Supplemental Material (SM)

Mercury methylation by the methanogen *Methanospirillum hungatei*

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This supplemental material includes:

1) Materials and Methods
2) References
3) Supplementary Figures S1 to S5

Materials and Methods

Mercury Methylation Experiments. Individual cultures for methylation experiments were pre-grown for 4-5 days until they reached exponential phase. Before tests, pre-grown cells of all strains were washed twice with fresh sulfide-free media under strictly anaerobic conditions using tightly sealed 50 ml centrifuge tubes (centrifuged for 20 min. at 3,000 rpm). Washed cells were then resuspended in a sulfide-free medium for each strain and used as inocula. A similar dilution of inoculum of each individual strain (roughly 10%) was used in Hg methylation experiments, e.g., roughly 7 ml of pre-grown culture which was subject to washing was added to 63 ml of fresh medium in a 130 ml serum bottle. Initial inoculation of each strain was adjusted to a similar cell density in different experiments by adjusting to final OD_{660} of 0.010-0.011 (about 2.8×10^4 cells ml^{-1}) for *M. hungatei* and to 0.020-0.022 (about 4.6×10^5 cells ml^{-1}) for *D. africanus* and others. Experiments were performed in triplicate. Heat killed abiotic controls, prepared by treating cultures at 80 °C for 1 h (1), and sterilized media blanks were included in all methylation experiments.

Potential Hg methylation rates were measured by spiking $^{203}HgCl_2$ (in 0.1 N HCl) kindly provided by Christy C. Bridges (Mercer University School of Medicine, Macon, GA). The radioisotope (specific activity, 2.504 μCi μg^{-1}) was injected into each serum bottle at 180.6~191.9 kBq L^{-1} (corresponding to 9.7~10.3 nM or 1.9~2.1 μg Hg L^{-1}). The final concentration of Hg in these incubation was similar to or lower than concentrations in previous methylation studies (2-5). A volume of 0.1 N NaOH similar to that of the injected ^{203}Hg substrate was immediately injected into the serum bottles to neutralize the added acid, and the cultures were thoroughly mixed by vortexing. Cultures were then statically incubated at either 32 or 37 °C in the dark. Ten ml aliquots were withdrawn with a syringe in daily intervals for MeHg

extraction and a similar volume of O_2-free 100% N_2 was added to the bottles to maintain constant pressure for five days.

Newly synthesized $CH_3{}^{203}Hg$ in cultures was separated from unreacted $^{203}HgCl_2$ by a toluene extraction method that was modified from previous studies (6-8). Samples were extracted on the day of sampling to minimize the effect of ^{203}Hg decay. Recoveries of MeHg ranged from 90 to 98% as determined by using cell cultures to which 2 ml of 3N HCl were added to terminate microbial activities followed by the addition of ~0.1 µCi ^{14}C-CH_3HgCl (specific activity, 60 mCi/mmol; radiochemical purity, 95.2%; Amersham Corp., Buckinghamshire, England), and employing the same extraction protocol. The radiotracer approach allows quick analysis of potential methylation rates at an ambient total Hg concentration and/or at trace levels (6, 9). Since previous tests showed the potential formation of unknown organic forms of ^{203}Hg (Mark Marvin DiPasquale, USGS, personal communication) and low levels of Hg(II) carryover into toluene are known (10), the formation of MeHg in *M. hungatei* cultures was confirmed by the detection of MeHg by EPA method 1630 (11).

Potential initial Hg methylation rates (fmol MeHg per µg protein day^{-1}) were calculated from the linear range of lines describing MeHg concentrations vs. time, either normalized by initial protein (for Fig. 1a) or by protein levels at each time point for Fig. 2. The initial concentration of cell proteins at inoculation was used in the calculations for Fig. 1a to allow a protein-normalized comparison among different treatments and with results obtained by others (5). The Hg methylation rates (Fig. 2) were calculated based on the initially linear range of methylation in 32 h for all strains and treatments except for *G. sulfurreducens* incubated at 32 °C and *M. hungatei* at 37 °C where 12 hrs measurements were used to calculate initial rates.

