMPTPHSFT  249
            LLR LAKGRPVSRTT+A  L+      + A L     T YD+ G+++  GL+L  T + F
Sbjct  27   LLRELAKGRPVSRTTLAGILDWPAERVAAVLEQATSTEYDKDGNIIGYGLTLRETSYVFE  86

Query  250  VCGNQLYTWCALDALMYPVALEQVAQVESHCPVTGIPVRMTATPTGVVDPSPAGVSLSMV  429
            +   +LY WCALD L++P  + + A+V SHC  TG PV +T +P+ +    PAG+++S+V
Sbjct  87   IDDRRLYAWCALDTLIFPALIGRTARVSSHCAATGAPVSLTVSPSEIQAVEPAGMAVSLV  146

Query  430  APSEQAGCCSVRNSFCSGVHFIssaeaaaswlslH---PEASIVSLEEAWQIGHAVMQQR  600
             P E A    VR SFC  VHF +S   A  W S H      +IVS+ EA+ +G     +
Sbjct  147  LPQEAA---DVRQSFCCHVHFFASVPTAEDWASKHQGLEGLAIVSVHEAFGLGQEFNRHL  203

Query  601  LSHGETSRP  627
            L    +  P
Sbjct  204  LQTMSSRTP  212
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Table S4: *Geobacter* strains with complete genome sequences available in NCBI containing homologs of *merA*. Sequence identify based on a BLASTP search using the translated sequence of the mercuric reductase from pDU1358 (WP_000149288.1) resulted in sequence identities ranging from 39-42% (similarity 54-55%). Furthermore, amino acid residues essential for mercuric reductase activity including the vicinal cysteine pair near the C-terminus were verified to be present in these homologs.[5,6]

Strain	***merA* homolog (NCBI RefSeq)**
Geobacter bremensis R1	WP_026843080.1
Geobacter bemidjiensis Bem	WP_012529076.1
Geobacter sp. M21	WP_012774348.1
Geobacter uraniireducens Rf4	WP_011937772.1
Geobacter sp. M18	WP_015721964.1
Geobacter sulfurreducens PCA	WP_010944034.1
Geobacter metallireducens GS-15	WP_004512764.1
Geobacter sulfurreducens KN400	WP_014552187.1
Geobacter sp. GSS01	WP_039647438.1
Geobacter daltonii FRC-32	WP_041267067.1
Geobacter sp. OR-1	WP_041973282.1
Geobacter pickeringii	WP_039739586.1

Table S5: Bacterial strains with complete genome sequences containing homologs of *hgcA* and *hgcB*. NCBI taxonomy IDs and accession numbers are listed for homologs of *hgcA* and *hgcB*.

Species	**NCBI txid**	***hgcA***	***hgcB***
Geobacter sulfurreducens PCA	243231	WP_010942086	WP_010942087
Geobacter metallireducens GS-15	269799	WP_004512129	WP_004512128
Desulfovibrio desulfuricans ND132	641491	WP_014321697	WP_014321698
Desulfobulbus propionicus DSM 2030	577650	WP_015723167	WP_015723166
Desulfovibrio aespoeensis Aspo-2	643562	WP_013515564	WP_013515565
Desulfovibrio africanus str. Walvis Bay	690850	WP_014258348	WP_014258346
Geobacter bemidjiensis Bem	404380	WP_041263054	WP_012529615
Geobacter daltonii FRC-32	316067	WP_012647565	WP_012647564
Geobacter sp. M18	443143	WP_041248385	WP_015719368
Geobacter sp. M21	443144	WP_015838335	WP_015838334
Geobacter uraniireducens Rf4	351605	WP_041245229	WP_011937421
Acetivibrio cellulolyticus CD2	509191	WP_040427875	WP_010243113
Acetonema longum APO-1 DSM 6540	1009370	WP_004098500	EGO62460
delta proteobacterium NaphS2	88274	WP_040874856	WP_006425356
delta proteobacterium MLMS-1	262489	WP_040868017	WP_007291819
Desulfitobacterium dehalogenans ATCC 51507 DSM 9161	756499	WP_014794563	AFM01082
Desulfitobacterium dichloroeliminans LMG P-21439	871963	WP_015261306	WP_015261307
Desulfitobacterium metallireducens DSM 15288	871968	WP_025248733	CP007032: 1755476..1755763
Desulfomicrobium baculatum DSM 4028	525897	WP_050762129	WP_012805587
Desulfonatronospira thiodismutans ASO3-1	555779	WP_008871053	WP_008871052
Desulfosporosinus orientis DSM 765	768706	WP_014185007	WP_014185008
Desulfosporosinus sp. OT	913865	WP_009624460	WP_009624459
Desulfosporosinus youngiae DSM 17734	768710	WP_007786010	WP_007786009
Dethiobacter alkaliphilus AHT 1	555088	WP_040379342	WP_008519316
Ethanoligenens harbinense YUAN-3	663278	WP_049776646	ADU26529
Methanocella paludicola SANAE	304371	BAI60790	BAI60789
Methanoregula boonei 6A8	456442	WP_011991429	WP_011991428
Methanosphaerula palustris E1-9c	521011	WP_012617701	ACL16383
Methanospirillum hungatei JF-1	323259	WP_011447905	WP_011447904
Syntrophobotulus glycolicus DSM 8271	645991	WP_013625504	WP_013625503
Syntrophus aciditrophicus SB	56780	WP_049750061	WP_041585151
uncultured *Desulfobacterium sp.*	201089	CBX26761	CBX26762
Methanocella arvoryzae MRE50	351160	WP_012035144	WP_012035145
Geobacter sulfurreducens KN400	663917	WP_010942086	WP_010942087
Geobacter metallireducens RCH3	691164	WP_004512129	WP_004512128
Desulfosporosinus acidiphilus SJ4	646529	WP_014826630	WP_014826631
Methanolobus tindarius DSM 2278	2221	WP_052324379	WP_023846499
Methanofollis liminatans DSM 4140	28892	WP_004039402	WP_004039396
Methanoregula formicica SMSP	593750	WP_048111164	WP_015284971
Methanomethylovorans hollandica DSM 15978	867904	WP_015324055	WP_015324054
Dehalobacter restrictus DSM 9455	871738	AHF10374	WP_025205892

Desulfomonile tiedjei DSM 6799	706587	AFM23739	WP_014808894
Desulfonatronum lacustre Z-7951 DSM 10312	935942	WP_051434252	WP_028570972
Desulfovibrio africanus DSM 2603	1121435	WP_027367778	WP_027367780
Desulfovibrio africanus PCS	1262666	WP_005985160	WP_005985173
Desulfotignum phosphitoxidans DSM 13687	1286635	WP_040012032	WP_006967327
Clostridium litorale DSM 5388	1121324	WP_052635851	WP_038261074
Dehalobacter sp. CF	1131462	WP_015045218	WP_015043156
Dehalobacter sp. DCA	1147129	WP_015043157	WP_015043156
Dehalobacter sp. E1	304767	WP_019225877	WP_019225878
Dehalobacter sp. UNSWDHB	1339256	WP_021315307	WP_021315263
Clostridium termitidis CT1112	1195236	WP_040761202	WP_004626264
Dehalococcoides mccartyi DCMB5	1193807	WP_015406907	AGG05960
Methanolobus psychrophilus R15	1094980	WP_015052522	WP_015052521
Methanocella arvoryzae MRE50	351160	WP_012035144	WP_012035145
Methanomassiliicoccus luminyensis B10	1080712	WP_019177913	WP_019177912

References

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