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Microbial Metal Tolerance in Bermuda Carbonate Sediments†

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The recovery of aerobic heterotrophic bacteria from Bermuda carbonate sediments on metal-supplemented media varied as much as 44-fold over a 15-cm depth. Distributional relationships with sulfate-reducing bacteria and sediment character indicated that metal tolerance was a function of metal bioavailability.

Heavy metals in the marine environment act as micronutrients or toxic agents for microorganisms (7, 9). The toxicity of a particular metal depends upon its concentration and speciation. Free metal ions generally are the most toxic (1, 6, 7, 21, 22). Toxicity may be reduced or eliminated when metals become associated with organic material (11, 17), are removed from solution as metal sulfides (3), or are involved in cation exchange reactions (2).

Jones (9) and Gonye and Jones (6) reported the stimulatory and inhibitory effects of metal amendments on the recovery of viable (plate-count) marine bacteria from estuarine, continental shelf, and open oceanic waters. Open-ocean isolates were more tolerant to nickel stress as evidenced by improved recovery on nickel-amended media (6). Even though the open ocean is metal sparse compared with near shore waters (4), the paucity of dissolved organic matter available for metal chelation at sea was cited as a mechanism for the increased interaction of open-ocean bacteria with free metal ions (6). Open-ocean isolates may have developed a mechanism for resisting free metal ions. The stimulation of bacterial populations by micromolar metal supplements to agar media was attributed more to the displacement of ions in the medium which were lower on the avidity series than to direct metal stimulation by the toxic supplement. However, some bacteria isolated from metal-polluted waters and sediments were more tolerant to metal stress than were those isolated from pristine environments (18, 19).

The present work was carried out as part of a multidisciplinary study of the biogeochemistry of Bermuda carbonate sediments (6a, 14, 16). We report that certain regions of Bermuda sediments harbored bacterial populations in which a large percentage of bacteria were stimulated by amendments of copper and nickel to growth media. Metal-induced recovery of aerobic heterotrophic bacteria varied with depth and revealed a relationship with sulfate-reducing bacteria.

Sediment samples were obtained from three locations in Bermuda with a hand-held sediment box corer (Plexiglas; 25 by 10 by 30 cm): Coot Pond (CP1), Mills Creek (MC1), and Devil's Hole (DH1) (14). Samples from CP1 and MC1 were collected in water less than 1 m deep at low tide. Sediments at CP1 consisted of poorly sorted calcium carbonate silts and sands. Mills Creek (MC1), the only estuary in Bermuda, contained sediments consisting of carbonate silts overlying a zone of saltwater peat. Devil's Hole (DH1) is a 25-m basin in Harrington Sound with clay-size carbonate sediments. This sample was collected by using SCUBA gear. The salinity at each site was approximately 35‰.

Horizontal sections (2 to 3 cm) of sediment were removed from cores in an N_2-filled glove bag, and homogenized portions were entered into 9-ml, sterile, 75% artificial seawater (13) dilution blanks which were prereduced (GasPak; BBL Microbiology Systems). After the samples were diluted, sulfate-reducing bacteria (SRB) were enumerated in duplicate on anaerobic spread plates (15). Aerobic heterotrophic bacteria (AB) were enumerated on duplicate spread plates of a low-nutrient medium (12) supplemented with order of magnitude variations of $CuCl_2$ or $NiCl_2$ between 10^{-6} and 10^{-3} M. Populations were compared with bacterial counts on unamended media. Colony-forming units were determined on a sediment dry-weight basis.

SRB populations increased from the surface to the 15-cm sediment depth at station CP1 (Table 1). AB populations varied with the sediment depth. Significantly greater percentages of AB were obtained in the presence of both 10^{-6} and 10^{-5} M $CuCl_2$ and $NiCl_2$ in the top 8 cm as

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TABLE 1. Recovery of AB from Coot Pond sediment on metal-supplemented media calculated as a percentage of counts on unamended media

Depth (cm)	Colony-forming units/g		% AB recovered on medium supplemented with (M):							
			Cu^{2+}				Ni^{2+}			
	SRB ($\times 10^{-3}$)	AB ($\times 10^{-6}$)	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
0–3	6.5	96	531	114	57	<0.1	114	91	45	0.17
3–6	40	12	525	183	10	<0.1	258	225	7	0.09
6–8	54	140	135	85	25	<0.1	164	107	48	0.07
8–10	51	280	89	103	18	<0.1	128	103	39	0.01
12–15	170	110	12	16	12	<0.1	12	11	4	0.001

compared with deeper sediments. At higher concentrations of $CuCl_2$ and $NiCl_2$, the metal ions were inhibitory. An inverse tendency was observed between the distribution of SRB populations and AB stimulated by the lower concentrations of copper and nickel.

In station MC1 sediments, measurable SRB were restricted to the top 8 cm of the carbonate-rich region (Table 2). AB populations varied between 2×10^9 and 4×10^9/ml in the top 6 cm, with a reduction of two orders of magnitude below 6 cm. The AB which developed on media amended with 10^{-6} to 10^{-4} M copper or nickel again were recovered in somewhat inverse proportion to the SRB populations, although the depth distributions were distinct from CP1. The recovery of AB on media supplemented with 10^{-6} M copper or nickel ions was greater than on unsupplemented controls at all sampling depths (Table 2). This recovery of metal-induced AB was apparent even when 10^{-4} M metal ions were added to the medium used to enumerate bacteria below the depth containing detectable SRB.

