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Transformation of Elemental Mercury by Bacteria

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The fate and impact of elemental mercury in closed bacterial cultures were examined. The quantity of elemental mercury oxidized by bacteria ranged from small amounts for *Pseudomonas aeruginosa*, *P. fluorescens*, *Escherichia coli*, and *Citrobacter* to essentially all of the added elemental mercury for *Bacillus subtilis* and *B. megaterium*. The percentage of the total mercury in the system associated with bacterial cells ranged from 18.6 to 43.2%. Growth of the two *Pseudomonas* species was inhibited by elemental mercury, whereas growth of the other cultures was not distinguishable from that in mercury-free controls. No methylmercury was formed by the six cultures within 48 h.

Recent research on the fate of mercury in the environment demonstrates that elemental mercury is a common product of biological and chemical transformation of a variety of mercurials. Pure cultures of bacteria produce elemental mercury from mercuric chloride (10), phenylmercuric acetate (5), ethylmercuric phosphate (5), methylmercuric chloride (5), and methylmercuric bromide (12). Mixed cultures in aqueous systems release up to 70% of added mercuric ion as elemental mercury when incubated either aerobically or anerobically (2). Small amounts of elemental mercury are abiotically released from sediments receiving mercuric ion (3).

The fate of elemental mercury and its impact on microorganisms in water are not well defined. Evaluation of the thermodynamics of mercury (6, 8) revealed that elemental mercury can be oxidized in most freshwaters, but rates of oxidation of elemental mercury in water were not reported. Jernelöv (8) suggested that the organic carbon in water should increase the rate of oxidation of elemental mercury. We (7) observed that elemental mercury is stable in a sterile, chemically defined growth medium for at least 48 h.

In mixed culture systems receiving elemental mercury (8), methylmercury, the organomercurial commonly found in fish, can be formed at rates comparable to those obtained when mercuric ion is added. Elemental mercury may be oxidized in the water, assimilated by the bacteria, and methylated within the cell. Alternatively, elemental mercury could be transported through the bacterial membrane prior to oxidation and methylation.

The objective of the current study was to determine the fate of elemental mercury added to pure cultures of bacteria. Phenomena examined included (i) oxidation and methylation of elemental mercury by bacteria, (ii) accumulation of mercury by bacteria, and (iii) toxicity of elemental mercury to bacteria.

MATERIALS AND METHODS

Organisms. Bacteria tested for their ability to transform elemental mercury were *Escherichia coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Citrobacter* sp., *Bacillus subtilis*, and *Bacillus megaterium*. Inocula were prepared by growing the test cultures in the appropriate medium for 24 h and by diluting with sterile buffer (1) prior to aseptic transfer to the test flasks.

Media. *E. coli*, *Citrobacter*, *P. fluorescens*, and *P. aeruginosa* were grown in basal salts medium (11) supplemented with 0.25% glucose, and the *Bacillus* species were grown in the basal salts medium supplemented with 0.1% yeast extract.

Growth system. A 500-ml volume of the desired growth medium was placed in each flask and sterilized. After cooling, elemental mercury was added by the procedure of Holm and Cox (7). After a 48-h exposure to elemental mercury, the mercury source was removed, samples were removed for initial chemical analysis, and the flasks were inoculated. The cultures were incubated for an additional 48 h (125 rpm, 25 C), after which samples were removed for chemical and microbiological analyses.

Chemical determinations. Total mercury, mercuric ion, elemental mercury, and methylmercury were quantitated in each culture, and total mercury was determined in bacterial cells harvested by centrifugation. A laboratory data control ultraviolet monitor (model 1235) was used for quantitating total mercury, mercuric ion, and elemental mercury. Elemental mercury was quantitated by analyzing the undigested samples directly without addition of a reducing agent; mercuric ion was determined by adding 1.0 ml of 10% stannous chloride in 50% HCl to each sample, incu-

bating 30 s, and quantitating the mercury as elemental mercury; the difference between the amount of mercury recovered as elemental mercury with and without the reducing agent was considered to be mercuric ion. Total mercury was determined by digesting each sample with aqua regia and oxidizing with permanganate (4).

Methylmercury was determined by the method of Longbottom et al. (9) with a Barber Coleman gas chromatograph equipped with a radium-226 electron capture detector. A Pyrex column (1 m by 6 mm) was empolyed for separation. The carrier gas (N_2) flow rate was 60 ml/min; the operating temperatures for the column, detector, and inlet were 140, 210, and 180 C, respectively.

