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84. HEAVY METAL RESISTANT BACTERIA ASSOCIATED WITH THE BLACK CLAM *VILLORITA CYPRINOIDES* VAR *COCHINENSIS* (HANLEY) AND WATER COLLECTED FROM COCHIN BACKWATER

K. R. Sreekumari and P. Lakshmanaperumalsamy *
School of Marine Science, Cochin University of Science & Technology, Cochin-682 016.
** Department of Environmental Sciences, Bharathiar University, Coimbatore-641 046*

ABSTRACT

A total of 147 strains of heterotrophic bacteria, isolated from water and the black clam *Villorlia cyprinoides* var *conchinensis* from Cochin backwater were subjected to heavy metal sensitivity tests for five heavy metals viz. mercury, zinc, cadmium, copper and lead. In general, the isolates from water showed higher resistance towards the heavy metals. Members of all the genera except Coryneform group showed similar trend of resistance. The strains of Coryneform group isolated from animals were more resistant than their counterparts isolated from water. Minimum inhibitory concentration (MIC) of the metals varied. It may be concluded from the results that the habitat of the organisms plays a unique role in the ecology of heavy metal resistant bacteria.

INTRODUCTION

The Cochin backwater, a part of the Vembanad lake which is attached with several rivers and a network of canal, receives effluents from a number of Industries. Large quantities of heavy metals are present in these effluents. It is true that most of these metals are essential in trace amounts for the normal metabolism of aquatic organisms. But excessive amounts of these metals have been proved lethal.

Considerable interest has been shown to know the effect of heavy metals on micro-organisms present in aquatic environment. Tolerance to elevated levels of heavy metal is evident in microorganisms isolated from metal contaminated environments (Gracia Toledo et al 1985). In Cochin backwater the effect of heavy metals on macroorganism such as fish, molluscs etc. have been studied (National Seminar on Mussel watch, Cochin 1986). However, similar attempt to understand the influence of heavy metals on the microbial load is not made. In the present study, the distribution of heavy metal resistant baeteria in water and in association with black clam of Cochin backwater was made.

MATERIAL AND METHODS

The black clam (*Villorita cyprinoides* var *cochinensis)* and the water samples were collected from Vembanad lake near the Kumbalam island and transported to the laboratory in an insulated ice box. Standard methods were followed for enumeration, isolation and identification of bacteria (Sreekumari and Lakshmanaperumalsamy 1986)

Metal sensitivity of selected bacterial isolates was tested on nutrient agar medium (Peptone 0.5%; Beef extract 0.5%; NaC1 1.5%; agar 2%). Filter sterilized salts of five metals (mercury, zinc, copper, cadmium and lead) were incorporated in the sterile molten basal medium at different concentrations. before dispensing into petriplates. Isolates enriched in nutrient broth (6 h) were spot inoculated on the metal incorporated medium. Plates were incubated at 37°C for 18-24 h. If no growth was seen after 24h, plates were reincubated for an additional 24 h. If growth was observed within 48 h, the isolate was treated as resistant to that concentration of the metal. The criteria used by Austin et al (1977) were further extended to other metals, for differentiation of the strains into sensitive and resistant forms (critical concentration was fixed as 10 ppm for mercury and 100 ppm for other metals). Minimum inhibitory concentrations (MIC) of the resistant strains were found out.

RESULTS AND DISCUSSION

All the isolates tested were found resistant to zinc and lead. Resiatance to mercury, copper and cadmium varied among isolates. 90.5% of the isolates were resistant to mercury; 97.3% to cadmium and 76.9% to copper (Table 1).

