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merA Gene Expression in Aquatic Environments Measured by mRNA Production and Hg(II) Volatilization†

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The relationship of *merA* gene expression (specifying the enzyme mercuric reductase) to mercury volatilization in aquatic microbial communities was investigated with samples collected at a mercury-contaminated freshwater pond, Reality Lake, in Oak Ridge, Tenn. Levels of *merA* mRNA transcripts and the rate of inorganic mercury [Hg(II)] volatilization were related to the concentration of mercury in the water and to heterotrophic activity in field samples and laboratory incubations of pond water in which microbial heterotrophic activity and Hg(II) concentration were manipulated. Levels of *merA*-specific mRNA and Hg(II) volatilization were influenced more by microbial metabolic activity than by the concentration of mercury. *merA*-specific transcripts were detected in some samples which did not reduce Hg(II), suggesting that rates of mercury volatilization in environmental samples may not always be proportional to *merA* expression.

The heavy metal mercury is an environmental contaminant of concern because of its high toxicity to living organisms. Inorganic mercury [Hg(II)] can be biotransformed to methylmercury (MeHg), which is both more toxic and more readily bioaccumulated. Methylation, together with degradation of MeHg and reduction of Hg(II), constitutes microbially mediated reactions in the geochemical cycling of mercury. Increased knowledge of these microbial activities could allow us to better control the cycling of mercury in the environment and to decrease levels of MeHg that are available for bioaccumulation (5).

A common mechanism of bacterial resistance to Hg(II) found among a large variety of gram-negative and gram-positive bacteria is detoxification by reduction to the elemental, volatile, and less toxic form, Hg^0. This reaction is catalyzed by the cytosolic, NADPH-dependent mercuric reductase that is encoded by the *merA* gene of the mercury resistance (*mer*) operon. Transcriptional activity of the *mer* operon (*merA*; the genes involved in transport functions, *merTP*; and the regulatory genes, *merRD*) is controlled by the regulatory protein MerR, which displays negative control features (repression) in the absence of Hg(II) and is a transcription activator in the presence of Hg(II) (28, 45). In vitro (36) and in vivo (11, 43) experiments have shown that nanomolar concentrations of Hg(II) are sufficient to induce high levels of *mer* transcription.

Mercury-reducing bacteria have been isolated from various environments, water (1, 25), sediment (29), agricultural soil (37), and clinical sources (18). Barkay et al. (2, 4) and Rochelle et al. (39) developed a series of DNA probes to investigate the abundance of *mer*-carrying bacteria in the environment, and Barkay et al. (3, 4) used these probes to study the role of *mer* genes in adaptation of marine and freshwater microbial communities to Hg(II). Hybridization with whole-community genome showed that the system encoded by transposon Tn*501* was dominant in the microbial community of a contaminated freshwater pond and was likely to contribute to observed Hg(II) reduction and subsequent volatilization (6). However, the presence of a specific gene does not mean that it is expressed in the environment and provides no information on its role in a specific ecological function. Sequences homologous to *mer* genes have been encountered in mercury-sensitive strains (7), and environmental conditions may not allow expression of *mer*. In addition, mercury-resistant bacteria carrying *mer*-like sequences are routinely found in environments that have never been exposed to mercury contamination (4, 44). Thus, detecting *mer* gene products (mRNA transcripts and polypeptides) in situ could verify that genes related to mercury biotransformations are expressed and add to our understanding of the role of *mer* in the geochemical cycling of mercury.

The purpose of this study was to explore the relationships between expression of indigenous *merA* genes and Hg(II) volatilization in a mercury-contaminated freshwater pond, Reality Lake (RL), in the vicinity of Oak Ridge, Tenn., and to study the effects of environmental factors on *merA* expression. The influence of Hg(II), MeHg, and temperature on *mer* expression was previously investigated in several pseudomonads that originated from RL (47). A considerable variation among isolates suggested that an erroneous impression of community function might arise from the study of isolated strains. Thus, we chose to measure levels of *merA* transcripts in RNA extracts directly isolated from field samples and microcosm incubations. Such an approach has been developed and successfully applied to detect *nptII* (neomycin phosphotransferase), *rbcL* (ribulose 1,5-bisphosphate carboxylase-oxygenase), and *xylE* (catechol 2,3-dioxygenase) transcripts from pure cultures and natural aquatic communities (33, 34) and for *merA* and *nahAB* (naphthalene dioxygenase) transcripts from soils seeded with *Pseudomonas putida* (48). Here we applied an improved mRNA extraction method (22) to study the influence of Hg(II) and nutrients on *merA* expression by relating *merA*

