APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1979, p. 478-485
0099-2240/79/09-0478/08$02.00/0

Role of Plasmids in Mercury Transformation by Bacteria Isolated from the Aquatic Environment

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Received for publication 14 June 1979

Eight mercury-resistant bacterial strains isolated from the Chesapeake Bay and one strain isolated from the Cayman Trench were examined for ability to volatilize mercury. Mercury volatilization was found to be variable in the strains tested. In addition, plasmids were detected in all strains. After curing, two of the bacterial strains lost mercury resistance, indicating that volatilization is plasmid mediated in these strains. Only two cultures demonstrated ability to methylate mercuric chloride under either aerobic or anaerobic conditions. Methylation of mercury, compared with volatilization, appears to be mediated by a separate genetic system in these bacteria. It is concluded that mercury volatilization in the estuarine environment can be mediated by genes carried on plasmids.

Elevated concentrations of mercury have been detected in the sediment of several estuaries (7, 18). Mercury-resistant bacteria isolated from these environments have been found to transform organic and inorganic mercurial compounds to Hg^0 and to methylate inorganic mercury.

The most completely documented pathway of detoxification of mercury is reduction from the inorganic (Hg^{2+}), or organomercurial, form to metallic mercury (Hg^0) (12, 19, 29). Mercury volatilization in bacteria has been shown to be plasmid mediated (37). Mercuric reductase, an enzyme involved in the conversion of Hg^{2+} to Hg^0, is coded by the *mer* gene carried on plasmids in *Escherichia coli* (11, 22, 29, 31), *Pseudomonas* spp. (4, 5), and *Staphylococcus aureus* (13, 21, 36). The significance of plasmids in resistance of bacterial populations to or metabolism of mercury or both can be demonstrated by the fact that the *mer* gene has been shown to be located on transposons (27) and associated with sex factors (4, 14, 29), thus enhancing the potential for occurrence in the environment. The amount of information available concerning the reduction of mercurials is much greater than that for the biochemical and genetic mechanisms involved in microbial methylation of mercury. The microbial processes of methylation remain to be elucidated.

In the Chesapeake Bay, sediment has been shown to contain between 0.015 and 0.860 mg of mercury per kg (18). Mercury-resistant bacteria have been isolated from Chesapeake Bay water and sediment (2, 6, 18), with *Pseudomonas* spp. comprising a significant proportion of the isolated heterotrophic, aerobic bacterial populations resistant to mercury (2, 18). Several of the mercury-resistant bacterial strains isolated from the Chesapeake Bay have been shown to volatilize mercury from phenylmercuric acetate or mercuric chloride (17, 18).

Strains of bacteria isolated from the clinical environment have been shown to possess plasmids associated with volatilization of mercury, but little information is available concerning the function of such plasmids in mercury-resistant bacterial populations of the natural environment. The present investigation was undertaken to determine if volatilization of mercury in estuarine bacterial populations was plasmid mediated and whether these plamids were also associated with the methylation of mercury.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains used in this study are listed in Table 1. Details of the method employed to isolate, classify, and identify *Bacillus* sp. F96, *P. fluorescens* B69, *P. fluorescens* B1, and *P. fluorescens* F63 have been reported elsewhere (1, 2). *Pseudomonas* sp. S58 and *Acinetobacter* sp. W45 were isolated on estuarine salts agar medium, composed of NaCl, 10 g; $MgSO_4 \cdot 7H_2O$, 4.75 g; KCl, 0.18 g; proteose peptone, 10 g; yeast extract, 1 g; distilled water, 1 liter (pH 7.0) and amended with 5 μg of Hg as $HgCl_2$ per ml, and identified and classified as described elsewhere (1, 2). Strain CC2B was isolated by using the medium described by Yamada and Tonomura (39), modified by addition of 0.18 g of KCl, 4.75 g of $MgSO_4 \cdot 7H_2O$ per liter, and 15 μg of Hg as $HgCl_2$ per ml. The inoculated medium was incubated under anaerobic conditions. Unidentified gram-negative rod 769 was isolated from the Cayman Trench by K. Ohwada et al. (manuscript in preparation). Throughout the study all

