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Phenotypic and Genotypic Adaptation of Aerobic Heterotrophic Sediment Bacterial Communities to Mercury Stress

TAMAR BARKAY† AND BETTY H. OLSON*

Environmental Analysis, Program in Social Ecology, University of California, Irvine, California 92717

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The effects of mercury contamination of lake sediments on the phenotypic and genotypic mercury resistance of the indigenous heterotrophic aerobic bacterial communities were investigated. Strong positive correlations between mercury sediment concentration and the frequency of the gene coding for mercury volatilization (*mer*) ($r = 0.96$) or the phenotypic mercury resistance ($r = 0.86$) of the studied communities suggested that the inheritance via selection or genetic exchange of the *mer* gene had promoted bacterial adaptation to mercury. Failure to detect the *mer* gene in one mercury-contaminated sediment where phenotypic expression was low suggested that other mechanisms of resistance may partially determine the presence of mercury-resistant organisms in mercury-contaminated sediment or that the mercury in this particular sediment was very chemically limited in its availability to the microorganisms.

Natural bacterial communities are very important in the functioning of the major elemental cycles. Because environmental pollution poses a risk to essential microbial functions, the adaptive response of natural bacterial communities to pollutants has been extensively studied. Microbially mediated carbon (5) and nitrogen (16) cycles as well as biotransformation of pollutants such as *p*-nitrophenol and methyl parathion (17) in affected natural samples has been used to describe adaptation. This phenomenon has also been assessed by estimating how community structure indices vary with the degree of pollution stress such as that from acid mine wastes on the communities of a freshwater lake (19) or cadmium on soil bacterial communities (2). The demonstration of adaptive traits in organisms isolated from impacted environments is most commonly used by microbial ecologists to study adaptation. If such adaptive traits are more abundant in organisms originating in polluted environments than in those from unpolluted ones, adaptation is suggested (8, 11, 18).

All of these approaches describe adaptation in phenotypic terms, i.e., they are based on the activities and functions of the communities or isolated strains. A more basic understanding of adaptation could be achieved if the molecular mechanisms which promote the inheritance of new phenotypes in natural bacterial communities were followed. Mechanisms such as gene transfer, legitimate and illegitimate recombination, transposition, and DNA rearrangements may promote adaptation in the natural environment (14). For example, the observation that many of the genes which code for degradation or detoxification of pollutants are carried on plasmids has led to the hypothesis that adaptation is assisted by plasmid spread in natural communities. Consequently, cryptic plasmids and R factors were found in natural bacteria isolated from polluted and unpolluted environments, and their molecular mass and distribution suggested a role in adaptation (4). These studies have provided circumstantial evidence that molecular mechanisms can and do play a role in the adaptation of natural bacterial communities to pollution stress. The work reported here further supports this premise by demonstrating an increase in the frequency of specific mercury resistance genes (*mer*) in bacteria isolated from mercury-contaminated sediments.

Onondaga Lake is a small natural lake which received mercury-laden wastes between 1946 and 1970. Wastes were discharged from a nearby chemical industry at a rate of 48.4 kg of Hg per day into Ninemile Creek and East Flume Mercury Source Investigation, Onondaga Lake Study Project 11060 FAY 4/71) (Fig. 1). Four sediment samples were collected from Onondaga Lake on 6 March 1984, and one sample was obtained from White Lake on 27 April 1984. During March sampling, the overlaying ice layer was broken with a spade to allow access to the sediment. The sediment surface was aseptically scraped (top 2 cm) and scooped into sterile glass jars. Samples were immediately placed on ice and processed on the same day upon arrival in the laboratory. Sediments were mixed with CO_2-free distilled water (obtained by bubbling N_2 for 30 min) to yield 1:1 (wt/vol) slurries, and pH readings were taken with a model 401 Ionalyzer pH meter (Orion Research, Inc., Cambridge, Mass.). Weighed sediment samples were placed in quartz boats and dried overnight at 110°C. The dry samples were weighed (dry weight) and combusted under a pure-oxygen atmosphere at 870°C for 5 min. The resulting ash was weighed (ash weight), and the weight loss during combustion was considered as the organic matter fraction of the sample. Samples for mercury analysis were stored at −20°C and analyzed by furnace combustion and plasma emission spectrometry (6) during the first week after sampling.

