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Role of the Bacterial Organomercury Lyase (MerB) in Controlling Methylmercury Accumulation in Mercury-Contaminated Natural Waters

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FIGURE 1. Relationship between the percent of Hg_T as MeHg and the Hg_T concentration in various surface waters from previously published studies (*8, 40, 57−66*). The line represents the best-fit linear regression of the data ($p < 0.001$).

The curious phenomenon of similar levels of methylmercury (MeHg) accumulation in fish from contaminated and pristine environments may be explained by the observation that the proportion of total mercury (Hg_T) present as MeHg is inversely related to Hg_T in natural waters. We hypothesize that this "MeHg accumulation paradox" is explained by the quantitative induction of bacterial enzymes that are encoded by the mercury resistance (*mer*) operon, organomercury lyase (MerB), and mercuric reductase (MerA) by inorganic Hg (Hg[II]). We tested this hypothesis in two ecosystems in New Jersey: Berry's Creek in the Meadowlands (ML) and Pine Barren (PB) lakes. Across all sites, an inverse correlation ($r^2 = 0.80$) between the concentration of Hg_T (ML, 113−4220 ng L^{-1}; PB, 0.3−5.4 ng L^{-1}) and the proportion of Hg_T as MeHg (MeHg in ML and PB ranged from 0.08 to 1.6 and from 0.03 to 0.34 ng L^{-1}, respectively) was observed. The planktonic microbial community in Meadowlands surface waters exhibited adaptation to mercury, the presence of *mer* genes and mRNA transcripts, and high rates of reductive demethylation ($k_{deg} = 0.19$ day^{-1}). In contrast, the microbial community of PB was not adapted to mercury and demonstrated low rates of oxidative demethylation ($k_{deg} = 0.01$ day^{-1}). These results strongly support our hypothesis and show that the degradation of MeHg by *mer*-encoded enzymes by the water column microbiota of contaminated environments can significantly affect the amount of MeHg that is available for entry into the aquatic food web.

Introduction

Mercury, a toxic heavy metal, is a widespread pollutant due to industrial contamination and atmospheric deposition in ecosystems remote from industrial or mining activities. The fate of Hg within aquatic ecosystems is dependent upon a large number of factors including physical, chemical, and biological processes (*1, 2*). Oxidized Hg transported to
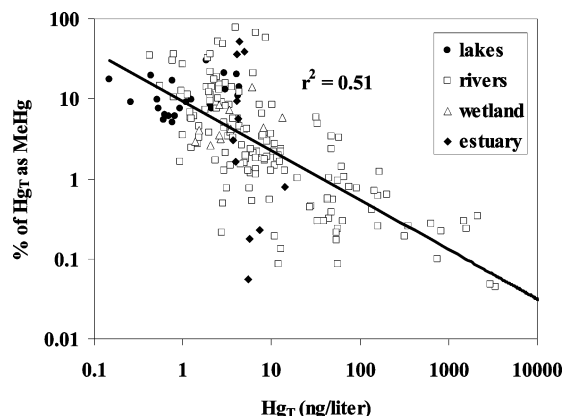
sediments has the potential to be methylated, primarily by activities of sulfate-reducing bacteria (*3, 4*). Methylmercury (MeHg) is of particular public health concern due to its bioaccumulation and biomagnification within the aquatic food web, posing health risks to humans who consume fish caught in contaminated waters (*5*).

Numerous studies have demonstrated that fish from highly contaminated industrially impacted waters and those from remote locations where atmospheric deposition is the sole source of Hg contain similar Hg concentrations (*6−8*). For example, Hg levels in predatory fish collected from the acidic lakes of the Pine Barrens, NJ (0.59−2.10 ppm) (*9*), are almost as high as those from industrially impacted sites in the Meadowlands, NJ (0.95−4.66 ppm) (MERI, manuscript in preparation), although the latter is more impacted by Hg contamination (see below). One explanation for this paradox is suggested by the observation of a lower proportion of total Hg (Hg_T) present as MeHg in highly contaminated as compared to pristine surface waters (Figure 1). Since most of the Hg in marine and freshwater fish is present as MeHg (*10, 11*), the "Hg accumulation paradox" may be explained by limited MeHg accumulation in Hg-contaminated surface waters, such that MeHg contributes a much smaller fraction of the Hg_T than in environments with low levels of Hg_T.

The amount of MeHg that is available for accumulation by aquatic biota is dependent upon the relative rates of MeHg production and degradation. Methylmercury is degraded by two microbially mediated processes (*12*) and photodegradation (*13*). Reductive demethylation is thought to be mediated by Hg-resistant bacteria that degrade MeHg to the volatile end products, CH_4 and Hg(0) (*14*). A second mechanism, oxidative demethylation, is believed to be a cometabolic byproduct of C1 metabolism, resulting in the production of CO_2, a small amount of CH_4, and possibly Hg(II) (*15*). We propose that reductive demethylation by Hg-resistant bacteria limits the amount of MeHg that accumulates in Hg contaminated surface waters.

Bacterial resistance to MeHg is encoded by the mercury resistance (*mer*) operon (*12, 16*) and involves the sequential action of two enzymes, organomercurial lyase (MerB) and mercuric reductase (MerA). MerB cleaves the C−Hg bond of MeHg to CH_4 and Hg(II), which is further reduced to gaseous Hg(0) by MerA. The expression of these genes is tightly controlled by Hg(II) through the action of a Hg(II)-responsive regulatory protein, MerR. As the bioavailable concentration

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TABLE 1. Summary of Hg$_T$ and MeHg Concentrations and Chemical/Physical Properties of Water Collected from the Meadowlands and Pine Barren Lakes

date	site	temp (°C)	pH	conductivity (μS/cm)	DOC (mg/L)	Hg$_T$ (ng/L)	MeHg (ng/L)
Aug 2000	MLU1	25.3	7.20	842		638 (83)[a]	0.22 (0.02)
	MLM	27.8	7.23	891	10.5−11.7	4220 (2320)	0.43 (0.30)
	MLD	27.5	7.22	905		1360 (250)	0.31 (0.20)
Apr 2001	MLU2	16.8	6.99	1105	5.8 (0.3)	12.0 (0.6)	0.04 (0.01)
	MLM	14.5	6.88	1010	7.0 (0.3)	264 (11)	0.69 (0.02)
	MLD	15.1	7.19	1003	6.8 (0.5)	272 (29)	0.75 (0.06)
Sep 2001	MLU2	20.6	7.08	189	8.3 (0.9)	15.6 (1.6)	0.20 (0.21)
	MLM	20.5	7.30	243	5.9 (0.6)	113 (20)	1.17 (0.77)
	MLD	20.5	7.33	337	5.7 (0.3)	289 (44)	0.48 (0.29)
Jul 2002	MLU2	23.4	6.59	1382	11.4 (1.9)	32 (19)	0.08 (0.02)
	MLM	26.4	6.60	5390	10.7 (3.8)	1230 (1200)	1.50 (1.04)
	MLD	26.3	6.89	5540	10.0 (3.4)	1250 (340)	1.60 (1.20)
May 2003	MLD	15.2	6.66	1593	nd[b]	130 (6.2)	nd
Aug 2000	PBH	25.9	4.30	38	7.6 (0.3)	5.4 (0.6)	0.27 (0.08)
	PBB	26.3	5.60	45	7.8 (0.2)	4.4 (0.1)	0.34 (0.08)
Apr 2001	PBO	10.2	3.92	47	nd	3.3 (0.2)	0.10 (0.01)
	PBA	12.0	4.09	61	4.9 (0.1)	1.5 (0.1)	0.03 (0.01)
Sep 2001	PBO	20.8	4.30	36	9.8 (2.0)	0.63 (0.004)	0.14 (0.03)
	PBA	22.7	4.61	53	10.0 (2.4)	0.35 (0.04)	0.12 (0.03)
May 2003	PBO	21.0	4.84	38	nd	4.1 (0.6)	nd

[a] Numbers in parentheses indicate ±1 standard deviation. [b] nd, not determined.

of Hg(II) or organomercurials increases, the mercury compound binds to MerR and induces transcription of a polycistronic message, encoding MerA and MerB (16). Because *mer*-mediated MeHg degradation results in the production of gaseous end products, activation of this system results in the elimination of Hg from the microbe's local environment (12). The induction of *mer* transcription by Hg is proportional to the amount of Hg present (i.e., the more Hg present, the higher the level of expression (17, 18)); therefore, the rate of reductive demethylation is likely dependent on the concentration of Hg.