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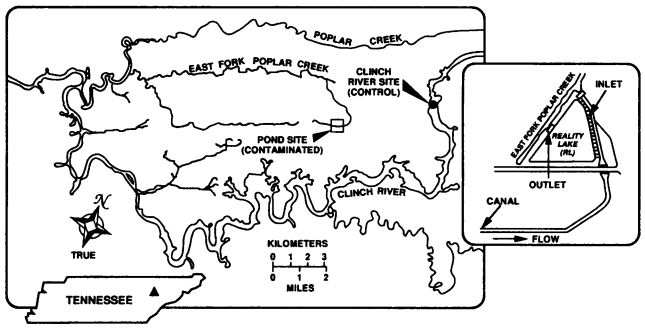

FIG. 1. Sampling locations in the vicinity of Oak Ridge, Tenn. Area map showing the contaminated and control study sites. (Inset) Sampling locations in the contaminated site.

transcript levels in indigenous bacterial communities to heterotrophic activity and Hg^0 volatilization.

MATERIALS AND METHODS

Study site and field sampling. RL, a mercury-contaminated freshwater pond in the vicinity of a nuclear weapons facility in Oak Ridge, Tenn., has been previously described (6) and is illustrated in Fig. 1. In addition to our previous sampling locations, RL inlet (inflow) and RL outlet (outflow), and the uncontaminated Clinch River (CR), we collected a sample (canal) from an open canal that delivers mercury-contaminated water from the nuclear weapons facility to RL.

RNA extraction and *merA* mRNA quantification. RNA extraction and quantification of *merA* transcripts were performed as described by Jeffrey et al. (22). Briefly, water samples were filtered onto 0.22-μm-pore-size cartridge filters (Sterivex-GS; Millipore Corp., Bedford, Mass.) submerged in an ice water bath. Liquid was removed from the cartridge, and filters were stored on dry ice or at −70°C until extraction. RL samples were filtered in the field, and frozen filters were transported to the laboratory. Cells were lysed by boiling the filter in NaCl-Tris-EDTA–sodium dodecyl sulfate–diethylpyrocarbonate, and RNA was extracted with a solution of guanidinium isothiocyanate, phenol-chloroform, and Sarkosyl (for details on composition of solutions, pH, temperature, and time of incubations, see reference 22). Further purification of RNA was achieved by two precipitation steps with isopropanol. Final precipitates were dissolved in 1 mM EDTA and treated with DNase or RNase as described by Pichard and Paul (33). RNA samples were blotted onto polyvinylidene fluoride membranes (Immobilon-N; Millipore Corp.) and hybridized with a ^{35}S-labeled single-stranded RNA probe. The probe was produced in vitro by transcription of the noncoding DNA strand of a 1.1-kb *Hin*dIII-*Eco*RI DNA fragment, encompassing almost two-thirds of the *merA* gene from Tn*501* cloned in a pGEM-3Z vector (Promega Corp., Madison, Wis.) and bacteriophage T7 RNA polymerase, as described by Jeffrey et al. (22). Hybridization was as described previously (23, 33). Hybridization results were quantified by radioactive counting, using a radioanalytical imaging system (β-scanner; Ambis Systems, Inc., San Diego, Calif.). Concentrations of target mRNA were determined by using a standard curve of in vitro-synthesized *merA* mRNA, as described before (22, 34).

$^{203}Hg^0$ volatilization assays. Volatilization assays were performed as previously described (6) except that $^{203}Hg(NO_3)_2$ [specific activity, 1.6 mCi mg of $Hg(II)^{-1}$; Buffalo Materials Research Center, State University of New York at Buffalo, Buffalo] was added to a final concentration of 435 ng of Hg(II) $liter^{-1}$, and charcoal absorbers (Mine Safety Applications Co., Pittsburgh, Pa.) were removed for $^{203}Hg^0$ analysis 1, 2, and 3 h after addition of the substrate.