Sediment samples agitated on a rotary shaker (150 rpm, 10 min) were serially diluted in an 0.85% NaCl solution, and 0.1-ml aliquots were spread on plate count agar (Difco Laboratories, Detroit, Mich.) for total aerobic heterotrophic bacterial counts. Enumeration of mercury-resistant bacteria was performed with medium amended with 50 μg of Hg per ml (as $HgCl_2$). Filter-sterilized mercury stock solution (10 mg of $HgCl_2$ per ml) was added to autoclaved plate count agar prior to dispensing. Inoculated plates were incubated for 4 days at room temperature before enumeration. The percent mercury resistance was calculated as the percent resistant bacteria of total aerobic heterotrophs. For each sediment sample, 100 colonies were isolated randomly from

* Corresponding author.
† Present address: Biotechnology and Microbial Ecology Branch, U.S. Environmental Protection Agency, Sabine Island, Gulf Breeze, FL 32561.

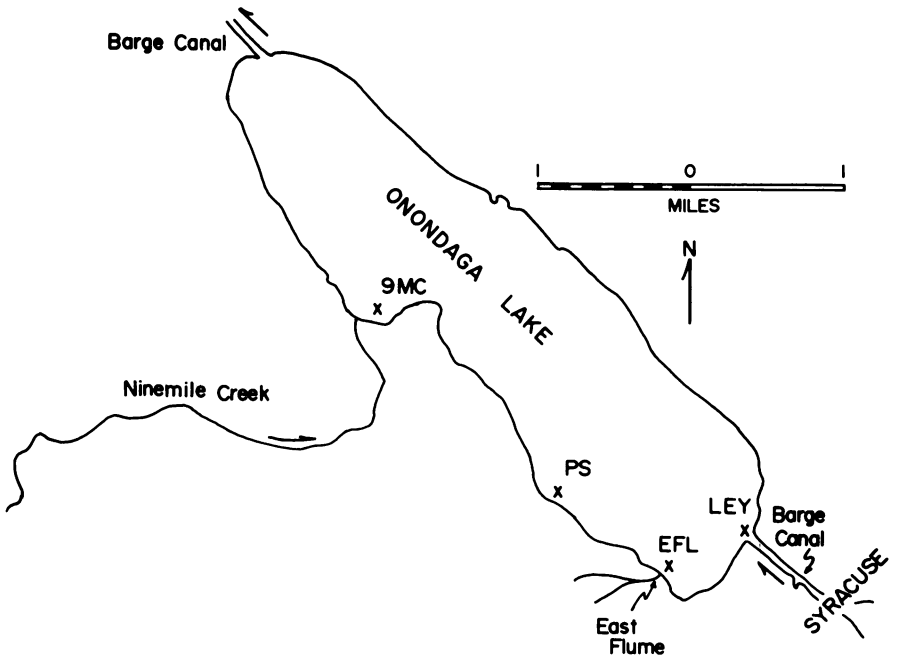

FIG. 1. Geographical map of Onondaga Lake and sampling locations.

plate count agar plates. These strains were Gram stained, and gram-negative organisms were maintained in the laboratory on plate count agar slants covered with sterile mineral oil for further study. These strains were identified to the genus level by the method of Ward et al. (18a). DNA-DNA hybridization with the *mer* probe (1) was carried out as previously described (1). Further work was not carried out with gram-positive bacteria because the *mer* probe was found to be nonspecific for the mercury resistance systems of these organisms (1).

