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Chemotactic Behavior of *Aeromonas hydrophila*

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Abstract. *Aeromonas hydrophila* exhibits chemotactic behavior to amino acids and carbohydrates. Ten isolates of *A. hydrophila* from water and infected fish showed the same basic response profile. Maximum chemotaxis to amino acids occurred at 10^{1} *M*, though concentrations of substrate as low as 10^{-3} *M* elicited a positive response. Carbohydrates induced a maximal chemotaxis at lower concentrations (10^{-2} *M*), with the threshold for responsiveness occurring at 10^{-4} *M*. Carbohydrates gave significant motility in *A. hydrophila* isolates, while amino acids did not. *Aeromonas hydrophila* isolates were maximally chemotactic to substrates they could metabolize. Motility and chemotaxis by *A. hydrophila* may explain spatial heterogeneity and concentration phenomena observed in natural aquatic environments.

Aeromonas hydrophila is a ubiquitously occurring bacteria in natural and polluted aquatic environments [13, 23]. It has been reported in high densities from open ocean waters [31], estuaries [17, 23], all types of freshwater habitats [23], including thermal environments [9, 22], and tropical rain forest watersheds [18]. In addition, *A. hydrophila* has been shown to cause disease in snails [28], fish [9, 10, 20, 29], lizards [27], alligators [12], amphibians [8], turtles [33], cattle [36], and more recently, humans [5, 35].

Temperature is an important environmental factor in regulating densities of *A. hydrophila* [9, 22]. *Aeromonas hydrophila* is always associated with water quality characteristics that also support phytoplankton populations [16, 18, 32]. Indeed, densities of *A. hydrophila* can be predicted in North Carolina reservoirs by models that incorporate ammonia, total nitrogen, chlorophyll A, orthophosphates, dissolved oxygen, and temperature [17, 19]. The association between phytoplankton and densities of *A. hydrophila* was also indicated through the observation of 100-fold increases in surface microlayer densities of *A. hydrophila* taken near aquatic macrophytes [16].

One of the possible mechanisms by which *A. hydrophila* may become differentially distributed in aquatic environments and/or by which *A. hydrophila* may locate susceptible hosts is through chemotaxis. This was suggested for bacteria in general by Chet and Mitchell [3] in their review of the ecological aspects of bacteria motility. Other work has shown that *A. hydrophila* is distinctly chemotactic to fish surface mucus [21]. Most *A. hydrophila* isolates have a single polar flagella, though peritrichous forms have been reported [7]. The monotrichous forms would most certainly contribute to a chemotactic capability for this bacterium. Since the basic chemotactic behavior of *A. hydrophila* has not previously been examined, we have attempted to define the basic chemotactic responsiveness in several environmental isolates of *A. hydrophila*.

Materials and Methods

Isolation of bacteria. During the course of several environmental investigations of *A. hydrophila* [11, 16, 24], random, single colonies were selected from count estimates. R-S medium [34], which is known to be 94% presumptive for *A. hydrophila*, was used for primary isolation. Yellow colonies were selected after 24 h incubation at 35°C. These isolates were further characterized and confirmed as *A. hydrophila* using API-20E (Analytab Products, Plainview, NY), oxidase tests, 0/129 sensitivity, serology, and fluorescent antibody (for details see Fliermans and Hazen [11] and Hazen et al. [24]). Isolates were taken from a variety of sources including water, sediment, fish, alligators, and humans. All isolates, after being completely characterized as *A. hydrophila*, were grown in carbohydrate-free media, dispensed to small vials with equal volumes of glycerin, and stored at −70°C until used. This method was found to preserve isolates for more than 2 years (Hazen, unpublished data).

Chemotaxis assay. The technique used was a modification of Adler's [1] technique [21]. Isolates were grown in 3% TSB for 24

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Table 1. Chemotactic index for amino acids[a]

Substrate	Concentration (*M*)				Motility
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
Asparagine	*1.36*	*1.37*	*1.28*	*1.17*	*1.27*
Valine	*1.75*	*1.67*	*1.15*	1.01	1.05
Lysine	*1.60*	*1.56*	*1.37*	1.09	0.82
Cysteine	*1.51*	*1.47*	*1.12*	1.09	1.05
Alanine	*1.42*	*1.34*	*1.17*	0.93	1.12
Ornithine	*1.70*	*1.63*	1.09	1.04	0.97
Proline	*1.52*	*1.38*	1.10	0.82	1.08
Glycine	*1.61*	1.05	1.00	0.94	0.95

[a] All values are the mean of ten determinations; standard deviations of the mean were always less than 0.15. Values in italics are significant as determined by analysis of variance. Chemotactic index = experimental cell counts/control cell counts.