TABLE 1. *Bacterial strains used in this study*

Identification and strain no.	Source[a]
Bacillus sp. F96	Sediment
Pseudomonas fluorescens F63	Water
Pseudomonas sp. S58	Sediment
Unidentified gram negative rod 769	Cayman Trench sediment
Pseudomonas sp. 244	Sediment
Enteric bacterium CC2B	Sediment
Pseudomonas fluorescens B69	Sediment
Acinetobacter sp. W45	Water
Pseudomonas fluorescens B1	Water

[a] Except where indicated, the strains listed were isolated from samples collected in the Chesapeake Bay.

cultures were maintained on modified Yamada medium (39) to which 10 μg of Hg as $HgCl_2$ per ml was added.

Bacterial volatilization and methylation of mercury. Bacterial cultures examined for volatilization and methylation of mercury were inoculated into the modified medium of Yamada and Tonomura (39), amended with 10 μg of Hg as $HgCl_2$ per ml, and incubated at 21°C. Cultures in the exponential phase of growth were diluted 1:100 in 150 ml of fresh medium containing 10 μg of Hg as $HgCl_2$ per ml. Samples were withdrawn after 0, 24, and 48 h for examination of total mercury concentration and total viable counts, i.e., colony-forming units. Samples for total mercury analysis were stored at −70°C after collection and before analysis. Bacterial cultures examined for methylation of mercury were centrifuged at 27,138 × *g* in a Sorvall RC2-B centrifuge for 20 min. The supernatant and pellet fractions were separated and frozen at −70°C before analysis for methylmercury.

Modified Yamada agar, prepared with 2% agar (Difco Laboratories, Detroit, Mich.), was used for the total viable counts. Determination of anaerobic volatilization or methylation or both was accomplished as described above, except that all flasks were continuously purged with nitrogen.

Mercury analysis. Total mercury was measured with an atomic absorption spectrophotometer (Perkin-Elmer, model 460, Norwalk, Conn.) equipped with a graphite furnace.

Methylmercury was extracted from supernatant and bacterial pellets (wet weight), using a modification of the Westöö method (20), and analyzed on the Zeeman Isotope Shift atomic absorption spectrophotometer (9).

Screening for plasmids. The agarose gel technique described by Meyers et al. (15) was used for detection of bacterial plasmids.

Preparation of DNA. Thirty-milliliter culture samples were grown for 24 h in modified Yamada broth. Cleared lysates, prepared by the method described by Guerry et al. (8), were incubated at 37°C with ribonuclease (Calbiochem, La Jolla, Calif.) at a final concentration of 10 μg/ml for 1 h. After ribonuclease treatment, the lysates were extracted twice with tris(hydroxymethyl)aminomethane (Tris; 50 mM)-saturated phenol and three times with chloroform–isoamyl-alcohol (50:1). Extracts, purified to yield a 260/280 optical density ratio of >2.0, were added to sodium acetate solution (final concentration, 0.3 M sodium acetate). Two volumes of 95% ethanol were added to the samples, after which they were stored overnight at −20°C. The deoxyribonucleic acid (DNA) preparation was centrifuged (48,000 × *g*) for 30 min at −10°C, and the supernatant was removed. The pellet was dried under nitrogen, redissolved in 0.2 ml of TES buffer (0.05 M NaCl, 0.005 M ethylenediaminetetraacetic acid, 0.03 M Tris, pH 8.0), and analyzed by gel electrophoresis.

