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Role of Chemotaxis in the Ecology of Denitrifiers

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A modification of the Adler capillary assay was used to evaluate the chemotactic responses of several denitrifiers to nitrate and nitrite. Strong positive chemotaxis was observed to NO_3^- and NO_2^- by soil isolates of *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Pseudomonas stutzeri*, with the peak response occurring at 10^{-3} M for both attractants. In addition, a strong chemoattraction to serine (peak response at 10^{-2} M), tryptone, and a soil extract, but not to NH_4^+, was observed for all denitrifiers tested. Chemotaxis was not dependent on a previous growth on NO_3^-, NO_2^-, or a soil extract, and the chemoattraction to NO_3^- occurred when the bacteria were grown aerobically or anaerobically. However, the best response to NO_3^- was usually observed when the cells were grown aerobically with 10 mM NO_3^- in the growth medium. Capillary tubes containing 10^{-3} M NO_3^- submerged into soil-water mixtures elicited a significant chemotactic response to NO_3^- by the indigenous soil microflora, the majority of which were *Pseudomonas* spp. A chemotactic strain of *P. fluorescens* also was shown to survive significantly better in aerobic and anaerobic soils than was a nonmotile strain of the same species. Both strains had equal growth rates in liquid cultures. Thus, chemotaxis may be one mechanism by which denitrifiers successfully compete for available NO_3^- and NO_2^-, and which may facilitate the survival of naturally occurring populations of some denitrifiers.

The process of denitrification, the anaerobic reduction of nitrate or nitrite to nitrous oxide or elemental nitrogen, has received a great deal of attention in recent years because it has been recognized as a major sequence in the nitrogen cycle and because it is responsible for significant losses of nitrogen from agricultural soils (21). To date, however, most of the research devoted to this subject has been restricted to biochemical or physiological studies, with little attention being paid to the ecology of the organisms responsible for denitrification (27). In the latter respect, it is known that the predominant denitrifiers are able to maintain relatively high populations in certain soils and that these denitrifying populations constitute ca. 20% of the organisms capable of anaerobic growth, as well as being 1 to 5% of the total heterotrophic community (27). Furthermore, the predominant denitrifiers have been identified to be a "very diverse group of gram-negative, motile bacteria," the majority of which are *Pseudomonas* species (13).

It is still not known, however, why the predominant denitrifiers are so successful in nature, especially since it appears that some denitrifiers survive better under aerobic conditions (25). It is apparent that the most important habitats for denitrification are those in which aerobic-anaerobic interfaces occur (27). Thus, nitrate produced in an aerobic zone diffuses to an adjacent anaerobic zone, leading to a concentration gradient, where it is quickly reduced by denitrifiers (5, 6, 15). Bacteria capable of carrying out denitrification that survive better in an aerobic zone, are located in an aerobic zone, or both, then, may not contribute significantly to the denitrification process, unless they are able to move to the site of nitrate in the anaerobic zone or to create local environmental conditions in the aerobic zone that allow for denitrification. Theoretically, pseudomonads could reach nitrate by exhibiting a positive chemotactic response (movement toward a chemical gradient [2]) to nitrate. Alternatively, the aerobic-anaerobic interface itself may migrate to the site of nitrate production (27). Thus, those denitrifiers located in an aerobic zone also could switch from aerobic respiration to denitrification as environmental conditions change from aerobic to anaerobic.

It seems likely, however, that chemotaxis would offer a strong competitive mechanism for denitrifiers, since chemotaxis would allow denitrifiers to actively reach nitrate by directed motility, rather than by random movement or diffusion of nitrate. Furthermore, it has been shown that bacteria capable of eliciting a chemotactic response usually find themselves at a selective advantage in unmixed environments (22), such as soil, where spatial or temporal chemical gradients persist (17). Likewise, chemotaxis appears to offer an ecological advantage to a number of other organisms in a variety of environments (3, 7, 8, 12, 20, 23, 24, 28). The present studies were initiated to examine the chemotactic responses of several denitrifiers to nitrate and nitrite.