We propose that enrichment of Hg-resistant bacteria and the Hg-dependent production of MerB is one possible explanation for the "mercury accumulation paradox" (Figure 1). Thus, in environments containing high concentrations of Hg$_T$, resistant microbes are enriched and Hg induces *mer* genes, resulting in MeHg degradation and reduced accumulation of MeHg in aquatic consumers. In contrast, in environments with low levels of Hg$_T$, the concentration of bioavailable Hg is insufficient to efficiently induce expression of *mer* operons in a smaller number of resistant bacteria, resulting in the subsequent accumulation of MeHg. Thus, activities of MerB in mercury-resistant bacteria limit the production of MeHg in highly contaminated waters.

Here we examined the relationships between the concentration of Hg$_T$ and the proportion of Hg$_T$ present as MeHg in the water column and *mer* expression and the reductive degradation of MeHg by the indigenous microbial community of two sites in New Jersey, the highly Hg-contaminated Berry's Creek in the Meadowlands (ML) and the Pine Barren lakes (PB) which only receive Hg inputs from the atmosphere.

Methods

Site Description and Field Sampling. Two study sites were chosen in New Jersey with different sources for Hg input: industrial contamination from chloralkali and Hg thermometer plants in Berry's Creek (ML) (19) and atmospheric deposition in Pine Barren lakes (PB) (20). Located on the western edge of the New Jersey Meadowlands, Berry's Creek flows through an upland marsh to the Hackensack River. Separated from the estuary by a tide gate, the freshwater portion of Berry's Creek is of neutral pH and low to moderate conductivity (Table 1). The Oswego, Absegami, and Harrisville Lakes are located east of Wharton State Forest in southern

New Jersey's Pinelands National Reserve. These impounded lakes collect low-pH and low-conductivity water (Table 1) from the forested coastal plain and feed the Mullica River and Great Bay estuary.

All ML samples were collected during low tide upstream of the tidal gate in Berry's Creek. Water was collected from four ML and two PB sites in August 2000, April 2001, September 2001, July 2002, and May 2003. Two PB lakes, Batsto (PBB) and Harrisville (PBH), sampled in August 2000, were subsequently replaced by lakes Oswego (PBO) and Absegami (PBA) as the latter are less impacted by human habitation and agricultural runoff. Berry's Creek sites included two upstream of the Hg point source, MLU1 (August 2000 sampling) and MLU2 (all other sampling dates), a site (MLM) adjacent to the source, and a site (MLD), ~100 m downstream from MLM. After the August 2000 sampling, MLU2 replaced MLU1 to obtain a larger gradient in Hg$_T$ concentrations (Table 1).

Salinity, pH, conductivity, temperature, and dissolved oxygen were measured in the field using the Checkmate II Deluxe Field System (Corning). DOC was analyzed in GF/F glassfiber filtered water in Seitzinger's laboratory (Marine and Coastal Sciences, Rutgers University) in August 2000 and April 2001 and by Nutrient Analytical Services Laboratory (Solomons, MD) in September 2001 and July 2002. Surficial water was collected in triplicate for Hg$_T$ and MeHg analyses using acid-cleaned glass I-Chem bottles prepared by clean techniques (R. Flett, personal communication). Acid-cleaned Teflon bottles and clean sampling protocols (21) were used to collect samples from PB lakes in April and September 2001. TraceMetal-grade HCl (all dates except July 2002) or BrCl (July 2002) were added to 0.2% and 0.1%, respectively, in the field for preservation. Water samples for the isolation of microbial biomass were collected as previously described (22) and maintained on ice until processed within 2−4 h of sampling.

Total Hg and Methylmercury Analyses. Hg$_T$ and MeHg samples collected in August 2000 and April 2001 were analyzed by Flett Research Ltd, Winnipeg, while those collected in September 2001, July 2002, and May 2003 were analyzed at Rutgers University following the methods of Bloom and Crecelius (23) on a cold vapor atomic fluorescence detector (Tekran 2500, Tekran Inc., Toronto, Ontario). All samples collected were analyzed in duplicate. Detection limits

for Hg_T ranged from from 0.33 to 2.3 ng/L for ML samples and 0.36 to 0.66 ng/L for PB samples. Methylmercury samples ($n = 2-4$) were analyzed as described by Horvat et al. (24) except that distillation was performed under argon gas flow to reduce interferences during derivitization (Riedel and Heyes, personal communication). Distillation recoveries for spiked environmental samples ($n = 12$) ranged from 67% to 123%. Reported MeHg concentrations were not corrected to compensate for distillation recoveries. Detection limits for MeHg averaged 0.057 ng/L.

Expression and Activity of MerA and MerB in *Pseudomonas stutzeri* OX. Strain OX (25) was grown aerobically on denitrification medium (DNM) (26) to midexponential growth (1×10^7 CFU/mL) and induced with 0, 0.4, 2, or 5 μM $HgCl_2$ while shaking. After a 10 min induction period, a 1.5 mL aliquot was removed for *mer* transcript analysis and placed immediately on ice to reduce mRNA degradation. The cells were centrifuged at 4 °C, and the pellets were stored at −80 °C. For resting cell MeHg degradation assays, the cultures were shaken for an additional 20 min to allow for protein synthesis prior to the addition of 200 μg/mL chloramphenicol (Sigma-Aldrich). The cells were washed and resuspended in Hg volatilization buffer (26) to an optical density at 660 nm (A_{660}) of 0.14. Washed cells were diluted 1:2 in buffer to a final cell density of 8.8×10^7 CFU/mL and dispensed (10 mL) into 25 mL flasks in triplicate. Sterile buffer was used as a negative control. ^{14}C-MeHg (90 nCi, 30 ng of Hg/mL; Amersham; spec. act = 0.3 Ci/g of Hg) was added to each flask, and the cells were incubated at 28 °C with shaking. The remaining ^{14}C-MeHg was monitored by counting 0.5 mL aliquots of the liquid phase over time in a Beckman LS 6500 liquid scintillation spectrometer.

RNA Extraction and Quantitation. Total RNA was extracted from filtered aquatic biomass according to the method described in Jeffrey et al. (22), except that lysis buffer lacked diethylpyrocarbonate (DEPC), and the crude extracts were resuspended and dialyzed against TE (10 mM Tris, 1 mM EDTA, pH 8) for 2−3 h. DEPC was not added to the lysis buffer to avoid inhibition in subsequent enzymatic processes. A 0.5 mL aliquot of the initial 4 mL lysate was frozen at −20 °C for DNA analysis. RNA from strain OX was similarly extracted, except that cell pellets were first resuspended in 350 μL extraction buffer (27) and lysed with 0.5 μg/mL Proteinase K (Fisher) while shaking at 100 rpm for 1 h at 37 °C.

Contaminating DNA was removed by treatment with RQ1 DNase (Promega), according to the manufacturer's instructions. The concentration of RNA in DNase-treated extracts was quantified by measuring SYBR Green II fluorescence (Molecular Probes) on a Turner Quantech fluorometer (Barnstead Thermoline) using a standard curve prepared with *Escherichia coli* W rRNA (Sigma-Aldrich). The concentration of RNA in standards was verified by spectrophotometric measurement at A_{260} (Ultrospec 3000, Pharmacia Biotech).

