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Chemotaxis in *Pseudomonas aeruginosa*

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A chemotaxis system for *Pseudomonas aeruginosa* was defined by using the method of Adler. Cells were attracted to compounds in the order ammonium chloride > amino acids > organic acids. Two sugars were assayed and elicited no response. Comparisons with other model systems are discussed.

In recent years investigations of bacterial chemotaxis have centered around three model systems, the peritrichously flagellated, fermentative organisms *Escherichia coli, Salmonella typhimurium,* and *Bacillus subtilis* (2, 5, 7). *Pseudomonas aeruginosa* was chosen for this study because it is markedly different from the above systems by being monotrichously flagellated and obligately respiratory. In addition, *P. aeruginosa* is an interesting and important bacterium because of its extraordinary nutritional diversity (10), its reasonably well-described genetic system (6), and its medical significance as an opportunistic pathogen (8). *P. aeruginosa* X from the Indiana University culture collection was used to determine the optimum conditions for chemotaxis by using the technique described by Adler (1). The chemotaxis and wash media contained 10^{-2} M potassium phosphate buffer (pH 7.0), 10^{-3} M $MgSO_4$, and 10^{-4} M dipotassium ethylenediaminetetraacetate. Terms and definitions are those used by Mesibov and Adler (9). The standard deviation for replicate determinations for a single assay was about 14%. The data reported are based on two or more averaged points (Table 1).

The rates of accumulation of bacteria in the capillaries in response to L-leucine are shown in Fig. 1. The initial rate was constant and dependent on the concentration of leucine, a finding similar to those described for *E. coli* (1) and *B. subtilis* (11). *P. aeruginosa,* however, exhibited no initial lag before entering the capillaries.

Figure 2 shows that the response to L-leucine was directly proportional to the bacterial concentration of the pond over a limited range. The same behavior was observed in *E. coli* and *B. subtilis* (1, 11). Adler (1) pointed out that the plateau is caused by some unknown limiting factor(s), such as the orifice size of the capillary or the sinking of the bacterial cloud outside the orifice.

Chemotactic responses for 10 compounds were investigated (Fig. 3 and Table 1). The concentration response curves for four amino acids in Fig. 3 show the classical *E. coli*-type response to attractants (1, 9). For these amino acids, the minimum threshold of 1.2×10^{-4} M was in the same range as the other model systems, whereas the peak responses were much greater for *P. aeruginosa.*

As with *E. coli* (1), metabolism of a compound by *P. aeruginosa* is not essential for taxis. DL-Trifluoroleucine (Table 1) is not metabolized by *P. aeruginosa* (K. Andrews, personal communication), yet it had the same threshold value as L-leucine and nearly the same peak response. Unexpectedly, the readily metabolized sugars galactose and glucose elicited no response above background (Table 1). These same sugars were good attractants for *E. coli* (3) and *Pseudomonas lacrymans* (4).

The presence of the amino group appears to have a strong effect on chemotaxis in *P. aeruginosa* (Table 1). When the amino group was replaced by hydrogen, the aspartate-succinate pair showed the same threshold, but succinate elicited a pronounced reduction in the peak response. For the glutamate-glutarate pair, gluta-

TABLE 1. *Chemotaxis toward 10 compounds by P. aeruginosa*

Compound	Threshold molarity	Maximum response	
		Peak (M)	No. of bacteria[a]
L-Arginine	7×10^{-7}	10^{-2}	791,000 (3)[b]
L-Aspartate	1.2×10^{-4}	5×10^{-2}	485,000 (6)
L-Glutamate	1.5×10^{-4}	10^{-1}	480,000 (5)
L-Leucine	2.0×10^{-6}	10^{-3}	458,000 (5)
DL-Trifluoroleucine	2.0×10^{-6}	10^{-2}	328,000 (2)
Succinate	1.9×10^{-4}	10^{-2}	43,000 (6)
Glutarate	1.2×10^{-5}	10^{-2}	232,000 (3)
Galactose	$>10^{-1}$		
Glucose	$>10^{-1}$		
Ammonium chloride	6.0×10^{-8}	10^{-4}	7,600 (2)

[a] Background values from 1,800 to 4,300 were subtracted from all appropriate responses.
[b] Number in parentheses is the number of replicate points averaged to determine the response value.