[^{3}H]thymidine and [^{14}C]leucine incorporation rates. Incorporation rates of [^{3}H]thymidine (specific activity, 81 Ci $mmol^{-1}$; ICN, Irvine, Calif.) and [^{14}C]leucine (specific activity, 310 mCi $mmol^{-1}$; ICN) were determined as described by Chin-Leo and Kirchman (9). Concentrations of thymidine and leucine were 10 and 40 nM, respectively, and incubations were carried out for 30 min. These conditions produce linear rates of incorporation with time. RL samples were incubated in the field at in situ temperature immediately after sampling. Cell-associated radioactivity was determined after filtration through 0.2-μm-pore-size, mixed cellulose acetate filters (Millipore GS) and a cold 5% trichloroacetic acid wash by liquid scintillation counting (Packard Tri-Carb TR; Packard Instruments, Downers Grove, Ill.).

Total and dissolved mercury determinations. Samples collected for mercury analysis were preserved by the addition of 0.5% (final concentration) bromine monochloride. Total mercury and dissolved mercury (filtered through a 0.45-μm-pore-size nylon filter [Cole-Parmer Instrument Co., Niles, Ill.]) in water samples were determined by reduction with $SnCl_2$ and gas phase detection of Hg^0 by cold vapor atomic fluorescence after a gold amalgamation step (19, 48). Prior to reduction with $SnCl_2$, water samples were treated with 0.5% bromine monochloride–0.1% hydroxylamine (13).

The following nomenclature describing mercury is used: Hg_t, all species detected by cold vapor atomic fluorescence; Hg_{dis}, all species detected in samples filtered through 0.45-μm-pore-size filters; Hg(II), all species with the +2 valence. The term mercury is used as a general term when the species of mercury is unknown.

Bacterial counts. Total direct counts were determined by 4′,6-diamidino-2-phenylindole staining as described by Porter and Feig (35). Heterotrophic plate counts were determined by plating diluted water samples on plate count agar (Difco Laboratories, Detroit, Mich.). Mercury resistance counts were determined on plate count agar amended with 10 μg of Hg(II) ml^{-1} (50 μM) as $HgCl_2$. CFU were counted after 2 days of incubation at 30°C.

Laboratory incubations. Ten liters of unfiltered RL outlet water was placed in 20-liter high-density polyethylene containers manufactured to exceed Environmental Protection Agency superfund standards for metal analysis (Fisher Scientific, Orlando, Fla.). Six treatments (each consisting of duplicate containers) involving combinations of two Hg(II) concentrations (as $HgCl_2$) and three yeast extract (YE) concentrations were set up: (i) no additions; (ii) Hg(II) added to 50 μg $liter^{-1}$; (iii) YE added to 0.1 mg $liter^{-1}$; (iv) YE (1 mg $liter^{-1}$); (v) Hg(II) (50 μg $liter^{-1}$) and YE (0.1 mg $liter^{-1}$); (vi) Hg(II) (50 μg $liter^{-1}$) and YE (1 mg $liter^{-1}$). The indigenous concentration of total mercury in the water [and thus in all incubations to which Hg(II) was not added] was 1.12 ± 0.01 μg $liter^{-1}$. To establish three different levels of microbial metabolic activity, samples were incubated at room temperature for 5 h after the addition of YE and prior to the addition of Hg(II). Samples were taken before the addition of Hg(II) (time [t] = 0) and at 30 min and 4, 8, 12, and 19 h for analyses of *merA* transcripts, remaining mercury, and [^{14}C]leucine incorporation rate. No 30-min samples were taken for incubations to which Hg(II) was not added. Control incubations, consisting of 5 liters of autoclaved outlet water, with or without addition of Hg(II), were sampled at t = 0 and at 12 and 36 h for abiological removal of mercury.