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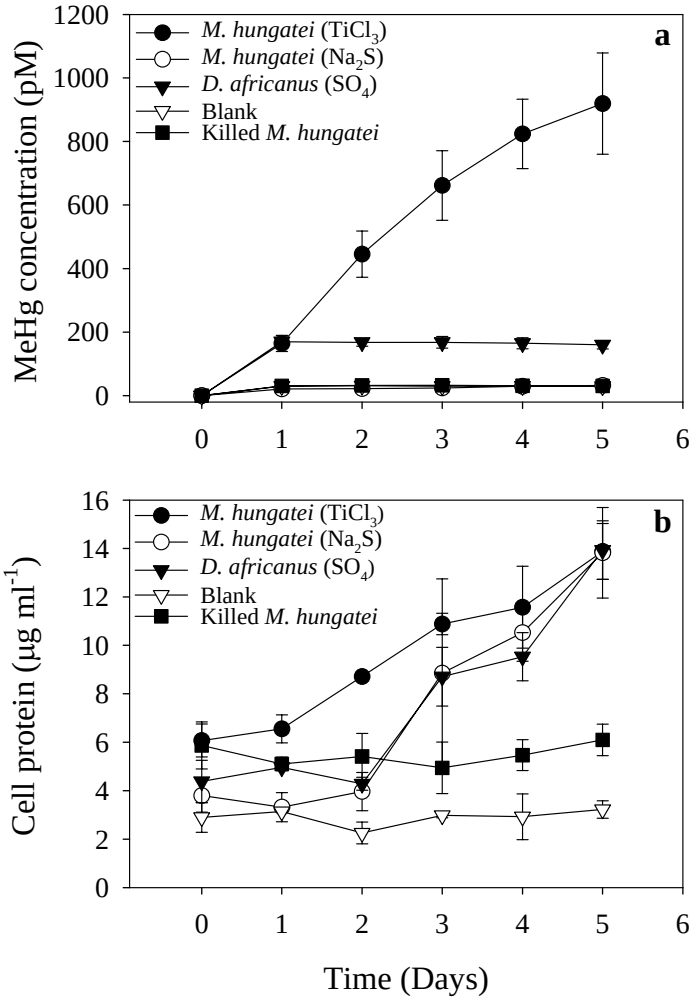

FIG. S1 Hg methylation by *M. hungatei* JF-1 and *D. africanus* DSM 2603. Synthesis of MeHg (pM) (a) and cell growth (protein concentrations, b) of *M. hungatei* in media with $TiCl_3$ or Na_2S as a reductant, and *D. africanus* in medium containing Na_2SO_4 as a terminal electron acceptor.