Agarose gel electrophoresis. Agarose gel electrophoresis was performed by using a vertical slab gel apparatus (Aquebogue Machine and Repair Shop, Aquebogue, N.Y.) with slab dimensions of 15.9 by 19.0 by 0.13 cm. Four milliliters of 7% acrylamide gel, which provided a supporting medium, was poured into the slab before addition of agarose. Acrylamide gel was prepared as follows. Acrylamide solution, 6.9 ml (acrylamide, 15 g; bisacrylamide, 0.75 g; 100 ml of distilled water), was mixed with 1.5 ml of sodium acetate electrophoresis buffer [0.04 M tris(hydroxymethyl)-aminomethane, 0.002 M ethylenediaminetetraacetic acid, and 0.02 M sodium acetate, adjusted to pH 8.0 with acetic acid], 7.95 ml of distilled water, 150 μl of 10% ammonium persulfate, and 10 μl of *N,N,N',N'*-tetramethylethylenediamine. The acrylamide gel was allowed to solidify for approximately 20 min, after which 0.7% agarose (wt/vol), dissolved in sodium acetate buffer at 65°C, was poured into the slab and a 12-tooth "comb" was inserted into the gel. After 30 min, the comb was removed and the slab was connected to the gel apparatus. Before loading the DNA, the gel was equilibrated for 30 min with the apparatus operating at 50 V. Samples of DNA were mixed with 10% (vol/vol) bromophenol blue dye solution (62% glycerol, 2.5% sodium dodecyl sulfate, and 1% bromophenol blue) and loaded onto the gel. Electrophoresis was run, in a sodium acetate buffer, at room temperature with the apparatus set at 100 V until the dye reached the interface between the acrylamide and agarose layers, ca. 3 to 4 h.

Detection of plasmids. After electrophoresis, the gels were submerged in ethidium bromide solution (Sigma Chemical Co., St. Louis, Mo.), prepared by adding ethidium bromide, 0.4 μg/ml, to distilled water. The gels were allowed to stand in the ethidium bromide solution for 30 min at 4°C, after which the gel was removed and placed on a long-wave ultraviolet (UV) light table (Ultra Violet Products C-62, San Gabriel, Calif.) and a photographic record was prepared, using a Polaroid model MP4 camera.

Molecular weight determination. Plasmids of known molecular weight were obtained from E. Lederberg (Plasmid Reference Center, Stanford, Calif.). The molecular weights for reference plasmids Sa, RP4, and R1 were 23×10^6, 34×10^6, and 62×10^6, respectively (15). The log of relative mobility (R_f), i.e., the ratio of plasmid to dye mobility in centimeters, and the log of molecular weight (MW) yielded a linear relationship: $\log MW = \log R_f \times (-3.02) + 5.55$. The

molecular weights of the plasmids found in the strains freshly isolated from the environment were thus calculated. At least one reference plasmid was always included in each molecular weight analysis.

Antibiotic and metal resistance. Antibiotic resistance tests were carried out following methods described by Allen et al. (1). Heavy metal resistance patterns were determined using a modification of the procedure described by Austin et al. (2). Modified Yamada medium was substituted for the test medium employed by Austin et al. (2).

Curing experiments. Curing experiments were carried out using the following agents: acridine orange, mitomycin C, and UV light. Curing by acridine orange was accomplished by following the method described by Willetts (38). Mitomycin C-treated cells were grown overnight in modified Yamada broth containing 2 μg of mitomycin C (Calbiochem, La Jolla, Calif.) per ml. To cure plasmids by UV light, cells were harvested when the cultures reached stationary phase of growth, centrifuged at 9,750 × *g* for 15 min, washed in a salts solution (NaCl, 10 g; $MgSO_4 \cdot 7H_2O$, 4.7 g; and KCl, 0.18 g distilled water, 1 liter), again centrifuged and suspended in the salts solution, final concentration of 10^8 cells per ml, and placed in quartz tubes. The cell suspension in the quartz tubes was exposed to 460 mW of UV light (Ultra Violet Products UVS-54) per cm. The quartz tubes were set at a distance of 15.0 cm from the UV source and were exposed to the UV light for 45 min. Controls consisted of cells treated, as described above, without exposure to the curing agents. After curing, cells were plated on solid modified Yamada medium without mercury. After growth was observed, the plates were replicated, in duplicate, onto fresh Yamada media, i.e., with Hg (10 μg/ml as $HgCl_2$) added and without Hg. Cultures which failed to grow on medium prepared with mercury were considered to be cured only after no growth was demonstrated in modified Yamada broth to which mercury had been added.

RESULTS AND DISCUSSION

Plasmid detection and curing. The molecular weights of plasmids in the mercury-resistant bacteria are given in Table 2. Plasmids were detected in all of the bacterial strains examined. The molecular weights of the plasmids varied from 1.6 × 10^6 for *P. fluorescens* F63 to 109.2 × 10^6 in the case of the unidentified gram-negative rod 769. Only three of the nine bacterial strains examined demonstrated a single plasmid.