TABLE 1. Results of measurements performed to determine the relationship of mercury volatilization to *merA* gene expression in field samples collected in Reality Lake, Oak Ridge, Tenn.[a]

Parameter measured	CR	RL		
		Canal	Inflow	Outflow
Mercury concn[b]				
Hg_t (μg $liter^{-1}$)	0.018 ± 0.001	1.66 ± 0.12	0.93 ± 0.01	1.12 ± 0.01
Hg_{dis} (μg $liter^{-1}$)	ND	1.22 ± 0.01	0.76 ± 0.01	0.14 ± 0.08
Heterotrophic activity				
TdR incorporation rate (pmol $liter^{-1}$ h^{-1})	3.3 ± 1.4	0.07 ± 0.07	1.3 ± 0.4	49.5 ± 0.2
Leu incorporation rate (pmol $liter^{-1}$ h^{-1})	231.6 ± 18.6	11.9 ± 18.9	75.3 ± 0.4	1729.9 ± 33.7
Hg(II) volatilization (% loss)[c]	0	0	2.2 ± 0.7	9.1 ± 0.8
Bacterial counts				
DAPI (cells ml^{-1})	ND	5.58×10^4	1.69×10^5	ND
Plate count (CFU ml^{-1})				
LB	1.20×10^3	4.00×10^1	2.50×10^3	1.96×10^3
LB + 50 μM $HgCl_2$	3.93×10^2	2.00×10^1	1.57×10^3	3.70×10^2
merA transcript abundance (pg $liter^{-1}$)	101 ± 2.2	39 ± 1.4	102 ± 3.1	265 ± 14.5

[a] Abbreviations: RL, Reality Lake; CR, Clinch River; ND, not determined; TdR, thymidine; Leu, leucine; DAPI, 4,6-diamidino-2-phenylindole. Results are reported as the means ± standard deviations of triplicate determinations.
[b] See Materials and Methods for definitions of Hg_t and Hg_{dis}.
[c] Percentage of Hg(II) lost 3 h after addition of $^{203}Hg(II)$. 0, loss by biologically active samples did not exceed activity of sterile controls.

Statistical analysis. Multivariate analysis of variance (or multiple regression) was performed to determine the effect of different factors [time and concentrations of Hg(II) and YE] on mRNA production, heterotrophic activity, and Hg(II) volatilization measured during laboratory incubations. Significance of difference between two means was established by Student's *t* test ($P < 0.05$) (46).

RESULTS

Field studies. Data obtained from each of the field samples are summarized in Table 1. Concentrations of Hg_t in contaminated samples (canal, inflow, and outflow) were between 0.9 and 1.7 μg $liter^{-1}$, with the highest in the canal (1.66 ± 0.12 μg $liter^{-1}$). Outflow and inflow had slightly lower concentrations of Hg_t (1.12 ± 0.01 and 0.93 ± 0.01 μg $liter^{-1}$, respectively). Total mercury was also detected in the CR control site but at a level (Hg_t = 0.018 ± 0.001 μg $liter^{-1}$) orders of magnitude lower than in the pond. Hg_{dis}, likely to represent a more bioavailable form of mercury, declined following release from the plant, with the canal having the highest concentration and the outflow having the lowest. The reverse was observed with the potential to volatilize $^{203}Hg(II)$. The highest activity was demonstrated by the outflow followed by the inflow, whereas no biological potential for Hg^0 evolution was observed with the CR and canal samples. Heterotrophic activity as determined by both [^{14}C]leucine and [^3H]thymidine incorporation rates varied by orders of magnitude among the different samples, with the highest activity in the outflow followed by CR, inflow, and canal. Data for direct cell counts are available only for the inflow and the canal, with the former having twice as many cells as the latter (Table 1). However, on two previous occasions, a higher number of cells (both heterotrophic plate counts and direct counts) had been observed in the outflow than in the inflow or the canal (22, 30). Total plate counts showed slightly higher counts for the inflow compared with the outflow and CR. Very few colonies were detected on plates inoculated with canal water. The proportion of Hg(II)-resistant CFU was higher in the inflow (63%) and in the canal (50%) and lower in the outflow (19%). CR contained a significant number of Hg(II)-resistant CFU (32% of total). Similar observations were made during additional samplings (22, 30). *merA* transcripts were detected in RNA extracts isolated from all samples including the control site (CR), where total mercury concentration was below levels known to induce *mer* transcription (11, 36). Furthermore, the *merA* mRNA concentration in CR was 2.5 times greater than that in the canal and similar to that in the inflow.