Two samples, 9MC and EFL, were obtained from Ninemile Creek and East Flume, respectively (Fig. 1). Both sites had very similar physicochemical parameters (Table 1). The EFL sediment sample contained 5.45 μg of Hg per g, and the 9MC sediment sample contained 0.88 μg of Hg per g. These values represent a decrease in mercury concentration since 1971, when surface sediment samples obtained from East Flume and Ninemile Creek contained 40 and 5 μg of Hg per g of sediment, respectively. The more highly polluted sample, EFL, had the highest number of mercury-resistant aerobic heterotrophs, representing 0.55% of the total aerobic heterotrophic community. Of 31 strains tested, 7 had DNA sequences homologous to the *mer* probe. Of the seven mer^+ strains (i.e., strains which hybridized with the *mer* probe), six were identified as *Pseudomonas* spp. and one was identified as *Alcaligenes* sp. 9MC sediment contained 2.23×10^6 CFU total aerobic heterotrophs and 2.00×10^2 CFU (0.009%) mercury-resistant organisms per g. Of the 100 isolates, 26 were tested for the presence of the *mer* gene. Three isolates (11.5%) were mer^+, and they were identified as *Pseudomonas* sp., *Acinetobacter* sp., and *Moraxella* sp.

One sample (LEY) was collected at Ley Creek, a creek at the southern end of Onondaga Lake (Fig. 1) where sediment mercury concentration was 5 μg/g in 1971. When sampled in March 1984, this sediment had a mercury content of 4.25 μg/g (Table 1). Thus, the mercury concentration in the sediment surface in Ley Creek appeared to have remained stable during the past 13 years. Only 0.024% of the aerobic heterotrophic community of the LEY sample was phenotypically resistant to mercury. Thus, although the mercury level in this sample was at the same magnitude as that in the EFL sample (5.45 μg/g), its mercury resistance level was more than 20 times lower (0.55% for EFL). Of 64 isolates tested, none hybridized with the *mer* probe. Thus, although the bacterial community of Ley Creek had developed in mercury-contaminated sediment and contained mercury-resistant organisms, albeit at a very low frequency, the *mer* gene could not be detected in the genomes of representative heterotrophs.

The fourth sample (PS) was obtained at a pumping station in a location between East Flume and Ninemile Creek (Fig. 1). Low mercury levels (1 μg/g of sediment) were recorded there in the past, and it was therefore selected as a relatively unimpacted area. Indeed, only 0.086 μg of Hg per g of sediment was found in the sample obtained in March 1984, a concentration similar to that of the control sample (WTL) and below the abundance of this element in the earth's crust, 0.5 μg/g (15). PS sediment microflora demonstrated low phenotypic resistance to mercury, with 6.00×10^1 CFU/g of sediment representing 0.073% of the total aerobic heterotrophs in this sediment. Of the 47 gram-negative strains isolated from PS sediment and hybridized with the *mer* probe, only 2 strains, a *Pseudomonas* sp. and a *Flavobacterium* sp., had DNA sequences homologous to the *mer* probe.

An additional sediment sample (WTL) was collected in April 1984 from White Lake, which neighbors Onondaga Lake. White Lake has never been exposed to mercury pollution, and its sediment contained a background mercury concentration, 0.11 μg/g. The total aerobic heterotrophic counts in WTL sediment were 5.9×10^4 CFU/g, the mercury-resistant aerobic heterotrophs were below the level of detection, $<10^1$ CFU/g (<0.017%), and only 2 strains (both identified as *Pseudomonas* sp.) of 43 tested (4.6%) hybridized with the *mer* probe.

