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Widespread Distribution of Ability to Oxidize Manganese Among Freshwater Bacteria

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Manganese-oxidizing heterotrophic bacteria were found to comprise a significant proportion of the bacterial community of Lake Washington (Seattle, Wash.) and Lake Virginia (Winter Park, Fla.). Identification of these freshwater bacteria showed that members of a variety of genera are capable of oxidizing manganese. Isolates maintained in the laboratory spontaneously lost the ability to oxidize manganese. A direct correlation was found between the presence of plasmid DNA and the ability of the organism to oxidize manganese.

The biological oxidation of manganese in freshwater ecosystems is generally attributed to either aquatic fungi (8) or members of the bacterial genera *Leptothrix, Pedomicrobium, Metallogenium* (10), *Hyphomicrobium* (9), and *Sphaerotilus* (6). Studies of marine bacteria (3, 7) lead to the conclusion that microbes from a large variety of other genera are also capable of oxidizing manganese. Our studies of the freshwater communities of Lake Washington, Seattle, Wash., and Lake Virginia, Winter Park, Fla., suggest that the ability to oxidize manganese is widespread among freshwater heterotrophs. The diversity of manganese-oxidizing bacteria and the instability of this trait in these organisms led us to investigate the possibility that a plasmid is involved in bacterial manganese oxidation.

Water samples collected aseptically with Cobet samplers (Hydro Products, San Diego, Calif.) were spread on K_1 agar plates containing 0.2% peptone (Difco Laboratories, Detroit, Mich.), 0.05% yeast extract (Difco), 0.02% $MnSO_4 \cdot H_2O$, and 1.5% Difco agar (W. Krumbein, personal communication). After incubation for 2 weeks, the plates were replica-plated and then stained with benzidine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.). Colonies that turned blue were scored as manganese oxidizers (4), and replicate colonies were streaked for isolation on mK_1, a modified form of K_1 medium, containing 0.015% peptone, 0.015% yeast extract, 0.02% $MnSO_4 \cdot H_2O$, and 1.5% agar. Isolates were identified according to *Bergey's Manual of Determinative Bacteriology* (2). These cultures were maintained on mK_1 agar slants stored at 4°C. Every 6 months the isolates were retested for the ability to oxidize manganese.

Enumeration of manganese-oxidizing bacteria in Lake Washington was performed over a period of 18 months. Representative results from seven sampling dates are shown in Table 1. The percentage of the heterotrophic bacterial population that could oxidize manganese varied throughout the year; no consistent seasonal or vertical patterns were found in their distribution. The percentage of the bacterial population in Lake Virginia capable of oxidizing manganese ranged from a high of 62% to a low of 6%, similar to the results found in Lake Washington, and also showed no pattern in the seasonal or vertical distribution of these organisms.

Identification of the isolates obtained from Lake Washington (Table 2) shows that manganese oxidation in freshwater can be carried out by a diverse group of organisms. All of the organisms isolated were chemoheterotrophs and appeared to have no obligate requirement for manganese oxidation. After growth for 6 months on mK_1 medium at 4°C, one of the organisms, a *Caulobacter* sp., isolated 8 June 1978, had lost the ability to oxidize manganese. By spring of 1981, of the isolates shown in Table 2 only the *Bacillus* sp. retained its ability to oxidize manganese.

Because of the widespread distribution and the spontaneous loss of this characteristic, we investigated the possibility that the ability to oxidize manganese was dependent upon the presence of a plasmid. Cultures were screened for the presence of plasmids by isopycnic centrifugation. Cells were grown in either nutrient broth (Difco) or mK_1 broth to late exponential phase. Triton X-100 was used to lyse the cells according to the procedure of R. Gill (personal communication). The DNA was precipitated with polyethylene glycol (5), placed in an ethidi-

TABLE 1. Occurrence of viable heterotrophs in Lake Washington capable of oxidizing manganese[a]