Two of the bacterial strains, *P. fluorescens* B69 and *Acinetobacter* sp. W45, were cured using mitomycin C (Fig. 1). *P. fluorescens* B69A lost the 34.3-Mdalton plasmid, retaining two plasmids, whereas B69F was cured of all three plasmids. *Acinetobacter* sp. W45 could be cured of only the 4.7-Mdalton plasmid. Attempts made to cure the other bacterial strains were not successful.

Volatilization of mercury. The total volatilization of mercury under aerobic conditions

TABLE 2. *Molecular weights of plasmids in the mercury-resistant bacterial strains*

Bacterial strain	Mol wt (× 10^6)		
	Plasmid	Plasmid	Plasmid
Bacillus sp. F96	89.9		
P. fluorescens F63	1.6	4.5	
Pseudomonas sp. S58	53.5		
Unidentified gram-negative rod 769	109.2		
Pseudomonas sp. 244	3.2	6.6	
Enteric bacterium CC2B	2.3	3.3	6.6
Acinetobacter sp. W45	2.2	4.7	81.9
P. fluorescens B69	18.5	34.3	39.5
P. fluorescens B1	11.3	16.3	20.4

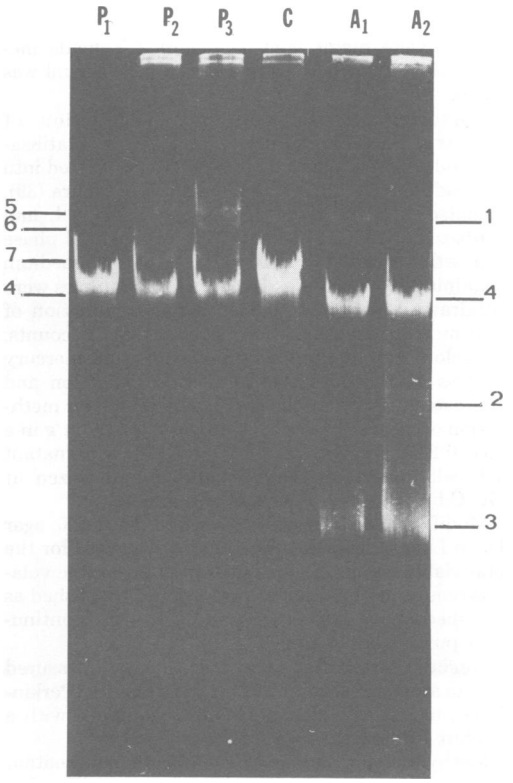

FIG. 1. *Gel patterns for plasmids of P. fluorescens B69, Acinetobacter sp. W45, and their cured derivative strains. Well designations are: (P_1) P. fluorescens B69 cured strain B69F; (P_2) P. fluorescens B69 cured strain B69A; (P_3) P. fluorescens B69; (C) E. coli W3110 chromosomal DNA; (A_1) Acinetobacter sp. W45 cured strain W45A; (A_2) Acinetobacter sp. W45. Band designations are: (1) 81.6-Mdalton plasmid DNA; (2) 4.7-Mdalton plasmid DNA; (3) 2.2-Mdalton plasmid DNA; (4) chromosomal DNA; (5) 39.5-Mdalton plasmid DNA; (6) 34.3-Mdalton plasmid DNA; (7) 18.5-Mdalton plasmid DNA.*

