Biodegradation
DOI 10.1007/s10532-015-9752-3

ORIGINAL PAPER

The effect of aqueous speciation and cellular ligand binding on the biotransformation and bioavailability of methylmercury in mercury-resistant bacteria

Udonna Ndu · Tamar Barkay · Amina Traore Schartup · Robert P. Mason · John R. Reinfelder

Received: 17 September 2015 / Accepted: 15 December 2015

Abstract Mercury resistant bacteria play a critical role in mercury biogeochemical cycling in that they convert methylmercury (MeHg) and inorganic mercury to elemental mercury, Hg(0). To date there are very few studies on the effects of speciation and bioavailability of MeHg in these organisms, and even fewer studies on the role that binding to cellular ligands plays on MeHg uptake. The objective of this study was to investigate the effects of thiol complexation on the uptake of MeHg by measuring the intracellular demethylation-reduction (transformation) of MeHg to Hg(0) in Hg-resistant bacteria. Short-term intracellular transformation of MeHg was quantified by monitoring the loss of volatile Hg(0) generated during incubations of bacteria containing the complete *mer* operon (including genes from putative mercury transporters) exposed to MeHg in minimal media compared to negative controls with non-*mer* or heat-killed cells. The results indicate that the complexes MeHgOH, MeHg-cysteine, and MeHg-glutathione are all bioavailable in these bacteria, and without the *mer* operon there is very little biological degradation of MeHg. In both *Pseudomonas stutzeri* and *Escherichia coli*, there was a pool of MeHg that was not transformed to elemental Hg(0), which was likely rendered unavailable to Mer enzymes by non-specific binding to cellular ligands. Since the rates of MeHg accumulation and transformation varied more between the two species of bacteria examined than among MeHg complexes, microbial bioavailability, and therefore microbial demethylation, of MeHg in aquatic systems likely depends more on the species of microorganism than on the types and relative concentrations of thiols or other MeHg ligands present.

Keywords Methylmercury · Bioavailability · Cysteine · Glutathione · Broad spectrum mercury resistance

U. Ndu (✉) · R. P. Mason
Marine Sciences Department, University of Connecticut, Groton, CT, USA
e-mail: udonna.ndu@duke.edu

T. Barkay
Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ, USA

A. T. Schartup
Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

U. Ndu · J. R. Reinfelder
Department of Environmental Sciences, Rutgers University, New Brunswick, NJ, USA

Introduction

The amount of mercury (Hg) in the biosphere has increased since industrialization due to human activities, and this increase has led to higher levels of methylmercury (MeHg) in aquatic organisms

Published online: 22 December 2015

consumed by humans (Driscoll et al. 2013). Consumption of fish and other seafood is the major MeHg exposure route for much of the human population (Bjerregaard 1996; Aschner and Aschner 1990), and there are substantial human health concerns related to this exposure (Ask et al. 2002; Harada 1978; Bakir et al. 1980). While much of the MeHg formed in aquatic systems is due to microbial processes, the amount of MeHg in aquatic ecosystems is controlled by its production and demethylation by both biological and abiotic processes (Morel et al. 1998). The largest bioconcentration step for aquatic food chains is between water and the base of the food web (Watras and Bloom 1992), and studies have shown that the bioconcentration factor varies widely across ecosystems. The most important factor influencing the accumulation of MeHg by microorganisms (bacteria and phytoplankton) has been shown to be the aqueous speciation of MeHg complexes (Ndu et al. 2012; Luengen et al. 2012). $MeHg^{+}$ forms stable aqueous complexes with several inorganic anions (OH^{-}, Cl^{-}, HS^{-}) and organic ligands, especially those containing reduced sulfur groups (e.g. thiols) (Rabenstein 1978; Berthon 1995). Thermodynamic modeling studies of pore water of common wetland soils and sediments indicate that organic thiols bind up to 50 % of dissolved mercury (Skyllberg 2008). Lower molecular weight thiols, like glutathione and cysteine, are a small subset of these organic thiols. While neutrally charged complexes of MeHg (e.g. MeHgCl and MeHgOH) have been shown to accumulate in microorganisms by passive diffusion (Mason et al. 1996; Benoit et al. 2001), the role of organic complexation, especially low molecular weight compounds, on MeHg accumulation is less clear.

