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Biotransformation of Mercurials by Intestinal Microorganisms Isolated from Yellowfin Tuna

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Mercury accumulated in tuna fish is mostly in the form of methylmercury (KAMPS et al. 1972, RIVERS et al. 1972, PAN-HOU & IMURA 1980). It is most probable that the accumulation of methylmercury is via the food chain. Yet, the possibility of mercury methylation in tuna fish cannot be ruled out, since we have reported that liver homogenates from some kinds of tuna fish, which contained higher level of methylmercury, have a relatively high ability to form methylmercury from inorganic mercury in vitro (IMURA et al. 1972).

Transformation of mercury compounds by some intestinal flora has been reported (ABDULLA et al. 1973, EDWARDS & MCBRIDE 1975, ROWLAND et al. 1975, 1977, 1978, 1980), and the role of this microbial function in fish intestine cannot be ignored when we consider the mechanism in accumulation of methylmercury in fish. The present investigation was undertaken to study whether transformation of mercury compounds can actually occur by the pure cultures of bacteria isolated from the yellowfin tuna intestinal tract.

MATERIALS AND METHODS

Organisms and culture Fourteen bacterial strains isolated from yellowfin tuna(*Thunnus albacares*) intestine were supplied by Dr. A. Gohda (The Kitasato Institute. Tokyo Japan). Organisms were grown aerobically at 20°C in a nutrient broth composed of 1% peptone, 0.5% meat extract, 0.5% $MgCl_2$, 3% NaCl, 0.01% $Fe_3(SO_4)_2$, 0.07% KCl, 0.26% $MgSO_4$ and 0.1% $CaSO_4$ (pH 7.2).

Determination of susceptibility of the bacteria to mercury compounds Organisms were grown overnight in the nutrient broth. Two-tenth mL of the culture (10^7 cells/mL) was added to 9.8 mL of the nutrient broth containing various concentrations of mercurials. The minimum inhibitory concentration of mercurials, which inhibit the increase of cell densities after 20 h incubation, was determined by measuring the turbidity of cultures at 660 nm.

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Demethylation and methylation of mercurials

Organisms were grown in a 100 mL of nutrient broth containing 40 µg of CH_3HgCl or 130 µg of $HgCl_2$ at 20°C. To absorb the evaporated mercury during aeration, the culture chamber was connected to a series of traps. The first and third traps contained 20 mL of 0.5% *L*-cysteine solution to retain methylmercury and inorganic mercury ion. The second trap contained a 20 mL of 5% $KMnO_4$ in 5% H_2SO_4 to oxidize metallic mercury that might escape from the first trap. After incubation, the mercury compounds in the culture chamber and in the traps were analysed. Methylmercury was measured by gas chromatography using an electron capture detector (IMURA et al. 1972). Total mercury was analysed by atomic absorption spectrometry after the samples were digested with concentrated nitric acid (IMURA et al. 1977).

RESULTS AND DISCUSSION

To select the practical mercury concentrations in the culture medium, the overnight culture of each bacterial strain was inoculated into the nutrient broth containing various concentrations of mercurials. Table 1 showed the minimum inhibitory concentrations of mercury compounds for the strained intestinal samples. Of the 14 cultures isolated from tuna intestine, 5 strains (strain No. 493, 494, 497, 498 and 501) appeared to have relatively higher resistance to mercurials. The concentrations of mercury in the culture medium in which all the bacteria can still grow, were selected. The suitable concentrations were 0.4 µg/mL for methylmercury and 1.3 µg/mL for mercuric chloride.

To determine the capacity of the intestinal bacteria to methylate inorganic mercury, the organisms were inoculated into the broth containing 1.3 µg/mL of $HgCl_2$. Methylmercury in the culture medium and the traps was determined after 24 or 48 h incubation. Under the condition employed, no detectable amount of methylmercury was formed by any bacterial strain. The sensitivity of the method for methylmercury measurements employed in this study was about 0.25 mmol/L methylmercury when 100 mL of culture medium was used for determination.

The bacteria having relatively higher mercury resistance were supposed to have an ability to decompose and volatilize the mercury compounds in culture medium. To examine this possibility the 5 strains which having higher resistance were screened for methylmercury decomposing activity. As shown in Table 2, after 24 h incubation, an appreciable loss of extractable methylmercury was observed in the cultures of *Enterobacter sp.* (strain No. 497) and of *Pseudomonas sp.* (strain No. 501).

