Supporting Information: Wiatrowski et al., "Novel reduction of mercury(II) by mercury-sensitive dissimilatory metal reducing bacteria"

Reduction of Hg(II) by *S. oneidensis* MR-1 is cell density dependent: A negative correlation ($r^2 = 0.889$) was observed between specific activity of Hg(II) reduction by MR-1 and cell density (Fig. S1A). When cell density exceeded approximately 4 µg protein mL^{-1}, corresponding to cell density of approximately 1×10^6 cells mL^{-1}, its effect was so profound that a drop in initial reduction rates was noted (Fig. S1B). We hypothesized that this effect was due to a decreased bioavailability of Hg(II) caused by sorption to cellular material, as was the case for induction of luminescence in a *mer-lux* based bioassay (1). To test this hypothesis, we added an increasing amount of autoclaved MR-1 cells to a constant number of live cells at a density corresponding to 0.35 µg protein mL^{-1}, at which the inhibitory effect of cell biomass was minimal (Fig. S1A and S1B). The initial rate of Hg(II) removal decreased as the amount of added autoclaved cells increased, while autoclaved cells had very little activity by themselves (Fig. S1C). Thus, the specific rate of Hg(II) reduction by MR-1 declines with increased cell density, an effect most likely attributed to binding of Hg(II) to cell biomass. Because of this effect, we opted to work with cell concentrations corresponding to 0.2 to 0.5 µg protein mL^{-1}, a range of biomass that gave the greatest degree of reproducibility. This protein

concentration represents a culture density of 1×10^5 cells mL^{-1}.
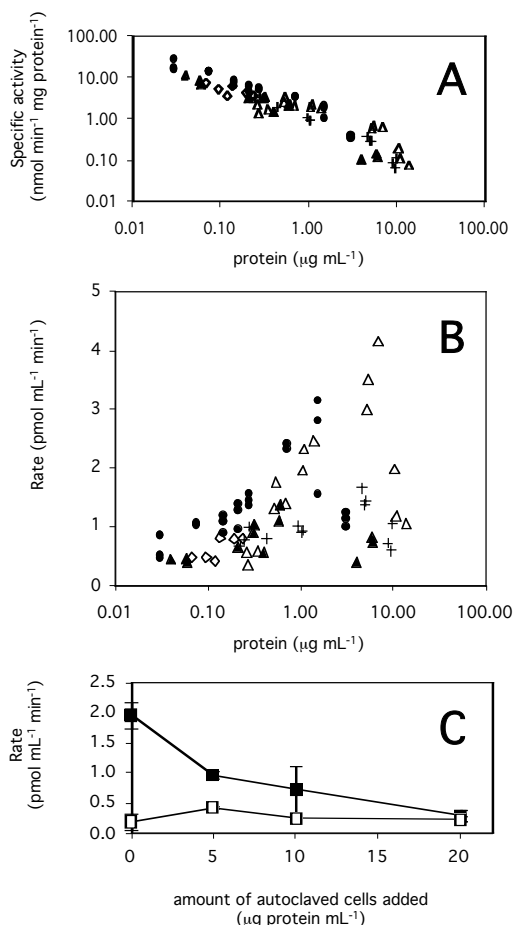

Figure S1: Effect of cell density of MR-1 on Hg(II) reduction. Specific activity **(A)** and rate **(B)** of Hg(II) loss from growth media are shown. Each class of symbol represents an independent experiment, and each data point represents a single sample. **(C)** Effect of dead biomass on Hg(II) reduction by a constant amount of live biomass ■ - live cells at a concentration of 0.35 μg protein mL^{-1}. □ - no live cells. Means and standard deviations of three replicates are shown. Errors of measurement were 0.08 pmol mL^{-1} min^{-1} for **(B)** and 0.1 pmol mL^{-1} min^{-1} for **(C)**. Mercury concentration was 0.3 μM and was analyzed using ^{203}Hg as a tracer.