Hg methylation rates in the anaerobic, Fe-reducing bacterium *Geobacter sulfurreducens* were higher when Hg^{2+} was bound to cysteine rather than chloride, but were lower in the presence of glutathione and penicillamine (Schaefer and Morel 2009). In contrast, it was found that thiols did not affect the accumulation of Hg(II) by the sulfate-reducing *Desulfovibrio desulfuricans* (Gilmour et al. 2011). It is not known whether MeHg is taken up in a similar manner to inorganic Hg. Using a *mer-lux* bioreporter to evaluate bioavailability of MeHg, it was reported that binding of cysteine with MeHg resulted in increased bioavailability in aerobic bacteria whereas increased binding with glutathione reduced bioavailability of MeHg (Ndu et al. 2012). It was also shown that higher molecular weight organic matter like humic acids reduced the bioavailability of MeHg when MeHg is bound to it. While these results demonstrated an effect of thiols on MeHg accumulation, they could not be used to determine the short-term uptake kinetics by which various complexes of MeHg are accumulated. The data presented in (Ndu et al. 2012) represents uptake in cells that do not have the Hg transporters and may not necessarily apply to Hg-resistant cells which have Hg transporters.

Here we examine the short-term uptake of MeHg into mercury-resistant bacteria to evaluate how speciation impacts uptake in these organisms. Broad spectrum Hg-resistant cells are able to transform MeHg to methane and the divalent mercuric ion (Hg^{2+}), and subsequently reduce Hg^{2+} to elemental mercury (Hg(0)) (Barkay et al. 2003). The transformation from MeHg to Hg(0) results from the combined activities of the enzymes MerB (organomercurial lyase) and MerA (mercuric reductase). Broad spectrum Hg-resistant cells also possess Hg transporters (MerT and MerE) in their cell membranes which act in conjunction with the periplasmic bound protein MerP to import Hg(II) and MeHg (Sone et al. 2010). With appropriate controls, the intracellular transformation rates of MeHg can be determined in broad spectrum, Hg-resistant bacteria by measuring the conversion of MeHg to volatile Hg(0) which can be purged from reaction vessels (Chien et al. 2010). In this study, we examined the effects of thiol complexation on the uptake of MeHg by quantifying the intracellular demethylation and reduction of MeHg in two broad spectrum Hg-resistant strains (*Escherichia coli* and *Pseudomonas Stutzeri* OX).

Materials and methods

Strains

In our assays, we used a strain of *E. coli* with broad spectrum mercury resistance obtained from the study of Chien et al. (2010). This strain contains two plasmids pHYR1A and pET-MB1B1. The plasmid pHYR1A was developed from the pHY300PLK vector and includes the *mer* operon genes (*E*, *P*, *T*, and *A*) from *B. cereus* RC607 under the control of the *mer* promoter. Mer E, and Mer T are transport proteins

while MerP is a perisplasmic binding protein that assists in the transport of Hg species by binding and bringing these species to the transporters which then convey Hg into cells. The formation of a tri-coordinate complex between Hg(II) and three cysteine residues in the high affinity binding site of the regulatory protein MerR is essential for the induction of the *mer* operon by Hg(II) (Helmann et al. 1990; Summers 1992). The pET-MB1B1 plasmid was developed from the PET-9a vector and includes *merB* under the control of the *lac* promoter. Thus in this strain, MerA is synthesized after induction with Hg(II) while MerB is synthesized upon induction by isopropyl β-D-1-thiogalactopyranoside (IPTG).

Pseudomonas Stutzeri OX was also used in our study. *P. stutzeri* OX is a natural strain that has 2 *mer* operons (a narrow spectrum *mer* operon and a broad spectrum *mer* operon) located on the 80-kb plasmid, pPB (Reniero et al. 1995, 1998). The plasmid pPB has a functioning transport system which includes genes for the transporters MerT and MerC, and the perisplasmic binding protein MerP (Reniero et al. 1995, 1998).