Bacterial viability. Cell counts for each of the cultures except those of the *Bacillus* species were obtained at 0 and 48 h by using tryptone glucose extract/1% yeast extract agar as plating media. Plate cultures were incubated for 48 h at 25 C before colonies were counted.

Statistical evaluation. Tables 1, 2, and 3 contain statistics for the experimental treatment. Included are the number of flasks (N), the mean, and standard deviation (S) of the N observations. A t test based on paired observations of the 0- and 48-h mercury concentration in each of the flasks was used to test the null hypothesis that bacteria do not transform mercury.

RESULTS AND DISCUSSION

During exposure to elemental mercury for 48 h, the medium containing yeast extract adsorbed about twice as much mercury as did the medium without yeast extract (0 time, Table 1). After the source was removed, elemental mercury was stable in a sterile, basal salts medium containing glucose, but was slowly oxidized in a basal salts medium supplemented with 0.1% yeast extract (Table 1). Because both media were incubated aerobically, this observation suggests that the nature of the organic carbon supplement is more important in the oxidation of elemental mercury than is the dissolved oxygen in the medium.

Growth of each of the test organisms in the test media increased the quantity of elemental mercury oxidized (Table 2). The t test revealed a significant decline ($\alpha = 0.05$) in elemental mercury concentration in the 48-h culture period. The amount of elemental mercury oxi-

TABLE 1. *Comparison of the stability of elemental mercury in two growth media*

Time[a]	Basal salts medium		Basal salts medium + yeast extract	
	Elemental mercury (μg/liter)	Total mercury (μg/liter)	Elemental mercury (μg/liter)	Total mercury (μg/liter)
0	57.0[b] (S = 6.1, N = 10)[c]	57.5 (S = 10.6, N = 10)	56.5 (S = 5.6, N = 9)	104.9 (S = 19.9, N = 9)
48	56.3 (S = 4.3, N = 10)	52.5 (S = 4.3, N = 9)	35.0 (S = 4.9, N = 9)	102.1 (S = 22.9, N = 9)

[a] Hours elapsed after removal of Hg° globule.
[b] Mean of N observations.
[c] S, Standard deviation; N, number of flasks.

TABLE 2. *Concentration of elemental mercury and mercuric ion in cultures incubated for 0[a] and 48 h*

Organism	Elemental mercury (μg/liter)		Mercuric ion (μg/liter)	
	0 h	48 h	0 h	48 h
P. aeruginosa	58.6 (S = 7.1, N = 6)	54.0 (S = 5.5, N = 6)	0.6 (S = 1.3, N = 6)	3.8 (S = 4.4, N = 6)
P. fluorescens	54.6 (S = 6.4, N = 8)	38.4 (S = 9.4, N = 7)	0.3 (S = 0.7, N = 7)	6.7 (S = 3.7, N = 7)
Citrobacter sp.	57.6 (S = 8.6, N = 4)	36.8 (S = 4.5, N = 4)	1.6 (S = 1.5, N = 4)	1.5 (S = 1.0, N = 4)
E. coli	59.2 (S = 2.8, N = 4)	34.0 (S = 9.0, N = 4)	1.1 (S = 1.5, N = 4)	2.1 (S = 2.8, N = 4)
B. subtilis	49.8 (S = 8.7, N = 4)	7.2 (S = 6.1, N = 4)	11.9 (S = 8.5, N = 4)	8.2 (S = 10.3, N = 4)
B. megaterium	57.9 (S = 7.1, N = 10)	0.1 (S = 0.2, N = 10)	9.6 (S = 6.7, N = 10)	1.8 (S = 2.3, N = 10)

[a] Samples for mercury analyses were removed prior to adding the inoculum. S, Standard deviation.