Analysis of open reading frames with homology to known mercuric reductase genes in *Geobacter* spp. BLAST-P searches performed using characterized MerAs from *Bacillus cereus* RC607 (accession number AAA83977) and *Shewanella putrefaciens* (accession number CAA89057) as queries recovered two proteins with homology to the active site of MerA from the PCA genome (2) encoded by genes annotated *merA-1* and *merA-2*, and one sequence from the GS-15 genome, encoded by a gene annotated *merA*.

(Genbank accession numbers gi2688060, gi2688155, and gi3739061 respectively). The possibility of functioning MerA in *Geobacter metallireducens* GS-15 and *Geobacter sulfurreducens* PCA was examined by scrutinizing their amino acid sequences in light of the known characteristics of active MerA (3). An alignment of these genes with MerA of strain RC607 (Fig. S2) reveals the presence or absence of residues that are essential for the functioning of MerA.

All MerA's found in these *Geobacter* spp. lack the N-terminus (*NmerA*), which, although not required for function *in vitro* (4), nonetheless is only absent in two biochemically characterized MerA's (5,6). Reduction of Hg(II) by PCA's MerA-1 is ruled out because this protein lacks the vicinal cysteine pair at the carboxy terminus (indicated by asterisks * in Fig. S1), which is required for charge regulation in the enzyme's active site(7).

PCA MerA-2 and GS-15 MerA have an irregularity in the region of the active site which may affect interactions with Hg(II); while all known MerA's have a valine at amino acid 208 [relative to RC607, indicated by an ampersand (&)], both *Geobacter* proteins have an isoleucine. By contrast, the more common substitution at this position is with leucine , as in most dihydrolipoamide dehydrogenases, which are flavin oxidoreductases related to MerA that do not have Hg(II) reduction activity.

The gene encoding mercuric reductase is typically a part of an operon that contains genes specifying other functions of the mercury resistance system in bacteria. GS-15 *merA* and PCA *merA-2* reside in a 30 kb region of synteny, which contains genes for the NADH dehydrogenase complex (nucleotides 175822 to 180354 in GS-15 and 3798645 to 3794113 in PCA), devoid of any known *mer* functions.

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                                                                                      60
Bacillus cereus RC607 MerA      MKKYRVNVQGMTCSGCEQHVAVALENMGAKAIEVDFRRGEAVFELPDDVKVEDAKNAIAD
G. metallireducens GS-15 MerA   ------------------------------------------------------------
G. sulfurreducens PCA MerA-2    ------------------------------------------------------------
G. sulfurreducens PCA MerA-1    ------------------------------------------------------------


                                                                                      151
ANYHPGEAEEFQSEQKTNLLKKYRLNVEGMTCTGCEEHIAVALENAGAKGIEVDFRRGEALFELPYDVDIDIAKTAITDAQYQPGEAEEIQ
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----------------------------------------------------------------------------MSDNFASPHDRD


                                                          &                           242
VQSEKRTDVSLNDEGNYDYDYIIIGSGGAAFSSAIEAVALNAKVAMIERGTVGGTCVNVGCVPSKTLLRAGEINHLAK-NNPFVGLHTSAS
--------------MPDDTHDLVILGSGSTAFAAALRAQSYGTRVLMVEKGVPGGTCINWGCVPSKTLIHAALFYQEGK-LGARLGLGECGG
--------------MSDKHDLIILGSGSTAFAAALRAHSRGARVLMVEKSVLGGTCINWGCVPSKTLIHGALFYQEGR-LGARLGLGECGN
LDERVRPPGWINPSPAPRYDLVVVGAGTAGLVCAAGEAGLGARVALVERHRLGGDCLNYGCVPSKALIRAARAAHDAGNGAPFGVTGCHGT