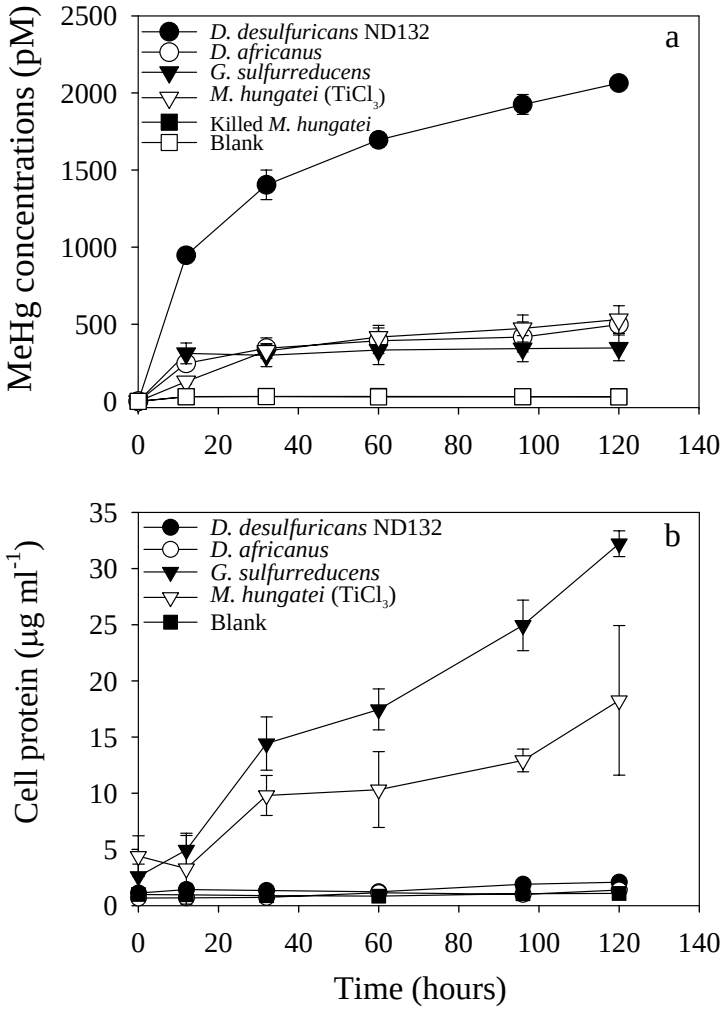

FIG. S2 Comparison of Hg methylation by *M. hungatei* JF-1 with activities of known methylating bacteria. MeHg concentrations (a) and cell growth (b) during incubation of *D. desulfuricans* ND132 (pyruvate as an electron donor and fumarate as an acceptor), *D. africanus* DSM 2603 (pyruvate and fumarate), *G. sulfurreducens* PCA (sodium acetate and ferric acetate) at 32 °C, and *M. hungatei* JF-1 at 37 °C. $TiCl_3$ was served as a reductant in all incubations.

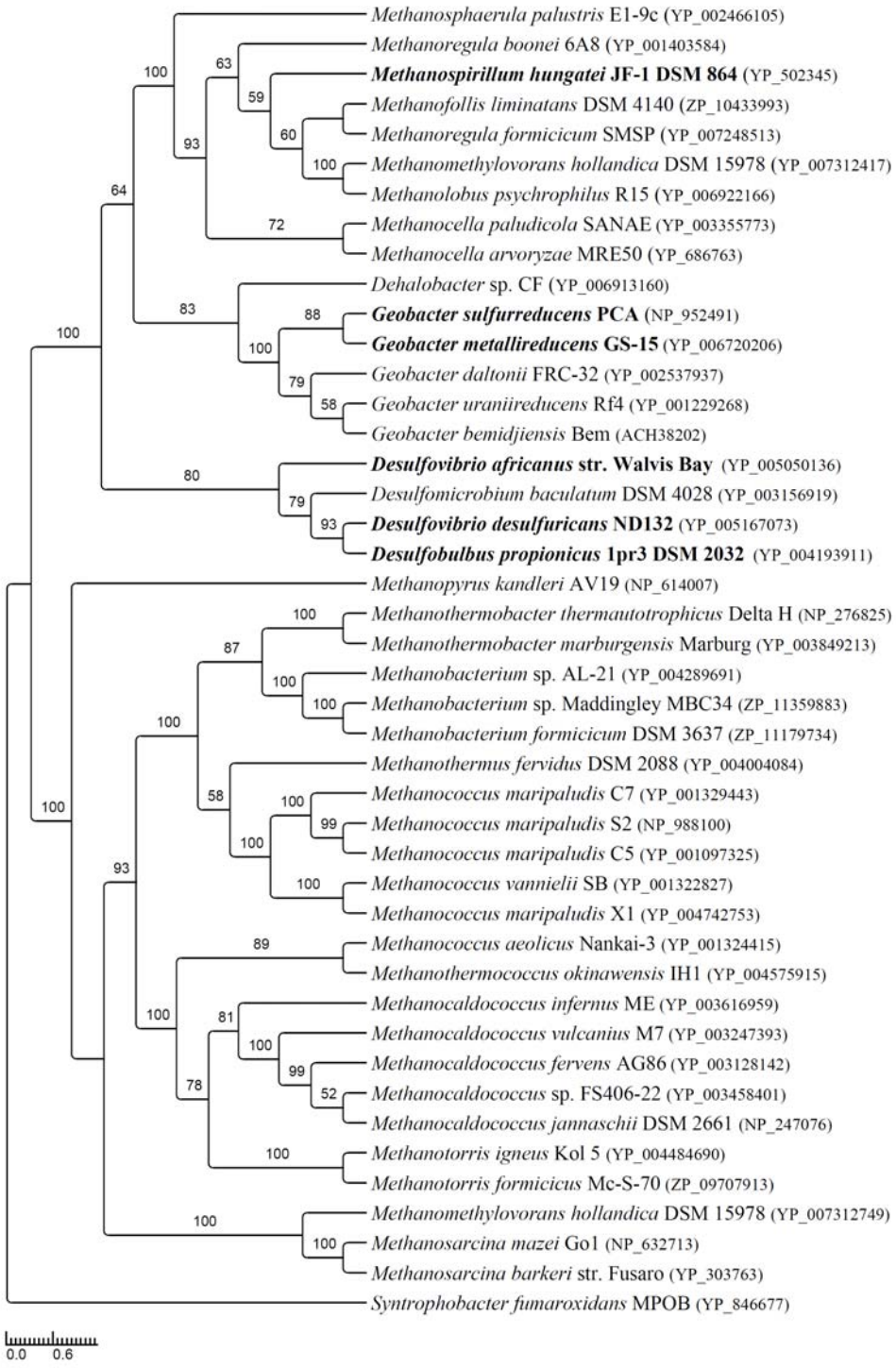

FIG S3 Maximum likelihood phylogeny of HgcA orthologs (or paralogs) from methanogens, and sulfate and iron reducing bacteria. Confirmed Hg methylators are highlighted in bold. Accession numbers are listed in parentheses. Numbers at branching points denote boot support as a percentage of 100 successful resampling.