Depth (m)	No. of bacteria recovered on sampling date[b]						
	6-24-77	9-27-77	12-13-77	3-14-78	6-22-78	9-28-78	12-12-78
0	2.6 (0.15)	2.2 (12.6)	6.8 (18.1)	1.83 (4.0)	5.53 (0.04)	0.4 (28.6)	4.0 (28.3)
10	2.03 (0.13)	1.63 (16.4)	4.65 (4.8)	2.52 (9.7)	6.00 (5.6)	1.13 (40.8)	—[c]
20	1.1 (6.2)	1.40 (12.4)	5.37 (6.6)	0.95 (2.4)	3.13 (52.9)	0.83 (46.9)	6.87 (48.5)
30	2.10 (14.5)	1.27 (27.7)	5.77 (69.0)	3.33 (56.1)	2.10 (11.3)	0.53 (30.6)	—
35	0.23 (3.2)	0.93 (8.9)	5.35 (28.5)	1.98 (12.8)	5.40 (8.8)	0.50 (38.5)	—
40	0.67 (—)	1.10 (11.4)	4.65 (35.4)	1.93 (13.0)	4.80 (24.8)	0.10 (9.7)	6.20 (12.9)
45	1.37 (5.7)	1.43 (13.9)	4.40 (4.5)	3.53 (23.5)	2.80 (21.6)	0.07 (7.4)	—
50	1.47 (5.2)	1.00 (2.2)	7.83 (7.3)	1.93 (28.5)	4.40 (40.9)	0.40 (23.5)	10.20 (28.7)
55	0.67 (—)	2.77 (19.9)	8.50 (4.9)	9.83 (5.5)	6.80 (36.6)	0.90 (35.6)	—
57	2.63 (21.6)	3.57 (18.7)	10.30 (17.6)	1.20 (2.7)	12.6 (8.8)	1.20 (34.6)	—

[a] The percentage of manganese-oxidizing bacteria to 10^2 viable heterotrophs per ml is shown in parentheses. Enumeration was performed on K_1 agar.

[b] —, Values are given as 10^2 viable heterotrophs per ml.

[c] —, No sample taken.

TABLE 2. Identification of manganese-oxidizing bacteria isolated from Lake Washington

Isolate	Date isolated	Depth (m)	Comments
Bacillus sp.	4-26-78	45	
Caulobacter sp.	7-20-78	20	Rod; light yellow colonies
Caulobacter sp.	8-8-78	20	Rod; bright yellow colonies
Caulobacter sp.	10-26-78	0	Rod; white colonies
Caulobacter sp.	6-8-78	55	Rod; orange colonies
Chromobacterium sp.	6-8-78	0	
Chromobacterium sp.	6-8-78	35	
Cytophaga sp.	1-31-79	0	Attacks starch
Hyphomicrobium sp.	4-26-78	0	Precipitates manganese
Pseudomonas sp.	6-8-78	0	
Pseudomonas sp.	6-8-78	0	

um bromide-cesium chloride gradient in a Type 65 fixed-angle rotor, and spun in a Beckman L2-65B Ultracentrifuge at 40,000 rpm for 50 h (1).

The DNA banding pattern of two isolates from Lake Washington, Mn-75 (*Cytophaga* sp.) and Mn-72 (gram-negative, motile rod), showed a marked difference between cultures grown in nutrient broth and those grown in mK_1 broth. For both cultures, the mK_1-grown cells showed two distinct bands of DNA, one located at the normal position of covalently closed circular DNA and the other corresponding to the position of open circular or linear DNA (1). Cells grown on nutrient broth showed only one band, that corresponding to open circular or linear DNA. Streak plates of these cultures performed before lysis and DNA isolation showed that whereas organisms grown in mK_1 broth continued to oxidize manganese, those grown in nutrient broth gave negative results when tested with benzidine dihydrochloride on either mK_1 or nutrient agar plates.

This study shows that the ability to oxidize manganese in freshwater ecosystems is not limited to a small group of genera, but rather that this characteristic is common to a large number of aquatic heterotrophs. The widespread distribution of this characteristic, and the simultaneous loss of plasmid DNA and the ability to oxidize manganese when cultures were grown on a nutrient medium, suggest that in some aquatic bacteria manganese oxidation may be related to the presence of a plasmid. The plasmid may either be directly involved in the oxidation process by providing essential gene products or may act indirectly, possibly by altering the microenvironment in such a way as to make the chemical oxidation of manganese favorable.

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