MATERIALS AND METHODS

Bacterial strains. The test organisms used in the experiments reported here were chosen to represent the predominant denitrifiers isolated from diverse soils from around the world (13). *Pseudomonas aeruginosa* JM42 was obtained from Larry Hochstein of this institution. *Pseudomonas fluorescens* 72 (ATCC 33512; here designated PF2), a soil isolate (13), *Pseudomonas stutzeri* 300 (ATCC 11607), and *P. stutzeri* 301 were kindly provided by Mary Firestone, University of California, Berkeley. A nonmotile strain of *P. fluorescens* (PF1) was provided by Susan Drabkin, San Jose State University, San Jose, Calif. *P. stutzeri* 1 was isolated from soil and characterized by standard methods.

Antibiotic-resistant strains were selected by spreading ~10^{10} cells on gradient plates of TSBYE agar (see below) to which 500 μg of filter-sterilized antibiotic (naladixic acid or neomycin-sulfate; Sigma Chemical Co., St. Louis, Mo.) per ml had been added to the bottom layer of agar. Single colonies were purified on nonselective TSBYE agar and tested for true antibiotic resistance.

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Culture media, growth conditions, and cell preparation. For some studies, the cells were grown in the synthetic medium described by Moulton and Montie (19), except that $FeCl_3 \cdot 6H_2O$ was replaced with $FeCl_2 \cdot 4H_2O$. Filter-sterilized glucose was added as the carbon source to the cooled, autoclaved medium at a final concentration of 0.2% (wt/vol). To test whether the chemotactic response of denitrifiers to nitrate (or nitrite) was dependent on a previous growth on nitrate, pseudomonads were grown with and without the addition of KNO_3 (final concentration, 10 mM) to the growth medium. KNO_3 was sterilized by filtration and added to the medium at the time of inoculation. Starter cultures were grown aerobically for 24 h at 30°C, and 0.5 ml was used to inoculate 20 ml of the chemically defined medium in 50-ml Erlenmeyer flasks. Then the cultures were incubated aerobically and anaerobically (4) at 30°C for 24 h. *P. aeruginosa* was grown similarly in a soil extract (SE; see below), without the addition of KNO_3, to test the ability of the strain to exhibit a chemotactic response when grown in a more natural growth medium. For the other experiments, bacteria were grown aerobically as above in tryptic soy broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.2% (final concentration) yeast extract (Difco) (TSBYE), with or without 10 mM of attractant as indicated. Growth of all strains tested, except PF1, on the above media yielded cells which were motile and chemotactic.

Cells were harvested by centrifugation at 3,000 × *g* for 10 min at 4°C, and the pellet was washed twice in chemotaxis medium which consisted of 0.01 M potassium phosphate buffer (pH 7.4), 1.0 mM $MgSO_4 \cdot 7H_2O$, and 0.1 mM disodium-EDTA (18). The final pellet was resuspended in chemotaxis medium to which 0.1% Triton X-100 had been added (final chemotaxis medium [FCM]) to prevent adhesion of pseudomonads to the capillary tubes (9). Preliminary studies showed that FCM did not inhibit motility or chemotaxis. The cells were suspended to give a final concentration of 1×10^6 to 2×10^6 bacteria per ml, which resembles natural population levels in soil (13).

For chemotaxis experiments, the bacteria were diluted in tryptone broth (Difco) and plated on TSBYE plates containing 2.0% agar to determine the number of bacteria per capillary. TSBYE agar plates containing cycloheximide (50 μg/ml) and naladixic acid (Sigma) or neomycin sulfate (Sigma) at 200 μg/ml were used to determine the number of antibiotic-resistant bacteria surviving after inoculation in soil (see below).