was observed to be equal to, or greater than, volatilization under anaerobic conditions. Variation observed in volatilization of mercury is ascribed to strain differences, viz., among the strains of *Pseudomonas*, the amount of Hg volatilized ranged from ca. 30 to 60% under aerobic conditions (Fig. 2). *P. fluorescens* B69, *Acinetobacter* sp. W45, enteric bacterium CC2B, *Pseudomonas* sp. 244, and *P. fluorescens* F63 volatilized approximately equal amounts of Hg, when incubated aerobically or anaerobically. Volatilization under anaerobic conditions, in some instances, exhibited different patterns, i.e., *P. fluorescens* B1 volatilized 40% of the mercury under aerobic conditions and less than 10% under anaerobic conditions. Thus, when volatilization of mercury under aerobic and anaerobic conditions was compared, the following conclusions could be drawn. In the case of *P. fluorescens* B1, *Pseudomonas* sp. S58, *Bacillus* sp. F96, and the unidentified bacterium 769, anaerobic volatilization of Hg was reduced, these organisms also being less efficient in volatilizing mercury under aerobic conditions, compared to the other strains examined in this study. Growth of all of the bacterial strains examined, except *Pseudomonas* sp. S58, was greatest during the first 24 h, as was the amount of mercury volatilized. The amount of Hg volatilized, on a per cell basis, was calculated by dividing net loss of mercury from the medium over a 24-h period by the total number of cells at the end of that growth period (Table 3). Mercury methylation was determined by extracting the methylmercury present in the growth media after 48 h of incubation. The only strains found to be capable of methylating Hg were *P. fluorescens* F63 (7.8×10^{-7} pg/cell) under aerobic conditions and enteric bacterium CC2B (1.7×10^{-5} pg/cell) under anaerobic conditions.

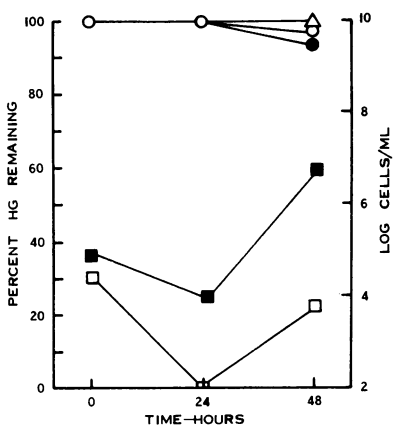

FIG. 2. *Mercury volatilization and growth of cured derivative strains B69A and B69F of P. fluorescens B69. Volatilization of mercury by cured strains B69A (○) and B69F (●). Uninoculated medium containing 10 μg of Hg per ml (△). Growth of B69A (□) and B69F (■).*

Volatilization of mercury mediated by plasmids. Loss of mercury resistance and the capability to volatilize mercury was found to be associated with loss of plasmids in *Acinetobacter* sp. W45 and *P. fluorescens* B69. Failure of the curing techniques employed in this study to cure the other bacterial strains of plasmids suggests that the plasmids associated with mercury resistance in these strains are stable.

Serological data provided by S. Silver's laboratory suggested that the loss of mercury resistance in the cured derivative strains of *P. fluorescens* B69 and *Acinetobacter* sp. W45 resulted from a loss of the mercuric reductase enzyme. Ouchterlony patterns showed precipitin reactions between crude preparations of parental strains B69 and W45 and antisera prepared against purified *E. coli* mercuric reductase. No such precipitin reactions were noted when crude extracts of the cured derivatives were tested. Thus, it is concluded that the mercuric reductases in *P. fluorescens* B69 and *Acinetobacter* sp. W45 are coded by genes located on specific plasmids carried by these strains. Plasmid mediation of reduction of mercuric compounds has

TABLE 3. *Mercury volatilization (micrograms per cell) under aerobic and anaerobic conditions*

Bacterial strain	Aerobic		Anaerobic	
	24 h	48 h	24 h	48 h
Bacillus sp. F96	1.5×10^{-9}		$>1.6 \times 10^{-4}$	
P. fluorescens F63	2.7×10^{-9}	4.8×10^{-10}	3.7×10^{-6}	4.2×10^{-8}
Pseudomonas sp. S58	1.0×10^{-8}	1.2×10^{-8}	4.0×10^{-7}	
Unidentified gram-negative rod 769	2.6×10^{-9}	5.6×10^{-10}	3.5×10^{-7}	4.2×10^{-6}
Pseudomonas sp. 244	6.6×10^{-9}	6.9×10^{-11}	2.7×10^{-6}	3.3×10^{-7}
Enteric bacterium CC2B	1.0×10^{-8}		1.5×10^{-8}	4.5×10^{-9}
Acinetobacter sp. W45	6.0×10^{-9}	4.2×10^{-11}	6.8×10^{-8}	4.1×10^{-9}
P. fluorescens B69	$>4.9 \times 10^{-7}$	1.2×10^{-9}	4.4×10^{-7}	6.4×10^{-7}
P. fluorescens B1	1.1×10^{-9}	1.6×10^{-10}	8.2×10^{-8}	1.6×10^{-8}

been documented for *E. coli* (11, 22, 31), *Pseudomonas* spp. (4, 5), and *Staphylococcus aureus* (36). Marine and estuarine bacteria possess related, if not identical, reduction mechanisms for mercury.

