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Sulfate-Reducing Bacterium *Desulfovibrio desulfuricans* ND132 as a Model for Understanding Bacterial Mercury Methylation▽†

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We propose the use of *Desulfovibrio desulfuricans* ND132 as a model species for understanding the mechanism of microbial Hg methylation. Strain ND132 is an anaerobic dissimilatory sulfate-reducing bacterium (DSRB), isolated from estuarine mid-Chesapeake Bay sediments. It was chosen for study because of its exceptionally high rates of Hg methylation in culture and its metabolic similarity to the lost strain *D. desulfuricans* LS, the only organism for which methylation pathways have been partially defined. Strain ND132 is an incomplete oxidizer of short-chain fatty acids. It is capable of respiratory growth using fumarate as an electron acceptor, supporting growth without sulfide production. We used enriched stable Hg isotopes to show that ND132 simultaneously produces and degrades methylmercury (MeHg) during growth but does not produce elemental Hg. MeHg produced by cells is mainly excreted, and no MeHg is produced in spent medium. Mass balances for Hg and MeHg during the growth of cultures, including the distribution between filterable and particulate phases, illustrate how medium chemistry and growth phase dramatically affect Hg solubility and availability for methylation. The available information on Hg methylation among strains in the genus *Desulfovibrio* is summarized, and we present methylation rates for several previously untested species. About 50% of *Desulfovibrio* strains tested to date have the ability to produce MeHg. Importantly, the ability to produce MeHg is constitutive and does not confer Hg resistance. A 16S rRNA-based alignment of the genus *Desulfovibrio* allows the very preliminary assessment that there may be some evolutionary basis for the ability to produce MeHg within this genus.

Mercury methylation is a natural microbial process that converts inorganic Hg(II) to the bioaccumulative toxin methylmercury (MeHg). Methylmercury contamination of food webs causes significant risk to people and other organisms near the top of food webs worldwide (1, 67). Although the biogeochemistry of MeHg production in the environment has been studied in detail for more than 3 decades, the biochemical mechanism of methylation in bacterial cells remains poorly understood, especially relative to MeHg demethylation by the organomercury lyase pathway (3) or the redox transformations of metal contaminants like uranium (28, 69) and chromium (50). As of yet, no metabolic pathway or gene that is common to methylators but absent in nonmethylators has been identified.

Methylmercury production is an anaerobic process that occurs in saturated soils, wetlands, decaying periphyton mats, aquatic bottom sediments, and anaerobic bottom waters (5, 57). Studies at a variety of ecological scales show that MeHg production is intimately linked to the sulfur and iron cycles. Many studies have demonstrated sulfate stimulation of MeHg production in freshwater sediments and wetlands (e.g., references 12, 36, 44, and 70), and many have found that Hg methylation occurs most readily in zones of microbial sulfate or ferric iron reduction (e.g., references 21, 35, 42, and 48).

However, the ability to produce MeHg is not a common trait of dissimilatory sulfate-reducing bacteria (DSRB) or Fe(III)-reducing bacteria (FeRB). Only a subset of the sulfate- and Fe(III)-reducing bacterial species tested have the ability to methylate Hg. Overall, this capacity has been tested with fewer than 50 bacterial strains. The order *Desulfovibrionales* has been most extensively examined, and about half of the examined species have the ability to produce MeHg (18, 27, 37, 47, 51, 62). Mercury-methylating DSRB are also found within the *Desulfobacterales* (6, 13, 27, 47, 64). In addition, several species of *Geobacter*, FeRB in the order *Desulfuromonales*, produce MeHg (28, 46), as well as *Desulfuromonas palmitatis* SDBY1, in the same order. Limited testing for Hg methylation outside the *Deltaproteobacteria* has focused on FeRB and DSRB in the *Gammaproteobacteria* and in the *Firmicutes*, none of which methylate Hg (46, 62, 64). To summarize, most organisms tested for methylation have been sulfate- or Fe(III)-reducing *Deltaproteobacteria*, and only about half of those tested have the ability to produce MeHg. No organisms outside the *Deltaproteobacteria* have been shown to produce MeHg, but fewer than 15 have been tested.

The ability of certain organisms to produce MeHg could be linked to a specific methyl-transferase pathway, to a Hg-specific uptake pathway, or to the biochemistry of Hg binding and movement within cells. In the late 1980s and 1990s, Richard Bartha's group studied the metabolic pathways leading to

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MeHg, using an estuarine DSRB, *Desulfovibrio desulfuricans* LS, which was isolated from a brackish New Jersey marsh (18). They proposed that Hg methylation in this organism occurred via transfer of a methyl group from methyl-tetrahydrofolate via methylcobalamin (MeB_{12}), with the methyl group originating either from C-3 of serine or from formate, via the acetyl-coenzyme A (CoA) synthase pathway (11, 15, 16). Since these pathways are not unique to *D. desulfuricans* LS, Bartha and colleagues proposed that the organism's ability to methylate mercury is most likely associated with the substrate specificity of its enzymes. Subsequent work confirmed that Hg methylation can occur independently of the acetyl-CoA pathway. Benoit et al. (6) demonstrated Hg methylation by *Desulfobulbus propionicus*, a DSRB that does not use the acetyl-CoA pathway. Ekstrom et al. (27) identified other Hg-methylating DSRB without the pathway. More recently, Ekstrom and Morel (26) showed that cobalt limitation inhibited MeHg production in *Desulfococcus multivorans*, an incomplete oxidizer that does use the acetyl-CoA pathway for major carbon metabolism. However, cobalt limitation did not affect Hg methylation in *Desulfovibrio africanus* (DSM 2603, strain Benghazi), an incomplete oxidizer that does not use that pathway, suggesting different methylation pathways in different organisms.

Differences in methylation rate among strains could also be due to differences in uptake pathways. The prevailing paradigm for Hg uptake by DSRB (5, 8, 23) is diffusion of small neutrally charged Hg complexes. However, Golding et al. (34) found that Hg uptake by *Vibrio anguillarum* and *Escherichia coli* strains modified with a *mer-lux* bioreporter system (which in this case did not include the Hg transport genes) was enhanced in the presence of a variety of small organic molecules, including amino acids. This result led to the hypothesis that Hg uptake may occur via a facilitated transport mechanism. Schaefer and Morel (66) showed that cysteine specifically enhanced Hg uptake and methylation in *Geobacter sulfurreducens* and proposed that *Geobacter* strains have a specific uptake mechanism for the Hg-cysteine complex.

Despite this progress, the mechanism of Hg uptake and methylation by bacteria remains unresolved. We have begun a concerted effort to identify the pathways and genes involved in MeHg production. *Desulfovibrio desulfuricans* ND132 was chosen for study because of its exceptionally high rates of Hg methylation in culture and its metabolic similarity to strain *D. desulfuricans* LS, the only organism for which methylation pathways have been partially defined. Before strain LS was lost, Pak and Bartha compared it to ND132 (58, 60). They noted that strain ND132 was similar in substrate utilization abilities but produced four times more MeHg than LS under the same lactate-sulfate culture conditions.

In developing a model organism for study, we hope to provide a well-characterized strain for further genetic and physiological study. Here, we describe the physiological characteristics of *D. desulfuricans* ND132, including antibiotic sensitivities important for genetic manipulation. We also provide basic information on ND132 MeHg production and degradation capabilities and explore how growth conditions affect these rates. Additionally, we explore Hg methylation within the genus *Desulfovibrio* in some detail. We examine the inducibility of Hg methylation and the role of methylation in Hg resistance among *Desulfovibrio* species. Finally, we summarize the available information on Hg methylation among *Desulfovibrio* species, including methylation rates for several new species. A potential evolutionary basis for methylation among *Desulfovibrio* species is explored through construction of a 16S rRNA gene-based phylogeny.

MATERIALS AND METHODS

Isolation of ND132. *Desulfovibrio desulfuricans* ND132 was isolated from mid-Chesapeake Bay bottom sediments sampled in May 1985, as part of a study of tin methylation (29, 32). The sampling site (near station R64; 38°33.86′N, 76°26.38′W) was on the western slope of the main bay channel in about 20 m of water, which is often below the oxycline in summer. The sediment was soft black silt, with an average pore water sulfide (top 10 cm) concentration of 0.52 mM. Bottom water salinity was 23 ppt. Most-probable-number (MPN) estimates for sulfate-reducing bacteria (SRB) yielded >6,000 cells/g wet sediment on lactate and >500 cells/g on acetate (29). Sites near this station have been repeatedly occupied for a variety of research and monitoring work, including measurement of sulfate reduction rates and other biogeochemical parameters (29, 42, 55, 63). Significant rates of tin (32) and mercury (42) methylation have been measured at the site.