Chemotaxis assays. The capillary assay for chemotaxis was performed by the principles of Adler (1), by the method of Freter and O'Brien (9). The bacterial suspensions (1 ml) were placed into small test tubes (12 by 75 mm). The capillaries (25-μl size Micro-Sampling Pipets; Corning Glass Works, Corning, N.Y.) were filled with an attractant dissolved in FCM and sealed at the upper end with grease. Control capillaries were filled with FCM alone and used to assess the background motility of the bacteria. Tryptone (final concentration, 1%) also was dissolved in FCM and used as a positive control attractant in each experiment. By means of adhesive tape applied to the outside of the test tubes, test and control capillaries were attached (one capillary per tube) so that the open ends of the capillaries were submerged 10 mm below the surface of the bacterial suspension. At this point the tubes were transferred to a 30°C "warm room" and incubated for 45 min. After the incubation period the contents of the capillaries were expelled into tryptone broth, serial 10-fold dilutions were made, and 0.1-ml samples were spread on TSBYE agar plates. Duplicate plate counts were made for each dilution to determine the number of bacteria per capillary tube. Each capillary assay was performed in duplicate, three separate times, and the results were averaged. To normalize for experimental or day-to-day differences in motility, the chemotactic ratio (R_{che}) was determined (3, 19) as: R_{che} = number of bacteria in attractant capillary/number of bacteria in control capillary. This value showed less variability between the different bacterial preparations than did the absolute accumulations (19). An R_{che} of 2.0 or greater was considered to be significant (19).

For soil chemotaxis experiments, 1 g of soil was mixed vigorously with 1 g of sterile distilled water for 30 s and allowed to briefly settle. Capillary tubes containing 10^{-3} M KNO_3 dissolved in either FCM or sterile distilled water, or sterile distilled water or FCM alone as controls, were submerged 20 mm into the soil-water suspensions, attached to test tubes with adhesive tape as above, and incubated at 30°C for 45 min. After the incubation period capillaries were removed, and their contents were expelled into tryptone broth. Serial 10-fold dilutions were then made, and 0.1 ml was spread on TSBYE agar containing 50 μg of cycloheximide per ml and a selective *Pseudomonas* agar (14).

The attractant chemicals were prepared fresh daily, sterilized by filtration through 0.22-μm filters (Millipore Corp., Bedford, Mass.), and diluted 10-fold in FCM to yield concentrations of 10^{-1} to 10^{-6} M.

TABLE 1. Chemotactic responses[a] of denitrifiers to various attractants

Organism	Attractant	Peak response		Threshold response	
		Molar concn (log)	R_{che}	Molar concn (log)	R_{che}
P. aeruginosa	NO_3^- [b]	−3	9.7	−4	1.4
P. fluorescens		−3	7.2	−5	3.0
P. stutzeri 300		−3	10.8	−5	1.5
P. stutzeri 1		−3	14.4	−5	1.9
P. aeruginosa	NO_2^- [b]	−3	5.8	−5	2.0
P. fluorescens		−3	21.5	−6	2.1
P. stutzeri 300		−3	10.5	−4	2.5
P. stutzeri 1		−3	16.8	−5	2.2
P. aeruginosa	NH_4^+ [b]	−2	2.0	−2	2.0
P. fluorescens		ND	ND	ND	ND
P. stutzeri 300		NR	NR	NR	NR
P. stutzeri 1		ND	ND	ND	ND
P. aeruginosa	Serine[c]	−3	30.8	−5	2.1
P. fluorescens		−2	15.5	−5	2.2
P. stutzeri 300		−2	16.8	−5	2.3
P. stutzeri 1		−2	16.1	−5	2.4
P. aeruginosa	SE[c]		7.3		
P. fluorescens			ND		
P. stutzeri 300			11.3		
P. stutzeri 1			ND		
P. aeruginosa	Tryptone (1%)[c]		4.3		
P. fluorescens			3.1		
P. stutzeri 300			8.4		
P. stutzeri 1			8.1		

[a] ND, Not determined; NR, negative response (see the text).
[b] Cells were grown in TSBYE broth containing 10 mM attractant.
[c] Cells were grown in TSBYE broth only.

Soils used and preparation. The soil used in the present study was a coarse sandy loam, with 1.0% organic matter and pH 6.5, collected from the Shaver Series (Log Creek Reference Study) at Sequoia National Park in California. Samples were collected from the top 6 cm of the soil surface with an Oakfield core sampler. Soil composities were made from three sites within the Shavers Series by mixing 20 cores taken from a 10- by 10-m area. After collection, the soils were partially air dried (to ca. 8% moisture content) and either used immediately or stored at 2 to 4°C.

SEs were prepared by making 1:2 soil-water suspensions and mixing them with a magnetic stirring bar for 24 h at 4°C. The soil-water suspensions were allowed to settle, and the resulting supernatants were sterilized by filtration through 0.22-μm Millipore filters. SEs were stored at 4°C and used within 5 days of preparation.