Thus, *merA* transcripts were most abundant and the highest Hg^0 volatilization activity was observed in the sample (outflow) that contained the most active microbial community (as indicated by heterotrophic activity) rather than in the sample with the highest Hg_t or Hg_{dis} concentrations (canal).

Laboratory incubations. Outflow water samples were supplemented with Hg(II) and/or YE to further examine relationships among *merA* transcription, Hg(II) concentration, and heterotrophic activity that were observed with field samples. Results for transcript production are presented in Fig. 2. In all incubations, and regardless of Hg(II) dose, time and nutrient concentrations significantly affected *merA* transcript levels ($P < 0.01$). Incubations with 0 and 0.1 mg of YE $liter^{-1}$ to which Hg(II) was not added showed a decrease in net *merA* transcript levels over time, and with a higher concentration of YE [1 mg $liter^{-1}$; Hg(II) not added] transcript concentration decreased at 19 h after an initial increase at 4 h (Fig. 2A). All incubations with 50 μg of Hg(II) $liter^{-1}$ exhibited an increase over time in net *merA* transcript levels. The magnitude of the increase in *merA* transcript concentration was directly related to the concentration of YE (Fig. 2B).

There was no detectable loss ($P = 0.575$) of mercury from incubations to which Hg(II) was not added (Fig. 3A). It should be noted that by analyzing Hg_t (with variability of analysis between 2 and 10%) we may have not detected low volatilization activities. Volatilization in RL may remove only a small proportion of Hg_t (Table 1 and reference 6). In incubations supplemented with Hg(II), the concentration of Hg(II) decreased with time ($P < 0.01$) and the level of nutrient amendment (and consequently, stimulation of metabolic activ-

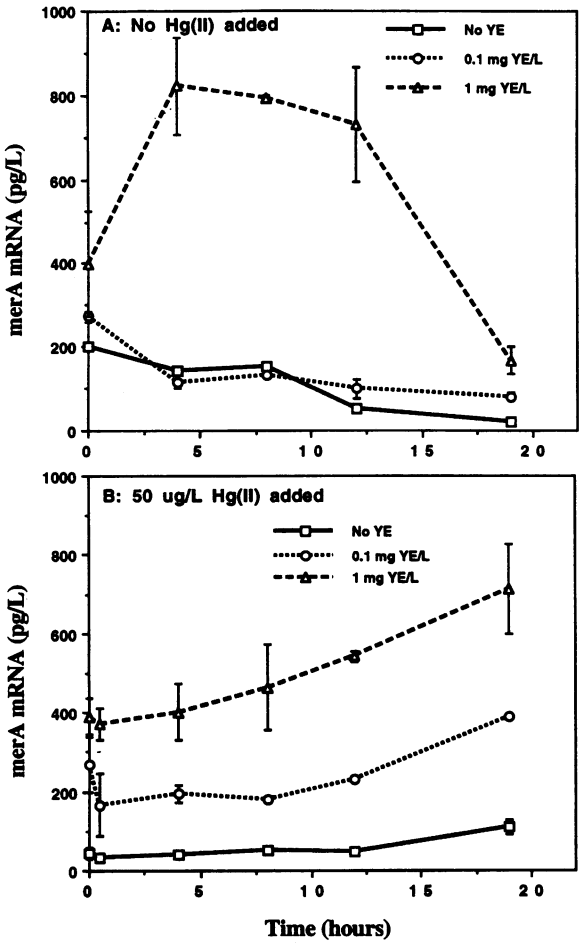

FIG. 2. Effect of nutrients and Hg(II) on *merA* mRNA accumulations in laboratory incubations. (A) Incubations with no addition of Hg(II); (B) incubations with the addition of 50 μg of Hg(II) liter^{-1}.