PCR Amplification of *merA* from Genomic DNA. Genomic DNA was extracted from aquatic biomass as described (28) and amplified by PCR in 25 μL reactions containing: $1\times$ buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM each A1s.F and A5-H1.R primers (Supporting Information), and 1 U Taq Polymerase (Fisher), following 35 cycles of 10 s at 94 °C, 30 s at 49 °C, and 60 s at 72 °C.

Quantification of *merA* and *merB* Transcripts−Strain OX. Purified RNA (100 ng) from strain OX was converted to cDNA in 50 μL reverse transcription (RT) reactions (Superscript II, Invitrogen) and random hexamers according to the manufacturer's instructions. Real-time quantitative PCR reactions (qPCR) were prepared [per 25 μL: 1 μL cDNA, 0.4 μM each OX-specific *merA* or *merB* primers (Supporting Information), and $1\times$ SYBR iQ Mix (Bio-Rad)] and amplified for 35 cycles of 94 °C for 10 s. and the appropriate annealing temperature for 60 s (Supporting Information), using an

iCycler iQ real-time PCR detection system (Bio-Rad). A standard curve with a known amount of purified pPB (29) DNA obtained by the alkaline lysis method (28) was constructed and quantitated by spectrophotometric measurement at A_{260}. Each analysis was done in triplicate. At the end of each qPCR reaction, a melt curve analysis confirmed the identity of the amplified DNA. Controls included reaction without cDNA and a demonstration that DNase treatment removed genomic DNA from RNA extracts, deduced from the absence of *mer*-specific products in PCR reactions to which 1 μL of a no RT control was added.

Detection of *merA* Transcripts in Aquatic Biomass. Three different methods were used: RNA:RNA hybridization, RT-qPCR, and RT-PCR. RNA:RNA dot blot hybridizations were carried out as previously described (22) except that a new *merA* riboprobe and moderate stringencies for posthybridization washes (28) were used. The new *merA* riboprobe was prepared as described (22) except that primers, r1.F and r2.R (Supporting Information), were used to amplify (3 min at 94 °C and 25 cycles of 45 s at 94 °C, 1 min at 58 °C, 3 min at 72 °C, and a final extension of 10 min at 72 °C) a 660 bp fragment from the 3′ end of *merA* in pEPA81* (30) that was then used to construct plasmid, pJMY2 (Yagi, unpublished results).

Hybridization signals were detected by a PhosphorImager SI and quantitated using ImageQuant v. 5.2 (Molecular Dynamics). Hybridization signals were compared against a standard curve of $1-200 \times 10^6$ copies of antisense pJMY2 *merA* transcripts alone (September 2001 and July 2002 waters) or combined with 250 ng of *E. coli* W rRNA (July 2002 and May 2003 waters). The phosphor-imaging signal from nonspecific hybridization of the ^{35}S-riboprobe to *E. coli* rRNA negative control blots was subtracted from the signals attained in the sample blots.

Reverse transcription of RNA from aquatic biomass was performed as described above for strain OX *mer* transcripts except that 200 ng of DNase-treated RNA was added to 20 μL RT reactions and subjected to both qPCR and PCR for detection of *merA* transcripts. qPCR and PCR reactions (25 μL) were prepared as described previously for strain OX and genomic DNA, respectively, except that 1 μL of cDNA, 0.6 μM A7s−n.F and A5-n.R (Supporting Information), and 1.5 mM $MgCl_2$ was added to each reaction and amplified for 45 cycles of 15 s at 94 °C and 60 s at 60 °C. These primers target the known diversity of *merA* sequences from Gram-negative bacteria (Ni Chadhain, in preparation). The qPCR calibration curve was established using pJMY2 cDNA from RT reactions containing from 3.8×10^3 to 3.8×10^7 copies of *merA* transcripts. PCR products were visualized in an ethidium bromide stained 1.5% agarose gel to verify the size of the amplicons. The detection limits for *merA* transcripts following RNA:RNA hybridization and RT-qPCR protocols were ~2 and 0.02 copies pg^{-1} of RNA, respectively.

Microbial Parameters. Total cell counts were determined for the August 2000 and September 2001 samplings by acridine orange direct count (31). Total culturable heterotrophs were quantified by spread plating serially diluted site water on LB (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl) plates supplemented with 25 μg/mL Netamycin (Sigma-Aldrich) to inhibit fungal growth. Hg-resistant (Hg^R) heterotrophs were quantified on the same medium supplemented with 50 μM $HgCl_2$. Diversity was estimated by the Shannon-Weaver diversity index (H') using morphological attributes of 67 colonies per sample as previously described (32).

^{14}C-MeHg Degradation by Aquatic Biomass. Water samples were collected from MLD and PBO. On the day of sampling, following a 2 h equilibration period at room temperature, site water (50 mL) was dispensed into serum bottles (159 mL) and sealed under air with Teflon stoppers. Triplicate live samples and an autoclaved control were spiked

with 10 nCi ^{14}C-MeHg (0.7 μg of Hg/L) and incubated statically at 22 °C. After ~5 and 16.6 h, 5 mL of 3 M NaOH was added to drive the produced $^{14}CO_2$ into the liquid phase as carbonate. Bottles were stored frozen at −20 °C until processed. To remove degradation products that accumulated during substrate storage, the ^{14}C-MeHg (100 nCi/mL, 8 mL) was purified 1 day prior to the experiment by four extractions into 1 mL methylene chloride and back-extraction of the pooled organic phase back into 8 mL of 0.001% HCl by evaporating the methylene chloride at 45 °C while bubbling with air (20 mL/min) (M. Marvin-DiPasquale, personal communication).

The amount of $^{14}CH_4$ and $^{14}CO_2$ produced as ^{14}C-MeHg demethylation products was quantified by the $^{14}CH_4$-combustion/$^{14}CO_2$-trapping technique (15). Due to the large volume of bottles used in this experiment, the samples were flushed for a total of 45 min with air and spiked 4 times with 1 mL of unlabeled CH_4 or CO_2 (100%) at 10 min intervals to drive all ^{14}C-labeled gases into trapping solutions. Recoveries of $^{14}CO_2$ and $^{14}CH_4$ were >95% and 91% ± 0.2% (n = 5), respectively (Supporting Information).

Values of ^{14}C-production were corrected for abiotic products obtained in autoclaved controls. The amount of $^{14}CH_4$ and $^{14}CO_2$ produced in MLD waters was linear (r^2 = 0.989, p < 0.001 and r^2 = 0.685, p < 0.05, respectively) rather than exponential with time, most likely due to the small change in substrate concentration during incubation. MeHg degradation rates (ng of Hg/L day^{-1}) were calculated by linear regression of ^{14}C-labeled gases produced with time. The 0 h time point was assumed to contain no ^{14}C-labeled gaseous products. Potential first-order rate constants, k_{deg} (day^{-1}), were calculated as

$$k_{deg} = \text{MeHg degradation rate}/C_{MeHg}$$

where C_{MeHg} is the concentration (ng of Hg/L water) of the added ^{14}C-MeHg. The ^{14}C-MeHg added was 170 and 5 times higher than ambient Hg_T concentrations in MLD and PBO waters, respectively. The half-life ($t_{1/2}$) of the ^{14}C-MeHg in these waters was calculated by $t_{1/2} = (\ln 2)/k_{deg}$.

Statistical Analysis. A two-tailed t-test (33) was used to determine the relative significance of all correlations.