Isolation was done on agar plates from lactate-sulfate enrichments made from the top 10 cm of sediment. It is important to point out that enrichment and isolation of this strain were done in medium without added Hg or Sn. The strain was initially selected for its ability to produce $MeSn^{3+}$ from inorganic tin. Since that time, the strain has been used to study the role of sulfur chemistry in Hg uptake and methylation (43), the uptake of thymidine by DSRB (30), and the potential for DSRB to reduce perrhenate (22).

PCR and sequencing. To test culture purity and to help identify isolates, 16S rRNA gene sequences were obtained. DNA was amplified directly from cell cultures, with standard RedMix (Gene Choice, Frederick, MD) protocols with universal forward (27F) and reverse (1541R) primers (300 nM each) and a PTC-200 DNA Engine thermal cycler (Bio-Rad Laboratories, Hercules, CA). When necessary, PCR products were purified from agarose bands with GeneClean (MP Biomedicals, LLC) or from liquid with Qiagen (Qiagen, Inc., Valencia, CA) DNA cleanup kits. Sequencing chemistry used standard BigDye v3.1 reaction protocols (Applied Biosystems, Inc., Foster City, California). Sequencing was performed at the Smithsonian Laboratory of Analytical Biology, with an ABI 3100 automated capillary DNA sequencer (Applied Biosystems).

SEM preparation and observation. Cells were prepared by fixing with 2.5% (vol/vol) anaerobic glutaraldehyde. After 2 h, fixed cells were placed onto a 0.22-μm-pore-sized Neopore filter. Filters were gently rinsed on a filter pad, 2 times with 0.1 M cacodylate buffer and 3 times with ultrapure water, and then covered with a second filter and dehydrated through an ethanol (EtOH) dehydration series. Dehydrated filters were placed between screens and critical point dried immediately (Tousimis Auto-Samdri; 815 Automatic Critical Point Dryer). The sandwiched membranes were then separated, mounted onto scanning electron microscopy (SEM) stubs and sputter coated (Emitech K575x Turbo Sputter Coater) with a thin layer of platinum on a rotating sample holder (20 kV, 45 s). The coated samples were then viewed with a Hitachi S-4700 field emission scanning electron microscope (FESEM) at a working distance of 5 to 6 mm, a voltage of 2.0 to 10.0 kV, and a magnification of approximately ×6,000.

Cultivation and maintenance of *D. desulfuricans* ND132. Cultures were maintained in the Gilmour laboratory on SRM medium (without Hg) (6) containing 20 mM Na_2SO_4, 20 mM Na lactate, salts (170 mM NaCl, 1.4 mM NaH_2PO_4, 19 mM NH_4Cl, 6.7 mM KCl, 1.5 mM $MgCl_2$, and 1.5 mM $CaCl_2$), 0.05% yeast extract (wt/vol), 25 nM selenate, 25 nM tungstate, 4.4 μM $FeCl_2$, 10 mM MOPS (morpholinepropanesulfonic acid) buffer, trace metals (see Table S1 in the supplemental material), and vitamins (Table S2) at pH 7.2. The medium was usually reduced with 100 μM Ti-NTA (freshly made from $TiCl_3$ and nitrilotriacetic acid [NTA] in saturated Na_2CO_3), and resazurin (0.001%) was used as a redox dye. Reductants were filter sterilized into medium tubes just before inoculation. Other reductants included thioglycolate-ascorbate (made from Na mercaptoacetic acid and Na ascorbate), and cysteine, both used at final concentrations of 100 μM.

In the Wall laboratory, strain ND132 was maintained on MOYLS4 medium (71) containing 40 mM Na lactate, 40 mM Na_2SO_4, 8 mM $MgCl_2$, 20 mM NH_4Cl, 0.6 mM $CaCl_2$, 2 mM K_2HPO_4-NaH_2PO_4, 30 mM Tris-Cl, 0.1× Thauers vitamins, 0.5× trace elements, 0.1% (wt/vol) yeast extract, $FeCl_2$-EDTA (0.06 mM and 0.12 mM, respectively) at pH 7.2 to 7.5. For ND132, the medium was typically modified to include 1% (wt/vol) NaCl, 1 mM cysteine and was reduced with 0.38 mM Ti-citrate. Prior to work described in this paper, purity was verified via single-colony isolation. To minimize phenotypic drift from repetitive cultur-

ing, all experiments used cells with fewer than three subcultures from frozen glycerol stocks. Cultures were routinely checked for aerobic contamination by streaking them on aerobic plates of complex medium containing glucose.

Metabolic by-product quantification. Sulfide was analyzed by ion-specific electrode after preservation in freshly made sulfide antioxidant buffer (24). Sulfate was measured by ion chromatography (25).

Mercury methylation and demethylation measurements. Methylmercury production and degradation in cultures were assessed using isotopically enriched stable Hg isotopes (39, 42, 46, 56). Throughout this report, the notation for individual Hg isotopes (e.g., ^{201}Hg), is used as shorthand for the excess concentration of a single enriched isotope above its natural abundance. MeHg production in cultures was assayed by measuring the amount of Me^{201}Hg produced from an inorganic $^{201}HgCl_2$ spike, while demethylation was assayed by measuring the loss of Me^{199}Hg spiked into a sample (38, 40, 53). Enriched Hg isotopes (^{199}Hg [91.95%] and ^{201}Hg [98.11%]) were obtained from Oak Ridge National Laboratory. Me^{199}Hg was synthesized from $^{199}HgCl_2$ using methylcobalamin (41). For most experiments, $^{201}HgCl_2$ and Me^{199}HgCl were added at 10 ng/ml of culture medium and tests were done in triplicate with uninoculated medium as a blank, unless specified otherwise. The stable isotope concentrations used were significantly above background Hg concentrations in the culture medium. For culture studies using 10 ng ^{201}Hg/ml, detection limits for MeHg production were on the order of 0.1 pg/ml or 0.001% methylation, based on the method detection limit for excess Me^{201}Hg. The average relative percent deviation (RPD) for duplicate analysis of methylation assays was 2%. For culture studies using a 10-ng-Me^{199}Hg/ml spike, the detection limit for MeHg degradation was on the order of 3%, based on the average RPD of duplicate sample analyses. The amount of dissolved gaseous mercury (DGM) produced by cultures and controls was assessed by purging the sealed cultures onto gold traps. Mercury loss to bottle walls was measured directly at the end of the incubations by filling empty bottles with 0.5% (70:40 concentrated nitric-sulfuric) acid plus 1% BrCl and analyzing the digest acid after >24 h.

Induction of Hg methylation. *D. desulfuricans* ND132 and another Chesapeake Bay *Desulfovibrio* isolate, T2, were grown through four batch culture cycles at four Hg concentrations (0, 0.5, 5, and 50 mg/liter) and then assayed for MeHg production. Mercury exposure and methylation assays were done in SRM medium (with lactate as the substrate and sulfate as the electron acceptor). Methylation was assayed during batch culture growth with 500 ng/ml added $HgCl_2$.

Mercury toxicity. Mercury toxicity was assessed during batch culture fermentative growth (without sulfate) with pyruvate as the carbon source. Mercury was added to medium as $HgCl_2$ before inoculation. Toxicity was assessed by following the optical density (OD) of cultures through time.

Hg and MeHg analysis. Hg and MeHg quantifications were carried out by isotope dilution inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer ElanDRC ICP-MS) coupled to standard digestion/distillation methods for trace-level Hg analysis and speciation, as previously described (42, 56). Briefly, total Hg samples were digested in 0.5% (vol/vol) digest acid (a mix of 70:40 concentrated nitric-sulfuric acids with 1% [wt/vol] BrCl). Digested samples were introduced into the ICP-MS following $SnCl_2$ reduction in a flow injection/gas-stripping system. MeHg was separated from the sample matrix via atmospheric pressure water vapor distillation, followed by ethylation, purging with nitrogen gas, trapping on Tenax, thermal desorption, separation by gas chromatography, and detection with ICP-MS.

The concentrations of natural abundance Hg and MeHg and of excess enriched Hg stable isotopes were quantified via isotope dilution (39). Isotope dilution ICP-MS uses an enriched Hg stable isotope spike as an internal standard in every sample, significantly improving the accuracy and precision of these multistep analytical methods. Blanks and suitable certified reference materials were run with every batch.