Competition experiments. Survival and competition between a motile, chemotactic strain of *P. fluorescens* (PF2) and a nonmotile strain of the same species (PF1) were examined by adding antibiotic-resistant cells to aerobic and anaerobic soils, by a modification of the method of Smith and Tiedje (25). Stationary-phase aerobic cultures were harvested by centrifugation (3,000 × *g* for 10 min), washed twice, and resuspended in sterile distilled water, and 50 μl of one or both suspensions was added to 20 g of soil in 50-ml test tubes to give a final density of 10^5 bacteria per g of soil. (Preliminary serial-dilution platings on a selective *Pseudomonas* agar [14] indicated that *Pseudomonas* population levels for these soils were 1×10^5 to 2×10^5 pseudomonads per g soil.) In one set of experiments the soil was brought to 100% moisture content by mixing 20 g of soil with 20 g of sterile distilled water, and the tube was sealed with a rubber stopper. Forty microliters of a 1 M solution of KNO_3, made fresh and sterilized by filtration, was added to the top of the soil before the tube was sealed. In another set of experiments, the soil was brought to 30% moisture content with sterile distilled water and left open to the atmosphere, without the addition of KNO_3. These experiments were referred to as anaerobic and aerobic, respectively (25). For some experiments, the bacterial test suspension was placed in the bottom of the test tube first, and then the soil was slowly layered over the top of the suspension. This was done to prevent excessive mixing of bacteria and soil. For other experiments, the entire contents, including bacteria and KNO_3 (when added), were mixed thoroughly. All tubes were incubated at 30°C. Pseudomonads were counted at various intervals by thoroughly mixing the entire contents of the tube, making serial 10-fold dilutions, and spreading 0.1 ml on TSBYE agar containing the appropriate antibiotic. Plates were incubated at 30°C for 72 h, and the number of bacteria per gram of soil was calculated.

RESULTS

Nitrate, nitrite, and other attractant responses. The ability of several denitrifiers to respond chemotactically to NO_3^-, NO_2^-, NH_4^+, serine, an SE, and tryptone is summarized in Table 1. The concentration response curves to nitrate (Fig. 1) for *P. aeruginosa*, *P. fluorescens* (PF1), and *P. stutzeri* (300) indicated that these bacteria were strongly attracted to 10^{-3} M nitrate. The threshold response (minimum concentration of an attractant needed to elicit an observable response in the capillary assay) for nitrate was determined to be 10^{-4} M for *P. aeruginosa* and 10^{-5} M for *P. stutzeri*. The concentration response curves to nitrite (Fig. 2) for *P. aeruginosa*, *P. fluorescens*, and *P. stutzeri* also indicated a peak response at 10^{-3} M nitrite for all strains tested. The threshold response to nitrite was 10^{-4} for *P. stutzeri* 300, 10^{-5} M for *P. stutzeri* 1 and *P. aeruginosa*, and 10^{-6} M for *P. fluorescens*.