Materials

A MeHg standard was obtained from Brooks Rand Corporation. Luria–Bertani (LB) medium containing kanamycin (50 μg mL^{-1}) and ampicillin (100 μg mL^{-1}) was the growth medium. M63 and M9 minimal media (Miller 1972) containing kanamycin (25 μg mL^{-1}) and ampicillin (50 μg mL^{-1}) were used in intracellular MeHg transformation experiments. Since one of our objectives was to study the effects of extracellular MeHg speciation on the intracellular transformation of MeHg, it was necessary to carry out experiments in a minimal medium where the concentrations of anions and ligands are more clearly defined. The M63 minimal medium consists of KH_2PO_4 (100 mM), $(NH_4)_2SO_4$ (15 mM), $FeSO_4.7H_2O$ (1.7 μM), $MgSO_4$ (1 mM), and glycerol (0.4 %) while M9 consists of Na_2HPO_4 (42 mM), KH_2PO_4 (22 mM), NaCl (9 mM), $MgSO_4$ (1 mM), NH_4Cl (18.7 mM), and glycerol (0.4 %). Vitamins and yeast were not added in minimal media so as to simplify the speciation of MeHg. Minimal media was not used for growth because *E. coli* grows very slowly in these media (Selifonova et al. 1993). Minimal media used for *E. coli* cultures was further amended with IPTG (0.1 mM) during the assay. The pH of both media was 7. Solutions of the thiols used in this study (cysteine and glutathione), were prepared just before each experiment by dissolution of these compounds in water that had been deoxygenated by bubbling with ultrapure nitrogen.

Mer assays

A single colony of the *E. coli* strain was grown aerobically in LB medium containing the appropriate antibiotics at 37 °C while shaking at 270 rpm overnight (O/N). A portion of the O/N culture was added to fresh LB medium and grown for 3 h with an IPTG concentration of 0.1 mM. Cells were then repeatedly washed with minimal medium (Ndu et al. 2012). *Pseudomonas stuzeri* OX was grown aerobically at 30 °C in LB medium. A portion of the O/N culture was added to fresh LB medium and grown until mid-log phase. The cells were then washed with M9 minimal medium as described above.

To examine the effect of speciation on the transformation of MeHg, the concentration of MeHg (19 nM, 186.6 nM) was kept constant while the concentration of the ligand (cysteine or glutathione) was fixed at 80 nM or 1000 nM. As 80 nM was shown by equilibrium calculations with Mineql to be sufficient to bind all MeHg (at 19 nM), short-term intracellular transformation experiments were performed with 80 nM thiol and 19 nM MeHg. MeHg was mixed with each ligand in 0.5 mL of water before the addition of cells with an optical density (660 nm) of 0.1 in 2 mL minimal media. The final volume at the start of the experiment was 2.5 mL and the optical density was 0.073 which corresponds to a cell number of 0.6×10^6 cells mL^{-1} obtained by direct counts. At specific time intervals, cell suspensions were bubbled with ultrapure nitrogen to remove Hg(0) and then preserved with bromine chloride (BrCl) solution (0.2 N) (Lawson and Mason 1998; EPA 2002). Following a 24 h digestion with BrCl, samples were treated with hydroxylamine, and analyzed for total mercury using the Hydra AA Automated Mercury Analysis System. The loss of mercury was calculated by subtracting the amount of mercury remaining from what was added, and is reported as percentage Hg loss. Assays were done in triplicate and the standard error is reported. A blank experiment and a negative control

using an *E. coli* strain that does not have any component of the *mer* operon were performed in like manner. For assays involving *P. stutzeri*, a negative control experiment was performed by using heat killed cells. Statistical analysis was performed by using a *t* test ($P < 0.05$) to compare means at equivalent time points.

Heat killed cells assay

In order to evaluate the role of non-specific binding of MeHg to cell membranes in the bioavailability of MeHg, intracellular MeHg transformation was measured in assays of live *P. stutzeri* cells incubated with variable amounts of heat killed cells. *P. stutzeri* cells from a moderately dilute culture ($O.D._{660} = 0.1$) were killed by incubating at 80 °C for 30 min. In these assays, a fixed amount of live cells (2 ml at $O.D._{660} = 0.1$) was added to a variable amount of dead cells (0, 1, 2, 3 mL at $O.D._{660} = 0.1$) before exposure to 19 nM MeHg in M9 media.

Results and discussion

Effect of speciation

The Hg-resistant strain of *E. coli* was able to transform most of the MeHgOH, MeHg-cysteine (MeHgCYS) and MeHg-glutathione (MeHgGSH) into purgeable Hg(0) over 12 h (Fig. 1) indicating that all three forms of MeHg were bioavailable to this bacterium. The loss of Hg represents the minimum amount of MeHg that entered the cells. Although MeHgOH and MeHgCYS displayed higher bioavailabilities than MeHgGSH (Fig. 1), the differences were not statistically significant. These results are consistent with those of Ndu et al. (2012) who, using a *mer*-promoter-linked bioluminescent (*mer-lux*) bio-reporter, showed that MeHgCl, MeHgOH, MeHgCYS and MeHgGSH were all bioavailable. Whereas Ndu et al. (2012) found that MeHgCYS was more bioavailable than MeHgOH and MeHgCl, the present study could not distinguish between the bioavailabilities of MeHgCYS and MeHgOH. A key difference between the strains used in this study and Ndu et al. (2012) is that the strains used in this study have Mer transporters (Mer E, T and C) and the perisplasmic protein (MerP) which might affect the nature of MeHg uptake into cells. A negative