Alignment and phylogenetic analyses of Hg methylation within *Desulfovibrio*. A 16S rRNA gene-based phylogenetic tree of *Desulfovibrio* was constructed. The tree was based on the aligned sequences of 49 type strains of *Desulfovibrio*, plus *Desulfobacter postgatei* as the reference and outgroup taxon, all available from the Ribosomal Database Project (RDP) on 10 October 2010 (17). To these were added 13 additional species of *Desulfovibrio* that have been tested for mercury methylation ability and for which 16S rRNA gene sequences were available (see Tables S2 and S3 in the supplemental material). We sequenced available cultures for which sequences were not published. The RDP alignment was used as a reference for the addition of these taxa in GENEIOUS version 5.1, and the final alignment was manually corrected. The alignments were trimmed to a common region that included 1,384 aligned bases for all taxa, except in the cases of strain *D. desulfuricans* strain LS and *D. africanus* ADR13, for which only partial length sequences (1,183 and 540 bp, respectively) were available. The partial sequence for *D. desulfuricans* LS, done in the 1990s, was provided by R. Devereux. It lacks 200 bp at the 3′ end and has a number of gaps. A full list of the source of all sequences used is given in Table S2.

The alignment was analyzed through a Bayesian phylogenetic approach as implemented in MrBayes version 3.1 (65). The program was set to use a HKY85 substitution model and a gamma rate distribution, to run in six parallel chains, and to save every 100th tree. A total of 2,100,000 generations of the chains in the Markov chain Monte Carlo (MCMC) simulation procedure were conducted. The average standard deviation (SD) of split frequencies between the MCMC chain runs was <0.01 upon termination of the analysis. Trees from the initial 100,000 generations were discarded as burn-in of the chains, and the remaining 2,000,000 are summarized in a 50% majority rule consensus tree with the branch posterior probabilities shown.

FIG. 1. Scanning electron microscopy image of *D. desulfuricans* ND132 showing a rod shape of variable length that has a tendency to curve.

RESULTS

Description and morphology. *D. desulfuricans* strain ND132 cells are Gram-negative, slightly curved rods with a relatively constant diameter of ~0.75 μm but variable lengths, averaging ~4 μm (Fig. 1). The strain is motile and produces bisulfate reductase (desulfoviridin) (61). It is nonsporulating based on visual observation of old cultures and was unable to grow after exposure to 90°C heat for 10 min. Phylogenetic analysis based on the 16S rRNA gene sequence (HQ693571) assigned the strain to the genus *Desulfovibrio* (*Deltaproteobacteria* → *Desulfovibrionaceae*).

Electron donors and acceptors. *D. desulfuricans* strain ND132 is an incomplete oxidizer, with limited metabolic flexibility (Table 1). The most robust growth was observed during sulfate respiration with either lactate or pyruvate as the carbon source. Fermentative growth occurred on pyruvate in the absence of sulfate, but with lower cell yields than during respiratory growth with pyruvate and sulfate. The organism was not able to grow on lactate alone. It was also able to utilize fumarate or formate during sulfate respiration. Growth on fumarate alone occurred after a long lag but achieved nearly the same cell densities as growth with pyruvate alone. All media containing formate also included 20 mM acetate as a carbon source, since *Desulfovibrio* species require an organic C_2 compound in addition to CO_2 for cell synthesis. Pyruvate synthesis by a reductive carboxylation of the activated acetate, acetyl-

TABLE 1. Electron donors and acceptors supporting the growth of *D. desulfuricans* ND132[a]

Substrate	Electron acceptor	Growth[b]
Respiration		
Lactate	Sulfate	+++
Pyruvate	Sulfate	+++
Malate	Sulfate	−
Fumarate	Sulfate	++
Succinate	Sulfate	−
Proline	Sulfate	−
EtOH	Sulfate	−
Cysteine	Sulfate	−
Alanine	Sulfate	−
Glycinebetaine	Sulfate	−
Choline	Sulfate	−
Formate-acetate	Sulfate	+
Lactate	Sulfite	++
Pyruvate	Sulfite	+
Formate-acetate	Sulfite	−
Lactate	Nitrate	−
Lactate	Fumarate	+
Pyruvate	Fumarate	+++
Formate-acetate	Fumarate	+
Lactate	Fe(III)	−*
Pyruvate	Fe(III)	−*
Pyruvate-acetate	Fe(III)	−*
Acetate	Fe(III)	−*
Fermentation		
Lactate		−
Pyruvate		+
Malate		−
Fumarate		+
Succinate		−
Proline		−
EtOH		−
Cysteine		−
Alanine		−
Glycinebetaine		−
Choline		−
Formate-acetate		−

[a] Growth in modified SRM (6) or MOY (71) medium, with potential organic substrates added at 30 to 60 mM concentrations, was tested. For formate, 20 mM Na-acetate was also added as the carbon source. Sulfate, sulfite, fumarate, and Fe(III) citrate (50 mM) were tested as potential electron acceptors. All media contained yeast extract. All tests were done in at least triplicate, and positive cultures were subcultured again in the same medium. Growth was measured once cells reached stationary phase.

[b] +++, $OD_{660} \geq 1.0$; ++, $OD_{660} \geq 0.5$; +, $OD_{660} \geq 0.1$; *, no reduction of Fe(III).

CoA, has been shown to be the first step in cell synthesis when cells were grown on hydrogen or formate (2, 68).

Unlike for the well-studied *Desulfovibrio vulgaris* (61), growth of ND132 on formate was poor. ND132 was unable to respire sulfate in the presence of other citric acid intermediates, amino acids, and other common DSRB substrates listed in Table 1. ND132 did not reduce Fe(III) chelated with citrate, given a variety of electron donors. As is typical of most *Desulfovibrio* species, ND132 did not grow on nitrate with lactate as the electron donor (52).

Importantly, ND132 was able to grow well using fumarate as an electron acceptor and pyruvate as the electron donor. At comparable substrate concentrations, ND132 growing on pyruvate-fumarate achieved an optical density about two-thirds of that of lactate-sulfate-grown cells. This growth mode allows experimentation with minimal generation of sulfide, a strong inhibitor of Hg methylation (6). At 32°C, in modified MOYLS4 medium, the optimal conditions for growth rate and yield were 40 mM (each) pyruvate and fumarate and 100 μM Ti-NTA as a reductant. Higher concentrations of pyruvate and fumarate did not enhance growth, nor did different ratios of pyruvate-fumarate (see Fig. S1 in the supplemental material).

Optimal growth parameters. *D. desulfuricans* strain ND132 is a salt-tolerant mesophile. The optimal NaCl concentration for growth was about 2% (wt/vol), although good growth was achieved over a range of 0% to 3% (see Fig. S2 in the supplemental material). This wide range of salt tolerance reflects the estuarine habitat from which the strain was isolated. The strain grew well over a pH range of 6.8 to 8.2, with an optimum pH of 7.8 (Fig. S2). The optimal growth temperature was 32°C. Growth rate was only slightly less at 30 and 37°C, but no substantial growth occurred at 45°C. Thiogylcolate-ascorbate, Ti-NTA, and cysteine (all 100 μM) were adequate reductants for growth on either lactate-sulfate or pyruvate-fumarate in liquid medium. Strain ND132 appears to be extremely sensitive to O_2. Single colonies failed to form in the anaerobic glove bag unless the agar plates were additionally placed in sealed containers with additional palladium catalyst.

Antibiotic resistance. Determination of antibiotic sensitivity profiles for a bacterium may offer insight into the genetic complement of the organism and give a useful foundation for future genetic studies. Strain ND132 was resistant to ampicillin (up to 200 μg/ml), whereas it was sensitive to all other antibiotics tested (the aminoglycosides, kanamycin, spectinomycin, gentamicin, and G418 or genetisin) within the chosen concentration ranges (see Fig. S3 in the supplemental material). Additionally, chloramphenicol (50 μg/ml) has previously been shown to reduce leucine uptake and MeHg production by this strain (37), demonstrating sensitivity to this antibiotic and suggesting involvement of new protein synthesis for MeHg production.

Methylmercury production and degradation. MeHg production and degradation by strain ND132 were measured simultaneously in batch culture by the addition of isotopically enriched $Me^{199}Hg$ and $^{201}HgCl_2$ to culture medium prior to inoculation, each at 10 ng/ml (Fig. 2). The experiment was done with both sulfidogenic and nonsulfidogenic media.

D. desulfuricans ND132 produced MeHg in both media, primarily during exponential growth (Fig. 2A and B). The level of MeHg production was higher during growth in nonsulfidogenic (pyruvate-fumarate) medium. In this medium, roughly 35% of the Hg added as enriched $^{201}HgCl_2$ was converted to $Me^{201}Hg$ during batch growth (Fig. 2A). In lactate-sulfate medium, when millimolar concentrations of sulfide were generated, less than 10% of the added $^{201}Hg(II)$ was converted to $Me^{201}Hg$ (Fig. 2B). Uninoculated culture medium was used to assess abiotic methylation from culture medium components. In both uninoculated controls, less than 1% of the added $^{201}HgCl_2$was converted to MeHg over 68 h. MeHg production was also measured in cell-free spent medium by adding $^{201}HgCl_2$ to filtrates from active cultures. $Me^{201}Hg$ production was similar to that observed in uninoculated medium, confirming that extracellular metabolites do not directly methylate Hg.

