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Mercury methylation coupled to iron reduction by dissimilatory iron-reducing bacteria

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HIGHLIGHTS

- Iron reduction occurred with DIRB. Hg(II) restrained dissimilatory Fe(III) reduction.
- Mercury methylation could be mediated by iron-reducing bacteria (DIRB).
- Mercury methylation rate was positively correlated with iron reduction rate.

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ABSTRACT

Iron reduction and mercury methylation by dissimilatory iron-reducing bacteria (DIRB), *Geobacter sulfurreducens* and *Shewanella oneidensis*, were studied, and the relationship of mercury methylation coupled to iron reduction was determined. The ability of both bacteria for reducing iron was tested, and Fe(III) reduction occurred with the highest rate when ferric oxyhydroxide was used as a terminal electron acceptor. *G. sulfurreducens* had proven to mediate the production of methylmercury (MeHg), and a notable increase of MeHg following the addition of inorganic Hg was observed. When the initial concentration of $HgCl_2$ was 500 nM, about 177.03 nM of MeHg was determined at 8 d after *G. sulfurreducens* inoculation. *S. oneidensis* was tested negligible for Hg methylation and only 12.06 nM of MeHg was determined. Iron reduction could potentially influence Hg methylation rates. The increase in MeHg was consistent with high rate of iron reduction, indicating that Fe(III) reduction stimulated the formation of MeHg. Furthermore, the net MeHg concentration increased at low Fe(III) additions from 1.78 to 3.57 mM, and then decreased when the added Fe(III) was high from 7.14 to 17.85 mM. The mercury methylation rate was suppressed with high Fe(III) additions, which might have been attributable to mercury complexation and low availability.

1. Introduction

Mercury (Hg) pollution has become a global concern due to its toxic properties. It contaminates water sources through atmospheric deposition, weathering of cinnabar, runoff from industrial sites and abandoned mines, and microbial production of acid mine drainage (Wiener et al., 2006). The greatest potential human health concern of Hg is the conversion of inorganic Hg to organic Hg compounds such as methylmercury (MeHg), which bioaccumulates in aquatic organisms and is a neurotoxin that damages the central nervous system in humans (Fitzgerald and Clarkson, 1991; Scheulhammer et al., 2007; Huguet et al., 2010). MeHg is highly toxic and actually more toxic than any other Hg species, such as elemental mercury (Hg^0) and mercuric ion (Hg(II)) (Fitzgerald and Clarkson, 1991), and bioaccumulative through the food chain. In addition, MeHg is water soluble, and readily transferred from sediment to water, and then to biota such as fish (Hammerschmidt et al., 2006; Lin and Jay, 2007).

MeHg concentrations in most sediments are controlled by in situ net microbial methylation (Benoit et al., 2003; Hammerschmidt and Fitzgerald, 2004). Environmental mercury methylation is an anaerobic microbial process generally driven by dissimilatory sulfate-reducing bacteria (DSRB) (Benoit et al., 2003) and magnified in organic rich sediments (Gray and Hines, 2009). Extensive research has linked sulfate-reducing with Hg methylation in a wide range of aquatic systems (Biswas et al., 2011; Bridou et al., 2011; Compeau and Bartha, 1985; Gilmour et al., 1998, 2011). Under sulfidic conditions in the presence of millimolar amounts of dissolved sulfide in organic-compound-rich marine sediments, rapid MeHg

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accumulation is coupled to fast sulfate reduction. In a field experiment, the addition of sulfate is shown to enhance the total concentration of MeHg in a sediment (Gilmour et al., 1998), which is interpreted as a net production of MeHg mediated by the activity of DSRB.

Although Hg methylation have been investigated in a variety of sedimentary environments under sulfate-reducing and methanogenic conditions, little is known about the capacity for microbial Hg transformation in sediments dominated by other terminal electron accepting processes (TEAP). Several studies have examined microbial Hg transformation in sediments amended with other potential electron acceptors such as nitrate, Fe(III), and Mn(IV) (Gilmour et al., 1998; Han et al., 2008), but these studies do not deal with whether these substrates are utilized as electron acceptors by microorganisms in the sediment.

The redox cycling of iron plays a major role in the biogeochemical cycling of many elements in natural systems (Lovley and Phillips, 1986; Liu et al., 2001; Salas et al., 2010). On the reductive side of the iron redox cycle, the dissimilatory microbial reduction of iron oxides has a major impact on the aqueous/solid phase geochemistry and behavior of natural compounds and contaminants in nonsulfidogenic subsurface sedimentary environments. Because these sediments contain iron minerals and the reduction of iron is the dominant TEAP, methylation is postulated to be due to the activity of iron-reducing bacteria. However, earlier pure-culture studies offer limited support for this hypothesis (Warner et al., 2003).