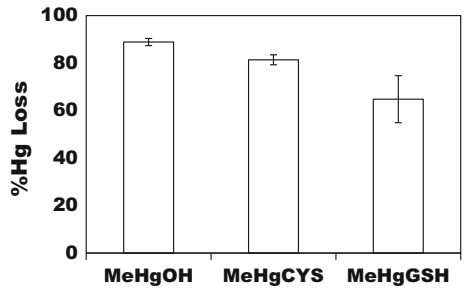

Fig. 1 Bioavailable fractions (portion converted to purgeable Hg(0)) of MeHgOH, MeHgCYS and MeHgGSH in a Hg-resistant strain of *E. coli*. Cells (O.D. = 0.07, cell number = 0.6×10^6 cells mL^{-1}) were exposed to 186 nM of MeHg in the absence of an organic ligand (MeHgOH) or in the presence of 1000 nM of cysteine or glutathione in M63 minimal medium for 12 h. MeHgOH, MeHgCYS and MeHgGSH represent methylmercury-hydroxide complex, methylmercury-cysteine complex and methylmercury-glutathione complex respectively. Equilibrium calculations show that all of the added MeHg was bound to thiol ligands in the presence of 1000 nM cysteine or glutathione

control experiment and a blank experiment showed that the loss of Hg in Hg-resistant bacteria is due to the activities of MerB and MerA (Fig. 2a, b), and without these enzymes there is very little MeHg transformation. When compared to other approaches used to examine uptake across the membrane (Schaefer and Morel 2009; Deng et al. 2013), the experimental approach used in this study offers the advantage of providing an estimate of MeHg uptake without a cell-surface wash.

Our subsequent short-term uptake experiments showed that the loss of MeHgOH was faster than that of MeHgCYS in *P. stutzeri* (Fig. 2a). Similarly, in *E. coli* slightly more MeHgOH was transformed in 1 h than MeHgCYS (Fig. 2b). It would appear that *P. stutzeri* transforms MeHg much faster than the *E. coli* strain given that *P. stutzeri* attained a maximum extent of transformation at about 1 h or less after exposure to MeHg while in *E. coli*, it took about 6 h to reach the maximum extent of transformation. Thus, *P. stutzeri* may be a better candidate for use in MeHg remediation bioreactors.

The differences in the transformation rates are consistent with our understanding of the uptake of MeHg by mercury-resistant microbes. If the rate of transfer of the MeHg from the ligand complex in solution to the transporter on the cell surface is the rate limiting step, or occurs at a similar rate to the