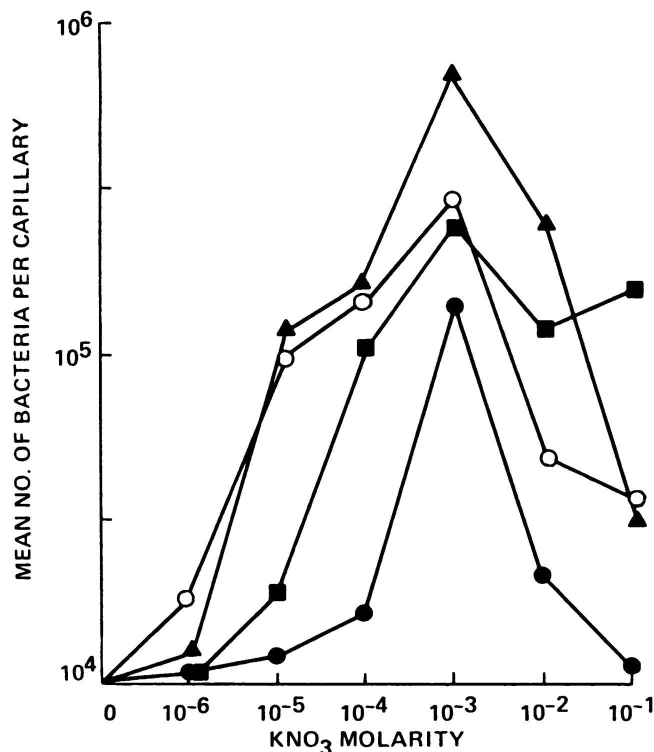

FIG. 1. Concentration response curves for chemotaxis of *P. aeruginosa* (●), *P. fluorescens* (▲), and *P. stutzeri* (■) to NO_3^-. Cells were grown in TSBYE broth containing 10 mM KNO_3 and were assayed for 45 min at 30°C. *P. aeruginosa* also was grown in an SE (○) and tested for its ability to respond chemotactically to NO_3^-. Each point is the average of triplicate assays, and backgrounds were subtracted.

The ability of the denitrifiers to move chemotactically to serine was tested, and the results showed that PF2 and all strains of *P. stutzeri* had peak responses at 10^{-2} M serine, whereas *P. aeruginosa* demonstrated its best response at 10^{-3} M serine. The threshold response for serine was determined to be at 10^{-5} M for all strains tested. It also was found that both *P. aeruginosa* and *P. stutzeri* demonstrated highly significant chemotactic responses to an SE (Table 1). When NH_4^+ was tested as a possible attractant, *P. aeruginosa* was found to be the only organism to demonstrate a positive chemotactic response. The peak and threshold response occurred at 10^{-2} M NH_4^+, with an R_{che} of 2.0. *P. stutzeri* 300 appeared to have a negative chemotactic response to NH_4^+, since significantly more bacteria were observed to accumulate in control capillaries than in test capillaries.

Conditions for chemotaxis to nitrate by denitrifiers. To determine whether denitrifiers could demonstrate a positive chemotactic response to nitrate under various conditions and whether such a response was dependent on previous growth on nitrate, *P. aeruginosa*, *P. stutzeri*, and *P. fluorescens* cells were grown aerobically and anaerobically in a chemically defined medium (with and without nitrate) and tested in the capillary assay (Fig. 3). All strains tested were significantly attracted to nitrate when grown aerobically or anaerobically, with or without nitrate. Thus, chemotaxis toward NO_3^- by the denitrifiers did not require previous

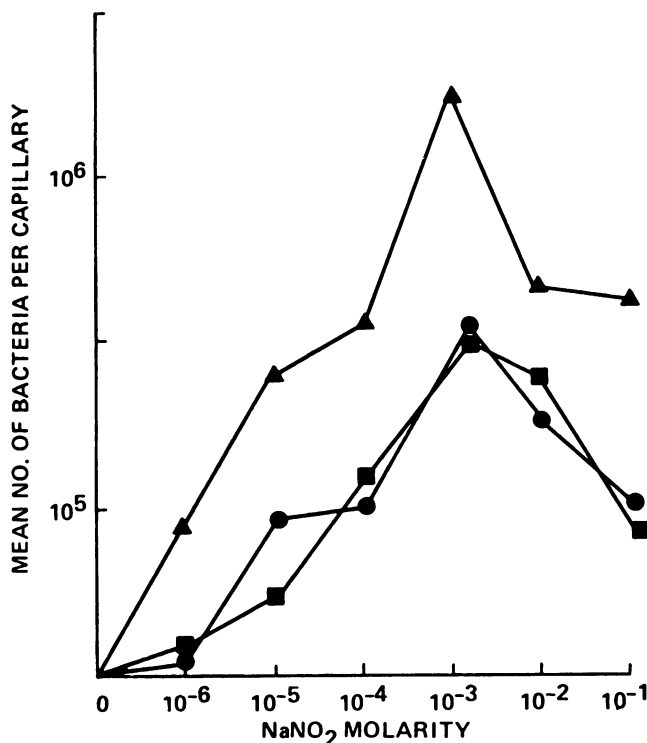

FIG. 2. Concentration response curves for chemotaxis of *P. aeruginosa* (●), *P. fluorescens* (▲), and *P. stutzeri* (■) to NO_2^-. Cells were grown in TSBYE broth containing 10 mM $NaNO_2$ and were assayed for 45 min at 30°C. Each point is the average of triplicate assays, and backgrounds were subtracted.