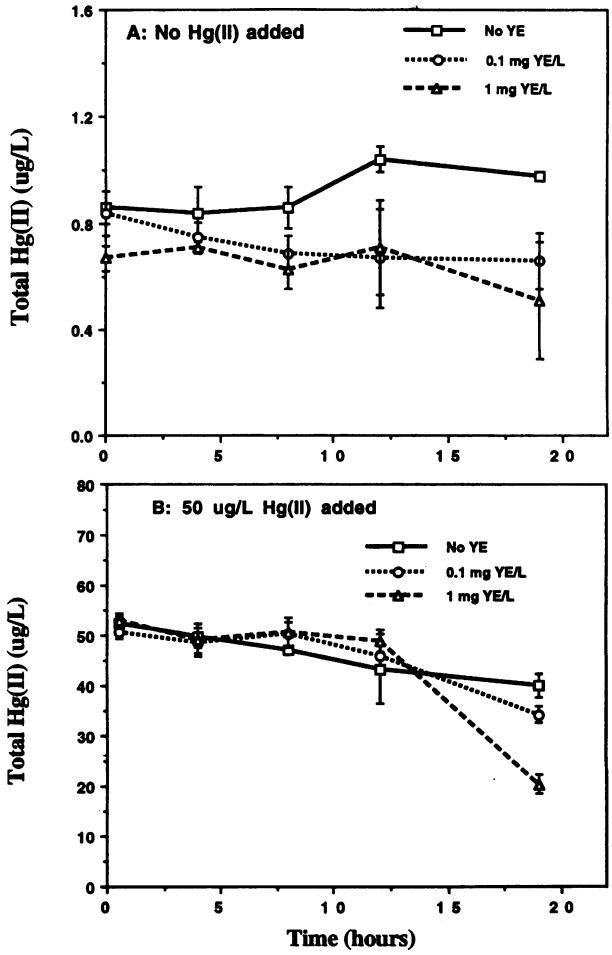

FIG. 3. Effect of nutrients and Hg(II) on mercury loss from laboratory incubations. (A) Incubations with no addition of Hg(II); (B) incubations with the addition of 50 μg of Hg(II) liter^{-1}. The concentration of mercury in incubations to which Hg(II) was not added was the amount present in RL water at the time of sampling (Hg_t = 1.12 ± 0.01 μg liter^{-1}).

ity) had no significant effect on this loss (P = 0.219; Fig. 3B). However, about 30% of the mercury was lost at the end of the experiment in incubations supplemented with the lowest YE concentrations (0 and 0.1 mg liter^{-1}), whereas 70% was lost in the incubation with the highest YE concentration (1 mg liter^{-1}). No change in Hg(II) concentrations was detected in control autoclaved samples after 36 h of incubation.

Heterotrophic activity was directly related to the concentration of YE in incubations that were not supplemented with Hg(II) ($P < 0.01$) (Fig. 4A). The addition of 50 μg of Hg(II) liter^{-1} almost totally inhibited [^{14}C]leucine incorporation, probably due to toxicity, regardless of YE concentration (Fig. 2B). However, in all incubations activity recovered after a lag period of 8 to 12 h, and once recovered, its magnitude was directly related to nutrient concentration ($P < 0.05$).

DISCUSSION

An improved mRNA extraction method (22) was applied to measure *merA* transcripts in environmental samples from a mercury-contaminated pond and to study the influence of the inducer, mercury, and nutrient concentrations on *merA* gene expression in laboratory incubations containing indigenous microbial communities. In vitro (36) and in vivo (11, 43) experiments have shown that the *mer* operon is an inducible system responding to subtle increases in Hg(II) concentrations. Ralston and O'Halloran (36) showed that an increase from 3.6 to 32 nM Hg(II) resulted in a ninefold increase in *mer* gene transcription. In our study, *merA* gene transcripts were detected in environmental samples containing 0.018 to 1.66 μg of Hg_t liter^{-1} (equivalent to 0.09 to 8.3 nM mercury; Table 1), suggesting that induction was possible in the natural water samples studied. Indeed, a previous study used *mer-lux* gene fusions to demonstrate that there were inducing concentrations of mercury in RL (43).