Table 2. Chemotactic index for carbohydrates[a]

Substrate	Concentration (*M*)				Motility
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
Maltose	*1.39*	*1.80*	*1.53*	1.10	*1.24*
Sucrose	*1.64*	*1.20*	*1.17*	0.94	0.80
Salicin	ND	*1.60*	*1.19*	1.03	1.20
Lactose	*1.25*	*1.22*	*1.31*	0.96	0.71
Mannitol	*1.61*	*1.53*	1.06	0.96	1.18
Raffinose	*1.34*	0.90	0.87	ND	1.32
Arabinose	1.07	0.97	0.98	ND	2.24
Xylose	1.07	0.97	ND	ND	1.23

[a] All values are the mean of ten determinations; standard deviations of the mean were always less than 0.15. Values in italics are significant as determined by analysis of variance. Chemotactic index = experimental cell counts/control cell counts.

h and harvested by centrifugation at 6000 *g* for 10 min at 4°C. The pellet was resuspended in an equal volume of PBS (pH 7), and centrifuged again; the washing step was repeated twice more. Final resuspension was in chemotactic buffer (KPB) after Adler [1], adjusting the cell density to 10^9 cells ml^{-1}. The bacterial suspension was dispensed in 0.25-ml quantities to 6 × 12 mm test tubes. Into each test tube containing cell suspension was introduced a capillary tube, closed at one end, containing the substrate to be tested [1]. After incubating the capillary tube in the bacterial suspension for 1 h at 35°C (35°C is the optimum growth temperature for *A. hydrophila*), the capillary tubes were removed. The contents were then washed into a diluting vial containing 10 ml of sodium azide–free isotonic diluting solution (Fisher Scientific Company, Fairlawn, NJ) and counted using a model ZF Coulter counter (Coulter Electronics, Hialeah, FL). Ten replicates were simultaneously studied for every substrate tested. Each substrate was tested at five different molarities (from 1 to 10^{-4}); also included was a KPB control and a motility test. All dilutions of substrates were made with KPB. The KPB control was performed exactly as described above for substrate tests except that the capillary tube was filled with KPB. The motility test consisted of a KPB control in which 0.01 ml of the undiluted test substrate was added directly to the bacterial suspension prior to incubation with the capillary tube containing KPB. Motility is here defined as the ability to increase activity in the absence of a specific point source of substrate. If motility occurs, the test counts in the capillary tube will significantly exceed those in the KPB control capillary.

Data analysis. All dilutions of each substrate, the KPB control, and motility test were examined for differences using analysis of variance. All counts were transformed with log (x) before analysis, to reduce heteroscedascity as determined by skew and kurtosis. Group means found to be significantly different were further differentiated from each other using a Student-Newman-Keuls multiple-range test. Any probability less than or equal to 0.05 was considered significant [37].

Results

Chemotactic responses to amino acids (Table 1) were greater than the chemotactic responses to carbohydrates (Table 2). However, responses to carbohydrates were much more variable (Fig. 1) than were responses to amino acids (Fig. 2). The motility test was positive for most carbohydrates, while only asparagine among the amino acids tested produced a positive motility test (Tables 1 and 2).

The sensitivity of the chemotactic responses of *A. hydrophila* was low, with a threshold concentration of 10^{-2} *M* (Table 3). *Aeromonas hydrophila* was most sensitive to asparagine. The carbohydrates elicited a significant response at lower concentrations of substrate (10^{-3} *M* or less).

No significant differences in chemotactic behavior occurred among isolates from infected fish (BSM, LN1, CR10, 849L, 33A) and water (HR, FT4, FT1, LNH, MR). If the strain being studied could metabolize (use as a carbon and/or energy source) the substrate under consideration, the minimum molarity that would elicit a significant response was significantly less than that of nonmetabolizable test susbstrates. When the substrate could be metabolized, the mean threshold concentration was $10^{-2.5}$ *M* ($N = 35$), versus $10^{-0.7}$ *M* ($N = 25$) for substrates that could not be metabolized.