The phenotypic and genotypic resistance to mercury of

TABLE 1. Physicochemical and biological characteristics of the studied sediment samples

Sample[a]	Sampling date	Hg concn (μg/g [dry wt]) in:		Temp (°C)	pH	% Organic matter[b]	Total bacterial counts (CFU/g [wet wt])	Phenotypic resistance (CFU of Hg-resistant strains/g)	Genotypic resistance (% of strains hybridizing with the *mer* probe)	No. of strains tested[c] for *mer*
		1972[d]	1984							
EFL	6 March 1984	40	5.45 ± 0.25	2	7.4	17.2	1.86×10^6	1.03×10^4	22.6	31
9MC	7 March 1984	5	0.88 ± 0.07	2	7.2	19.6	2.23×10^6	2.00×10^2	11.5	26
LEY	6 March 1984	5	4.25 ± 0.05	1	7.2	9.5	2.97×10^5	7.00×10^1	<1.6	64
PS	6 March 1984	1	0.086 ± 0.005	3.5	7.7	4.9	8.18×10^5	6.00×10^1	4.2	47
WTL	27 April 1984	ND[e]	0.11 ± 0.01	11	6.4	ND	5.24×10^4	$<1 \times 10^1$	<4.6	43

[a] Sample designations are as described in the text.
[b] Percent organic matter = [(dry weight − ash weight)/dry weight] × 100.
[c] All the viable gram-negative strains from each sediment sample.
[d] Source: U.S. Environmental Protection Agency Mercury Source Investigation, Onondaga Lake Study Project 11060 FAY 4/71.
[e] ND, Not determined.

the studied heterotrophic bacterial communities was correlated with the mercury concentration of the sediment samples by Pearson's standard correlation coefficient. The percent hybridization and the log percent bacterial mercury resistance were used for these analyses. The LEY sample *mer* frequency and the WTL sample percent mercury resistance and *mer* frequency were below the level of detection; therefore, these sites were not included in the analyses. Positive correlations were obtained for *mer* frequency ($r = 0.96$, $n = 43$) and for bacterial resistance ($r = 0.87$, $n = 54$). These results suggest that mercury resistance is common in the indigenous bacterial communities of contaminated sediments and that it is mediated by mercury resistance genes which code for the reduction of mercury. However, the *mer* gene could not be detected in the genomes of aerobic heterotrophs isolated from one sediment sample where mercury concentrations remained stable since 1971 (LEY). This observation could be explained by (i) the small numbers of organisms examined or (ii) the presence of a different chemical species of mercury in LEY sediment which may have induced resistance by an alternative mechanism at a very low frequency or which may have been unavailable to microorganisms. Protection from mercury through another segment of the microbial community inactivating the compound by microbially produced H_2S (12) and by a cell envelope barrier (13) has been described. A change in the chemical form of the mercury in the LEY sediment sample cannot be explained by the measured physicochemical characteristics (Table 1), but certainly the percent organic matter was substantially lower than in the EFL and 9MC sediment samples. A similar lack of phenotypic bacterial resistance to Cd, Pb, and Zn from soils contaminated with these metals by historic smelting has been reported (10). This study found that extractable levels of these metals were statistically signficantly lower than for matched sites at which bacterial resistance was noted. Data of this nature demonstrates the importance of understanding the role that physicochemical factors play in microbial adaptation at the molecular level. Although the stable level of mercury in LEY sediment may have resulted from the absence of microbially mediated mercury reduction (as indicated by DNA-DNA hybridization with the *mer* probe), such an explanation is premature in light of several other possibilities, such as mechanical sediment perturbations and settling of new layers of sediment.

The positive correlations of *mer* frequency with sediment mercury concentrations provide a possible explanation at the molecular level for the commonly observed increase in mercury and other metal resistance in bacterial communities which develop under metal stress in aquatic sediments (8, 18, 19) and soils (3, 11). Although all of these studies hypothesized that resistance genes promoted these phenomena, the data presented here are the first direct evidence that a specific gene system is present in stressed natural bacterial communities. A similar observation was recently reported by Nevo et al. (9), who demonstrated that marine shrimp and gastropods isolated from mercury-contaminated sediments had higher frequencies of mercury-tolerant allozymes than did organisms isolated from unpolluted sediments. The known molecular details of the mercury reduction (i.e., resistance) system which have been revealed in laboratory studies (7) could be used for further analysis of the molecular events involved in the adaptation of natural bacterial communities to mercury stress.

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