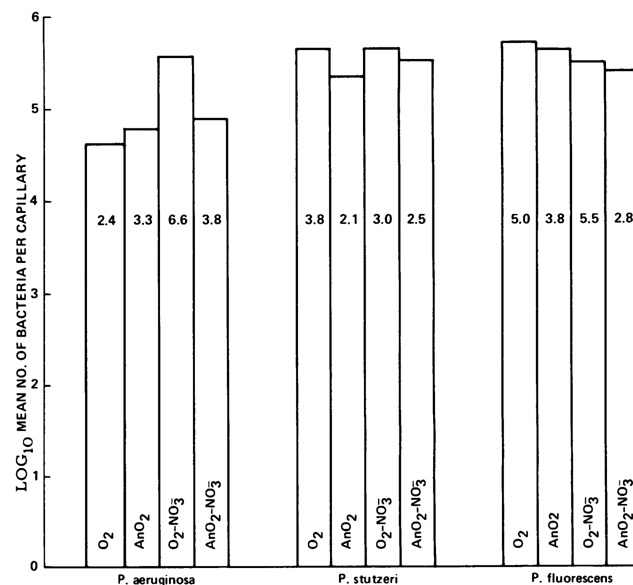

FIG. 3. Chemotaxis of *P. aeruginosa*, *P. stutzeri*, and *P. fluorescens* to 10^{-3} M NO_3^-. Cells were grown in a chemically defined medium (see the text) aerobically (O_2), anaerobically (AnO_2), aerobically with 10 mM KNO_3 in the medium (O_2-NO_3^-), or anaerobically with 10 mM KNO_3 in the medium (AnO_2-NO_3^-) and were assayed for 45 min at 30°C. Each bar represents the average of duplicate assays, and backgrounds were subtracted. The numbers within the bars indicate the average R_{che}'s for each set of experimental conditions.

growth on nitrate. The best response, however, was usually observed when the cells were grown aerobically with nitrate in the growth medium. *P. fluorescens*, for instance, had R_{che}'s of 5.5 and 5.0 for aerobic growth with and without nitrate and R_{che}'s of 3.8 and 2.8 for anaerobic growth with and without nitrate, respectively. Similar responses to nitrate were observed with *P. aeruginosa* and *P. stutzeri*. The best response (R_{che}, 6.6) for *P. aeruginosa* was observed after aerobic growth with nitrate in the growth medium, whereas *P. stutzeri* demonstrated its best response (R_{che}, 3.8) after aerobic growth without nitrate. *P. aeruginosa* and *P. stutzeri* also were grown in a chemically defined medium (without NO_2^-) and tested for their ability to respond chemotactically to nitrite. Similarly, it was found that chemoattraction to NO_2^- by both denitrifiers was not dependent on previous growth on NO_2^- (data not shown).

The ability of nitrate and nitrite (10^{-3} M) to elicit chemotactic responses by SE-grown cells of *P. aeruginosa* is summarized in Table 2. The concentration response curves for nitrate (Fig. 1) and nitrite (data not shown) indicated that cells of *P. aeruginosa* grown in an SE were strongly attracted to NO_3^- (R_{che}, 9.8) and NO_2^- (R_{che}, 6.2), with a peak response for both chemicals at 10^{-3} M. Threshold responses for SE-grown *P. aeruginosa* were observed to occur at 10^{-6} and 10^{-5} M for nitrate and nitrite, respectively. SE-grown cells of *P. aeruginosa* also were shown to be strongly attracted to SE (R_{che}, 8.2) and tryptone (R_{che}, 4.4). It is interesting to note that SE-grown cells of *P. aeruginosa* always demonstrated stronger responses to all test attractants (cf. Tables 1 and 2).

It also should be noted that it is not known whether the in vitro results of the present study adequately reflect the sensitivity of NO_3^- and NO_2^- attraction in natural environments, although the average pseudomonad R_{che} for 10^{-3} M NO_3^- from all three soil sites tested (see below) was similar to the in vitro results.