TABLE 1. *Percentage of frequency of heavy metal resistant heterotropic bacteria*

Metals	Total	Water	Animal
Mercury	133	37	96
	(90.5)	(94.9)	(88.9)
Zinc	147	39	108
	(100.0)	(100.0)	(100.0)
Cadmium	143	39	104
	(97.3)	(100.0)	(96.3)
Copper	113	31	82
	(76.9)	(79.5)	(75.9)
Lead	147	39	108
	(100.0)	(100.0)	(100.0)

The percentage frequency of resistance of heterotrophic bacterial isolates to various metals on a samplewise analysis showed that isolates from water exhibited higher resistance to mercury, cadmium and copper than those from animal samples. Since Cochin backwater receives heavy metals from effluents of various factories, higher percentage of metal resistant bacteria might have occurred. Also, the periodical input of heavy metals might have affected the bacterial strains to get them trained for higher level of tolerance. Similar findings were reported from various geographical locations (Cook and Goldman, 1976; Austin et al. 1977; Mills and Colwell 1977). The presence of more heavy metal resistant bacteria in water than animal is not in agreement with the findings of Pradeep (1986) who reported higher percentages of metal resistant *Vibrio parahaemolyticus* strains associated with plankton, prawns and fishes. The bivalves are well known for the accumulation of heavy metals at higher proportions in the various organs of the body. The incidence of lesser percentage of metal

TABLE 2. *Generawise analysis of percentage frequency of heavy metal resistant heterotrophic bacteria*

Genera	Sample	Hg	Zn	Cd	Cu	Pb	Total
Pseudomona	W	100.0	100.0	100.0	100.0	100.0	9
	A	86.4	100.0	95.5	77.3	100.0	22
Vibrio	W	100.0	100.0	100.0	75.0	100.0	4
	A	84.0	100.0	96.0	72.0	100.0	25
Aeromonas	W	100.0	100.0	100.0	100.0	100.0	2
	A	100.0	100.0	100.0	95.2	100.0	21
Enterobacteriaceae	W	100.0	100.0	100.0	90.9	100.0	11
	A	92.9	100.0	92.9	57.1	100.0	14
Alcaligenes	W	100.0	100.0	100.0	0.0	100.0	1
	A	75.0	100.0	100.0	87.5	100.0	8
Acinetobacter	W	100.0	100.0	100.0	100.0	100.0	1
	A	50.0	100.0	100.0	50.0	100.0	2
Elavobacterium	W	—	—	—	—	—	—
Cytophaga group	A	100.0	100.0	100.0	100.0	100.0	1
Moraxella	W	100.0	100.0	100.0	100.0	100.0	1
	A	100.0	100.0	80.0	80.0	100.0	5
Bacillus	W	80.0	100.0	100:0	80.0	100.0	5
	A	66.7	100.0	100.0	33.3	100.0	3
Coryneforum group	W	66.7	100.0	100.0	33.3	100.0	3
	A	100.0	100.0	100.0	66.7	100 0	3
Micrococcus	W	100.0	100.0	100.0	100.0	100.0	2
	A	100.0	100.0	100.0	100.0	100.0	4

resistant bacteria shows that there may be some synergistic antagonistic activity which may be playing an imporant role between the host microenvironment and the bacteria and this requires extensive study.

Generawise analysis of percentage frequency of heavy metal resistance showed that except Coryneform group of bacteria, all the genera encountered in the study, showed similar pattern i. e., water samples contained more heavy metal resistant bacteria than those from animal samples (Table 2). This may be attributed to the fact that the bacteria sharing a common environment may share a common mode of development of hevy metal resistance.

Out of 147 strains subjected to sensitivity studies on heavy metals, none of them were found to be resistant to a single metal species alone. All the strains showed multiple resistance. Maximum resistance was found towards all the five metals tested followed by four and three metals respectively. The percentage of multiple resistivity was in the order of 72.8%, 21.1% and 6.1% respectively (Table 3). The minimum inhibitory concentration (MIC) for all the strains tested were also worked out, The maximum percentage showed MIC to the tune of 25 ppm, 300 ppm, 200 ppm, 150 ppm, and 1000 ppm for mercury, Zinc. Cadmium, copper and lead respectively.

TABLE 3. *Multiple resistance of heterotrophic bacteria*

Resistant to heavy metals	Resistance to number of heavy metals					
	5	4	3	2	1	Total
Number of isolates	107	31	9	0	0	147
Percentage	72.8	21.1	6.1	0.0	0.0	

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