Results

Hg(II) Induces Expression and Activity of MerB. As a proof-of-principle for the Hg(II)-dependent expression of MerB and degradation of MeHg, we demonstrated that exposure of a bacterium carrying a broad spectrum *mer* operon to increasing concentrations of Hg(II) resulted in increases in both *merA* and *merB* transcript abundance (Figure 2A) and in MeHg degradation rates (Figure 2B). *P. stutzeri* strain OX is resistant to inorganic and organic Hg (25) and carries two discrete *mer* operons, a narrow-spectrum *Tn501*-like operon and a broad-spectrum *Tn5053*-like operon.

Following a brief induction period, the abundance of *merA* and *merB* transcripts in cultures grown to midexponential phase and induced with 0, 0.4, 1.0, 2.0, or 5.0 μM Hg(II) increased with increasing concentration of the inducer (Figure 2A). In the absence of Hg(II), 610 *merA* and 100 *merB* transcripts per picogram of total cellular RNA were detected. Between 0 and 1.0 μM Hg(II), transcripts of both genes increased by less than 2-fold with more dramatic increases occurring at inducing concentrations of >1.0 μM Hg(II). At 2 μM Hg(II) transcript abundance increased over 10-fold for *merA* and over 8-fold for *merB*; at 5 μM Hg(II) a 30-fold increase in *merA* transcripts (17 000 copies pg^{-1} RNA) and over a 10-fold increase in *merB* transcripts (1100 copies pg^{-1} RNA) was noted (Figure 2A). Thus, Hg(II) quantitatively induced expression of both *merA* and *merB,* and *mer* induction in strain OX follows the previously described
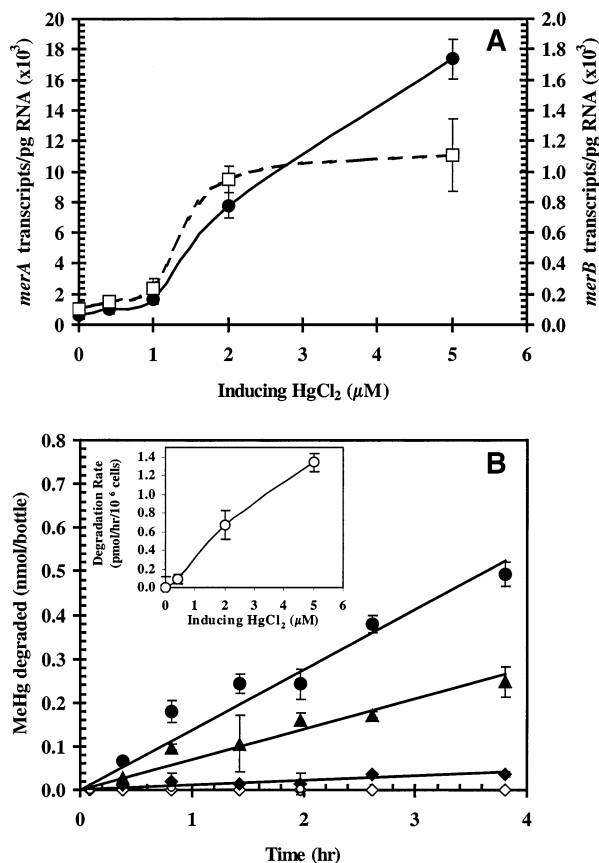

FIGURE 2. Exposure of midexponential cells of strain OX to increasing concentrations of Hg(II) induces expression of MerA and MerB, as demonstrated by an increase in (A) *merA* (●) and *merB* (□) transcript abundance, and (B) the rate of ^{14}C-MeHg degradation. Exposure to (◇) 0; (◆) 0.4; (▲) 1.0; (●) 5.0 μM HgCl$_2$ during induction. (inset) Rates of ^{14}C-MeHg degradation vs inducing Hg(II) concentrations.

ultrasensitive threshold response of *mer* promoters (17, 18). Interestingly, at inducing Hg(II) concentrations >2 μM, *merA* transcript abundance continued to increase while *merB* transcripts leveled off. This is likely due to differences in transcription kinetics between *merB* and *merA,* which is not surprising as *merB* is transcribed from its own promoter (29). *merA* transcripts were consistently 7- to 15-fold more abundant than *merB* transcripts at a given inducing Hg(II) concentration, which may be expected as strain OX contains two copies of *merA* and a single copy of *merB* (29).

Rates of ^{14}C-MeHg degradation increased with inducing Hg(II) concentrations, as seen by loss of ^{14}C-substrate from the liquid medium in resting cell assays (Figure 2B), corresponding to patterns observed for *mer* transcript abundance (Figure 2A). No MeHg degradation was observed in uninduced and sterile controls, and exposure to as little as 0.4 μM Hg(II) resulted in a slow but detectable rate of MeHg degradation. Increasing the Hg(II) concentration from 0.4 to 2 μM resulted in a 6-fold increase in the MeHg degradation rate, and a further increase to 5 μM yielded another doubling in activity (Figure 2B, inset). The data shown in Figure 2B is the first demonstration of MeHg degradation by strain OX, which is important because the MerB in this strain shares only a partial homology with other bacterial MerB enzymes (25, 34).

These results demonstrate a direct quantitative relationship between inducing Hg(II) concentrations, *merA* and *merB* transcript abundance, and the rate of MeHg loss from assay solutions. Therefore, the rate of MeHg degradation is directly related to the concentration of the inducer, Hg(II), and *merA*

abundance reflects transcription of both *merA* and *merB* in this bacterium.

Characterization of Surficial Waters. A variety of chemical/physical parameters were measured at each site at the time of collection (Table 1). ML and PB sites varied in pH and salinity, while concentrations of DOC were similar. PB lakes have low salinity and are naturally acidic (average pH 4.5) due to the growth of pine trees in the region, lack of buffering capacity in the sandy soils and sediments, and the effects of acid deposition. In contrast, ML waters have neutral pH (average pH 7.0), and tidal influences from the downstream estuary results in slight increases in conductivity. Direct bacterial cell counts of water samples collected in August 2000 and September 2001 indicated that the total cell numbers were only slightly higher in ML (1.4×10^6 cells/mL) than in PB (0.4×10^6 cells/mL) waters. Differences in temperature, pH, salinity, and DOC influence the bioavailability of Hg(II) (*2,35−39*) and may confound direct comparisons between PB and ML sites. A site upstream of the major source of Hg contamination in Berry's Creek (MLU2) was therefore included in the study. Physical−chemical parameters at MLU2 were similar to those of MLM and MLD, yet Hg$_T$ and MeHg concentrations were significantly lower (Table 1).

Hg$_T$ and MeHg Concentrations. A wide range of Hg$_T$ concentrations was observed in ML (12−4220 ng/L) and PB (0.35−5.4 ng/L) waters (Table 1). In contrast, a much narrower range of MeHg concentrations (0.03−1.6 ng/L) was detected across all sites, with only slightly lower concentrations in PB than in ML waters. The difference in concentration ranges for the two forms of Hg leads to an inverse correlation ($r^2 = 0.80$; $p < 0.001$) between the proportion of Hg$_T$ as MeHg and the Hg$_T$ concentration (Figure 3A). Thus, the trend reported here for New Jersey sites is similar to that observed in a variety of aquatic systems worldwide (Figure 1). When Hg$_T$ and MeHg concentrations are plotted in order of increasing Hg$_T$ concentrations (Figure 3B), it is evident that in ML samples MeHg increased with Hg$_T$ concentrations ($r = 0.73$, $p < 0.001$), as observed in other locations (*35*). However, across all sites Hg$_T$ increased over 4 orders of magnitude while the corresponding increase in MeHg was only 2 orders of magnitude, suggesting that the inverse relationship between the proportion of Hg$_T$ as MeHg and Hg$_T$ concentration is primarily driven by high Hg$_T$ concentrations. In PB lakes, where Hg$_T$ and MeHg concentrations were within ranges reported in other lakes that are impacted by atmospheric deposition of Hg (*40*), no clear relationship emerged between Hg$_T$ and MeHg concentrations ($p < 0.5$) (Figure 3B), possibly due to the small sample size included in this study.