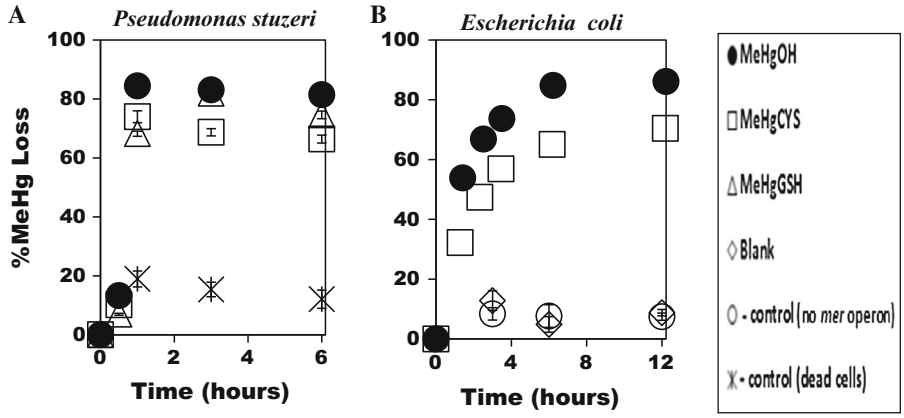

Fig. 2 **a** Transformation rates of MeHgOH, MeHgCYS and MeHgGSH into purgeable Hg(0) by Hg-resistant *Pseudomonas stuzeri* (O.D. = 0.07, cell number = 0.6×10^6 cells mL^{-1}) exposed to 19 nM of MeHg with or without 80 nM of cysteine or glutathione in minimal medium. The negative control experiment was performed under the same experimental conditions but with heat killed *P. stutzeri* cells. MeHgOH, MeHgCYS and MeHgGSH represent methylmercury hydroxide complex, methylmercury-cysteine complex and methylmercury-glutathione complex, respectively. **b** Transformation rates of MeHgOH, and MeHgCYS into purgeable Hg(0) by Hg-resistant *Escherichia coli* suspensions (O.D. = 0.07, cell number = 0.6×10^6 cells mL^{-1}) exposed to 19 nM of MeHg with or without 80 nM of cysteine or glutathione in minimal medium. The negative control experiment was performed under the same experimental conditions but with an *E. coli* strain that lacked the *mer* operon. MeHgOH, and MeHgCYS represent methylmercury hydroxide complex, and methylmercury-cysteine complex respectively

internalization reaction, then the speciation in the solution would have an impact on the transformation rate. Additionally, it would be expected that inorganic complexes would be transformed more rapidly than organic complexes. This is exactly what we found. If the internalization reaction was the rate limiting step, then the complexation in solution and the rate of transfer of the MeHg to the surface site on the transporter would occur at a more rapid rate, regardless of the speciation in solution. The observed differences strongly indicate that the transfer of the MeHg from the solution complexes to the transporter occur at a rate slower than or comparable to the rate of uptake and internal demethylation.

Evaluation of the biosensor approach

As previously described in Ndu et al. (2015), for proper interpretation of the results of the assays based on Hg(0) production, other processes that could produce Hg(0) from the added MeHg within the medium or at the surface/within cells must be taken into account as such processes would influence the net amount of Hg(0) in the medium. Examination of the negative controls shown in Fig. 2a, b, which are suspensions of cells without the *mer* operon or dead cells, indicates that there is some loss of MeHg occurring that is independent of the activities of Mer enzymes, with a maximum amount of 10–18 %. Abiotic (photochemical) demethylation is likely producing this Hg(0) as any inorganic Hg(II) produced by demethylation would likely be further reduced to Hg(0). This abiotic degradation is significantly lower than values obtained from other assays indicating that the majority of the Hg(0) mercury is formed as a result of the activities of MerB and MerA.

There is also the possibility for the transformation of Hg(0), primarily through oxidation to Hg(II), which would lead to an underestimate in the degree of transformation. Hg(0) has been shown to oxidize to Hg(II) through biotic and abiotic pathways (Gu et al. 2011; Zheng et al. 2012, 2013; Yamamoto et al. 1995). If there was net oxidation in the medium then estimates of bioavailability could be higher than derived from the assays reported here. However, Smith et al. (1998) compared Hg(0) oxidation rates in aerobic bacteria to Mer induced reduction rates in the same bacteria and found that Hg(0) oxidation rates were more than an order of magnitude lower than rates of Hg(II) reduction catalyzed by Mer enzymes. Furthermore, estimates from another previous study (Yamamoto et al. 1995) indicated that under oxic

conditions, the rate of abiotic oxidation of Hg(0) in the presence of cysteine was about 0.23 % after 10 h, or 0.02 %/h which will have little or no impact on the results of this study. Hence, for these reasons, Hg(0) oxidation is not thought to affect the validity of the results obtained in this study.

Effect of membranes

A portion of the MeHg added to all incubations, and/or the inorganic Hg(II) formed by the demethylation of MeHg, was subsequently not reduced to Hg(0) by the Hg-resistant bacteria used in this study (Figs. 1, 2). This pool of MeHg and/or inorganic Hg(II) was likely rendered unavailable to Mer enzymes by binding to cellular ligands within the bacteria (Schaefer and Morel 2009). To examine the importance of binding to cellular sites in limiting the overall extent of Hg(0) formation, an experiment with Hg-resistant *P. stutzeri* was conducted in which varying amounts of heat killed cells (80 °C for 30 min) were added to a fixed amount of live cells. If non-specific binding of MeHg and Hg(II) was an important sink for Hg besides its conversion to Hg(0), then the initial rate of loss of MeHg from the medium, and the formation of Hg(0), would be slower in the presence compared to the absence of dead cells. This is what we found (Fig. 3). This suggests that a portion of MeHg is bound to surfaces which render it not readily bioavailable, and