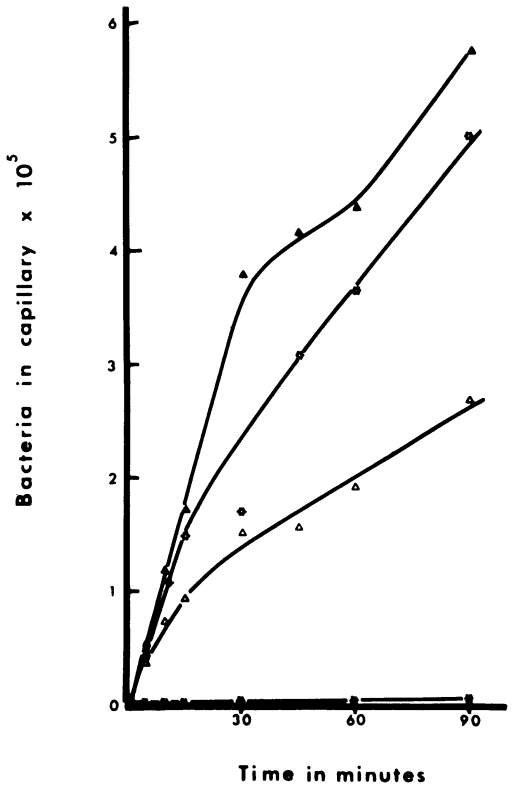

FIG. 1. *Rates of accumulation of bacteria in capillaries containing various concentrations of* L-*leucine in chemotaxis medium. Bacterial cells were grown to mid-exponential phase at 30°C in a medium containing (per liter of distilled water):* KH_2PO_4, *2.72 g;* $(NH_4)_2SO_4$, *1.0 g;* $MgSO_4 \cdot 7H_2O$, *0.58 g;* $CaCl \cdot 2H_2O$, *0.067 g;* $(NH_4)_6Mo_7O_{22} \cdot 4H_2O$, *0.2 mg;* $FeSO_4 \cdot 7H_2O$, *0.02 g; nitrilotriacetate, 0.2 g; and succinate, 10 g. Cells were harvested and washed twice by centrifugation at 3,020 × g for 10 min at room temperature. The cells were gently resuspended in 10 ml of chemotaxis medium to a final concentration of about 6 ×* 10^7 *bacteria per ml. A 0.2-ml amount of this suspension was used to fill the pond in the chemotaxis apparatus (1). Capillary tubes (1-μl Drummond Microcaps) were filled with chemotaxis medium containing an attractant by the method of van der Drift and de Jong (11) and inserted into the pond. Assays were run for specified periods of time at 30°C. The capillaries were then removed, rinsed, crushed in ice-cold 0.1% tryptone broth diluent, and plated on 1% tryptone agar for standard plate counts. Attractant concentrations were:* 10^{-4} *M* L-*leucine* (△), 10^{-3} *M* L-*leucine* (▲), 10^{-2} *M* L-*leucine* (☆), *and background (no attractant)* (★).

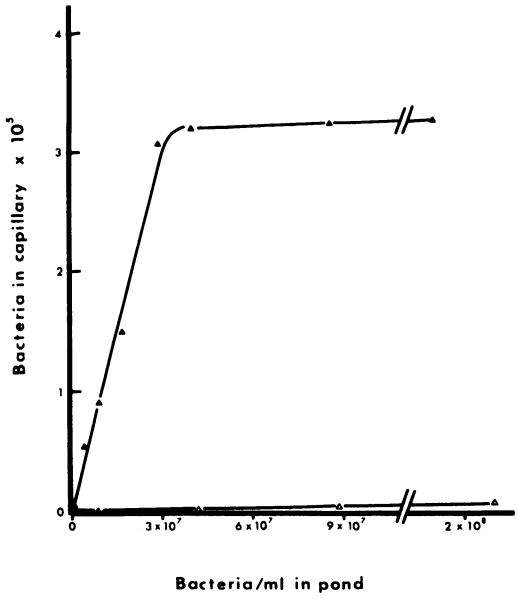

FIG. 2. *Effect of bacterial pond concentration on the response to* 10^{-3} *M* L-*leucine* (▲) *and background* (△) *in a 30-min assay.*

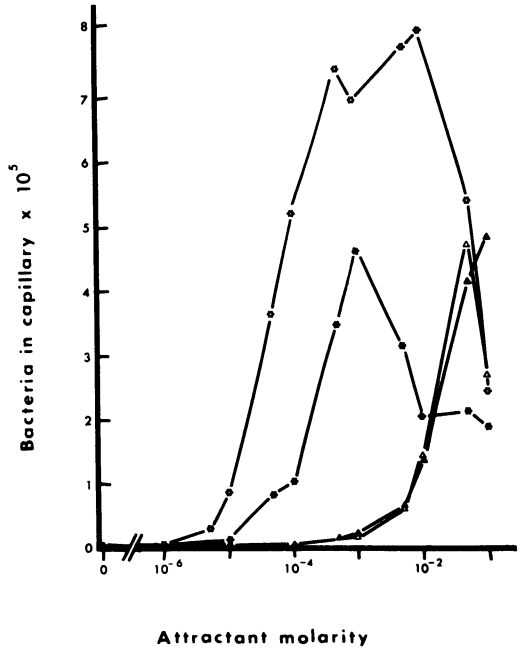

FIG. 3. *Concentration response curves for P. aeruginosa toward* L-*arginine* (☆), L-*leucine* (★), L-*aspartate* (△), *and* L-*glutamate* (▲) *in a 30-min assay.*

rate was almost 10 times more attractive than glutamate, as determined by threshold values. It also elicited a pronounced peak response reduction. When ammonium chloride was tested, the calculated threshold was the lowest of the compounds surveyed (i.e., the strongest attractant), and the peak response was barely detectable. It should be noted that the number of different chemoreceptors involved in the above

responses is not known at this time.

From preliminary observations shown in Table 1, it appears that *P. aeruginosa* is strongly attracted to amino-nitrogen compounds. Compounds that are solely carbon sources may or may not be attractants, and the compounds that are attractants elicit reduced accumulations compared with their amino acid analogs. Surprisingly, ammonium chloride, which serves solely as a nitrogen source, had the lowest threshold of any compound tested. Further study of the relationship between attractant metabolism and the interaction between nitrogen and carbon compounds relative to chemotaxis may reveal insights into the ecological evolution of *P. aeruginosa*.

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