Microbial Adaptation to Mercury in ML and PB. Adaptation of microbial communities in Berry's Creek and PB lakes to life in the presence of toxic concentrations of mercury was examined using the criteria of Barkay (*41*). In water samples collected in August 2000, a higher proportion of the total culturable heterotrophs was resistant to Hg(II) in the ML (9%; $2−4 \times 10^3$ Hg(II) resistant [Hg^R] CFU/mL), as compared to PB waters (<5%; <80 Hg^R CFU/mL). In addition, *merA* genes were readily detected by PCR in DNA extracted from microbial biomass from MLU1, MLM, and MLD sites, while none were detected in extracts of either PBB or PBH waters (Figure 4). Whereas the Shannon-Weaver diversity indices (H′) for total heterotrophs were similar in both sites (H′ = 2.7−2.8), a higher diversity of Hg^R heterotrophs from ML waters (H′ = 2.1−2.7) than from PB lakes (H′ = 0) was observed. A diversity index of 0 indicates a single colony morphology in the sample. Thus, the microbial community in Berry's Creek, but not that of PB lakes, was adapted to mercury toxicity as indicated by the enrichment of Hg(II)-resistant bacteria, presence of *merA* genes in the microbial
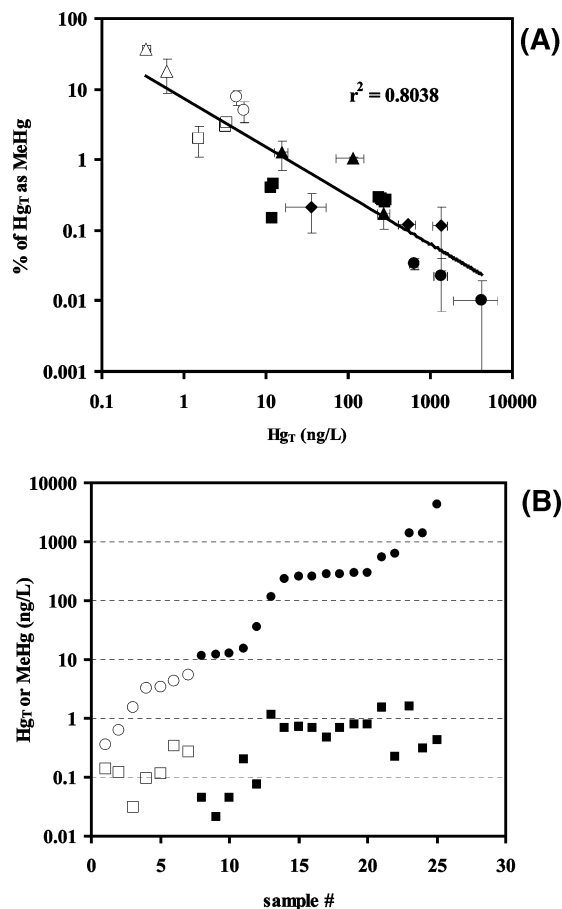
FIGURE 3. (A) Relationship between the proportion of Hg$_T$ as MeHg and the concentration of Hg$_T$ in Pine Barrens and Meadowlands surface waters. (○) Water collected Aug 2000, (□) Apr 2001, (△) Sep 01, (◇) Jul 02. Closed symbols represent ML, and open symbols represent PB waters. (B) Plot showing the concentrations of Hg$_T$ (○, ●) and MeHg (□, ■) in water samples ordered by increasing Hg$_T$ concentration. Closed symbols are ML samples; open symbols are PB samples.
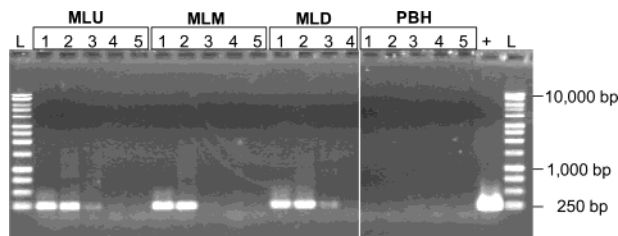
FIGURE 4. Gel electrophoresis showing *merA* PCR amplification products from ML and PB waters. L, 1 kB ladder (Promega); 1−5, indicate 10-fold serial dilutions (undiluted to 10^{-5}, respectively) of DNA extracts added to PCR reactions; +, positive control pHG103 (*67*).

biomass, and high diversity of resistant culturable heterotrophs.

***merA* Transcripts in Natural Waters.** *merA* was used as our target molecule for evaluating *mer* transcription in aquatic biomass. Due to the high degree of sequence diversity in a small number of *merB* sequences (*34*), it is not feasible at present to design primers or probes for this locus. Transcripts of *merA* were detected in RNA extracts of microbial biomass from the two study sites (Table 2). Two-thirds of RNA extracts from ML (21/40) and only one of three PB extracts contained *merA* transcripts. The abundance of *merA* transcripts in a sample collected in PBO in May 2003 (37 ± 15 copies pg^{-1} of RNA) was 3-fold lower than in the MLD sample (July 2002),

TABLE 2. Summary of Results for Detection and Quantitation of *merA* Transcripts in Aquatic Biomass by RNA:RNA Dot Blot Hybridization and RT-(q)PCR

date collected	site	*merA* transcripts (copies/pg RNA) by RNA:RNA hybridization	*merA* transcripts (copies/pg RNA) by RT-qPCR	ratio of samples where *merA* was detected by RT-PCR[a]
Apr 2001	MLU	nd[b]	nd	0/4
	MLM	nd	nd	0/2
	MLD	nd	nd	1/4
	PBO	nd	nd	1/1
Sep 2001	MLU	2900 (310)[c]	2.2 (0.2)	6/6
	MLM	1800 (1400)	6.0 (1.5)	6/6
	MLD	1800 (700)	3.8 (1.6)	6/6
	PBA	nd	nd	0/2
July 2002	MLU	1200 (450)	nd	2/4
	MLM	740 (370)	nd	0/4
	MLD	120 (40)	nd	0/3
May 2003	MLD	170 (90)	nd	0/2
	PBO	37 (15)	nd	n/a[d]

[a] This ratio indicates the number of samples where *merA* was detected by RT-PCR over the number of samples analyzed that were not RT-PCR inhibited. Each RT reaction contained 100−200 ng of total RNA extracted from aquatic biomass. [b] nd, not determined. [c] Numbers in parentheses indicate ±1 standard deviation. [d] n/a, not applicable as no samples could be successfully RT-PCR amplified.

with the lowest number of transcripts, 120 ± 40 copies pg^{-1} of RNA. In ML samples, transcripts were most abundant in September 2001 as indicated by both RT-qPCR and hybridization. However, a 2 orders of magnitude difference was observed between results of the two methods most likely due to the higher specificity of the RT-qPCR as compared to hybridization with a uniformly labeled 660 bp riboprobe. Furthermore, RT-qPCR showed an increased abundance of *merA* transcripts in samples collected downstream of the contamination outfall (MLM and MLD) relative to MLU, correlating well with the higher concentrations of Hg_T in these waters (Table 1). However, this trend was not replicated in hybridization results (Table 2). Although our quantitative detection of transcripts was lacking, a general trend indicating higher levels of *mer* expression in ML relative to PB waters does emerge from the presented data (Table 2). Issues associated with the quantitative detection of rare and transient transcripts in environmental biomass are addressed elsewhere (Yagi et al., manuscript in preparation).