Discussion

Aeromonas hydrophila exhibited significant chemotaxis to some amino acids at concentrations as low as 10^{-3} *M*. Adler [1] and others [2, 4, 6, 26, 30] report sensitivities as low as 10^{-6} *M* for amino acids by *Escherichia coli* and other bacteria; however, the statistical significance of some of these results is ambiguous. Maximal chemotaxis by *A. hydrophila* usually occurred at the highest concentration of substrate that was tested; however, maximal responses occasionally occurred at lower concentrations. This pattern is similar to that observed by

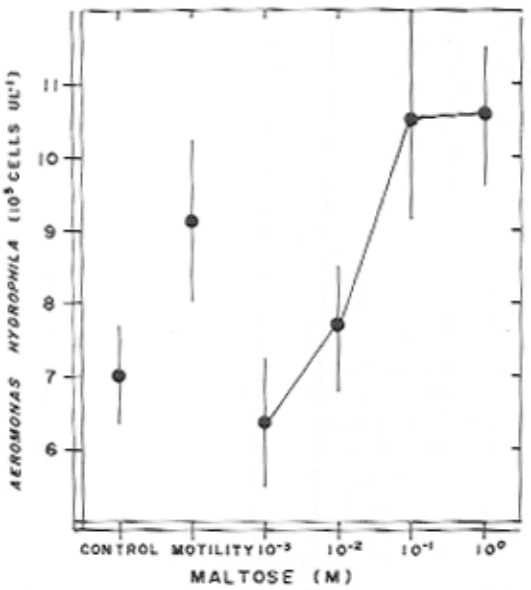

Fig. 1. Chemotactic response profile of *Aeromonas hydrophila* to different concentrations of maltose. All points represent the mean of ten determinations; bars are one standard deviation.

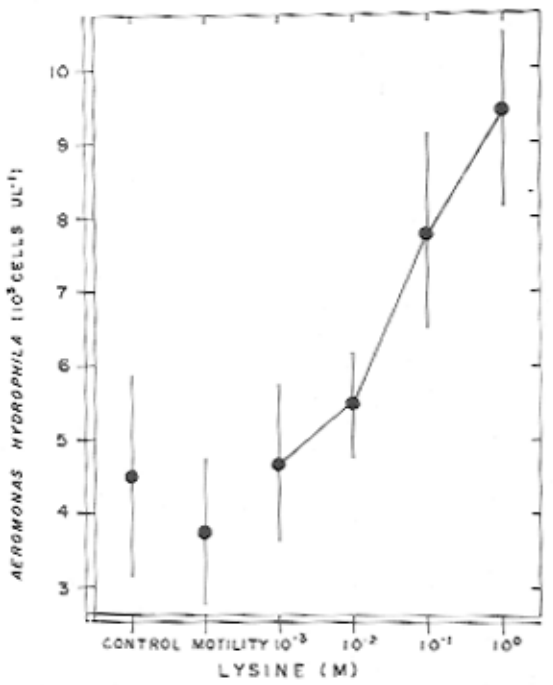

Fig. 2. Chemotactic response profile of *Aeromonas hydrophila* to different concentrations of lysine. All points represent the mean of ten determinations; bars are one standard deviation.

Adler [1] for *E. coli*. Thus, the range of concentrations of substrate that elicited chemotaxis by *A. hydrophila* is similar to that observed for *E. coli* except that *A. hydrophila* is less sensitive. The weak-acid repellent taxis of bacteria reported by Kihara and Macnab [25] may explain the results for some of the substrates at low concentrations. Adler [1] and others [14, 15] also demonstrated that a substance doesn't have to be metabolized or transported to be chemotactic in bacteria. However, *A. hydrophila* is more responsive chemotactically to substances that it can metabolize. The apparent chemotaxis of *A. hydrophila* to carbohydrates is largely attributable to an increase in motility in the presence of carbohydrates and is not a true chemotactic response. Lauffenburger et al. [26] point out in their mathematical model of random motility and bacterial growth that even random motility could promote dispersal from nutrient-poor regions and prevent dispersal from nutrient-rich regions. Though chemotaxis to substances that could not be metabolized was demonstrated for *A. hydrophila*, significantly better responses to substances that could be metabolized demonstrate the dependence of *A. hydrophila* motility and chemotaxis on an adequate energy source; this observation confirms the prediction made by Lauffenburger et al. [26].