Soil chemotaxis studies. The ability of nitrate to attract bacteria from soil was tested by submerging capillary tubes containing 10^{-3} M nitrate in soil-water suspensions and determining the total number of bacteria and pseudomonads which had entered the capillary after 45 min of incubation. All soil sites tested showed that the indigenous microflora responded significantly to nitrate (Table 3), with soil site 1 showing the best quantitative response. The average R_{che} for the total indigenous microflora from this site was 10.7, whereas the pseudomonad R_{che} for this site was 16.3. R_{che}'s for the total indigenous soil microflora for sites 2 and 3 were 2.3 and 2.0, respectively. The pseudomonad R_{che}'s for these sites again were higher than for the total indigenous microflora. Site 2 had a pseudomonad R_{che} of 5.8, whereas soil site 3 had a pseudomonad R_{che} of 3.0. For all three soil sites, ca. 62% of the total number of bacteria entering the test capillaries were found to be *Pseudomonas* spp. (Table 3).

TABLE 2. Chemotactic responses of *P. aeruginosa* cells grown in an SE

Attractant	Peak response		Threshold response	
	Molar concn (log)	R_{che}	Molar concn (log)	R_{che}
NO_3^-	−3	9.8	−6	1.9
NO_2^-	−3	6.2	−5	2.2
SE		8.2		
Tryptone (1%)		4.4		

TABLE 3. Chemotactic responses of indigenous soil microflora to 10^{-3} M NO_3^-

Soil site	Log_{10} total no. of viable bacteria per g of soil	Log_{10} total no. of *Pseudomonas* spp. per g of soil	Log_{10} mean no. of total bacteria per test capillary[a]	Total R_{che}	Log_{10} mean no. of *Pseudomonas* spp. per test capillary[a]	*Pseudomonas* R_{che}	% of chemotactic organisms which were *Pseudomonas* spp.
1	7.1	5.0	5.1	10.7	4.7	16.3	62.5
2	7.1	4.9	4.2	2.3	4.0	5.8	62.0
3	7.0	4.9	4.6	2.0	4.4	3.0	61.4
Avg	7.05	4.9	4.8	5.0	4.5	8.4	62.0

[a] The mean background accumulation in control capillaries was subtracted.

Soil survival and competition studies. To test whether chemotaxis could offer a selective advantage for denitrifiers in soil, a motile, chemotactic strain of *P. fluorescens* (PF2) and a nonmotile strain (PF1) of the same species were inoculated separately or together into soil (under both aerobic and anaerobic conditions) and quantitatively counted at various intervals after inoculation. Preliminary studies showed that both strains had similar generation times when grown in liquid cultures (data not shown). In unmixed anaerobic and aerobic soils, PF2 was found to survive significantly better than PF1 ($P < 0.001$, Table 4). For instance, 40% of the initial PF2 inoculum was recovered after 5 days of anerobic incubation, whereas only 3% of the initial PF1 inoculum could be recovered from the same soil. Similarly, for the same aerobic soil it was found that 70 and 6% of the initial inoculum were recovered for PF2 and PF1, respectively. When the bacteria and soils were mixed, however, both denitrifiers survived significantly better (Table 4). PF2 increased its net survival in the anaerobic soil from 40 to 150%. Likewise, in mixed aerobic soil PF2 increased its net survival from 70 to 200% of the initial inoculum. Mixing the soil and bacteria also allowed PF1 to significantly increase its ability to survive in aerobic and anaerobic soils (Table 4). Both strains survived better in aerobic soils (25).

DISCUSSION

The present study has demonstrated that several denitrifiers, namely *P. aeruginosa*, *P. fluorescens*, and *P. stutzeri*, were strongly attracted to NO_3^- and NO_2^-. In addition, it was found that the chemoattraction of these denitrifiers to nitrate and nitrite did not depend on previous growth on NO_3^- and NO_2^- and could occur when the bacteria were grown aerobically or anaerobically. Conceivably then, positive chemotaxis to sites containing NO_3^- or NO_2^-, or both, may be one mechanism which allows denitrifiers to successfully compete for nitrate and nitrite, facilitating the survival of naturally occurring populations of some denitrifiers. Consistent with this are the findings that the majority of bacteria from soil attracted to NO_3^- were *Pseudomonas* spp. and that a motile, chemotactic denitrifier survived significantly better in both aerobic and anaerobic soils than did a nonmotile strain of the same species.

