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Effect of Temperature on Growth and Activity of *Aeromonas* spp. and Mixed Bacterial Populations in the Anacostia River

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During the winter months, total bacterial counts in the water column and in the sediment in the Anacostia River were two- to eightfold higher than at other times of the year, whereas *Aeromonas* spp. decreased in number by several orders of magnitude. This significant decrease in number in the Anacostia River during the cold months of the year can be explained by the low metabolic activity of *Aeromonas* at low temperatures.

Aeromonas spp. are frequently isolated from soil, water, and human feces (11). The ecology of *Aeromonas* in the aquatic environment has been the subject of several studies recently published (3, 5) and reported (O. P. Daily, S. W. Joseph, R. I. Walker, C. R. Lissner, and R. J. Seidler, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, B79, p. 30; C. E. Warnes, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, N80, p. 177). A unique occurrence of primary soft-tissue infection caused by two species of *Aeromonas*, *A. hydrophila* and *A. sobria*, in a professional diver conducting SCUBA operations in the Anacostia River has recently been reported (7). The Anacostia River site at which a diver training school is located has been monitored for *Aeromonas* spp., as well as for total viable, heterotrophic bacterial populations in the water column and sediment. The incidence, as well as concentration, of *Aeromonas* spp. recovered from divers and their gear was found to be related to the number of *Aeromonas* spp. in the water column (9).

The objective of the investigation reported here was to examine factors influencing the survival of *Aeromonas* spp. in the Anacostia River, in order to determine those controlling seasonal incidence of *Aeromonas* spp. in the natural environment.

Water samples were collected with a sterile Niskin sampler (General Oceanics, Miami, Fla.) 1 m below the surface and 1 m above the sediment. Sediment was collected using a small, hand-operated grab sampler (Wildlife Supply Co., Mackinaw, Mich.). Water and sediment samples were returned to the laboratory for examination immediately after collection, with less than 2 h lapsing before bacteriological analyses were carried out.

Total viable, aerobic, heterotrophic counts were made by spread plating on plate count agar (8). For direct counts, the procedure of Hobbie et al. was employed (6). *Aeromonas* spp. were isolated as previously described (9).

An *Aeromonas* sp. strain isolated from Anacostia River water and unfiltered Anacostia River water was incubated overnight in tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C. Cells were harvested, washed twice with 20 ml of freshly filtered river water, and suspended in filtered river water. Glucose and acetate uptake were determined by the method of Vaccaro and Jannasch (10), with some modifications as described by Cavari and Hadas (2).

Water and sediment samples were collected over a 2-year period at the Anacostia River diving training site. Both direct and viable counts of the aerobic, heterotrophic bacteria were made, as well as counts of *Aeromonas* spp. Comparison of the total numbers of bacteria, obtained by direct and viable-count measurements, found in the warm summer months with results obtained for the colder months of the winter revealed an increase of about two- to eight-fold in the winter, compared with the summer. At the same time, the *Aeromonas* spp. counts decreased by several orders of magnitude in both the water column and sediment. A similar effect of temperature on *Aeromonas* has been reported by Hazen (4) and Warnes (Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, N80, p. 177). The number of *Aeromonas* spp. in the Anacostia River in the summer months was very large, compared to the concentrations of other bacterial species found in natural waters. Interestingly, several other sites in the United States have been shown to harbor high numbers of *Aeromonas* (9).

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Experiments performed to determine the cause of the observed decrease in counts of *Aeromonas* spp. during the cold months of the year employed pure cultures of an *Aeromonas* sp. strain which had originally been isolated from the Anacostia River. A mixed culture obtained from Anacostia River water was also employed. The cells were incubated at 4 and 25°C. At zero time, after 1 h, and at 48 h, samples were taken for enumeration of viable cells. As can be seen from Table 1, transfer of the Anacostia River mixed bacterial culture from a rich growth medium to filtered river water did not result in a significant change in viable count within the 1-h period of incubation at 4 and 25°C. At 25°C, the viable count increased ca. 10-fold after incubation for 48 h.