Considering the ubiquitous nature of the distribution of *A. hydrophila*, it is likely that some of the heterogeneity observed in aquatic environments, especially the association with phytoplankton which would represent a rich nutrient source [16], is due to the chemotactic behavior of *A. hydrophila*. It remains to be shown, however, whether this is a valid premise. Isolation of various compounds from

Table 3. Minimum substrate concentration for significant chemotactic index for strains of *Aeromonas hydrophila* by substrate[a]

	Isolate									
Substrate	BSM	HR	FT4	FT1	LNH	LN1	CR10	849L	MR	33A
Maltose	2	2	3*	3*	2*	2*	3	3	2*	2*
Raffinose	3*	3*	3*	3*	4	ND	3*	3*	3*	1*
Arabinose	4*	ND	4*	4	4*	ND	3*	4*	4*	1*
Xylose	4*	4*	4*	4*	3*	3*	3*	4*	3*	1*
Lysine	3	3	2	2	2	ND	2	3	2	3
Ornithine	2	2	2	2	4	ND	3	2	2	2

[a] All values are the reciprocal log molarities of the smallest molarity that could elicit a significant response. * Significant motility test.

natural aquatic environments and testing of their capability to stimulate chemotaxis by *A. hydrophila* is needed in order to confirm or reject this hypothesis.

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Literature Cited

1. **Adler, J.** 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. Journal of General Microbiology **74**:77–91.
2. **Alweiss, B., Dostal, J., Carey, K. E., Edwards, T. F., Fretter, R.** 1977. The role of chemotaxis in the ecology of bacterial pathogens of mucosal surfaces. Nature **266**:448–450.
3. **Chet, I., Mitchell, R.** 1976. Ecological aspects of microbial chemotactic behavior. Annual Review of Microbiology **30**:221–239.
4. **Currier, W. W., Strobel, G. A.** 1976. Chemotaxis of *Rhizobium* spp. to plant root exudates. Plant Physiology **57**:820–823.
5. **Davis, W. A., Kane, J. G., Garaguis, V. G.** 1978. Human *Aeromonas* infections: a review of the literature and a case report of endocarditis. Medicine (Baltimore) **57**:267–277.
6. **De Jong, M. H., van der Drift, C.** 1978. Control of the chemotactic behavior of *Bacillus subtilis* cells. Archives of Microbiology **116**:1–8.
7. **Eddy, B. P.** 1960. Cephalotrichous, fermentative gram-negative bacteria: the genus *Aeromonas*. Journal of Applied Bacteriology **23**:216–249.
8. **Emerson, H., Norris, C.** 1905. "Red Leg"—an infectious disease of frogs. Journal of Experimental Medicine **7**:32–60.
9. **Esch, G. W., Hazen, T. C.** 1978. Thermal ecology and stress: a case history for red-sore disease in largemouth bass *(Micropterus salmoides)*. In: Thorpe, J. H., Gibbons, J. W. (eds.), Energy and environmental stress in aquatic systems. DOE Symposium Series CONF-771114. Springfield, VA: NTIS.
10. **Esch, G. W., Hazen, T. C.** 1980. Stress and body condition in a population of largemouth bass, implications for red-sore disease. Transactions of the American Fisheries Society **109**:532–536.
11. **Fliermans, C. B., Hazen, T. C.** 1980. Immunofluorescence of *Aeromonas hydrophila* as measured by fluorescence photometric microscopy. Canadian Journal of Microbiology **26**:161–168.
12. **Gorden, R. W., Hazen, T. C., Esch, G. W., Fliermans, C. B.** 1979. Isolation of *Aeromonas hydrophila* from the American alligator, *Alligator mississippiensis*. Journal of Wildlife Diseases **15**:239–243.
13. **Grabow, W. O., DuPreez, M.** 1979. Comparison of m-Endo, LES, MacConkey and Teepol media for membrane filtration counting of total coliform bacteria in water. Applied and Environmental Microbiology **38**:351–358.
14. **Hazelbauer, G. L., Engstrom, P.** 1981. Multiple forms of methyl-accepting chemotaxis proteins distinguished by a factor in addition to multiple methylation. Journal of Bacteriology **145**:35–42.
15. **Hazelbauer, G. L., Engstrom, P., Harayama, S.** 1981. Methyl-accepting chemotaxis protein III and transducer gene trg. Journal of Bacteriology **145**:43–49.
16. **Hazen, T. C.** 1979. The ecology of *Aeromonas hydrophila* in a South Carolina cooling reservoir. Microbial Ecology **5**:179–195.
17. **Hazen, T. C.** 1983. A model for the density of *Aeromonas hydrophila* in Albemarle Sound. Microbial Ecology **9**:137–153.
18. **Hazen, T. C., Aranda, C. F.** 1981. The relationship between the distribution and abundance of bacteria and water quality in the Rio Mameyes watershed. Seventh Natural Resources of Puerto Rico Symposium. pp. 87–111. Department of Natural Resources, Commonwealth of Puerto Rico, San Juan.
19. **Hazen, T. C., Esch, G. W.** 1983. Effect of effluent from a nitrogen fertilizer factory and a pulp mill on the distribution and abundance of *Aeromonas hydrophila* in Albemarle Sound, North Carolina. Applied and Environmental Microbiology **45**:31–42.
20. **Hazen, T. C., Esch, G. W., Raker, M. L.** 1981. Agglutinating antibody to *Aeromonas hydrophila* in wild largemouth bass. Transactions of the American Fisheries Society **110**:514–518.
21. **Hazen, T. C., Esch, G. W., Dimock, Jr., R. V., Mansfield, A.** 1982. Chemotaxis of *Aeromonas hydrophila* to the surface mucus of fish. Current Microbiology **7**:371–376.
22. **Hazen, T. C., Fliermans, C. B.** 1979. Distribution of *Aeromonas hydrophila* in natural and man-made thermal effluents. Applied and Environmental Microbiology **38**:166–168.
23. **Hazen, T. C., Fliermans, C. B., Hirsch, R. P., Esch, G. W.** 1978. Prevalence and distribution of *Aeromonas hydrophila* in the United States. Applied and Environmental Microbiology **36**:731–738.
24. **Hazen, T. C., Raker, M. L., Esch, G. W., Fliermans, C. B.** 1978. Ultrastructure of red-sore lesions on largemouth bass *(Micropterus salmoides)*: the association of the peritrich *Epistylis* sp. and the bacterium *Aeromonas hydrophila*. Journal of Protozoology **25**:351–355.
25. **Kihara, M., Macnab, R. M.** 1981. Cytoplasmic pH mediates pH taxis and weak-acid repellent taxis of bacteria. Journal of Bacteriology **145**:1209–1221.
26. **Lauffenburger, D., Aris, R., Keller, K. H.** 1981. Effects of random motility on growth of bacterial populations. Microbial Ecology **7**:207–227.
27. **Marcus, L. C.** 1971. Infectious diseases of reptiles. American Veterinary Medicine Association **159**:1626–1631.
28. **Mead, A. R.** 1969. *Aeromonas liquefaciens* in the leukodermia syndrome of *Achatina fulica*. Malacologia **9**:43.
29. **Miller, R. M., Chapman, W. R.** 1976. *Epistylis* and *Aeromonas hydrophila* infections in fishes from North Carolina reservoirs. Progressive Fish-Culturist **38**:165–168.
30. **Okon, Y., Cakmakci, L., Nur, I., Chet, I.** 1980. Aerotaxis