Both field samples and bioassay results indicate that *merA* transcript levels are more strongly influenced by availability of growth substrates than by mercury concentration. Although *mer* transcription is positively related to the concentration of Hg(II) in vitro (36) and in vivo (11, 22, 43), no relationship between *merA* mRNA levels and Hg_t and Hg_{dis} concentrations was observed in our study. On the other hand, a clear positive relationship was observed between transcript levels and metabolic activities (Table 1). For example, outflow samples exhibited the lowest Hg_{dis} concentration but the highest *merA*

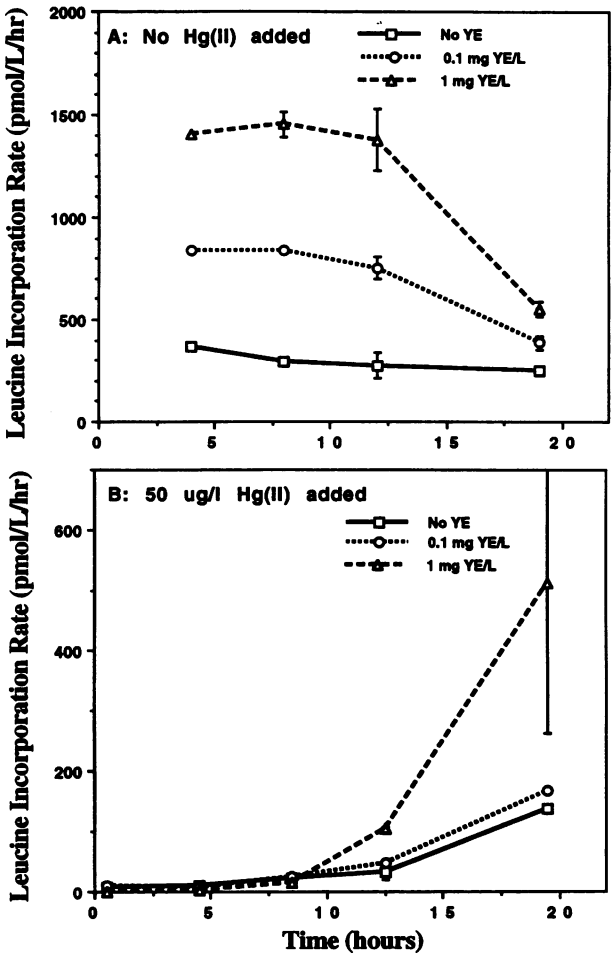

FIG. 4. Effect of nutrients and Hg(II) on [^{14}C]leucine incorporation rate in laboratory incubations. (A) Incubations with no addition of Hg(II); (B) incubations with the addition of 50 μg of Hg(II) liter^{-1}.

transcript and heterotrophic activity levels (Table 1). After the initial incubation period in the laboratory, *merA* transcript levels were directly related to the YE concentrations and to the [^{14}C]leucine incorporation rate (see time zero values in Fig. 2 and Fig. 4A). The response of *merA* transcription to the addition of 50 μg of Hg(II) liter^{-1} was a combination of both toxicity and induction. Heterotrophic activity and *merA* expression were totally inhibited 30 min after the addition of Hg(II) (Fig. 4B). Mercuric chloride at 1 μg liter^{-1} was previously reported to inhibit thymidine incorporation and glutamate incorporation and respiration by aquatic microbial communities (26). However, time and extent of recovery in Hg(II)-amended incubations were directly related to the concentration of the YE amendment (Fig. 2B and 4B). Similarly, in incubations to which Hg(II) was not added, but which contained Hg_t at an inducing concentration, *merA* transcript accumulation and [^{14}C]leucine incorporation rate were positively related to YE amendment (Fig. 2A and 4A). Thus, in the presence of inducing concentrations of mercury, the availability of growth and energy sources seems to determine the level of *mer* transcription. This is not surprising because macromolecule synthesis is highly sensitive to availability of growth substrates (24). Our results suggest that in the contaminated RL the availability of growth and energy sources, rather than the concentration of the inducer of the *mer* operon, mercury, was the limiting factor for *mer* transcription. Comparisons of results of studies that employed pure cultures (11, 17) or purified *mer* transcription components (36) and our findings (this study and reference 22) are therefore impossible because the former were performed with unlimiting supplies of growth substrates or cofactors.