                                                                                      333
NVDLAPIVKQKNDLVTEMRNEKYVNLIDDYG-FELIKGESKFVNENTVEVNGNQITAKRFLIATGASSTAPNIPGLDEVDYLTSTSLLELK
TVVLERLMARKDQVVGHLRQTKYLDILQDVPGLQLVKGTGRFLGPDRLEVGDREIRSERFLVAVGGDPRVPRIPGLESTPFLTSRGTLLLK
AVDLAPLMTRKEEVVKHLRTTRYLDILRNTPGLEIAKGTGRFLGSGRLEVVDQVYRCDRYLVAVGGTPRIPKIPGLESTPFLTSRGALLLK
GVDGAAVMERMRRLRAEIGRHDAAVRFRDLG-VHVFFGQGSFISRNALEVDGRRLNFVHAAVCTGARAAAPPVPGLAEAGYLTNETIFSLA


                                                                                      424
KVPNRLTVIGSGYIGMELGQLFHNLGSEVTLIQRSERLLKEYDPEISEAITKALTEQGINLVTGATYERVEQDGDIKKVHVEING-KKRII
TIPQSLVIIGGGVIAVEMGQMFQRLGAKVTILEHGPRIIGPVEPEPALAVRDFLRAEGMKIVCRTTICLAAQDGAGVRVEAERDG-ERVSF
RFPASLIIIGGGVIAVELGQMFQRLGTRVTILEHGPRILAPIEPEPALAIRNVLRDEGMEIICHSPVCAVSGDGSAVSVEVERED-GRRTY
TLPARLAVIGGGPIGCELAQAAARLGSSVTVIEAAPEIIPREDTDAAALVRHALERDRVSFLTAAAVVGVERR-SGARTLIVRQGDQSHEV


                                                                                      515
EAEQLLIATGRKPIQTSLNLHAAGVEVGSRGEIVIDDYLKTTNSRIIYSAGDVTPGPQFVYVAAYEGGLAARNAIGGLN-QKVNLEVVPGVT
TAEKLLVATGTAPATNGIGLELAGVETDPRGFVTVDERMRTTAPGIWAAGDCTGGMMIATVGAREGIVAVDDMLNPGCGCSMDFLSAPMAI
TAEKLLIAVGTTPATRGIGLELAGVETDGRGFVTVDERMRTTAPGIWAAGDCTGGMMIATVGAREGIIAVDDMFATGCGCAMDHLSVPMAI
TADEILVGACRTPNIEGLGLERAGIVADPLRGVRVNDRLRTDNPRVYAAGDICSPYRFTHAADAMARIVVANALFGAR-QRFSTQIIPWCT


                                                                                      606
FTSPSIATVGLTEQQAKEKGYEVKTSVLPLDAVPRALVNRETTGVFKLVADAKTLKVLGAHVVAENAGDVTYAATLAVKFGLTVGDLRETM
FTDPEVGMVGHTEEGAKAAGFDVVVNVMPVAAIPKAHVTGHTAGVIKMVADKATGRLLGVHLACHRGADIINEAALAIRFRATVEDLANAL
FTDPEVGAVGYTEQGARDAGLDPIVSILPVSAIPKAHVTGHTAGVIKLVAERATGRLLGAHLACHRGAELINEAALAIRLKALFEDLANAL
YTDPEVAHVGLYEREAGERGLAVDTLTVPLTEVDRALLDGEDEGFARVHLKRGTDRIVGATIVARHAGEMLNELTLAMSAGLGLSAIGRSI


                              **              647
APYLTMAEGLKLAVLTFDKDVSKLSCCAG------------
HVYPSMGEGLRLCAQGFSRDISRLSCCAE------------
HVYPSIGEGLRLCAQGFTRDVSKLSCCAE------------
HPYPTQAEAIKKLADAWNRTRLTPGVKRLMGIMLTLRRLWR
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Fig S2: Alignment of proposed MerA sequences from *Geobacter* spp. Alignments were created using the AlignX module in the Vector NTI ADVANCE software program (Invitrogen, Carlsbad, CA), and visualized using BOXSHADE (K. Hoffman and M. Baron www.ch.embnet.org/software/BOX_form.html). Black type and white background — nonconserved residues. White type and black background — identical residues. Gray background with black type — similar residues.

LITERATURE CITED:

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