and chemotaxis of *Azospirillum brasilense:* a note. Microbial Ecology **6:**277–280.

31. **Peele, E. R., Singleton, F. L., Deming, J. W., Cavari, B., Colwell, R. R.** 1981. Effects of pharmaceutical wastes on microbial populations in surface waters of the Puerto Rico dump site in the Atlantic Ocean. Applied and Environmental Microbiology **41:**873–879.
32. **Rippey, S. R., Cabelli, V. J.** 1980. Occurrence of *Aeromonas hydrophila* in limnetic environments: relationship of the organism to trophic state. Microbial Ecology **6:**45–54.
33. **Shotts, E. B., Gaines, J. L., Martin, C., Prestwood, A. K.** 1972. *Aeromonas*-induced deaths among fish and reptiles in an eutrophic inland lake. Journal of the American Veterinary Medicine Association **161:**603–607.
34. **Shotts, E. B., Rimler, R.** 1973. Medium for the isolation of *Aeromonas hydrophila*. Applied Microbiology **26:**550–553.
35. **Trust, T. J., Chipman, D. C.** 1979. Clinical involvement of *Aeromonas hydrophila*. Canadian Medical Association Journal **120:**942–947.
36. **Wohlegemuth, K., Pierce, R. L., Kirkbride, C. A.** 1972. Bovine abortion associated with *Aeromonas hydrophila*. Journal of the American Veterinary Medicine Association **160:**1001.
37. **Zar, J. H.** 1974. Biostatistical analysis. Englewood Cliffs, NJ: Prentice-Hall.