The cured derivatives, B69A and B69F, appeared to develop an alternative mechanism for mercury resistance. These strains demonstrated a decrease in cell number during the first 24 h of incubation, followed by a marked increase. Growth of the mercury-resistant cells occurred within 48 h after exposure of the cured strain to mercury and was not associated with volatilization of mercury (Fig. 2). The minimal inhibitory concentration of Hg demonstrated by several substrains of the 48-h culture of B69A was 4 μg of Hg per ml. The minimal inhibitory concentration for B69 and B69A was 20 and 0.1 μg of Hg per ml, respectively. Thus, it appears that the reverting B69A strains are intermediate between the resistant parental strain B69 and the sensitive cured derivative B69A with respect to resistance to mercury. The cured strain, W45A, was unable to grow in the presence of mercury under aerobic and anaerobic conditions. The cured derivatives, B69A and B69F, reported here are the first strains for which resistance to mercury is found to arise from a mechanism other than volatilization. Wang et al. (35) reported increased sensitivity to mercury for *E. coli*, a result of alteration of cell permeability to mercury compounds. It is hypothesized that the increased resistance noted for strains B69A and B69F is associated with alteration of cell membrane permeability, with the result that mercury is excluded from the cell. Such a mechanism has been described for resistance to cadmium (13). Obviously, chemical analyses of the membranes will be required to confirm this hypothesis.

Volatilization rates. Significant loss of mercury from the culture medium occurred within 24 h for all strains examined (except *Pseudomonas* sp. S58 and *Bacillis* sp. F96), with barely detectable volatilization occurring between 24 and 48 h, in general. Rapid rates of volatilization have been reported by other investigators for clinical isolates (5, 22, 29, 36). Induced strains of *E. coli* K-12 harboring plasmids have been shown to volatilize 100% of added ^{203}Hg within 90 min (22). Rapid volatilization of mercury has also been reported for *P. putida* (MER), in which 90% of ^{203}Hg is released after incubation for 10 min (5). In the case of the strains reported here, different patterns of mercury volatilization were observed. Gel precipitin reactions observed for the mercuric reductases of *P. fluorescens* B69 and *Pseudomonas* sp. 244 by S. Silver and colleagues (personal communication) lead us to conclude that differences in volatilization rates may be associated with structural and functional differences in the bacterial reductases. It is also possible that the transport systems of these strains may be sufficiently different to account for the variability observed in rate of volatilizations. Association of a transport function in the MER operon with volatilization of mercury has been suggested by Summers and Silver (30). Volatilization of mercury, at the cellular level, can be influenced by the genetic makeup of the host bacterium, where the same plasmids harbored by different cells are associated with differences in kinetics of mercury volatilization (30).

Volatilization of mercury and growth. Significant bacterial growth for all the strains occurred within 24 h, with a moderate increase in growth recorded at 48 h, except for *Pseudomonas* sp. S58 and *Acinetobacter* sp. W45. It should be noted that as mercury is volatilized, it is, therefore, lost from the medium, providing a less toxic or inhibitory environment for the bacteria. Theoretically, bacterial growth should be enhanced as mercury is released from the medium. *Acinetobacter* sp. W45 provides a good example of such a situation, since a substantial increase in cell number was recorded at 24 to 48 h after 50% of the mercury had been volatilized.

Bacillus sp. F96 exhibited a different type of volatilization, whereby rapid volatilization of mercury occurred after an initial lag in growth between 0 and 24 h. Interestingly, the number of cells did not increase after 24 h, even though volatilization of mercury showed a significant increase.

The amount of mercury volatilized, on a per cell basis, appears to be greater under anaerobic conditions (Table 3). Growth is limited under anaerobic conditions, and, in these studies, an inverse relationship was noted between total number of viable cells and amount of mercury volatilized on a per cell basis.