^{14}C-MeHg Demethylation in MLD and PBO Waters. Potential rates and identification of demethylation end products were performed to examine whether MeHg was degraded reductively or oxidatively in MLD and PBO waters. Data are presented for the May 2003 sampling (Figure 5). Similar results were observed on two earlier occasions (data not shown). Reasonable estimates of demethylation potentials can be calculated because, unlike inorganic Hg(II), MeHg does not appear to age in sediment, as adding MeHg as a tracer appears to be as available to demethylating organisms as the native MeHg pool (*42*); therefore, accurate estimates of first-order rate constants in aqueous samples are most likely feasible. In MLD waters, 13.2% of the added ^{14}C-MeHg was degraded in 16.6 h (Figure 5), resulting in a degradation rate of 135 ng of Hg L^{-1} day^{-1} and a $k_{deg} = 0.19\ day^{-1}$. Methane was the dominant demethylation end product, contributing $85 \pm 0.9\%$ of the total ^{14}C volatile products, in agreement with the detection of *mer* transcripts in these waters (Table 2). Rates of ^{14}C-MeHg degradation in PBO waters were more than 20 times lower (6.8 ng of Hg L^{-1} day^{-1}; $k_{deg} = 0.01\ day^{-1}$) than in MLD water with ^{14}CO$_2$ being the dominant end product ($90 \pm 2\%$). These rate constants correlate to a half-life of 3.6 and >72 days of the added ^{14}C-MeHg in MLD and PBO waters, respectively. The amount of ^{14}CH$_4$ produced in PBO waters was negligible and did not increase when
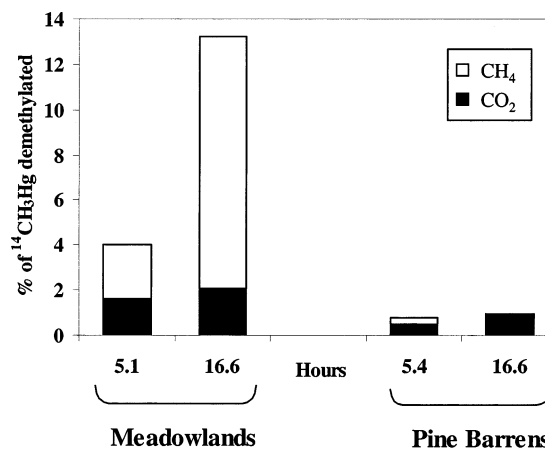

FIGURE 5. Total ^{14}CH$_4$ and ^{14}CO$_2$ produced after incubation of MLD and PBO waters with 10 nCi ^{14}C-MeHg for 5 and 16.6 h. Error bars representing ±1 standard deviation are too small to be seen.

incubated beyond 5 h. The Hg_T concentrations in the MLD samples were almost one-half that observed in previous sampling events; however, they were >30 times that observed in PBO (Table 1). These results suggest that MerB-mediated reductive demethylation dominated in MLD waters whereas demethylation in PBO was oxidative and at low rates.

Discussion

The results presented here support our hypothesis that the inverse relationship between the proportion of Hg_T as MeHg (%MeHg) and the concentration of Hg_T (Figures 1 and 3A) is a result of increased MeHg degradation rates due to induction of *mer* operon functions in Hg contaminated waters. This is supported by observations indicating that microbial biomass in ML but not in PB waters was (i) enriched for Hg^R bacteria, (ii) contained and expressed *mer* genes (Figure 4 and Table 2, respectively), and (iii) reductively demethylated MeHg (Figure 5). The role of MerA in the reduction and volatilization of mercury in natural waters has long been known from direct enzymatic studies (*43, 44*) and from the relationships of *merA* transcript abundance to Hg(0) production (*45*). Here, the scope of these findings is extended to the degradation of MeHg by demonstrating how expression and activity of MerB enhances MeHg degradation to CH$_4$ in polluted waters.

An alternative explanation for the proportionately high levels of MeHg in environments with low Hg_T is enhanced methylation (ref *35* and Figure 3B). Lake acidification, as is the case in PB lakes, stimulates Hg(II) transport into bacterial cells (*36*) and methylation rates (*46−48*). Existing data and our own unpublished results showing low methylation rates in the two study sites render this explanation unlikely. Pak and Bartha (*49*) showed that in PB lake sediments the rate of MeHg formation ($k_{meth} = \sim 0.002\ day^{-1}$) was low relative to demethylation ($k_{deg} = 0.1−0.2\ day^{-1}$), although extremely high Hg(II) and MeHg concentrations were added to sediment incubations (1.0 and 0.1 ppm, respectively). In estuarine sediments collected immediately downstream of MLD below the tidal gate, methylation was negligible ($k_{meth} < 2 \times 10^{-5}\ day^{-1}$; ref *50* and Schaefer, unpublished data). These low methylation potentials are likely due to high sulfide concentration (*50*) limiting Hg(II) bioavailability to methylating microbes (*35*).

Demethylation in the water column by aerobic microorganisms may be a critical process in reducing the amount of MeHg available for entry into the aquatic food web. Methylmercury produced in anoxic sediments is transported to the water column where it is absorbed by the unicellular microbes that constitute the base of the food web (*51*).

Therefore, MeHg degradation in this compartment is likely to limit MeHg accumulation and biomagnification. Thus far, few demethylation studies have focused on surface waters (*48, 52, 53*). To the best of our knowledge, the issues of reductive vs oxidative demethylation and the role of MerB-mediated degradation have not been addressed in aerobic water column samples. The effect of MerB-mediated MeHg degradation and the immediate reduction of the initial product, Hg(II), to Hg(0) by MerA is a net loss of mercury from the system via the volatilization of Hg(0). On the other hand, the end product of oxidative demethylation is thought to be Hg(II) (*15*), the substrate for methylation. A clear difference in rates and pathways for MeHg degradation was observed for the aquatic biomass with low rates of oxidative demethylation in PB and high rates of reductive demethylation in ML waters (Figure 5). With the likely low methylation rates in both PB and ML, the methylation/demethylation cycle is shifted toward net methylation in PB lakes, resulting in increases in %MeHg. However, in ML waters with active MerB-specified MeHg degradation and Hg(0) volatilization, the methylation/demethylation cycle shifts toward net losses from the water column, resulting in reduced proportions of Hg_T as MeHg with increasing Hg_T (Figure 3A).

The k_{deg} of MeHg in ML waters ($k_{deg} = 0.19$ day^{-1}) was ~10 times greater than in PB waters ($k_{deg} = 0.01$ day^{-1}) and in those reported previously for lakes with low Hg levels ($k_{deg} = 0.001-0.02$ day^{-1} (*48, 52*) as reported in ref *47* and <0.001−0.13 day^{-1} (*53*)). Our results suggest that the half-life of MeHg in PB, >72 days, is longer compared to that in ML, 3.6 days, allowing for entry into the aquatic food web for a significantly longer period of time in the former.

Three approaches to relate *merA* expression to Hg_T concentration were used (Table 2), and none provided quantitative data of high quality. Numbers of transcripts detected by both RT-qPCR and hybridization were at the lowest range of the corresponding calibration curves, limiting sensitivity of detection. Sensitivity might have been further compromised if concentrations of Hg(II) were low and at a range where induction of *mer* increases linearly rather than exponentially (Figure 2A), by *merA* sequence variability, and by RT-PCR inhibition by cellular and humic substances that are coextracted with nucleic acids from aquatic biomass. These problems are exacerbated by the short half-life of *mer* transcripts, about 1 min as shown by pure culture studies (*54*), and the transitory nature of Hg(II) bioavailability in natural waters (*37*). Therefore, the alternative approach of measuring specific rates of *mer* operon-encoded enzymes, as achieved for MerA (*43, 44*), may be more appropriate for evaluating *merB* expression in environmental biomass. If so, an assay for the activity of MerB in natural waters will need to be developed.