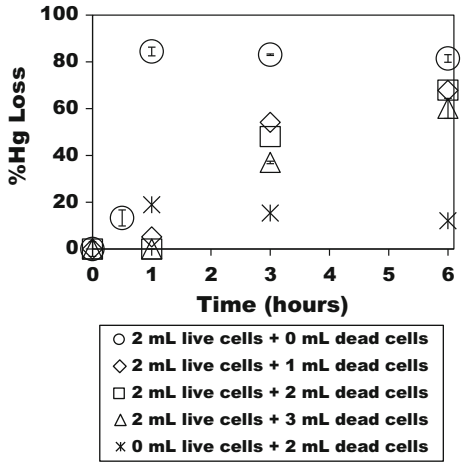

Fig. 3 MeHg transformation to purgeable Hg(0) by Hg-resistant *Pseudomonas stuzeri* OX exposed to 19 nM of MeHg in the presence or absence of heat-killed cells in M9 minimal medium. MeHgOH is methylmercury-hydroxide complex. The number of live cells is 0.6×10^6 cells

therefore it is not converted to Hg(0) by the *mer* genes. This conclusion is in agreement with past studies (Rasmussen et al. 1997) which found that increasing cell density reduced *mer-lux* bioassay sensitivity to Hg(II). It has also been reported that live phytoplankton cells accumulated more MeHg than in dead cells (Luengen et al. 2012), indicating that uptake into phytoplankton was dominated by active processes. However, there was a gradual increase in the demethylation-reduction rate of MeHg with time in the medium containing heat killed cells which would suggest that the pool of mercury attached to dead cells increasingly becomes available for transformation as the dead cells degrade. Thus, binding with the surfaces of dead cells is not irreversible and these surfaces can act as a reservoir of MeHg in these incubations. We suggest that as more MeHg is taken up into live cells depleting the medium concentration, MeHg bound to dead cells is released into solution.

Environmental relevance

Our study demonstrates that MeHg-thiol complexes are acceptable substrates for MeHg transformation in Hg-resistant bacteria. MeHgCYS and MeHgGSH complexes are environmentally important because in organic matter-rich systems, these complexes are present at sufficient concentrations that they could be the dominant complexes delivering MeHg and Hg to microbes (Schaefer and Morel 2009; Schartup et al. 2013). For example, MeHg-thiol complexes were reported to be the dominant MeHg complexes in a eutrophic fluvial wetland located in Baie St. Francois, Canada (Zhang et al. 2004). The present results suggest that the microbial bioavailability of MeHg in such systems will depend on the types and relative concentrations of the thiols and other reduced sulfur ligands present. The rates of MeHg accumulation and transformation varied more between the two species of bacteria examined than between MeHg complexes. Thus, the present results also suggest that the microbial bioavailability of MeHg, and therefore microbial demethylation rates in aquatic systems depends more on the species of microorganism than on the types and relative concentrations of thiols or other MeHg ligands present.

Furthermore, our results suggest that even if MeHg is taken up by facilitated or active processes, as is the case with the bacteria examined here, the speciation of MeHg in the aquatic solution is still important, and

uptake will not just be dependent on the uptake pathway and the organism involved, but will also depend on the solution speciation. This is an important finding as such a conclusion may not be apparent given that inorganic Hg has a very high water exchange constant. Clearly, even for active uptake of MeHg, solution speciation needs to be considered, as the differences examined here between uptake and transformation of MeHg bound to inorganic complexes and low molecular weight thiols was measurable (Fig. 2a) and the differences would be even more marked if MeHg was bound to high molecular weight dissolved natural organic compounds.

Hg-resistant bacteria play a critical role in MeHg cycling. MeHg is formed at the hypoxic-anoxic interface (Fitzgerald et al. 2007) in sediments by some anaerobic microorganisms such as sulfate reducing bacteria (Compeau and Bartha 1985), iron reducing bacteria (Fleming et al. 2006) and methanogens (Yu et al. 2013). Some of this MeHg is adsorbed to sediments (Feyte et al. 2010), demethylated and reduced to Hg(0) by aerobic Hg-resistant bacteria, or accumulated in cells that do not have broad spectrum mercury resistance. The Hg(0) formed by Hg-resistant bacteria can be released to the atmosphere, or oxidized back to Hg(II) by abiotic (Gu et al. 2011) or biologically-facilitated processes (Colombo et al. 2013, 2014). The rates of these transformations are therefore important because they control the amount of MeHg that may be accumulated in aquatic food chains and pose significant health risks to humans.