Recent research suggests that dissimilatory iron-reducing bacteria (DIRB) may play a role in environmental methylation (Warner et al., 2003; Fleming et al., 2006). Three strains of iron-reducing bacteria have been reported to methylate mercury at apparent rates similar to those reported for active sulfate reducers (kerin et al., 2006). Fleming et al. (2006) indicate that the sediments from Clear Lake, CA, which contain dissolved iron and show the signatures of iron reduction, continue to produce MeHg even in the presence of molybdate concentrations sufficient to fully inhibit sulfate reduction. The iron-reducing bacterium isolated from these sediments, *Geobacter* sp. strain CLFeRB, is able to methylate mercury in pure cultures at the rates comparable to those of sulfate-reducing bacteria.

In the present study, we aimed to screen a phylogenetically diverse group of DIRB cultures for Hg-methylating capability in order to provide insights into in situ biological methylation controls and to further investigate the phylogenetic distribution of methylating bacteria. Two well-characterized iron-reducing bacteria, *Geobacter sulfurreducens* PCA and *Shewanella oneidensis* MR-1, were employed in Fe(III) reduction and mercury methylation experiments.

2. Materials and methods

2.1. Chemicals

Mercuric chloride ($HgCl_2$, purity >95%) and methylmercury (MeHg, purity >95%) standards were purchased from Merck (Darmstadt, Germany). A stock solution was prepared with MeHg dissolving into methanol, and stored in brown bottles at 4 °C. Methanol was chromatographically pure; water was Mili-Q ultrapure; and the other reagents were of analytical grade.

2.2. Bacterium sources

Geobacter sulfurreducens PCA and *Shewanella oneidensis* MR-1 were provided by Oak Ridge National Laboratory (ORNL). The bacteria were inoculated directly from frozen stocks into Luria–Bertani (LB) broth and grown overnight in a 15 °C incubator shaking at 125 rpm. The cultures were washed with a defined medium to remove traces of LB.

2.3. Growth media and culture conditions

Two types of media were used to isolate and propagate iron-reducing bacteria. *G. sulfurreducen* medium: NH_4Cl 1.5 g L^{-1}, NaH_2PO_4 0.6 g L^{-1}, KCl 0.1 g L^{-1}, $NaHCO_3$ 2.5 g L^{-1}, CH_3COONa 0.82 g L^{-1}, and sodium fumarate 8.0 g L^{-1}, Wolfe vitamin mixture 10 mL L^{-1}, and trace element mixture 10 mL L^{-1} (ATCC Medium 1957). *S. oneidensis* medium: NaCl 5.0 g L^{-1}, beef extract 5.0 g L^{-1}, peptone 10.0 g L^{-1}, and pH 7.0. Different forms of iorn or $HgCl_2$ were added in the media as electron acceptors or donors. The final (postautoclaving) pH of each medium was adjusted to 7.0 using either 1 M HCl or 1 M NaOH.

Prior to inoculation, the medium was filter-sterilized with a 0.2 μm PES vacuum filtration system (Nalgene), and then added to serum bottles. The bottles were purged with pure N_2, plugged with butyl-rubber stoppers and crimp sealed. Washed cells were injected into the sealed serum bottles with a syringe using a 21-gauge needle to achieve a final cell concentration of approximately 1×10^8 cells mL^{-1}. Triplicate cultures were incubated at 30 °C and sampled at defined intervals for up to 480 h.

2.4. Fe(III) reduction experiments

Ferric chloride ($FeCl_3$), ferric oxyhydroxide (synthetic $Fe(OH)_3$) and ferric citrate were respectively used to perpare the Fe(III) reduced medium, whose initial concentration was 17.85 mM. For the ferrihydrite medium, ferric oxyhydroxide was synthesized as described elsewhere (Lovley and Phillips, 1986). *G. sulfurreducens* and *S. oneidensis* were grown through the batch culture in these media, and then the concentration of Fe(II) was measured for estimating the reduction rates of Fe(III).

Inorganic Hg was added to the medium for assessing the effect of Hg(II) on dissimilatory Fe(III) reduction. The medium contained ferric oxyhydroxide at a concentration of 3.57 mM, and $HgCl_2$ was added at 0, 0.5, 1.0, 1.5, and 2.0 μM. After the inoculation of bacteria and incubation at 30 °C, the contents of Fe(II) were measured.

2.5. Mercury methylation experiments

MeHg production was assayed by measuring the amount of MeHg produced from an inorganic Hg spike during batch culture growth through a stationary phase. *G. sulfurreducens* and *S. oneidensis* were grown through seven batch culture cycles at seven Hg concentrations (0, 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 μM) and then assayed for MeHg production. The toxicity of mercury exposure was assessed by following the cell density of cultures through time. For each concentration and growth condition, triplicate assays and abiotic controls were prepared. Abiotic controls were composed of autoclaved medium spiked with inorganic $HgCl_2$.

Mercury exposure and methylation assays were conducted in the defined medium with acetate as the electron acceptor and Fe(III) reduced medium with different forms of iron as the electron acceptors. Methylation was assayed during batch culture growth with 500 nM $HgCl_2$ added.

2.6. Cell density measurement

Cell density was monitored by measuring optical density at 610 nm with a Shimadzu spectrophotometer and by determining direct counts by epifluorescence. To determine direct counts, a 1-mL aliquot of culture sample was preserved with 100 mL of 37% formaldehyde (final concentration, 3.4%). Cells were then stored