Here we demonstrate a role for *mer*-specified activities in degradation of MeHg in natural surface waters. The input of MeHg to the water column is however determined by processes that occur in anoxic sediments (*12, 35*), and it is in this environment that *mer* activities may exert significant control over net MeHg accumulation. Complete *mer* genes were identified in genome sequences of some anaerobes. We previously showed that a denitrifying bacterium expressed *mer* genes under anaerobic conditions, albeit at higher Hg-(II) concentrations than that required under aerobic conditions (*26*). Furthermore, examination of data compiled from literature sources (*55−57*) shows that the choice between demethylation pathways in sediments is controlled by redox and Hg concentrations, with the reductive pathway dominating in aerobic incubations or under anaerobic incubations of highly contaminated sediments (*26, 56*). These observations suggest that *mer*-specified activities may play a significant role in the cycling of mercury in contaminated anoxic sediments. An understanding of the pathways of MeHg degradation in both water column and sediments is essential for efforts to reduce MeHg production and accumulation in aquatic food webs.

Supporting Information Available

Information on quality control testing of $^{14}CH_4$-combustion/$^{14}CO_2$-trapping method and primer sets used for PCR amplification is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Barkay, T. The Mercury Cycle. In *Encyclopedia of Microbiology*, 2nd ed.; Academic Press: San Diego, 2000; Vol. 3, p 171.

(2) Morel, F. M. M.; Kraepiel, A. M. L.; Amyot, M. The chemical cycle and bioaccumulation of mercury. *Annu. Rev. Ecol. Syst.* **1998**, *29*, 543.

(3) Compeau, G. C.; Bartha, R. Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. *Appl. Environ. Microbiol.* **1985**, *50*, 498.

(4) Gilmour, C. C.; Henry, E. A.; Mitchell, R. Sulfate stimulation of mercury methylation in freshwater sediments. *Environ. Sci. Technol.* **1992**, *26*, 2281.

(5) Clarkson, T. W. The toxicology of mercury. *Crit. Rev. Clin. Lab. Sci.* **1997**, *34*, 369.

(6) DiFrancesco, D. T.; Shinn, R. C. *New Jersey Mercury Task Force Report*; New Jersey Department of Environmental Protection: Trenton, NJ, 2002; Vol. II (Exposure and Impacts).

(7) Björklund, I.; Borg, H.; Johansson, K. Mercury in Swedish lakes—Its regional distrubtion and causes. *Ambio* **1984**, *13*, 118.

(8) Kannan, K. R. G. S., Jr.; Lee, R. F.; Windom, H. L.; Heitmuller, P. T.; Macauley, J. M.; Summers, J. K. Distribution of total mercury and methyl mercury in water, sediment, and fish from South Florida estuaries. *Arch. Environ. Contam. Toxicol.* **1998**, *34*, 109.

(9) NJDEP *Preliminary assessment of Hg_T concentrations in fish from rivers, lakes, and reservoirs in New Jersey*; New Jersey Department of Environmental Protection and Energy: Trenton, NJ, 1994.

(10) Bloom, N. S. On the chemical form of mercury in edible fish and marine invertebrates tissue. *Can. J. Fish. Aquat. Sci.* **1992**, *49*, 1010.

(11) Watras, C. J.; Bloom, N. S. Mercury and methylmercury in individual zooplankton: implications for bioaccumulation. *Limnol. Oceanogr.* **1992**, *37*, 1313.

(12) Barkay, T.; Miller, S. M.; Summers, A. O. Bacterial mercury resistance from atoms to ecosystems. *FEMS Microbiol. Rev.* **2003**, *27*, 355.

(13) Sellers, P.; Kelly, C. A.; Rudd, J. W. M.; MacHutchon, A. R. Photodegradation of methylmercury in lakes. *Nature* **1996**, *380*, 694.

(14) Spangler, W. J.; Spigarelli, J. L.; Rose, J. M.; Miller, H. M. Methylmercury: bacterial degradation in lake sediments. *Science* **1973**, *180*, 192.

(15) Marvin-DiPasquale, M. C.; Oremland, R. S. Bacterial methylmercury degradation in Florida Everglades peat sediment. *Environ. Sci. Technol.* **1998**, *32*, 2556.

(16) Brown, N. L.; Stoyanov, J. V.; Kidd, S. P.; Hobman, J. L. The MerR family of transcriptional regulators. *FEMS Microbiol. Rev.* **2003**, *27*, 145.

(17) Ralston, D.; O'Halloran, T. Ultrasensitivity and heavy-metal selectivity of the allosterically modulated MerR transcription complex. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3846.

(18) Rasmussen, L. D.; Turner, R. R.; Barkay, T. Cell-density-dependent sensitivity of a *mer-lux* bioassay. *Appl. Environ. Microbiol.* **1997**, *63*, 3291.

(19) Lipsky, D. S.; Koepp, S. J. Mercury levels in organisms in proximity to an old chemical site (Berry's Creek, Hackensack Meadowlands, New Jersey, USA). *Mar. Pollut. Bull.* **1986**, *17*, 219.

(20) Murphy, E. A.; Dooley, J.; Windom, H. L.; Smith, R. G., Jr. Mercury species in potable groundwater in southern New Jersey. *Water Air Soil Pollut.* **1994**, *78*, 61.

(21) Gill, G. A.; Fitzgerald, W. F. Picomolar mercury measurements in seawater and other materials using stannous chloride reduction and two-stage gold amalgamation with gas-phase detction. *Mar. Chem.* **1987**, *20*, 227.

(22) Jeffrey, W. H.; Nazaret, S.; von Haven, R. Improved method for recovery of mRNA from aquatic samples and its application to detection of *mer* expression. *Appl. Environ. Microbiol.* **1994**, *60*, 1814.

(23) Bloom, N. S.; Crecelius, E. A. Determination of mercury in seawater at sub-nanogram per liter levels. *Mar. Chem.* **1983**, *14*, 49.

(24) Horvat, M.; Liang, L.; Bloom, N. S. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples: Part II. Water. *Anal. Chim. Acta* **1993**, *282*, 153.

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

(26) Schaefer, J. K.; Letowski, J.; Barkay, T. *mer*-mediated resistance and volatilization of Hg(II) under anaerobic conditions. *Geomicrobiol. J.* **2002**, *19*, 87.

(27) Hurt, R. A.; Qiu, X.; Wu, L.; Roh, Y.; Palumbo, A. V.; Tiedje, J. M.; Zhou, J. Simultaneous recovery of RNA and DNA from soils and sediments. *Appl. Environ. Microbiol.* **2001**, *67*, 4495.

(28) *Current Protocols in Molecular Biology*; Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K., Eds.; John Wiley and Sons: Cambridge, 2000.

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

(30) Barkay, T.; Gillman, M.; Liebert, C. Genes encoding mercuric reductases from selected Gram-negative aquatic bacteria have a low degree of homology with *merA* of transposon Tn*501*. *Appl. Environ. Microbiol.* **1990**, *56*, 1695.

(31) Hobbie, J. E.; Daley, R. J.; Jasper, S. Use of Nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* **1977**, *33*, 1225.

(32) Barkay, T.; Tripp, S. C.; Olson, B. H. Effect of metal-rich sewage sludge application on the bacterial communities of grasslands. *Appl. Environ. Microbiol.* **1985**, *49*, 333.

(33) Rosner, B. *Fundamentals of Biostatistics*, 5th ed.; Duxbury: Pacific Grove, 2000.

(34) Pitts, K. E.; Summers, A. O. The roles of thiols in the bacterial organomercurial lyase (MerB). *Biochemistry* **2002**, *41*, 10287.

(35) Benoit, J.; Gilmour, C.; Heyes, A.; Mason, R. P.; Miller, C. Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. In *Biogeochemistry of environmentally important trace elements*; Chai, Y., Braids, O. C., Eds.; American Chemical Society: Washington, D.C., 2003; p 262.