Methylmercury was also substantially degraded during batch culture growth and in uninoculated controls (Fig. 2C and D). About two-thirds of the added $Me^{199}Hg$ added was lost during

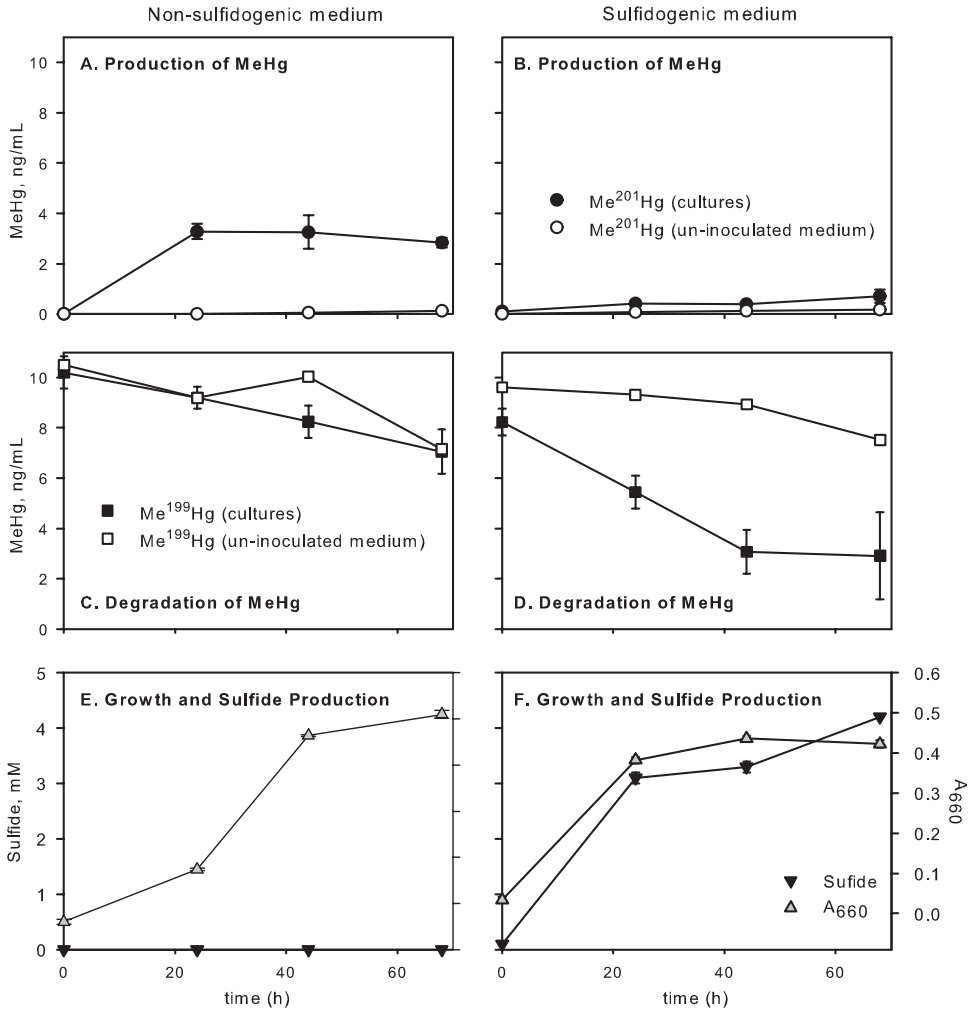

FIG. 2. Methylmercury production (A, B) and degradation (C, D) during batch culture growth of strain ND132 on pyruvate-fumarate medium (A, C, E) and lactate-sulfate medium (B, D, F). Media were amended with enriched $^{201}HgCl_2$ and $Me^{199}HgCl$ before inoculation, both to 10 ng/ml. In panels A and B, $Me^{201}Hg$ concentrations represent MeHg production, while in panels C and D, $Me^{199}Hg$ concentrations show simultaneous MeHg degradation. Error bars represent the standard deviations of results from three replicate cultures. Optical density and sulfide concentrations are shown for cultures in panels E and F.

batch culture growth on lactate-sulfate medium (Fig. 3D), while about 20% was lost in the corresponding uninoculated control. On pyruvate-fumarate medium, MeHg was degraded to the same extent (about 30%) in both cultures and uninoculated controls (Fig. 2C). The degradation of MeHg in uninoculated culture medium, in the dark, highlights the well-known lability of this compound and the need for appropriate controls when assessing demethylation in cultures and in the environment. It is notable that the sulfidogenic cultures degraded more MeHg than did uninoculated controls, while the nonsulfidogenic cultures did not. Chemical disproportionation of MeHg by sulfide (19, 69) is one potential explanation for the loss of MeHg in sulfidogenic cultures. We conducted a separate, matched experiment to test for abiotic MeHg degradation by sulfide in these media and found <3% MeHg degradation in uninoculated medium in the presence of up to 10 mM sulfide over 32 h at 27°C (see Fig. S4 in the supplemental material). We interpret these results to mean that demethylation is at least in part an active microbial process in this strain. However, the mechanism and extent of MeHg degradation by DSRB need further attention.

To evaluate the products of methylation and demethylation, and to ensure that the sampling design captured all of the Hg and MeHg in the experiments, we determined the mass balances for inorganic Hg and MeHg in the cultures (Fig. 3). We took filtered and unfiltered samples from the cultures and controls for Hg and MeHg analysis over time. At the end of the incubations, we purged the remaining medium in each bottle onto a gold column to trap any gaseous Hg that had formed. After the culture bottles were emptied, Hg that had sorbed to the glass bottle walls was measured by adding digestion acid to the empty bottles and then determining Hg in the acid.

Figure 3A and B show the mass balances for Hg added to cultures and controls as inorganic ^{201}Hg. About 2 μg (as Hg) of $^{201}HgCl_2$ was added to each 200-ml culture bottle prior to inoculation (leftmost bars in each panel). The remaining bars show the distribution of ^{201}Hg or MeHg after 68 h of incubation (corresponding to the last data point in Fig. 2). Other than

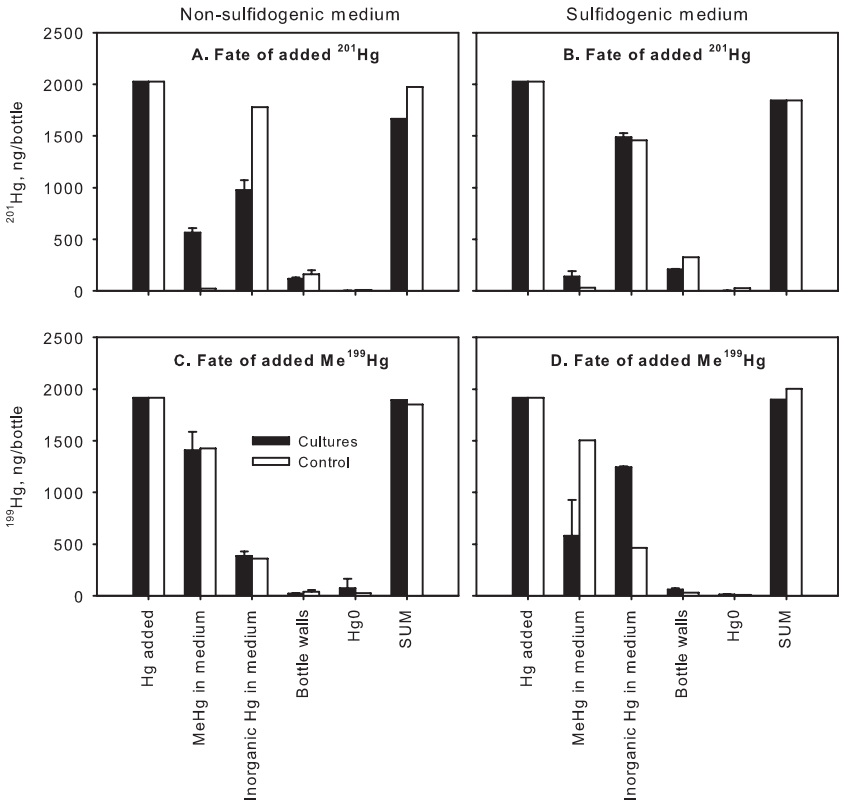

FIG. 3. Mass balance of Hg and MeHg in strain ND132 culture bottles after 68 h. Panels A and C are from pyruvate-fumarate cultures; panels B and D are from lactate-sulfate cultures. Panels A and B show the fate of inorganic ^{201}Hg added to cultures; panels C and D show the fate of Me^{199}Hg added to cultures. Data correspond to the last time point in Fig. 2. Closed bars represent averages of results from triplicate cultures, with standard deviations. Open bars represent uninoculated medium controls (single bottles). Each bar represents the total amount of Hg or MeHg in each phase in the 200-ml culture bottles. Left to right: total Hg or MeHg added to bottles, MeHg in culture medium (unfiltered), inorganic Hg in culture medium (unfiltered), total Hg on bottle walls, Hg^0, and the sum of all measured ^{201}Hg or ^{199}Hg at 68 h.

conversion to MeHg, the Hg added as inorganic ^{201}Hg remained mainly as inorganic Hg in culture medium in both sulfidogenic and nonsulfidogenic media. In this figure, the bars showing Hg and MeHg in culture medium include both the filterable and the particulate phases. Between 5 and 15% of the added ^{201}Hg(II) had sorbed to the glass bottle walls (fourth set of bars). Less than 1% of ^{201}Hg was converted to DGM in either medium and in uninoculated controls. After 68 h growth, the sum of all measured fractions was within 10% of the amount of added ^{201}Hg in all but one of the bottles (rightmost bar in each panel).