Acknowledgments We thank G. Endo for providing bacterial strains. This project was supported by grants from NSF-EAR (Geobiology & Low Temperature Geochemistry, EAR-0952291) and the U.S. Department of Energy, Office of Science (BER, DE-SC0007051), and a Hatch/McIntyre-Stennis grant through the New Jersey Agricultural Experiment Station.

Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

References

Aschner M, Aschner JL (1990) Mercury neurotoxicity: mechanisms of blood-brain barrier transport. Neurosci Biobehav Rev 14:169–176

Ask K, Akesson A, Berglund M, Vahter M (2002) Inorganic mercury and methylmercury in placentas of Swedish women. Environ Health Perspect 110:523–526

Bakir F, Rustam H, Tikriti S, Al-Damluji SF, Shihristani H (1980) Clinical and epidemiological aspects of methylmercury poisoning. Postgrad Med J 56:1–10

Barkay T, Miller SM, Summers AO (2003) Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol Rev 27:355–384

Benoit JM, Gilmour CC, Mason RP (2001) Aspects of bioavailability of mercury for methylation in pure cultures of Desulfobulbus propionicus (1pr3). Appl Environ Microbiol 67:51–58

Berthon G (1995) Critical evaluation of the stability constants of metal complexes of amino acids with polar side chains (Technical Report). Pure Appl Chem 67:1117–1240

Bjerregaard P (1996) Cardiovascular disease and environmental pollutants: the Arctic aspect. Arctic Med Res 55(Suppl 1):25–31

Chien MF, Narita M, Lin KH, Matsui K, Huang CC, Endo G (2010) Organomercurials removal by heterogeneous merB genes harboring bacterial strains. J Biosci Bioeng 110:94–98

Colombo MJ, Ha J, Reinfelder JR, Barkay T, Yee N (2013) Anaerobic oxidation of Hg(0) and methylmercury formation by Desulfovibrio desulfuricans ND132. Geochim Cosmochim Acta 112:166–177

Colombo MJ, Ha J, Reinfelder JR, Barkay T, Yee N (2014) Oxidation of Hg(0) to Hg(II) by diverse anaerobic bacteria. Chem Geol 363:334–340

Compeau GC, Bartha R (1985) Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. Appl Environ Microbiol 50:498–502

Deng G, Zhang T, Yang L, Wang Q (2013) Studies of biouptake and transformation of mercury by a typical unicellular diatom Phaeodactylum tricornutum. Chin Sci Bull 58:256–265

Driscoll CT, Mason RP, Chan HM, Jacob DJ, Pirrone N (2013) Mercury as a global pollutant: sources, pathways, and effects. Environ Sci Technol 47:4967–4983

EPA (2002) Method 1631, revision E: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry United States Environmental Protection Agency, Office of Water

Feyte S, Tessier A, Gobeil C, Cossa D (2010) In situ adsorption of mercury, methylmercury and other elements by iron oxyhydroxides and organic matter in lake sediments. Appl Geochem 25:984–995

Fitzgerald WF, Lamborg CH, Hammerschmidt CR (2007) Marine biogeochemical cycling of mercury. Chem Rev 107:641–662

Fleming EJ, Mack EE, Green PG, Nelson DC (2006) Mercury methylation from unexpected sources: molybdate-inhibited freshwater sediments and an iron-reducing bacterium. Appl Environ Microbiol 72:457–464

Gilmour CC, Elias DA, Kucken AM, Brown SD, Palumbo AV, Schadt CW, Wall JD (2011) Sulfate-reducing bacterium Desulfovibrio desulfuricans ND132 as a model for understanding bacterial mercury methylation. Appl Environ Microbiol 77:3938–3951

Gu B, Bian Y, Miller CL, Dong W, Jiang X, Liang L (2011) Mercury reduction and complexation by natural organic matter in anoxic environments. Proc Natl Acad Sci USA 108:1479–1483

Harada M (1978) Congenital Minamata disease: intrauterine methylmercury poisoning. Teratology 18:285–288

Helmann JD, Ballard BT, Walsh CT (1990) The MerR metalloregulatory protein binds mercuric ion as a tricoordinate, metal-bridged dimer. Science 247:946–948

Lawson N, Mason RP (1998) Accumulation of mercury in estuarine food chains. Biogeochemistry 40:235–247

