Indian Journal of Marine Sciences
Vol. 20, September 1991, pp. 229-231

Studies on Cu-tolerant bacteria in the Vellar estuary, southeast coast of India

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Received 4 March 1991, revised 29 July 1991

Sediment samples harboured more Cu-tolerant bacteria than seawater samples. Morphological and biochemical characteristics of the selected isolates from each sample were studied. The production of amylase, lipase and protease was also attempted. The genera such as *Vibrio, Micrococcus, Corynebacterium* and *Pseudomonas* were commonly encountered. Cu-tolerant forms may be used as a tool to control the Cu pollution in the environment.

All organisms require certain heavy metals including, Co, Cu, Fe, Mn and Zn, either for their nutrition, or for any other physiological functions. Among these, the heavy metal Cu has been investigated with regard to its toxicity and effects on biological processes more than any other single metal. The present study portraits Cu-tolerant bacteria in Vellar estuary.

Water samples for bacteriological examinations were collected in sterilized McCartney bottles. The sediment samples were collected using sterile spatula and transferred into new polythene bags. All samples were immediately transported to the laboratory and the bacteriological analysis was made using serial dilution technique within 2-4 h of collection.

For the enumeration of total heterotrophic bacteria (THB), appropriate dilutions were selected and 1 ml of sample was transferred to sterile petri plates. ZoBell's marine agar 2216 e medium (Hi Media, Bombay) supplemented with Cu as $CuSO_4$ salt (25 and 50 $\mu g.ml^{-1}$) and without Cu as control were made, poured into plates and mixed with the sample thoroughly. Plates in triplicate were incubated aerobically in an inverted position at room temperature ($28° \pm 2°C$) for 3 to 5 d. After the incubation period the total number of colony forming units (CFU) were counted. The well developed and different morphologically characterized colonies were randomly isolated and identified up to the generic level using taxonomic key of Simidu and Aiso[1]. Amylolytic, proteolytic and lipolytic activities of the bacterial isolates were also tested[2].

Population densities of THB and Cu-tolerant THB in water and sediment during different months are given in Table 1. THB grown at Cu concentration of 0 $\mu g.ml^{-1}$ varied between 22000 and 71000 $CFU.ml^{-1}$ in water and from 620000 to 3600000 $CFU.g^{-1}$ in sediment.

No appreciable difference was observed between the density of bacterial population grown at low (25 $\mu g.ml^{-1}$) and higher (50 $\mu g.ml^{-1}$) Cu concentration. In water the Cu-tolerant bacterial numbers fluctuated from 9000 to 30000 at 25 $\mu g.ml^{-1}$ and from 4000 to 55000 $CFU.ml^{-1}$ at 50 $\mu g.ml^{-1}$ Cu concentrations. The Cu-tolerant THB populations in sediment samples ($CFU.g^{-1}$) fluctuated from 70000 to 160000 at 25 $\mu g.ml^{-1}$ and from 70000 to 190000 at 50 $\mu g.ml^{-1}$. The THB population and Cu-tolerant forms were higher in sediment samples than in water samples.

The ratio in number of copper tolerant bacteria to THB was very high in water and sediment samples. The range for the water sample was 0.03-0.77 whereas for sediment sample was very low and fluctuated from 0.05 to 0.30. In both water and sediment samples, maximum ratio was observed in December (monsoon) and January respectively.

The generic composition and physiological groupings of randomly selected bacterial isolates is given in Table 2. Among the gram-positive bacteria *Micrococcus* was predominant followed by *Corynebacterium* and the gram-negative bacteria *Vibrio* was dominant. Rest of them were *Pseudomonas, Bacillus, Achromobacter* and *Flavobacterium/Cytophaga* group. About 225 strains of Cu-tolerant bacteria were isolated from both the concentrations. Forms belonging to the genera *Vibrio, Micrococcus, Corynebacterium, Pseudomonas* were predominant, followed by *Achromobacter* and *Flavobacterium/Cytophaga* group.