The concentration of mercury employed in the study reported here, 10 μg/ml, was selected because the concentration of total mercury recorded for estuarine and marine environments is reported to be in the range of <0.2 to 350 mg/kg, the concentration being greater at more polluted sites (24, 33). Bacteria can grow in the presence of, and volatilize, higher concentrations of Hg than that employed in this study (11, 32,). For example, *Pseudomonas* sp. 244 is capable of growth in a medium containing 100 μg of Hg as $HgCl_2$ per ml (3). Under aerobic conditions, it is possible for rapidly growing cultures to volatilize all of the available mercury, with the mercury itself becoming a limiting factor. Under anaero-

bic conditions, in all of the experiments reported here, the potential for volatilization was affected by physiological factors rather than the concentration of available mercury.

All of the bacterial strains examined in this study were found to be capable of volatilizing mercury, but only two of the strains were capable of methylating mercury under aerobic or anaerobic conditions. Bacterial strains methylating mercury comprise a group distinct from those reducing inorganic mercury. The majority of the bacterial strains capable of methylating mercury are reported to be members of the *Enterobacteriaceae* (10, 34).

Resistance to heavy metals mediated by plasmids. The resistance patterns for heavy metals other than mercury demonstrated by bacteria resistant to mercury are summarized in Table 4. All of the mercury-resistant strains exhibited resistance to at least one other metal. Metal resistance, in increasing frequency, ranged from Co < Cd < Zn < Ni < Cr < Pb; i.e., most of the strains were unable to grow in the presence of cobalt, whereas all were capable of growth in the presence of lead (Table 4). After curing, *P. fluorescens* B69A retained the metal resistance pattern of its parent strain, with the exception of being unable to grow in the presence of Cd^{2+} and Hg^{2+} (Table 4).

The other cured derivative, B69F, lost the three plasmids harbored by the parental strain (Table 2) and was, thereafter, unable to grow in the presence of nickel and zinc. The metal resistance patterns for the cured strains and the agarose gel profiles showing the plasmids present in the strains provide evidence that suggest that the genes involved in resistance to nickel and zinc are not located on the same plasmid as that for resistance to mercury. Nickel resistance has been shown to be plasmid mediated in other bacteria (23). Increased sensitivity to chromium was observed for the cured derivative strain, W45A, compared with its parent strain, *Acinetobacter* sp. W45. Thus, genes for both chromium and mercury resistance may be located on the 4.7-Mdalton plasmid found in the parental strain.

Linkage between genes associated with heavy metal resistance has been reported by several workers (16, 25, 28). Plasmid mediation of cadmium resistance in gram-negative bacteria has not heretofore been reported (28). Interestingly, the penicillinase plasmids of *Staphylococcus aureus* are known to carry the genes responsible for resistance to cadmium, mercury, and other heavy metals (19). Mechanisms for mercury and for cadmium resistance that are known to be mediated by plasmids are distinctly different. For example, mercury resistance occurs via volatilization (36), whereas cadmium resistance involves exclusion of the metal from the cell (13).

Plasmid mediation of drug resistance. All of the bacterial strains examined in this study were resistant to several antibiotics, except unidentified rod 769, which was resistant only to penicillin (Table 5). *Pseudomonas* sp. F63 and the enteric bacterium CC2B exhibited resistance to a large number of antibiotics. The majority of the strains examined were found to be sensitive to streptomycin, but resistant to penicillin (Table 5).

The antibiotic resistance pattern of the cured strain of *P. fluorescens* B69A was found to be exactly the same as that of its parent, whereas the cured strain B69F demonstrated a markedly different antibiotic resistant pattern from that

TABLE 4. *Heavy metal resistance patterns recorded for the bacterial strains employed in this study*[a]