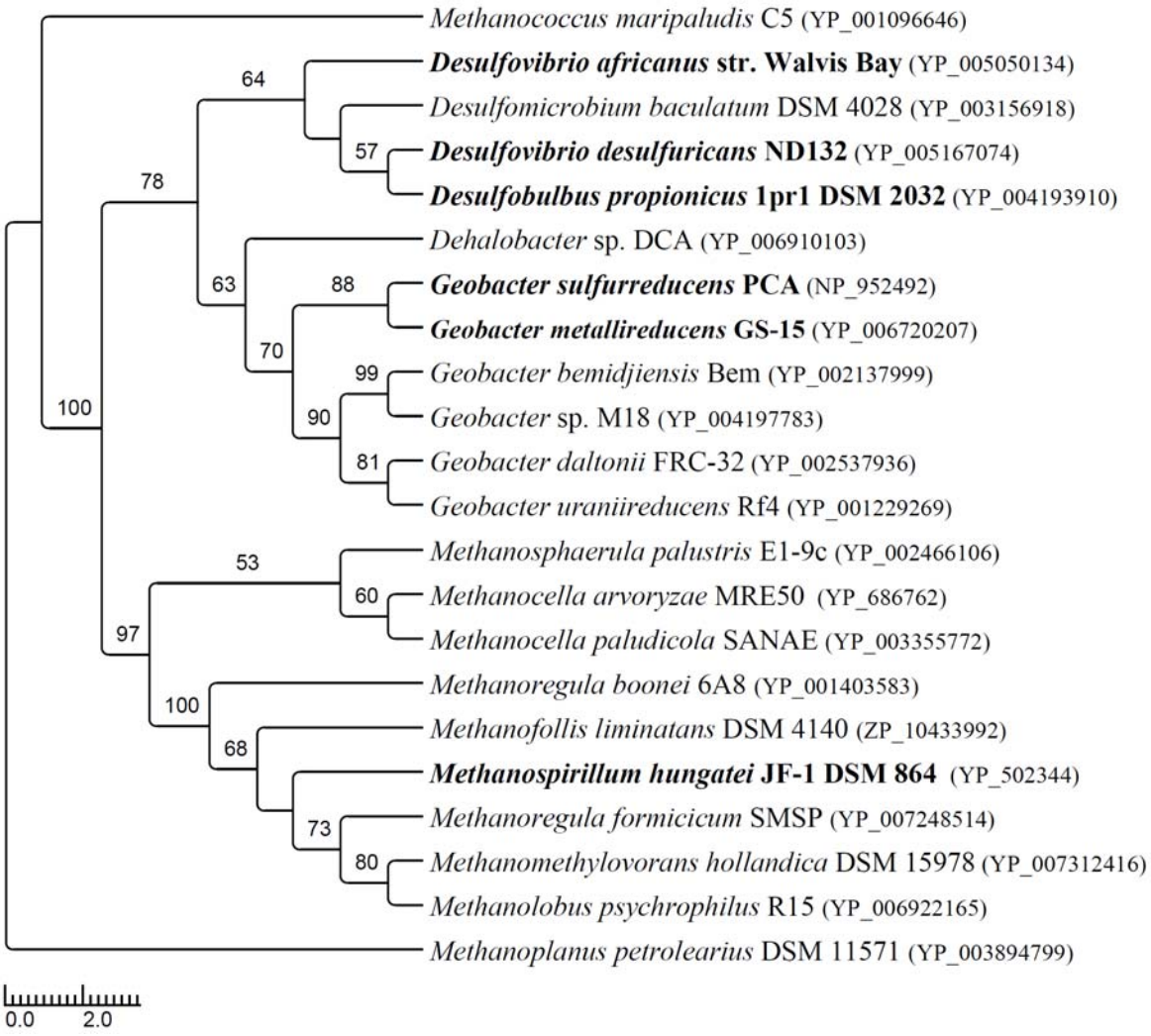

FIG S4 Maximum likelihood phylogeny of HgcB orthologs (or paralogs) from methanogens, and sulfate and iron reducing bacteria. Confirmed Hg methylators are highlighted in bold. Accession numbers are listed in parentheses.