Luengen AC, Fisher NS, Bergamaschi BA (2012) Dissolved organic matter reduces algal accumulation of methylmercury. Environ Toxicol Chem 31:1712–1719

Mason R, Reinfelder JR, Morel FMM (1996) Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. Environ Sci Technol 30:1835–1845

Miller JH (ed) (1972) Experiments in molecular genetics. Cold Spring Harbor, NY

Morel FMM, Kraepiel AML, Amyot M (1998) The chemical cycle and bioaccumulation of mercury. Annu Rev Ecol Syst 29:543–566

Ndu U, Mason RP, Zhang H, Lin S, Visscher PT (2012) Effect of inorganic and organic ligands on the bioavailability of methylmercury as determined by using a *mer-lux* bioreporter. Appl Environ Microbiol 78:7276–7282

Ndu U, Barkay T, Mason RP, Traore Schartup A, Al-Farawati R, Liu J, Reinfelder J (2015) The use of a mercury biosensor to evaluate the bioavailability of mercury-thiol complexes and mechanisms of mercury uptake in bacteria. PLoS ONE 10(9):e0138333. doi:10.1371/journal.pone.0138333

Rabenstein DL (1978) The aqueous solution chemistry of methylmercury and its complexes. Acc Chem Res 11:100–107

Rasmussen LD, Turner RR, Barkay T (1997) Cell-density-dependent sensitivity of a *mer-lux* bioassay. Appl Environ Microbiol 63:3291–3293

Reniero D, Galli E, Barbieri P (1995) Cloning and comparison of mercury- and organomercurial-resistance determinants from a *Pseudomonas stutzeri* plasmid. Gene 166:77–82

Reniero D, Mozzon E, Galli E, Barbieri P (1998) Two aberrant mercury resistance transposons in the *Pseudomonas stutzeri* plasmid. pPB. Gene 208:37–42

Schaefer JK, Morel FMM (2009) High methylation rates of mercury bound to cysteine by *Geobacter sulfurreducens*. Nat Geosci 2:123–126

Schartup AT, Mason RP, Balcom PH, Hollweg TA, Chen CY (2013) Methylmercury production in estuarine sediments: role of organic matter. Environ Sci Technol 47:695–700

Selifonova O, Burlage R, Barkay T (1993) Bioluminescent sensors for detection of bioavailable Hg(II) in the environment. Appl Environ Microbiol 59:3083–3090

Skyllberg U (2008) Competition among thiols and inorganic sulfides and polysulfides for Hg and MeHg in wetland soils and sediments under suboxic conditions: Illumination of controversies and implications for MeHg net production. J Geophys Res 113:G00C03. doi:10.1029/2008JG000745

Smith T, Pitts K, McGarvey JA, Summers AO (1998) Bacterial oxidation of mercury metal vapor Hg(0). Appl Environ Microbiol 64:1328–1332

Sone Y, Pan-Hou H, Nakamura R, Sakabe K, Kiyono M (2010) Roles played by MerE and MerT in the transport of inorganic and organic mercury compounds in gram-negative bacteria. J Health Sci 56:123–127

Summers AO (1992) Untwist and shout: a heavy metal-responsive transcriptional regulator. J Bacteriol 174:3097–3101

Watras CJ, Bloom NS (1992) Mercury and methylmercury in individual zooplankton: Implications for bioaccumulation. Limnol Oceanogr 37:1313–1318

Yamamoto M, Hou H, Nakamura K, Yasutake A, Fujisaki T, Nakano A (1995) Stimulation of elemental mercury oxidation by SH compounds. Bull Environ Contam Toxicol 54:409–413

Yu RQ, Reinfelder JR, Hines ME, Barkay T (2013) Mercury methylation by the methanogen *Methanospirillum hungatei*. Appl Environ Microbiol. doi:10.1128/AEM.01556-13:

Zhang J, Wang F, House JD, Page B (2004) Thiols in wetland interstitial waters and their role in mercury and methylmercury speciation. Limnol Oceanogr 49:2276–2286

Zheng W, Liang L, Gu B (2012) Mercury reduction and oxidation by reduced natural organic matter in anoxic environments. Environ Sci Technol 46:292–299

Zheng W, Lin H, Mann BF, Liang L, Gu B (2013) Oxidation of dissolved elemental mercury by thiol compounds under anoxic conditions. Environ Sci Technol 47:12827–12834