Figure 3C and D show the mass balances for Me^{199}Hg added to cultures and controls. About 2 μg (as Hg) of Me$^{199}HgCl_2$ was added to each 200-ml culture bottle prior to inoculation (leftmost bars in each panel). Me^{199}Hg was degraded to inorganic ^{199}Hg(II), with less than 5% of Me^{199}Hg converted to DGM in either medium. After 68 h of growth, the sum of all measured fractions was within 5% of the added Me^{199}Hg in all of the culture bottles. Although dimethylmercury was not explicitly measured, it would have been included in the MeHg fraction, since our samples were preserved in HCl acid, which readily converts dimethylmercury to MeHg in aqueous solution (4). This mass balance helps confirm that the declines in MeHg concentration were due to degradation (biotic and abiotic) and not to other loss mechanisms (like sorption to bottle walls or reduction to elemental Hg^0).

We also measured the distribution of Hg and MeHg between filterable and particulate phases within the culture medium (Fig. 4). These data provide information on bioavailability, sorption of Hg and MeHg to cells, fate of MeHg formed by cells, and chemical precipitation of Hg in uninoculated control medium. Even in uninoculated medium, more than half of the inorganic ^{201}Hg was initially found in the particulate phase (Fig. 4C and D), although there was no visible precipitate (Fig. 4C and D). In cultures, less than 10% of inorganic Hg remained in the filterable phase. Interestingly, this was true of both sulfidic and nonsulfidogenic cultures, suggesting that sulfide precipitation is not the driving mechanism. We interpret this to mean that there was rapid precipitation of inorganic Hg in the culture medium and, additionally, rapid association of inorganic Hg with cells. Potential Hg-containing precipitates include Ca and Mg phosphates and any TiO_2 formed from the Ti-NTA medium reductant. Initially, almost all of the Me^{201}Hg produced from inorganic ^{201}Hg by strain ND132 was filterable (Fig. 4E and F), demonstrating that most MeHg produced by cells is excreted. Over time, the Me^{201}Hg produced by cultures moved slowly into a particulate phase. The Me^{199}Hg added to cultures remained primarily in the filterable phase throughout the time course (data not shown).

Role of growth mode and reductants in methylation. We examined the role of substrates, electron acceptors, and reduc-

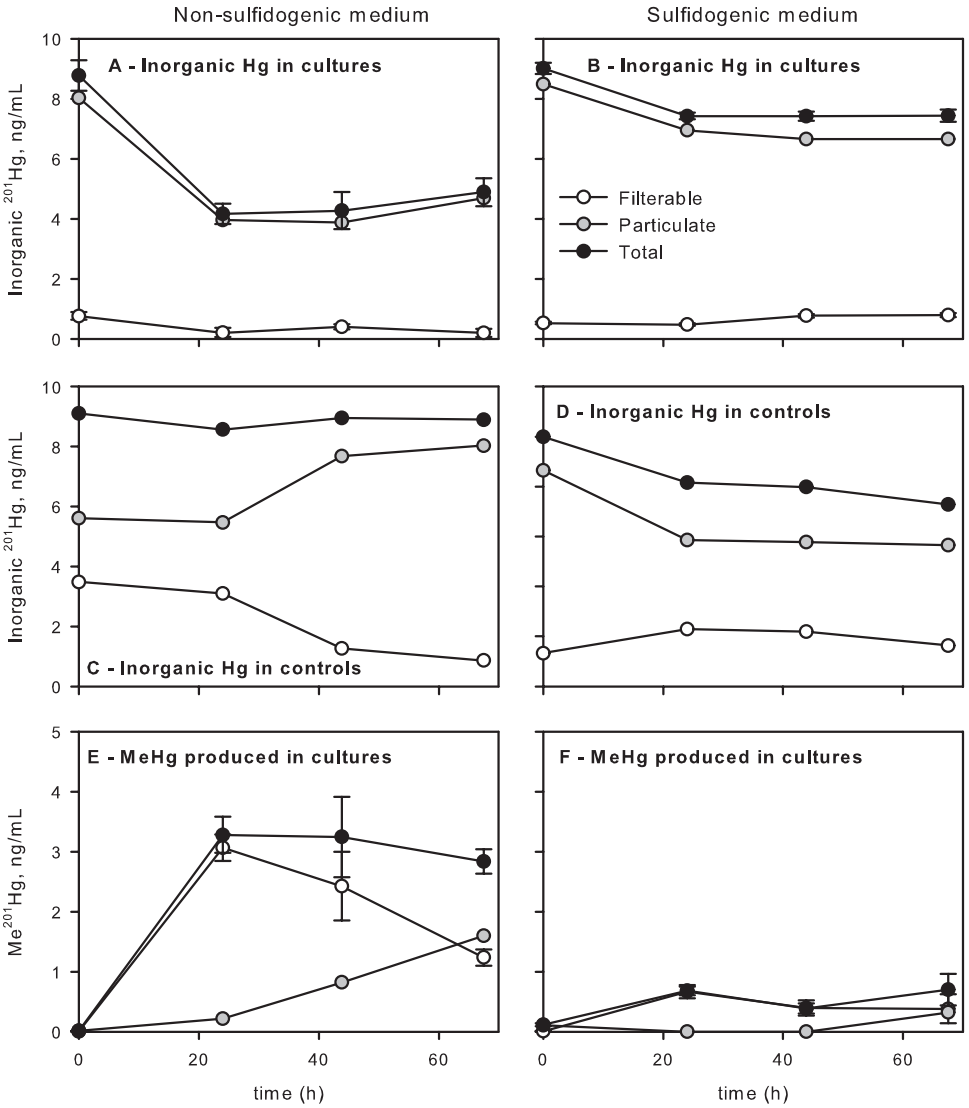

FIG. 4. Distribution of Hg and MeHg between the filterable and particulate phases in cultures and controls over time in batch cultures. "Filterable" refers to any Hg or MeHg that passes a 0.2-μm-pore-sized filter and is therefore not adsorbed to cells or bottle walls. Data correspond to the experiments represented in Fig. 2 and 3. (A and B) Inorganic ^{201}Hg in cultures. (C and D) Inorganic ^{201}Hg in uninoculated control medium. (E and F) Me^{201}Hg formed from inorganic ^{201}Hg in cultures. Data points show the averages of results from triplicate cultures, with standard deviations, or from single controls. Hg and MeHg were measured explicitly in the filtered and unfiltered samples; particulate data were obtained by subtraction. The level for inorganic Hg was calculated as the difference between the levels for total and MeHg.

tants in MeHg production by measuring MeHg production under different culture conditions (Fig. 5). The choice of organic substrate had limited effect on MeHg production. However, as expected (6, 7), sulfidogenic growth resulted in an almost 10-fold decrease in MeHg production relative to the level for growth without sulfide production. The choice of reductant had little impact on net MeHg production, at least during batch culture growth (Fig. 5, right).

Mercury concentration dependence of MeHg production. We also explored the relationship between Hg concentration in culture medium and net MeHg production. A concentration dependence experiment was done with batch cultures, and MeHg concentration was examined once cultures reached stationary phase. Under these test conditions, net MeHg production by strain ND132 growing on lactate-sulfate medium was a strong positive function of the log of the total added Hg concentration (Fig. 6).

Hg methylation is not inducible. We tested the inducibility of methylation in two Hg-methylating DSRB isolates, *D. desulfuricans* ND132 and another Chesapeake Bay *Desulfovibrio* isolate, T2. Cultures were exposed to four different Hg concentrations for multiple generations, after which time MeHg production rates were measured at a fixed mercury concentration. Preexposure to Hg did not affect rates of MeHg production by the strains tested (Fig. 7). For both strains, neither MeHg production nor growth was affected by preexposure to any of the Hg concentrations tested (analysis of variance [ANOVA]; single factor, alpha <0.05). Mercury methylation is a constitutive activity in these organisms. This small study also highlights the resistance of DSRB to Hg.