More than 60% of bacterial isolates belonging to the genera *Achromobacter* and *Flavobacterium/Cytophaga* group exhibited amylolytic activity, whereas in *Vibrio* and *Corynebacterium* only 50% of the isolates exhibited amylolytic activity (Table 2). More than 40% of the isolates belonging to the genera *Pseudomonas* and *Flavobacterium/Cytophaga* group was capable of hydrolysing gelatin, whereas only in *Vibrio* majority of the isolates (43.3%) exhibited lipolytic activity. This was followed by the isolates of *Achromobacter, Micrococcus* and *Corynebacterium*. The isolate belonging to the genera *Bacillus* exhibited all the 3 activities – amylolytic, gelatinolytic and lipolytic.

Estuarine waters and sediments are generally rich with organic matter. Hence the bacterial densities are always very high in these samples. Cu-tolerant bacteria occurred in all the samples during the entire period of study. It has also been pointed out by Bianchii and Colwell[3] that even a metal concentration of < 1 ppm can increase or produce heavy metal resistant bacteria in the environment.

In Vellar estuary[4] Cu is present at considerable level (14-24 ppm) and it may be responsible for the production of Cu-tolerant forms in this estuary. Cu is readily sorbed by bacteria in seawater[5] and it is toxic under anaerobic conditions than under aerobic conditions[6]. High occurrence of Cu-tolerant forms in monsoon may be due to the transportation of the Cu-tolerant forms that were present in the paddy fields into the estuary along with flood water.

The dominance of *Vibrio, Micrococcus* and *Corynebacterium* groups, which are mesophilic is probably due to high temperature and their association to the particulate organic matter[7]. Cu-tolerant bacteria were found mainly belonging to

Table 1—Number of total and Cu-tolerant bacteria in seawater and sediments

Months	Seawater (CFU.ml^{-1}) Cu concn. (μg.ml^{-1}) in media				Sediment (CFU.g^{-1}) Cu concn. (μg.ml^{-1}) in media			
	0 (THB) (A)	25	50 (B)	B/A	0 (THB) (A)	25	50 (B)	B/A
Oct. '88	55000	21000	8000	0.15	3600000	140000	170000	0.05
Nov.	34000	30000	26000	0.76	1500000	91000	72000	0.05
Dec.	71000	11000	55000	0.77	1000000	70000	140000	0.14
Jan. '89	43000	13000	4000	0.09	630000	160000	190000	0.30
Feb.	22000	9000	7000	0.32	620000	120000	70000	0.11
Mar.	270000	19000	9000	0.03	870000	130000	130000	0.15

THB = Total heterotrophic bacteria

Table 2—Generic composition and physiological groupings of the bacterial isolates (values in parentheses are percentage of isolates)

Genera	Total	Generic composition Cu concn. (μg.ml^{-1}) in media			Physiological groupings		
		0	25	50	Amylolytic activity	Gelatinolytic activity	Lipolytic activity
Micrococcus	93 (31.3)	15 (16.1)	45 (48.4)	33 (35.5)	30 (32.3)	27 (29)	18 (19.4)
Corynebacterium	48 (16.2)	9 (18.8)	21 (43.8)	18 (37.5)	24 (50)	15 (31.3)	6 (12.5)
Bacillus	3 (1.0)	3 (100)	0	0	3 (100)	3 (100)	3 (100)
Vibrio	90 (30.3)	18 (20)	48 (53.3)	24 (26.7)	45 (50)	33 (36.7)	39 (43.3)
Pseudomonas	21 (7.1)	3 (14.3)	9 (42.9)	9 (42.9)	6 (28.6)	9 (42.9)	0
Achromobacter	15 (5.1)	9 (60)	3 (20)	3 (20)	9 (60)	3 (20)	3 (20)
Flavobacterium/ Cytophaga group	27 (9.1)	15 (55.6)	9 (33.3)	3 (11.1)	18 (66.7)	15 (55.6)	0

the genera *Vibrio, Micrococcus, Corynebacterium* and *Pseudomonas* of which Vibrios were dominant. The inclusion of higher Cu concentration of the medium did not eliminate a particular taxonomic group completely.

The number of bacteria capable of hydrolysing various substrates is most sensitive indicator of pollution. Babich and Stotzky[8] and Tyler[9] demonstrated that the degradation of easily decomposable substrates like glucose and protein was less affected by heavy metals compared with more complex substrates like cellulose and starch. In the present study, however, such differences were not observed in the effect of functional groups of bacteria. Degradation experiments with more complex substrates like cellulose and chitin may provide useful information on the effect of Cu on the bacterial forms.

Authors thank the Director and the authorities of Annamalai University for providing facilities.

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