Surprisingly, *merA* transcripts were also detected in the CR control sample, although the level of Hg_t (0.018 ± 0.001 μg liter^{-1} or 0.090 ± 0.005 nM) was at a range reported to be too low to induce *mer* expression (11, 36, 43). The detection of *merA* transcripts in the CR microbial community may have several explanations. Nonspecific hybridization between our *merA* probe and other structurally similar genes (e.g., those encoding lipoamide dehydrogenase and glutathione reductase [16]), although theoretically possible, was unlikely. Hybridization between *merA* of Tn*501* and the glutathione reductase gene (*gor*) of *Pseudomonas aeruginosa* was demonstrated by Perry et al. (32), using a 57-bp oligonucleotide encompassing the most conserved region of these genes, those of the active sites of the two enzymes. However, except for these highly homologous regions, the nucleotide sequences of the reductases are not conserved enough to allow nonspecific reactions with our 1.1-kb RNA probe under the stringency conditions used. The homology value between pair sequences of the DNA template of our probe and the *gor* genes of *Escherichia coli* and *P. aeruginosa* and the *lpd* gene of *Pseudomonas fluorescens*, obtained by sequence alignment using the Clustal program (21) and visualization by MASE software (14), never exceeded 58.5%. For cross-hybridization to occur, a melting temperature of no greater than 37°C (calculated for RNA-RNA hybrid formation) should have been used (12°C lower than the melting temperature used in our study). Furthermore, RNA isolated from controls consisting of uninduced *E. coli* JM109 carrying the *mer* operon from pDU1358 and *P. aeruginosa* PAO25 carrying Tn*501* did not hybridize with the *merA* probe.

Micromolar concentrations of heavy metals such as Cd(II), Zn(II), Ag(I), Au(I), and Au(III) were shown to induce *mer* transcription (10, 36). We did not monitor the concentrations of these metal ions in the CR sample, and in the absence of these data one cannot rule out the possibility of induction by these alternative inducers. Similarly, the possibility of constitutive *mer* expression needs to be tested. Isolates from the CR sample could also possess *mer* operons inducible by lower concentrations of Hg(II) than those reported to induce the operons of Tn*21* and Tn*501*.

It has been proposed that by using mRNA as the hybridization target to monitor expression of specific genes, actual activities of microbial communities could be evaluated (12, 34). The results reported here clearly indicate that the presence of mRNA transcripts may not mean that the reactions which are mediated by the corresponding polypeptides occur in the environment. The production of *merA* transcripts in the absence of Hg(II) volatilization was noted with field samples and laboratory incubations. No potential for biological Hg(II) volatilization was detected in the canal and CR samples, although *merA* transcripts were detected in these samples (Table 1). These results suggest that the presence of *merA* transcripts in an environmental sample does not necessarily indicate biological Hg(II) volatilization. It is possible that other factors essential for the activity of the mercuric reductase were not available. Bogdanova et al. (7) reported the isolation of strains with intact reductases that failed to reduce Hg(II) due to the absence of transport functions. In addition, several researchers have shown that the operon is induced by lower Hg(II) concentrations than those recognized as a substrate

by the reductase. Induction of *mer* occurs at the nanomolar Hg(II) concentrations (11, 43), whereas the K_m of the mercuric reductase is in the 8 to 14 μM Hg(II) range, depending on the bacterial origin of the enzyme (8, 15, 38). Thus, it is possible that within a certain range of Hg(II) concentrations induction may occur, yet Hg(II) reduction cannot be detected. This could be the case in the canal and CR samples and with the RL outlet water that was employed in laboratory incubations. Although outlet samples showed a potential for $^{203}Hg(II)$ reduction immediately after sampling (Table 1), it is likely that by the time the incubations were initiated (approximately 24 h after sampling), the concentration of bioavailable mercury was significantly reduced. We observed a rapid loss in Hg_{dis} (42) and in $SnCl_2$ reducible mercury [assumed to correspond to Hg(II) (40)] in RL water during storage.

Our study demonstrated in situ *merA* gene expression by indigenous bacterial communities and the influence of environmental factors acting either directly [Hg(II) availability] or indirectly (level of nutrients) on *mer* transcription. However, whether or not changes in *merA* transcript levels were determined by an increase in the number of Hg(II)-resistant cells (27), an increase in gene copies per cell (31), or an increase of *merA* transcription (17), or all of these together, is not revealed by our results. These would require the measurement of *mer* expression on a per-cell basis in situ and could be achieved by the combination of highly specific RNA probes with microscopic observations (20).

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