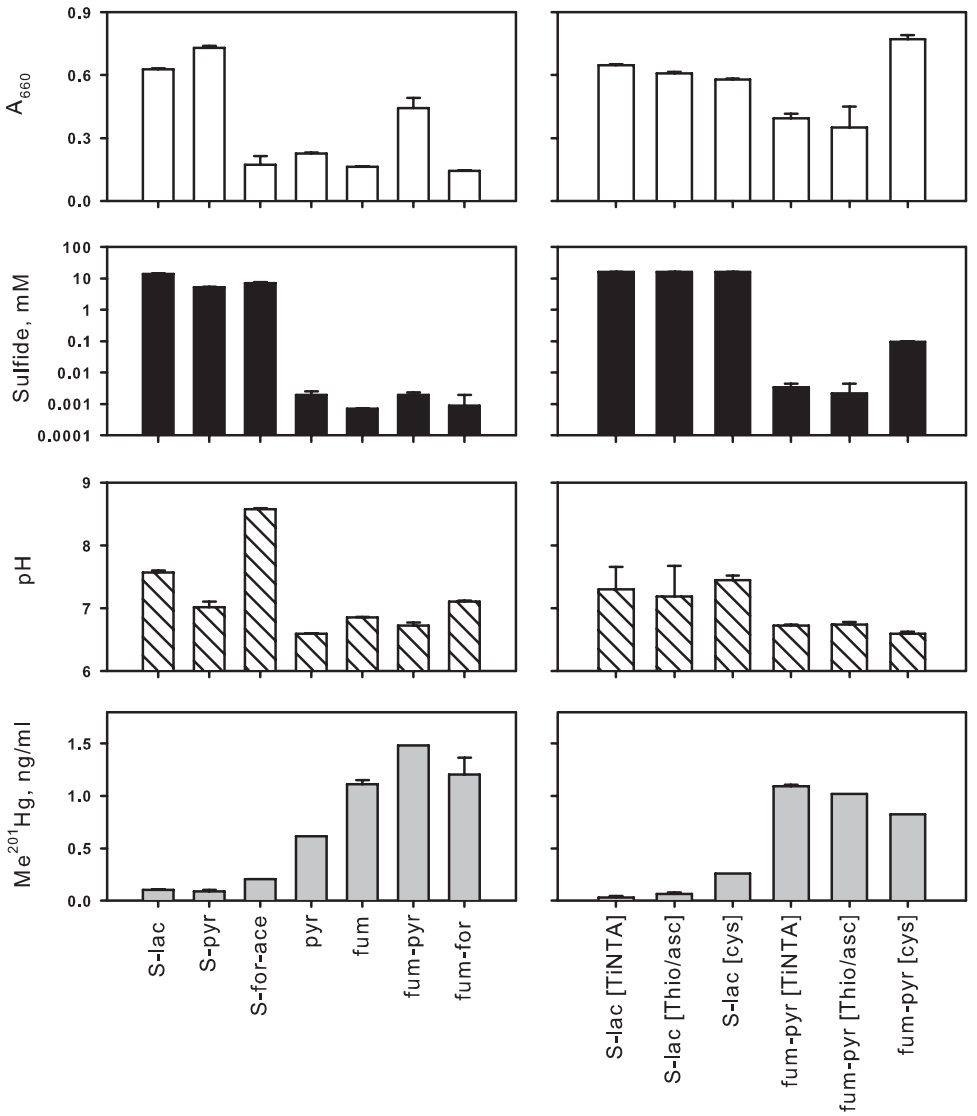

FIG. 5. Influence of substrates, electron acceptors, and medium reductants on growth and MeHg production by *D. desulfuricans* ND132. Data were collected at the end of batch growth on 40 mM substrates. Left panels show growth with a range of electron donors and acceptors, all reduced with 100 μM Ti-NTA; "S" represents sulfate (40 mM). Right panels show growth on sulfate-lactate (S-lac), sulfate-pyruvate (S-pyr), sulfate-formate-aceate (Sfor-ace), pyruvate (pyr), fumarate (fum), fumarate-pyruvate (fum-pyr), or fumarate-formate (fum-for) medium with Ti-NTA, thioglycolate-ascorbate ([Thio/asc]), or cysteine ([cys]) as the medium reductant (all added to give 100 μM). Error bars represent the standard deviations of results from three replicate growth tubes, with levels for blanks (uninoculated culture medium) subtracted where appropriate.

The ability to produce MeHg does not confer Hg resistance. To test whether the ability to produce MeHg confers Hg resistance on DSRB species, we compared the toxicities of Hg(II) to four estuarine DSRB species, two of which are capable of MeHg production and two of which are not. *D. desulfuricans* ND132 was no more or less resistant to inorganic Hg (based on growth) than two other estuarine *Desulfovibrio* strains that are not capable of Hg methylation (Fig. 8). One of the Hg-methylating strains tested (T2) was more sensitive to Hg than were the two nonmethylating strains tested. A study in which the aqueous concentration and complexation of Hg were controlled and modeled would provide stronger evidence, but these results suggest that the ability to produce MeHg does not confer Hg resistance. This makes sense given that MeHg is more toxic to bacteria than is inorganic Hg (45).

Mercury methylation capability among *Desulfovibrio* strains. In order to better understand the distribution of strains capable of producing MeHg within the genus *Desulfovibrio*, the Hg methylation ability of several *Desulfovibrio* strains was tested (Table 2). The Hg methylation capability of many of these organisms has not previously been published, with the exceptions of *Desulfovibrio desulfuricans* subsp. *desulfuricans* ATCC 13541 (47), *D. africanus* DSM 2603 (27) and *D. vulgaris* DSM 644 (62). Among the strains from culture collections, only *D. africanus* DSM 2603 produced MeHg concentrations significantly above that of uninoculated medium controls.

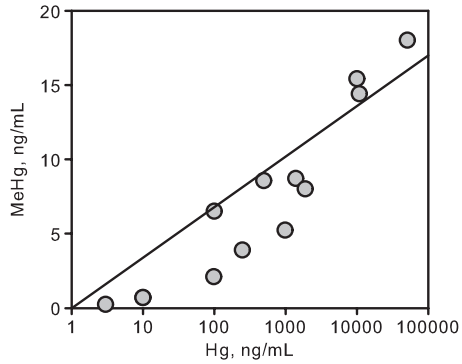

FIG. 6. Concentration dependence of MeHg production on inorganic Hg(II) added to medium by strain ND132 growing sulfidogenically on lactate-sulfate medium. All data were taken from batch cultures once cells had reached stationary phase. Mercury was added to culture medium prior to inoculation. The final optical densities (A_{660}) of cultures ranged from 0.4 to 0.7. The regression line fit to the data forced the intercept through zero: MeHg = 3 × (log Hg concentration).

Both Chesapeake Bay strains produced MeHg, although at very different rates.

It can be difficult to compare reported methylation rates among organisms, because medium chemistry, growth phase, and mercury concentration all affect methylation rates, as described here for strain ND132. However, King et al. (47) showed that MeHg production rates differ among Hg-methylating bacterial species. We also found significant difference among strains grown on the same medium (Table 2).

With the addition of the strains listed in Table 2, 19 *Desulfovibrio* strains have been tested for their ability to produce MeHg. Of those, about half (nine) do so. More *Desulfovibrio* strains have been tested than strains in any other single genus. Table S3 in the supplemental material provides a compilation of literature reports of Hg methylation by *Desulfovibrio* strains.

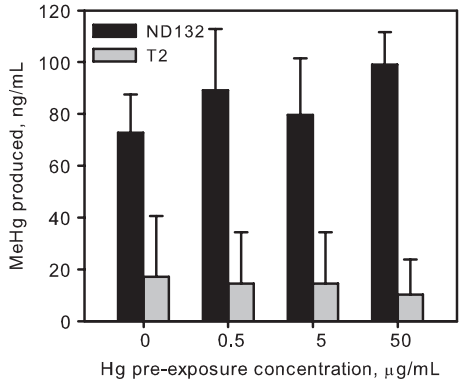

FIG. 7. Preexposure of two DSRB cultures to mercury did not increase MeHg production rates. *D. desulfuricans* ND132 and *D. desulfuricans* T2 were exposed to 0, 0.5, 5, or 50 μg Hg/ml through four batch culture cycles and then assayed for MeHg production from batch cultures with 500 ng/ml added $HgCl_2$. Bars show the amount of MeHg produced by cells preexposed to different Hg concentrations. Each bar represents the average and standard deviation of results from 4 methylation assays. Cell growth was not affected by preexposure to Hg, as assessed by the final optical density and the growth rate in the methylation assays (data not shown).