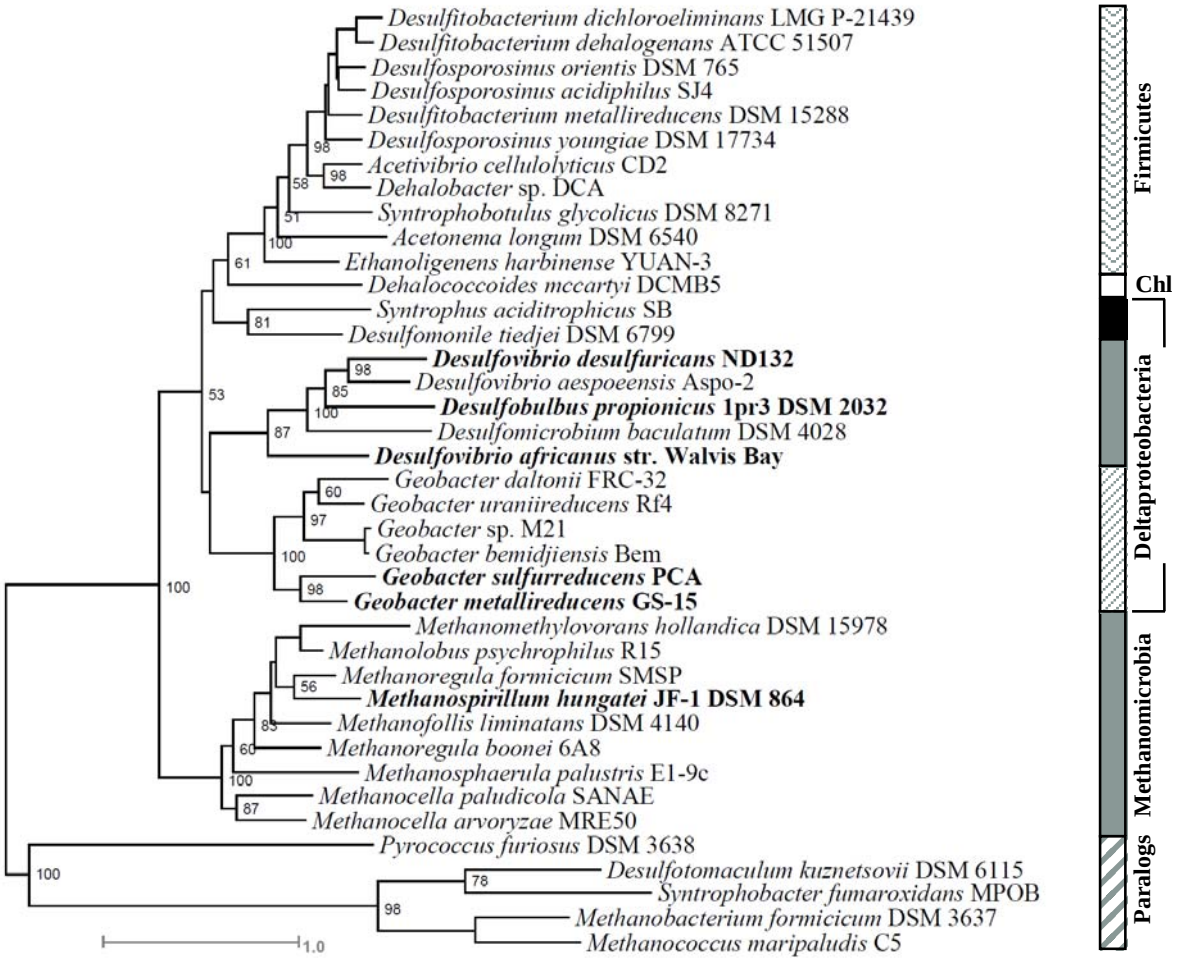

FIG S5 Maximum likelihood phylogeny of concatenated HgcA and HgcB from known Hg methylating species (shown in bold) and orthologs identified by homology to the proteins of *M. hungatei* JF-1. The bar on the right identifies taxa at the phylum/class level; Chl stands for Chloroflexi and the Deltaproteobacteria bracket distinguishes iron reducers, sulfate reducers, and syntrophs. The tree is out-grouped by paralogs of Hgc proteins belonging to the CdhD family. The bar at the bottom indicates branch length corresponding to 1 substitution per 100 amino acids.