The same treatment, when applied to the *Aeromonas* sp. isolate, resulted in an immediate decrease in the cell count of about 2 logs. Incubation at 25°C for 48 h resulted in an increase in the viable count of about 2 logs, but when incubation was continued at 4°C for 48 h, an additional decrease of about 50% in cell number was observed. The difference in survival of mixed populations of river bacteria and *Aeromonas* spp. at low temperatures may arise from differences in cell activity. River bacteria and the *Aeromonas* spp. isolates were assayed for heterotrophic activity at 4 and 25°C by measuring rates of uptake and mineralization of ^{14}C-labeled glucose and acetate.

In the case of mixed cultures of river bacteria, the rate of glucose uptake was 3.7-fold higher at 25°C than at 4°C (Table 2). Similar results were reported for *Pseudomonas fluorescens*, which demonstrated a V_{max} 3.2-fold higher in summer than in winter (1). With *Aeromonas* spp., however, 8.4-fold higher activity was noted (Table 2). This finding demonstrates a greater sensitivity of *Aeromonas* spp. to changes in temperature, compared with the mixed bacterial populations studied. Because of the observed response of *Aeromonas* spp. to temperature, activity measured at 4° C was ca. 10-fold lower than the activity of the mixed bacterial population from the Anacostia River, when glucose uptake and mineralization were measured (Table 2). An even more dramatic observation was that of acetate uptake, in which case no uptake was detected at 4°C (Table 2). It appears, therefore, that at low temperatures, the *Aeromonas* spp. are unable to supply energy requirements for maintenance and thus are not recovered during the winter months.

It can be concluded, therefore, that when *Aeromonas* spp. enter bodies of water in temperate environments, via sewage effluent or other sources, a significant decrease in the number of viable cells can occur during those seasons of the year when the water temperature is low. Based on heterotrophic activity measurements, the rate of nutrient uptake by *Aeromonas* will be too low at temperatures below ca. 15°C to compete with psychrotolerant microorganisms present in water and sediment of the natural aquatic habitat. At temperatures below 4°C, the rate of nutrient uptake by *Aeromonas* strains in

TABLE 1. *Total viable counts of Anacostia River bacteria and of Aeromonas spp. after transfer from a rich medium to filtered river water*

Incubation temp (°C)	Counts of river bacteria after time of incubation (h):			Counts of *Aeromonas* sp. after time of incubation (h):		
	0	1	48	0	1	48
4	4.0×10^5	3.2×10^5	1.05×10^5	4.65×10^5	1.0×10^3	5.5×10^2
25	4.0×10^5	1.05×10^5	1.26×10^6	4.65×10^5	1.5×10^3	2.3×10^5

TABLE 2. *Comparison of rates of glucose and acetate uptake by river bacteria and by Aeromonas spp. incubated at two different temperatures*

Isolate	Incubation temp (°C)	Glucose expt			Acetate expt		
		Viable count ($\times 10^5$)	Glucose uptake		Viable count ($\times 10^5$)	Acetate uptake	
			V_{max} ($\mu g \cdot liter^{-1} \cdot h^{-1}$)	V_{max}/TVC[a] ($\times 10^5$)		V_{max} ($\mu g \cdot liter^{-1} \cdot h^{-1}$)	V_{max}/TVC ($\times 10^5$)
River bacteria	4	5.2	22.0	4.23	2.9	0.79	0.27
	25	5.1	80.0	15.69	2.9	15.9	5.48
Aeromonas sp.	4	0.9	0.37	0.41	4.65	0	0
	25	0.9	3.10	3.44	4.70	25.6	5.45

[a] TVC, Total viable count.

general will be too low to meet their energy requirement for cellular maintenance, which is very likely the reason for their significantly reduced incidence in Anacostia River water and sediment.

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