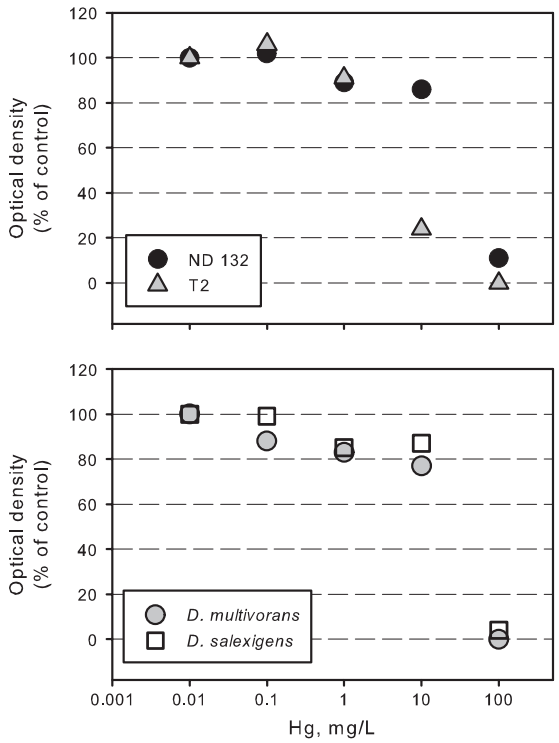

FIG. 8. The ability to produce MeHg does not confer Hg resistance. Mercury toxicity, as assessed by optical density at the end of batch culture growth, is shown for four estuarine DSRB species. The strains in the top panel are capable of MeHg production, whereas the strains in the bottom panel are not. All cultures were grown fermentatively on pyruvate. Each data point represents the average of results from three separate cultures. The standard deviation for each point averaged about 5%. OD_{660} readings were corrected for OD in medium blanks with matched Hg concentrations.

Phylogenetic distribution of the ability to produce MeHg. We constructed a 16S rRNA gene-based phylogeny of the genus *Desulfovibrio* in order to explore the evolutionary basis for MeHg production within this group (Fig. 9). *Desulfovibrio* is a large diverse genus, with 49 type strains listed in the RDP database (17). Our alignment included the 15 strains for which Hg methylation has been tested and for which sequences were also available (including strains presented here and those in the literature), plus all of the *Desulfovibrio* type strains. The analysis yielded four well-supported deep-branching main clades plus a number of less-well-supported smaller groups.

Most of the well-studied *Desulfovibrio desulfuricans* type strains, including *D. vulgaris* Hildenborough and *D. desulfuricans* strains Essex 6 and G20, fall into two related groups, labeled "Desulfuricans" groups 1 and 2 in Fig. 9. Interestingly, *D. desulfuricans* LS also aligned in this clade. However, only a partial (<1,200-bp) and discontinuous sequence is available for this lost strain, and its placement should be interpreted with some caution. Most of the strains tested for methylation to date fall in this group, but other than strain LS, there are no identified Hg methylators.

Strain ND132 falls into a group that is dominated by marine and halophilic strains (labeled the "Halophile" group in Fig. 9). This group includes another closely related methylator, *Desulfovibrio* strain BerOc1, isolated from a Mediterranean

TABLE 2. Mercury methylation capabilities of several *Desulfovibrio* species

Species or strain	Source	Medium	MeHg produced (ng/ml)[a]	
			Avg (SD)	Abiotic control
D. desulfuricans aestuarii Sylt3	ATCC 29578	Sulfate-lactate	0.040 (0.02)	0.020
D. desulfuricans subsp. *desulfuricans* Essex 6	DSM 642	Sulfate-lactate	0.001 (0.000)	0.001
D. desulfuricans subsp. *desulfuricans* El Agheila Z	DSM 1926	Sulfate-lactate	0.001 (0.001)	0.001
D. desulfuricans G20	J. Wall	Sulfate-lactate	0.001 (0.001)	0.001
D. gigas	DSM 1382	Sulfate-lactate	0.000 (0.001)	0.020
D. salexigens	DSM 2638	Sulfate-lactate	0.24 (0.32)	0.08
D. africanus	DSM 2603	Sulfate-lactate	0.51 (0.052)	0.02
D. africanus	DSM 2603	Pyruvate-fumarate	3.4 (0.79)	0.003
D. vulgaris Hildenborough	DSM 644	Sulfate-lactate	0.03 (0.02)	0.01
Desulfovibrio sp. strain T2	Chesapeake sediments	Sulfate-lactate	1.5 (0.3)	0.02
Desulfovibrio sp. strain X2	Chesapeake sediments	Sulfate-lactate	0.35 (0.1)	0.10
D. desulfuricans ND132	Chesapeake sediments	Sulfate-lactate	0.70 (0.26)	0.16
D. desulfuricans ND132	Chesapeake sediments	Pyruvate-fumarate	3.3 (0.30)	0.05

[a] Methylation was assayed by measuring MeHg at the end of batch growth, i.e., once cultures reached stationary phase, on medium containing 10 ng Hg/ml, added as $HgCl_2$ prior to inoculation. All assays were performed in triplicate, and averages are presented with standard deviations (SD).

estuary (62). However, the group also includes a strain without the ability to produce MeHg. Weakly related to this group is *D. africanus.* Both the type strain and strain ADR13, isolated from a French estuary (62), are Hg methylators. They are both weakly related to Hg-methylating strain T2 from Chesapeake Bay.

A group of species with the ability to utilize a broader set of substrates than is common in other *Desulfovibrio* species clusters loosely together in the "Substrate" group. This group contains Chesapeake Bay Hg-methylating strain X2. No other species in the group have been tested.

DISCUSSION

Phylogenetic distribution of Hg-methylating organisms. About half of the bacterial species known to produce MeHg are in the genus *Desulfovibrio*, but only about half of the *Desulfovibrio* species tested so far have the ability to produce MeHg. All of the other known Hg methylators are also *Deltaproteobacteria*. Ranchou-Peyruse et al. (62) constructed a 16S rRNA-based phylogeny of Hg methylators in the *Deltaproteobacteria* and concluded that, within the *Deltaproteobacteria*, there is no obvious taxonomic structure associated with the ability to methylate Hg.

Because so much bacterial diversity is unaccounted for in testable pure cultures, and since all of the known type strains of the genus have not been tested, a fully statistically supported ancestral state reconstruction for Hg methylation (e.g., using ModelTest or other methods) was not possible. However, our more specific alignment of Hg methylators within the genus *Desulfovibrio* suggests a potential, but unidentified, evolutionary basis for Hg methylation. Most of the commonly studied *Desulfovibrio* strains, including those for which full genomic sequences are available, aligned into a clade that contains few (if any) Hg methylators, while most of the strains with the ability to methylate Hg fall, at least loosely, into a group that is dominated by halophiles. This finding merits further investigation but must be interpreted with caution since several of the lineages within our analysis have relatively weak support, and the fraction of *Desulfovibrio* strains tested for Hg methylation remains low. As more *Desulfovibrio* genome sequences become available, it will be interesting to see how our alignment holds up with multiple-gene phylogenies. Further, since few organisms outside the *Deltaproteobacteria* have been tested for methylation, it is premature to assume that this ability is confined to a few clades within *Deltaproteobacteria*. To summarize, most of the Hg-methylating bacteria identified to date fall within a few clades in the *Deltaproteobacteria*, including two clades in the genera *Desulfovibrio* and *Geobacter* plus a few scattered species in the *Desulfobacteraceae* and the *Desulfovibrionaceae.*

Mercury methylation and MeHg demethylation by ND132. *D. desulfuricans* ND132 has the ability to produce MeHg and to degrade it, as first reported by Pak and Bartha (58, 59). The net rate of MeHg production depends on the ratio of rate constants for each process and the substrate pools. This is the same balance observed in most anaerobic sediments, where demethylation rate constants generally exceed methylation rate constants, but the substrate pool for methylation is much larger than the MeHg concentration, so that on balance a small percentage of the total Hg pool is found as MeHg (e.g., references 42 and 57).

As expected, gross methylation rates were substantially lower in the presence of sulfide. The strong inhibition of net MeHg production by sulfide has repeatedly been demonstrated to occur in cultures and natural sediments (9, 20, 31, 35, 54). Interestingly, the demethylation rates observed in strain ND132 were much higher in sulfidogenic cultures. Thus, the net MeHg production level is lower in sulfidogenic cultures because of both lower production and higher degradation. Abiotic controls containing sulfide did not significantly demethylate MeHg, suggesting no direct effect of sulfide on demethylation. The mechanism of MeHg degradation by species that do not contain the *mer* operon is unknown. Although sulfide-mediated abiotic degradation has been proposed, our data suggest that demethylation is a metabolic process, albeit one that is significantly affected by medium chemistry and MeHg bioavailability.