TABLE 1. Minimum inhibitory concentrations of mercury compounds for intestinal bacteria isolated from yellowfin tuna

Strain No.	Genus	Mercurials (μg/mL)	
		$HgCl_2$	CH_3HgCl
452	*Vibrio*	4.0	1.6
453	*Vibrio*	4.0	0.8
454	*Enterobacter*	4.0	0.8
455	*Enterobacter*	4.0	0.8
456	*Vibrio*	8.0	0.8
493	*Micrococcus*	16.0	6.4
494	*Micrococcus*	16.0	6.4
495	*Vibrio*	8.0	3.2
496	*Enterobacter*	8.0	1.6
497	*Enterobacter*	16.0	6.4
498	*Vibrio*	16.0	6.4
499	*Enterobacter*	8.0	1.6
500	*Streptococcus*	4.0	0.8
501	*Pseudomonas*	32.0	12.8

TABLE 2. Decomposition of methylmercury by the intestinal bacteria isolated from yellowfin tuna

Strain No.	Genus	CH_3HgCl remaining		CH_3HgCl recovery
		ng/mL	%	(% of added*)
-	-	397	99	-
493	*Micrococcus*	380	95	98
494	*Micrococcus*	401	100	101
497	*Enterobacter*	324	82	99
498	*Vibrio*	391	98	97
501	*Pseudomonas*	270	68	97

* Recovery was determined after incubation of 0.4 μg/mL of CH_3HgCl with the stationary growth phase of each organism for 10 min.

This experimental result suggested a possibility that the two strains were able to decompose methylmercury. Further, to characterize the reaction products the culture chamber was connected to a series of traps and the total mercury in the chamber and the traps was analysed. By 24 h incubation, 31% of methylmercury added was lost from the chamber of *Pseudomonas sp.* as shown in Table 3. Of the total mercury which was added as methylmercury to the medium, 82% was recovered from the medium and 17% was trapped in the second trap in which the vaporized elemental mercury was to be oxidized and trapped with $KMnO_4$-H_2SO_4 solution. However, no detect-

able amount of mercury was lost from the culture medium when the organism was heated at 100°C for 10 min before inoculation. These results suggested that the linkage between carbon and mercury was cleaved and subsequently reduced by the organism resulting in the formation of volatile mercury. Table 4 also showed that when $HgCl_2$ was added instead of CH_3HgCl to the culture medium, 52% of the total mercury added was recovered from the second trap. This result confirmed that the organism was capable of reducing inorganic mercury. The major product of transformation was metallic mercury since it was released as a volatile product from the culture medium and trapped in $KMnO_4$-H_2SO_4 solution.

TABLE 3. Decomposition and volatilization of methylmercury by the *Pseudomonas sp.* (strain 501) isolated from yellowfin tuna intestine

Incubation time (h)		Recovery			
		Methylmercury		Total mercury	
		µg*	%	µg*	%
0	Medium	31.2	100	31.4	100
24	Medium	21.4	69	25.6	82
	Trap 1	N.D.	-	0.1	0.3
	Trap 2	N.D.	-	5.2	17
	Trap 3	N.D.	-	0.4	1.3

* Calculated in terms of mercury/100 mL.
N.D.=less than 1.2 ng.

TABLE 4. Volatilization of inorganic mercury by the *Pseudomonas sp.* (strain 501) isolated from yellowfin tuna

Incubation time (h)		Recovery	
		µg*	%
0	Medium	96.2	100
24	Medium	46.5	48
	Trap 1	0.1	0.1
	Trap 2	50.0	52
	Trap 3	0.2	0.2

* Calculated in terms of mercury/100 mL.

The methylmercury accumulated in tuna fish has been generally considered to be the culmination of a complex food chain beginning with methylation of inorganic mercury in sediments. It has been known that methylmercury can be formed by microorganisms in mammalian intestinal tract (ABDULLA et al. 1973, EDWARDS &

MCBRIDE 1975, ROWLAND et al. 1975, 1977, 1978, 1980) and recently RUDD et al. (1980) reported that methylmercury could be formed also by fish intestinal contents. In the present study, no detectable amount of methylmercury could be formed by any pure cultures of bacteria isolated from tuna intestine under the conditions tested. In contrast, some of these organisms were capable of decomposing methylmercury with the concomitant formation of metallic mercury. The process of demethylation by the organisms in the intestine of tuna fish may play an important role in preventing the deposition of methylmercury in tuna fish.

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