TABLE 3. *Accumulation of mercury by bacteria during a 48 h growth period*[a]

Organism	Total mercury (μg/liter)	Cell-associated mercury	
		μg/liter	%
P. aeruginosa	47.4 (S = 3.87, *N* = 5)	No growth	
P. fluorescens	56.4 (S = 10.4, *N* = 6)	10.5 (S = 10.6, *N* = 4)	18.6
Citrobacter sp.	45.9 (S = 10.7, *N* = 4)	12.3 (S = 4.0, *N* = 4)	26.8
E. coli	57.6 (S = 2.8, *N* = 2)	13.5 (S = 2.1, *N* = 2)	23.4
B. subtilis	100.8 (S = 14.5, *N* = 4)	43.1 (S = 26.4, *N* = 3)	42.7
B. megaterium	116.4 (S = 22.2, *N* = 10)	50.3 (S = 19.6, *N* = 7)	43.2

[a] S, Standard deviation.

dized ranged from small amounts (*P. aeruginosa*) to large quantities (*B. megaterium*). The small amount of oxidation by the *Pseudomonas* species and *E. coli* was expected, because previous reports (10, 13) showed that these species reduced mercuric ion to elemental mercury.

The direct action of bacteria is not required to increase the rate of oxidation of elemental mercury in the growth systems. This was shown by introducing elemental mercury into a sterile filtrate from a 48-h culture of *B. megaterium* that had not been exposed to mercury. After exposure to elemental mercury for 48 h, the sterile filtrate contained more total mercury (217 μg/liter) than did the basal salts/yeast extract controls (Table 1) in which bacteria had not been cultured (104.9 μg/liter).

The elemental mercury oxidized by the bacteria was not quantitatively recovered as mercuric ion (Table 2). Much of the oxidized mercury in the growth systems may be complexed and thus protected from reduction by stannous chloride.

Consequently, only *P. fluorescens* and *B. megaterium* significantly changed the concentrations of mercuric ion between the 0- and 48-h sampling times (paired *t* tests, $\alpha = 0.05$; Table 2). *P. fluorescens* increased the concentration of mercuric ion; *B. megaterium* decreased it. The differences between these two cultures may be due either to the different growth kinetics (*P. fluorescens* grew poorly in the presence of elemental mercury and therefore provided less cellular carbon to bind the mercuric ion), or to the different growth media employed for the growth of the two organisms.

Elemental mercury was not transformed to methylmercury in these cultures during 48-h incubation periods (detection limit, 0.6 μg/liter). This differs from results of Vonk and Sijpesteijn (14), who reported that some of these same genera generate small amounts of methylmercury from mercuric chloride. The different results may be explained by the use of different media, mercury sources, incubation periods, and analytical procedures in their study (14) and ours.

A portion of the mercury that was added to these systems accumulated in the bacterial cells (Table 3). In these six systems, the percentage of the total mercury in the medium associated with the bacterial biomass ranged from 18.6 to 43.2%. Generally, those organisms growing in the basal salts medium contained less mercury than those growing in media containing yeast extract. The concentration factors for accumu-

TABLE 4. *Mercury concentration factors for bacteria grown in a basal salts medium containing elemental mercury*

Organism	Elemental mercury in medium (μg/g)	Mercury in cells[a] (μg/g)	Concn factor[b]
E. coli	0.0574	11.25	196
P. fluorescens	0.0546	65.63	1,202
Citrobacter	0.0576	12.81	222

[a] Mercury content of cells was estimated by measuring the mercury concentration in the pellet centrifuged from a 48-h culture of bacteria, assuming that 10^8 bacteria weigh 1 μg (wet weight).

[b] The concentration factor was obtained by dividing the mercury concentration of the cells by the mercury concentration of the medium.

TABLE 5. *Population changes of bacteria in media with and without elemental mercury*

Organism	Bacteria/ml			
	Control		Elemental mercury	
	0 h	48 h	0 h	48 h
P. aeruginosa	1.8×10^6	1.3×10^9	1.7×10^6	5.0×10^8
P. fluorescens	6.1×10^5	1.7×10^9	4.6×10^5	1.6×10^8
Citrobacter	3.7×10^6	8.4×10^8	4.8×10^6	9.5×10^8
E. coli	4.5×10^6	1.1×10^9	3.0×10^6	1.1×10^9

lation of mercury were 222, 196, and 1202 for *Citrobacter*, *E. coli*, and *P. fluorescens*, respectively (Table 4).

Elemental mercury does affect the growth of some bacteria. Table 5, a summary of population changes between the 0- and 48-h incubation periods, shows that elemental mercury killed *P. aerugenosa* and decreased the growth rate of *P. fluorescens*. The other cultures grew similarly to the controls. These observations demonstrate that the impact of elemental mercury on organisms in a complex aquatic system is not likely to be accurately predicted from the results of a study of only a few microbial cultures.

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