We observed that MeHg production by strain ND132, under otherwise fixed conditions, was a function of the log Hg concentration (Fig. 6). Put another way, the fraction of Hg converted to MeHg decreases significantly as Hg concentrations

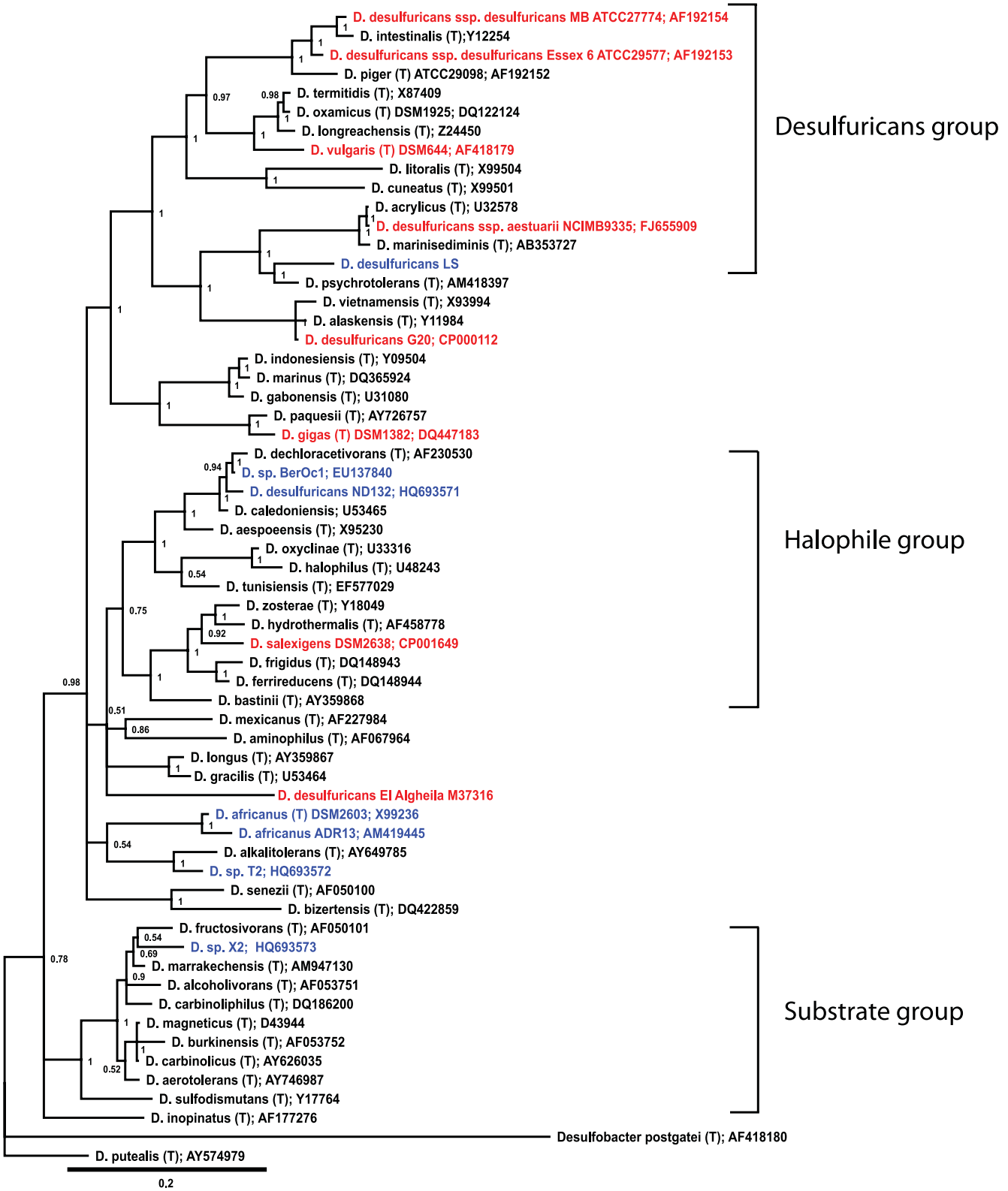

FIG. 9. 16S rRNA gene phylogeny for the genus *Desulfovibrio.* The tree was based on the aligned gene sequences of 16S rRNA from 49 type species of *Desulfovibrio*, plus 13 additional species of *Desulfovibrio* that have been tested for mercury methylation ability (Table 2; see also Table S2 in the supplemental material). The tree contains all *Desulfovibrio* strains for which Hg methylation ability has been reported to date, including those previously reported by other investigators (where sequences were available) (Table S2) and those reported here (Table 2). Type strains are indicated by "(T)." Strains able to produce MeHg are shown in blue, those unable to do so are in red, and strains that have not been tested are black. Positive methylation was defined as MeHg production significantly in excess of available (usually abiotic) controls. Alignment and phylogenetic methods are given in detail in the text. *Desulfobacter postgatei* was used as a reference and outgroup taxon.

increase. King et al. (49) also found a nonlinear relationship in estuarine sediment slurries and attributed it to first-order Michaelis-Menten kinetics. They hypothesized that the relationship was due to saturation of methylating enzymes in SRB. However, this relationship does not necessarily mean that Hg methylation follows Michaelis-Menten kinetics or that Hg uptake becomes saturated at higher Hg concentrations (48). It is more plausible to attribute the relationship to the solubility and speciation of inorganic Hg in the culture medium (10). Many researchers have hypothesized that Hg is available only to cells from the aqueous phase (8, 56) or perhaps bound to small organics that are taken up by cells (3, 33, 34, 66). Mercury is highly reactive to surfaces and particles, and the concentration in solution is not usually a linear function of the total Hg concentration.

Status of understanding the Hg methylation process. The ability to produce MeHg is constitutive, i.e., not inducible by prior exposure to Hg. However, the rate of methylation by any organism depends heavily on the bioavailability of Hg in culture medium for uptake. Like *Desulfobulbus propionicus* (6, 37), strain ND132 is an example of a DSRB that can produce MeHg when growing on a variety of electron acceptors. In our hands, all DSRB strains with the ability to methylate Hg have done so under all culture conditions tested, while strains that did not methylate Hg could not be induced to do so by changing conditions. From these results, we infer that methylation is not inducible nor is it linked directly to the sulfate electron transport pathway.

Our data show that Hg methylation occurs inside cells but that MeHg is rapidly exported out of cells. Our tests with spent culture medium confirm that extracellular metabolites do not methylate Hg. Mercury complexation dramatically influences uptake and methylation. In order to assess and compare Hg methylation rates among bacterial species and across growth conditions, it is necessary to know how Hg has partitioned among cells, medium, and bottle walls. Some measure of important aqueous Hg ligands, including thiols, dissolved organic matter, and sulfide, is also critical. Although it is clear that Hg complexation affects Hg uptake by DSRB, competing hypotheses for Hg uptake mechanisms by *Deltaproteobacteria* have not been resolved; indeed, uptake mechanisms may differ among Hg-methylating organisms (8, 66).

The ability to produce MeHg does not confer Hg resistance. Thus, Hg methylation did not evolve as a mechanism to protect cells from Hg toxicity. Rather, Hg methylation is most likely a metabolic mistake. Choi et al. (16) showed that MeB_{12} is the proximate methylating agent for Hg in *Desulfovibrio* strain LS, with the methyl group likely originating through the acetyl-CoA pathway. However, we now know that this pathway is not common to all Hg methylators, so small differences in enzymes within this pathway probably do not control Hg methylation ability among strains. We remain ignorant of whether differences in methylation ability among bacterial species are driven by differences in methyl transfer pathways, uptake mechanisms, metal-thiol housekeeping within cells, or something we have not yet suspected.

***Desulfovibrio desulfuricans* ND132 as a model organism.** *D. desulfuricans* ND132 makes an ideal organism for the study of Hg methylation because it exhibits exceptionally high rates of MeHg production but otherwise appears to be a relatively typical anaerobic, mesophilic estuarine *Desulfovibrio* strain. Phylogenetically, ND132 falls into a large group of salt-tolerant *Desulfovibrio* strains that are relatively distant from the best-studied strains in the genus. The organism has a wide range of salt and pH tolerance. Unlike many DSRB strains, ND132 has the ability to grow well using fumarate as an alternative electron acceptor to sulfate, allowing study of methylation during rapid growth while avoiding sulfide inhibition of methylation. The newly finished genome for strain ND132 (14) is the first complete genome that we are aware of for a *Desulfovibrio* strain that generates MeHg. The genome sequence will allow comparison with the many available full-genome sequences of other *Desulfovibrio* species and other DSRB and FeRB and present an unparalleled opportunity for follow-on studies that might include comparative transcriptomic and proteomic studies.

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