Bacterial strain	Resistance patterns						
	Cd (100)[b]	Co (100)	Cr (100)	Pb (100)	Hg (10)	Zn (100)	Ni (100)
Bacillus sp. F96	S	R	R	R	R	R	S
P. fluorescens F63	R	S	R	R	R	R	R
Pseudomonas sp. S58	S	S	S	R	R	S	S
Unidentified gram-negative rod 769	S	S	S	R	R	S	S
Pseudomonas sp. 244	±	±	R	R	R	±	R
Enteric bacterium CC2B	R	R	R	R	R	R	R
Acinetobacter sp. W45	S	S	R	R	R	S	R
Acinetobacter sp. W45A	S	S	±	R	S	S	R
P. fluorescens B69	R	S	R	R	R	R	R
P. fluorescens B69A	S	S	R	R	S	R	R
P. fluorescens B69F	S	S	R	R	S	S	S
P. fluorescens B1	R	R	R	R	R	R	R

[a] R, Growth on metal containing medium; S, no growth on metal containing medium; ±, questionable growth on metal containing medium.

[b] Numbers in parentheses are final concentrations (micrograms per milliliter), after addition to modified Yamada medium. See Materials and Methods.

TABLE 5. *Antibiotic resistance patterns of the bacteria resistant to mercury*[a]

Bacterial strain	Resistance patterns[b]							
	Tetracycline (30 μg)	Chloramphenicol (30 μg)	Nalidixic acid (30 μg)	Polymyxin B (300 U)	Penicillin (10 U)	Streptomycin (10 μg)	Bacitracin (10 U)	Erythromycin (15 μg)
Bacillus sp. F96	S	S	S	R	R	S	R	S
P. fluorescens F63	R	R	R	S	R	S	R	R
Pseudomonas sp. S58	S	S	R	S	S	S	S	S
Unidentified gram-negative rod 769	S	S	S	S	R	S	S	S
Pseudomonas sp. 244	R	±	S	S	R	S	R	S
Enteric bacterium CC2B	R	R	R	S	±	R	R	R
Acinetobacter sp. W45	S	R	S	S	R	S	R	±
Acinetobacter sp. W45A	S	R	R	S	R	S	R	S
P. fluorescens B69	S	R	S	±	R	S	R	R
P. fluorescens B69A	S	R	S	±	R	S	R	R
P. fluorescens B69F	S	R	R	R	S	R	R	R
P. fluorescens B1	S	R	±	R	R	±	R	R

[a] Resistant to mercury at a concentration of 10 μg/ml. See Table 4. R, Resistant; S, sensitive; ±, very slight (questionable) growth observed.

[b] Sensi-Discs (BBL Microbiology Systems, Cockeysville, Md.) were employed to determine resistance to the antibiotics.

of the parental strain (Table 5). Sensitivity to penicillin and resistance to nalidixic acid and streptomycin were detected after loss of the 39.5- and 18.5-Mdalton plasmids in *P. fluorescens* B69F. These data suggest that the majority of the genes conferring antibiotic resistance in *P. fluorescens* B69 are located on the chromosome, unless of course the screening procedure failed to detect other plasmids carried by this strain. It is concluded that the genes conferring resistance to mercury are not linked with those conferring drug resistance in *P. fluorescens* B69. This conclusion agrees with that presented by Chakrabarty (4) and Stanisich (26), who demonstrated that the plasmids MER and Hg-r occurring in *Pseudomonas* spp. carry mercury genes but no drug markers. Interestingly, the mercury-sensitive derivative of *Acinetobacter* sp. W45 became resistant to nalidixic acid upon curing of its 4.7-Mdalton plasmid. No other change in the antibiotic resistance pattern was detected. The genes conferring resistance to several of the other antibiotics tested may reside on the 2.2- and 31.9-Mdalton plasmids harbored by *Acinetobacter* sp. W45 or on the chromosome (Table 2).

In summary, plasmids carrying genes conferring resistance to heavy metals occur with surprisingly high frequency within bacterial populations found in the natural environment. Plasmids may, in fact, provide a fundamental and highly significant capability for a rapid response to introduction of allochthonous substrates for these bacteria, where genetic versatility and rapid response to environmental alteration can dictate survival.

ACKNOWLEDGMENTS

This work was supported, in part, by National Science Foundation grants OCE-76-82655 and DEB 72-02227 and Sea Grant project 04-8-MO1-73.

We thank S. Silver and T. Kinscherff for assistance in determining the presence of mercuric reductase in bacterial strains isolated from the Chesapeake Bay.

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