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Volatilization of Mercury Compounds by Methylmercury–Volatilizing Bacteria in Minamata Bay Sediment

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Minamata Bay has been heavily polluted by high mercury concentrations which gave rise for a long time to methylmercury poisoning, Minamata disease (Kutsuna 1968; Irukayama 1977). The mercury still exists in the sediments of the Bay (Kumamoto Prefecture Government 1973; Kumagai and Nishimura 1978). The fish and the shellfish living in the Bay contain high methylmercury concentrations as compared with those in other marine environments. The population of mercury-resistant bacteria in the sediments of Minamata Bay is larger than that in the sediments of other marine environments (Nakamura *et al.* 1986).

The mercury-resistant bacteria isolated from a marine environment have been found to transform organic and inorganic mercury compounds into mercury vapor (Hg^0) (Nelson *et al.* 1973; Spangler *et al.* 1973; Olsen *et al.* 1979). The mercury-resistance confirmed in various bacterial genera has been shown to be plasmid-mediated volatilization (Clark *et al.* 1977; Weiss *et al.* 1977; Robinson and Tuovinen 1984). However, there has been little definitive information on the volatilization of organic mercury by the bacteria living in the mercury-polluted environment.

It is important to know what bacterial transformations of mercury have been taking place and how the mercury-resistant bacteria may be playing a role in the mercury cycle in the marine environment of Minamata Bay. The object of the present study is to clarify the characteristics of the methylmercury-volatilizing bacteria in the sediments of Minamata Bay and of the volatilization of various mercury compounds by these bacteria.

MATERIALS AND METHODS

Sediment samples were collected in October 1985 from two stations in Minamata Bay near the drainage outlet of the acetoaldehyde plant of Minamata Factory by using sterile cylinders (55 cm diameter). The topmost 3 cm of the sediments were used for assessing total mercury contents and for isolating the bacteria.

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The basal medium was artificial sea water medium (modified ZoBell 2216 E medium) as described previously (Nakamura *et al.* 1986). About 1000 strains of methylmercury-resistant bacteria were isolated on agar plates containing 0.1 μg/mL of CH_3HgCl after incubation at 25°C for 48 h. These isolates were screened for the ability to volatilize methylmercury by measuring mercury loss in liquid media containing methylmercury (0.1 μg/mL) after incubation for 48 h at 25°C. The total mercury content in the medium was analyzed by a flameless atomic adsorption spectrophotometer with mercury analysis vaporizer (Tanaka *et al.* 1974).

The methylmercury-volatilizing strains were classified in accordance with the taxonomic scheme proposed by Shewan *et al.* (1960) and "Bergye's Manual of Determinative Bacteriology" (Buchanan and Gibbons 1974). The minimal inhibitory concentrations (MICs) of mercury chloride ($HgCl_2$), methylmercury chloride (CH_3HgCl), ethylmercury chloride (C_2H_5HgCl), propylmercury chloride (C_3H_7HgCl), and phenylmercuric acetate ($C_6H_5HgOCOCH_3$) against the methylmercury-volatilizing strains were determined by the agar dilution method. MICs of mercury compounds were taken as the next concentration beyond the last concentration at which growth was observed on solid medium in the presence of sequential concentrations of mercury compounds. The mercury compounds and their concentrations (μg/mL) used for the volatilization were as follows : $HgCl_2$, 5 ; CH_3HgCl, 0.25 ; C_2H_5HgCl, 0.1 ; C_3H_7HgCl, 0.1 ; $C_6H_5HgOCOCH_3$, 1. The mercury contents in the sediments were directly analyzed by the same method as used in analyzing the total mercury content in the medium.

RESULTS AND DISCUSSION

The total mercury concentration and the percentage of methylmercury-resistant bacteria for the total bacterial counts in the sediment of Minamata Bay are as shown in Table 1. The percentage of methylmercury-resistant bacteria for the total bacterial counts was calculated from the separate counts on methylmercury-free agar plates. The total mercury concentrations in the sediment of two stations in Minamata Bay were 32.4 μg/mL and 23.5 μg/mL, respectively. One observation made was the tendency for the percentage of methylmercury-resistant bacteria to be higher where the mercury concentration in the sediment was also higher. ; a similar tendency was reported by Nakamura *et al.* (1986).

The percentage and generic composition of the methylmercury-volatilizing bacteria are shown in Table 2. A total of 1068 bacterial strains (965 non-swarmer and 103 swarmer) isolated from the agar plates containing 0.1 μg/mL methylmercury were screened for methylmercury volatilizing ability. Of these strains, only 80 strains (7.5%) found to volatilize methylmercury by measuring mercury loss in liquid media containing methylmercury. These strains were composed of 60 *Bacillus* species (75.0%), 15 *Pseudomonas* sp. (18.8%), 3 *Moraxella* sp. (3.7%), and 2 unclassified (2.5%). The two unclassified strains were gram-

Table 1. Total mercury concentrations and percentage of CH_3HgCl-resistant bacteria in the sediments of Minamata Bay.

	Mercury[a] (μg/g)	C.F.U.[b]	CH_3HgCl (μg/mL)			
			0.01	0.025	0.05	0.1
Station 1	32.4	2.2×10^3	81.8[c]	63.6	54.5	2.3
Station 2	23.5	6.0×10^3	76.6	48.3	36.7	3.7

[a] Total mercury concentrations in sediment (/ dry weight).
[b] Colony-forming units.
[c] Percentage of CH_3HgCl-resistant bacteria for the total bacterial count.

Table 2. Generic composition of CH_3HgCl-volatilizing bacteria in the sediments of Minamata Bay.