The tendency for an inverse relationship between metal-induced bacteria recovery and SRB was not observed in DH1 sediments (Table 3). The largest concentration of SRB was found at 0 to 3 cm, as was the highest percentage of AB recovered on metal-amended media.

Plating techniques have been criticized for underestimating the true abundance of bacteria (8, 24). We employed this method to detect a relative physiological response.

A significant correlation between the distribution of viable-count SRB and the rate of sulfate reduction was obtained in these Bermuda sediments (6a). The most obvious explanation for the depth variations in the percentage of metal-resistant bacteria and SRB abundance at CP1 and MC1 is the removal of bioavailable metal ions by sulfide precipitation due to sulfate reduction. Metal sulfides per se are not toxic to bacteria (3). The sedimentary pore waters at CP1 and DH1 are supersaturated with metal sulfides (16). If metal availability is important in determining the resistance of sediment bacteria to metal stress, the enhanced recovery of bacteria at 6 to 10 cm in MC1 sediments is indicative of metal remobilization in the peat zone (Table 2). Conversely, the fine-grained sediments in the DH1 sample were homogeneous throughout, and metal-induced bacteria recovery did not increase in deeper sediments devoid of detectable SRB. This latter result would be expected if metals near the sediment surface were removed largely during sulfate reduction, eventually buried, and not remobilized.

Although copper and nickel values for these specific cores are not available, others (W. B. Lyons, P. B. Armstrong, and H. E. Gaudette, manuscript in preparation) found that the sediments at MC1 contained copper concentrations which decreased from 149 $\mu g \cdot g^{-1}$ in the top 2 cm to 35 $\mu g \cdot g^{-1}$ at 15 cm. These were the highest values noted from four locations sampled in Bermuda. Others (18, 19) have demonstrated the increased occurrence of metal-resist-

TABLE 2. Recovery of AB from Mills Creek sediment on metal-supplemented media calculated as a percentage of counts on unamended media

Depth (cm)	Colony-forming units/g		% AB recovered on medium supplemented with (M):							
			Cu^{2+}				Ni^{2+}			
	SRB ($\times 10^{-3}$)	AB ($\times 10^{-6}$)	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
0–3	8.0	2100	276	26	0.46	<0.1	300	30	0.76	<0.1
3–6	100.0	3400	141	15	2.1	<0.1	171	10	1.3	<0.1
6–8	43.0	15	446	73	5.8	<0.1	367	80	18.0	<0.1
8–10	<2.0	49	632	224	177.0	<0.1		110	161.0	<0.1
12–15	<2.0	80	192	84	3.1	<0.1	192	46	0.8	0.08

TABLE 3. Recovery of AB from Devil's Hole sediment on copper-supplemented medium calculated as a percentage of counts on unamended medium

Depth (cm)	Colony-forming units/g		% AB recovered on medium supplemented with Cu^{2+} (M):		
	SRB ($\times 10^{-3}$)	AB ($\times 10^{-6}$)	10^{-6}	10^{-5}	10^{-4}
0–3	15	180	129	190	34
3–6	9.8	0.87	80	32	7.0
9–12	2.3	0.24	58	51	4.2
15–18	<1.0	0.15	59	29	0.41
24–27	<1.0	0.09	35	54	0.76

ant bacteria in metal-polluted sediments. The finding that the highest percentage of metal-resistant AB at MC1 was found at 8 to 10 cm (Table 2), whereas the highest copper concentrations for this area were at 0 to 2 cm, suggests the importance of metal speciation and bioavailability of metal ions in determining the response of bacteria to metal stress.

MC1 contained ~15% organic matter by weight (5) as opposed to ~1.5% at CP1 (5) and ~1.0% at DH1 (23). MC1 pore water contained twice as much dissolved organic carbon as did CP1 pore waters (18 versus 9 mg · $liter^{-1}$, respectively) (14). The high organic content of MC1 sediments provided an increased potential for metal complexation and remobilization.

In some instances, the recovery of Bermuda sediment bacteria on metal-amended media exceeded 500% of the control. This recovery is markedly larger than any previously reported for estuarine, neretic, or oceanic water samples (6, 9, 18, 19) or for clastic (terrigenous origin) estuarine sediments (18, 19). This response varied greatly with depth. It seems unlikely that the growth of these bacteria was limited by a lack of copper or nickel in the medium, since trace contamination of growth media at levels greater than 10 $\mu g \cdot liter^{-1}$ is not uncommon (10). Although organic constituents can complex metals in growth media, Ramamoorthy and Kushner (20) provided evidence that these metals are still available for bacterial uptake even though metal toxicity may be reduced or eliminated. A more plausible explanation for the stimulatory action of metal amendments is the displacement hypothesis (6, 9). Stimulatory cations displaced by more reactive metal ions probably were required by both metal-sensitive and metal-resistant bacteria, the stimulatory effect on the former being cancelled by metal toxicity. Therefore, the variation in percent recovery of AB was due to differences in relative metal resistance with sediment depth.

Bacteria residing in Bermuda sediments varied greatly in their response to metal supplements. Because of interactions among sulfide precipitation, organic matter character, and speciation of metal ions, depth variations in microbial metal tolerance should be considered when examining the effects of metal stress on sedimentary bacteria.

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