(36) Kelly, C. A.; Rudd, J. W. M.; Holoka, M. H. Effect of pH on mercury uptake by an aquatic bacterium: implications for Hg cycling. *Environ. Sci. Technol.* **2003**, *37*, 2941.

(37) Barkay, T.; Gillman, M.; Turner, R. R. Effects of dissolved organic carbon and salinity on bioavailability of mercury. *Appl. Environ. Microbiol.* **1997**, *63*, 4267.

(38) Compeau, G.; Bartha, R. Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions. *Appl. Environ. Microbiol.* **1984**, *48*, 1203.

(39) Steffan, R. J.; Korthals, E. T.; Winfrey, M. R. Effects of acidification on mercury methylation, demethylation, and volatilization in sediments from an acid-susceptible lake. *Appl. Environ. Microbiol.* **1988**, *54*, 2003.

(40) Watras, C. J.; Back, R. C.; Holvorsen, S.; Hudson, R. J. M.; Morrison, K. A.; Wente, S. P. Bioaccumulation of mercury in pelagic freshwater food webs. *Sci. Total Environ.* **1998**, *219*, 183.

(41) Barkay, T. Adaptation of aquatic microbial communities to Hg^{2+} stress. *Appl. Environ. Microbiol.* **1987**, *53*, 2725.

(42) Hintelmann, H.; Harris, R.; Heyes, A.; Hurley, J. P.; Kelly, C. A.; Krabbenhoft, D. P.; Lindberg, S.; Rudd, J. W. M.; Scott, K. J.; St. Louis, V. L. Reactivity and mobility of new and old mercury deposition in a boreal forest ecosystem during the first year of the METAALICUS study. *Environ. Sci. Technol.* **2002**, *36*, 5034.

(43) Ogunseitan, O. A. Protein method for investigating mercuric reductase gene expression in aquatic environments. *Appl. Environ. Microbiol.* **1998**, *64*, 695.

(44) Siciliano, S. D.; O'Driscoll, N. J.; Lean, D. R. S. Microbial reduction and oxidation of mercury in freshwater lakes. *Environ. Sci. Technol.* **2002**, *36*, 3064.

(45) Nazaret, S.; Jeffrey, W. H.; Saouter, E.; von Haven, R.; Barkay, T. *merA* gene expression in aquatic environments measured by mRNA production and Hg(II) volatilization. *Appl. Environ. Microbiol.* **1994**, *60*, 4059.

(46) Winfrey, M. R.; Rudd, J. W. M. Environmental factors affecting the formation of methylmercury in low pH lakes. *Environ. Toxicol. Chem.* **1990**, *9*, 853.

(47) Gilmour, C. C.; Henry, E. A. Mercury methylation in aquatic systems affected by acid deposition. *Environ. Pollut.* **1991**, *71*, 131.

(48) Xun, L.; Campbell, N. E. R.; Rudd, J. W. M. Measurements of specific rates of net methyl mercury production in the water column and surface sediments of acidified and circumneutral lakes. *Can. J. Fish. Aquat. Sci.* **1987**, *44*, 750.

(49) Pak, K.-R.; Bartha, R. Mercury methylation and demethylation in anoxic lake sediments and by strictly anaerobic bacteria. *Appl. Environ. Microbiol.* **1998**, *64*, 1013.

(50) Berman, M.; Bartha, R. Control of the methylation process in a mercury-polluted aquatic sediment. *Environ. Pollut. B* **1986**, *11*, 41.

(51) Mason, R. P.; Reinfelder, J. R.; Morel, F. M. M. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ. Sci. Technol.* **1996**, *30*, 1835.

(52) Korthals, E. T.; Winfrey, M. R. Seasonal and spatial variations in mercury methylation and demethylation in an oligotrophic lake. *Appl. Environ. Microbiol.* **1987**, *53*, 2397.

(53) Matilainen, T.; Verta, M. Mercury methylation and demethylation in aerobic surface waters. *Can. J. Fish. Aquat. Sci.* **1995**, *52*, 1597.

(54) Gambill, B. D.; Summers, A. O. Synthesis and degradation of the mRNA of the Tn21 *mer* operon. *J. Mol. Biol.* **1992**, *225*, 251.

(55) Oremland, R. S.; Culbertson, C. W.; Winfrey, M. R. Methylmercury decomposition in sediments and bacterial cultures: involvement of methanogens and sulfate reducers in oxidative demethylation. *Appl. Environ. Microbiol.* **1991**, *57*, 130.

(56) Marvin-DiPasquale, M.; Agee, J.; McGowan, C.; Oremland, R. S.; Thomas, M.; Krabbenhoft, D.; Gilmour, C. C. Methyl-mercury degradation pathways: a comparison among three mercury-impacted ecosystems. *Environ. Sci. Technol.* **2000**, *34*, 4908.

(57) Hines, M. E.; Horvat, M.; Faganeli, J.; Bonzongo, J.-C. J.; Barkay, T.; Major, E. B.; Scott, K. J.; Bailey, E. A.; Warwick, J. J.; Lyons, W. B. Mercury biogeochemistry in the Idrija River, Slovenia, from above the mine into the Gulf of Trieste. *Environ. Res.* **2000**, *83*, 129.

(58) Babiarz, C. L.; Hurley, J. P.; Hoffmann, S. R.; Andren, A. W.; Shafer, M. M.; Armstrong, D. E. Partitioning of total mercury and methylmercury to the colloidal phase in freshwaters. *Environ. Sci. Technol.* **2001**, *35*, 4773.

(59) Balogh, S. J.; Huang, Y.; Meyer, M. L.; Johnson, D. K. Episodes of elevated methylmercury concentrations in prairie streams. *Environ. Sci. Technol.* **2002**, *36*, 1665.

(60) Bonzongo, J.-C. J.; Heim, K. J.; Warwick, J. J.; Lyons, W. B. Mercury levels in surface waters of the Carson River−Lahontan Reservoir system, Nevada: influence of historic mining activities. *Environ. Pollut.* **1996**, *92*, 193.

(61) Ganguli, P. M.; Mason, R. P.; Abu-Saba, K. E.; Anderson, R. S.; Flegal, A. R. Mercury speciation in drainage from the New Idria mercury mine, California. *Environ. Sci. Technol.* **2000**, *34*, 4773.

(62) Kelly, C. A.; Rudd, J. W. M.; Bodaly, R. A.; Roulet, N. P.; St. Louis, V. L.; Heyes, A.; Moore, T. R.; Schiff, S.; Aravena, R.; Scott, K. J.; Dyck, B.; Harris, R.; Warner, B.; Edwards, G. Increases in fluxes of greenhouse gases and methyl mercury following flooding of an experimental reservoir. *Environ. Sci. Technol.* **1997**, *31*, 1334.

(63) St. Louis, V. L.; Rudd, J. W. M.; Kelly, C. A.; Beaty, K. G.; Flett, R. J.; Roulet, N. T. Production and loss of methylmercury and loss of total mercury from boreal forest catchments containing different types of wetlands. *Environ. Sci. Technol.* **1996**, *30*, 2719.

(64) Meuleman, C.; Leermakers, M.; Baeyens, W. Mercury speciation in Lake Baikal. *Water Air Soil Pollut.* **1995**, *80*, 539.

(65) Mason, R. P.; Sullivan, K. A. Mercury and methylmercury transport through an urban watershed. *Water Res.* **1998**, *32*, 321.

(66) Mason, R. P.; Lawson, N. M.; Lawrence, A. L.; Leaner, J. J.; Lee, J. G.; Sheu, G.-R. Mercury in the Chesapeake Bay. *Mar. Chem.* **1999**, *65*, 77.

(67) Griffin, H. G.; Foster, T. J.; Silver, S.; Misra, T. K. Cloning and DNA sequence of the mercuric- and organomercurial-resistance determinants of plasmid pDU1358. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 3112.