No. of tested strains	No. of CH_3HgCl-volatilizing bacteria	Bacterial genera	
1068 (non-swarmer-965) (swarmer-----103)	80 (7.5 %)	*Bacillus*	60 (75.0 %)
		Pseudomonas	15 (18.8 %)
		Moraxella	3 (3.7 %)
		unclassified	2 (2.5 %)

negative rods with peritrichous flagella. The distribution rate of MICs for various mercury compounds determined on 80 methylmercury-volatilizing strains from Minamata Bay sediments is shown in Table 3. The MICs of $HgCl_2$ and $C_6H_5HgOCOCH_3$ against these strains are distributed in narrow ranges : $HgCl_2$, 80-160 μg/mL ; $C_6H_5HgOCOCH_3$, 4-8 μg/mL. On the other hand, those of CH_3HgCl, C_2H_5HgCl and C_3H_7HgCl are widely distributed. It is hypothesized that the differences depend on the solubility of mercury compounds in lipids in bacterial membranes as well as on the binding of the compounds to sulfhydryl groups of proteins and enzymes, as reported by Berlin (1979).

The volatilization of mercury compounds by the methylmercury-volatilizing bacteria is shown in Table 4. All of the methylmercury-volatilizing bacteria were able to volatilize each of the mercury compounds used in this study. The mercury resistant plasmid of *Pseudomonas aeruginosa* and *Escherichia coli* is

Table 3. Distribution of Minimal Inhibitory Concentration (MICs) of mercury compounds against CH_3HgCl-volatilizing bacteria.

	MIC (μg/ml)	*Bacillus* (60)[a]	*Pseudo-monas* (15)	*Mora-xella* (3)	Unclassified (2)
$HgCl_2$	40	0.0[b]	6.7	0.0	0.0
	80	11.7	0.0	0.0	0.0
	160	83.4	93.3	100.0	100.0
	320	5.0	0.0	0.0	0.0
CH_3HgCl	0.25	13.3	21.0	33.3	0.0
	0.5	35.0	46.7	33.3	100.0
	1	26.7	20.0	33.3	0.0
	2	23.3	13.3	0.0	0.0
	4	1.7	0.0	0.0	0.0
C_2H_5HgCl	0.25	3.3	6.7	0.0	0.0
	0.5	38.4	53.3	0.0	50.0
	1	26.7	6.7	100.0	50.0
	2	31.6	33.3	0.0	0.0
C_3H_7HgCl	0.25	5.0	20.0	0.0	0.0
	0.5	41.7	33.3	0.0	100.0
	1	20.0	20.0	0.0	0.0
	2	20.0	13.4	75.0	0.0
	4	1.3	13.3	25.0	0.0
$C_6H_5HgOCOCH_3$	0.5	0.0	0.0	0.0	50.0
	2	16.7	26.7	0.0	0.0
	4	5.0	6.8	0.0	0.0
	8	78.3	66.7	100.0	50.0

[a]Number of strains
[b]Percentages of strains

divided into two classes of resistance : (1) "narrow-spectrum" resistance plasmids that are resistant to Hg^{++} and (2) "broad-spectrum" resistance to Hg^{++}, CH_3HgCl, C_2H_5HgCl, and C_3H_7HgCl and $C_6H_5HgOCOCH_3$. (Clark *et al* and Weiss *et al.* 1977). The volatilization of C_3H_7HgCl by bacteria had not yet been reported. It was confirmed in this study that 80 methylmercury-volatilizing bacteria removed mercury from liquid culture containing C_3H_7HgCl. It is probable that mercury-resistant bacteria remove C_3H_7HgCl as well as CH_3HgCl and C_2H_5HgCl through volatilization.

The methylmercury-volatilizing bacteria were isolated on agar plates containing 0.1 μg/mL of CH_3HgCl and screened for the ability to volatilize methylmercury. The percentages of methylmercury-volatilizing bacteria for the total bacterial count were low as compared to those of $HgCl_2$-volatilizing bacteria as reported by Nakamura *et al.* (1986). Spangler *et al.* (1973) reported

Table 4. Volatilization (%) of mercury compounds by CH_3HgCl-volatilizing bacteria isolated from Minamata Bay sediments.

	Bacillus (60)[a]	*Pseudomonas* (15)	*Moraxella* (3)	Unclassified (2)
$HgCl_2$	76.8±1.0[b]	77.5±2.2	73.2±7.8	83.9±1.6
CH_3HgCl	88.5±0.3	88.1±0.8	87.0±3.6	89.1±0.0
C_2H_5HgCl	77.9±0.6	76.6±2.4	81.5±0.9	74.3±2.0
C_3H_7HgCl	77.9±0.6	73.9±1.5	81.2±0.9	77.8±3.3
$C_6H_5HgOCOCH_3$	44.6±0.8	47.2±1.2	44.9±3.2	44.6±1.1

[a]Number of tested strains
[b]Mean loss ± SD (%)

that 30 of 207 (14.5%) bacteria, isolated from the sediment of Lake St Clair, were found to be methylmercury-volatilizing strains. The generic composition of those methylmercury-volatilizing bacteria were similar to that of bacteria in the sediment of Minamata Bay. These results suggest that mercury does not play a large role in causing bacteria in the sediment of Minamata Bay to develop their capacity for methylmercury-resistance through volatilization.

There are many reports concerning methylmercury formation in aquatic environments (Jensen and Jernelöv 1969; Andren and Harris 1973; Olsen and Cooper 1976). However, methylmercury is not detectable in the sediment of Minamata Bay. The methylmercury-volatilizing bacteria may play an important role in the mercury cycle of Minamata Bay.

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