TABLE 4. Survival of a nonmotile and chemotactic strain of *P. fluorescens* in aerobic and anaerobic soils

Contents mixed	Aerobic incubation				Anaerobic incubation			
	Final mean log_{10} no. of bacteria per g of soil[a]		% of initial inoculum		Final mean log_{10} no. of bacteria per g of soil[a]		% of initial inoculum	
	PF1	PF2	PF1	PF2	PF1	PF2	PF1	PF2
+	4.8	5.3	70	200	4.3	5.1	20	150
−	3.8	4.8	6	70	3.4	4.6	3	40
−	NI[b]	4.7	NI	60	NI	4.5	NI	30
−	3.8	NI	6	NI	3.3	NI	2	NI

[a] Strains PF1 and PF2 were inoculated into aerobic and anaerobic soils together or separately as indicated, and cultured 5 days later.
[b] NI, Not inoculated.

It is believed that the ability of PF2 to outgrow PF1 in aerobic and anaerobic soils was due to the ability of PF2 to demonstrate a positive chemotactic response to certain chemicals (possibly NO_3^- or NO_2^- in anaerobic soils) favorable for cell growth, rather than superior growth kinetics or random motility simply moving PF2 to a position of greater substrate concentration. It has been shown that both active chemotaxis and motility are required for efficient bacterial movement in soil (26). Furthermore, in natural environments containing an indigenous microflora, random motility usually prevents cells from accumulating in favorable growth regions (16). In such an instance, random motility reduces the population size of an organism by bringing it to a region with a lower nutrient concentration (16, 17). Pilgram and Williams (22), for example, showed that a motile, chemotactic strain of *Proteus mirabilis* attra cted by certain amino acids outgrew a motile, nonchemotactic mutant in a semisolid, amino acid medium, although both strains grew equally well in broth. Similarly, Freter et al. (10, 11) found that a motile, chemotactic strain of *Vibrio cholera* outgrew a randomly motile, nonchemotactic mutant (derived from the chemotactic parent) in the mouse intestine. In pure culture, however, random movement of the bacterium in any direction away from the initial point of inoculation can only bring it to a position of greater substrate concentration (16). It appears, then, that in certain environments motility is beneficial to survival only when it is guided by chemotactic stimuli, whereas it may be a liability in the absence of such stimuli (10, 11, 16, 17). Chemotaxis thus may aid denitrifiers to seek out and closely approach NO_3^- and NO_2^- (or other substrates) in certain habitats, such as at sediment surfaces or sites adjacent to aerobic zones where nitrate is being produced.

The ability of denitrifiers to move chemotactically through soil, however, probably varies from day to day and may not be possible under certain environmental conditions (26). The rate of bacterial spreading in soil has been shown to decrease as soil moisture decreased (26). Nevertheless, chemotaxis, singly and collectively with other survival mechanisms (see reference 27), may affect the ability of denitrifiers to succeed in certain environments, as well as compete for indigenous NO_3^- and NO_2^-. Consequently, the importance of chemotaxis should not be underestimated since it may well supply that extra increment of fitness which is responsible for the

"widespread occurrence and relatively high densities of denitrifiers found in nature" (27).

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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*. J. Gen. Microbiol. **74**:77–91.
2. **Adler, J.** 1975. Chemotaxis in bacteria. Annu. Rev. Biochem. **44**:341–355.
3. **Barak, R., I. Nur, and Y. Okon.** 1983. Detection of chemotaxis in *Azospirillum brasilens*. J. Appl. Bacteriol. **53**:399–403.
4. **Betlach, M. R., and J. M. Tiedje.** 1981. Kinetic explanation for accumulation of nitrite, nitric oxide, and nitrous oxide during bacterial denitrification. Appl. Environ. Microbiol. **42**: 1074–1084.
5. **Biggar, J. W.** 1978. Spatial varability of nitrogen in soils, p. 201–211. *In* D. R. Nielsen and J. G. MacDonald (ed.), Nitrogen in the environment, vol. 1. Nitrogen behavior in field soil. Academic Press, Inc., New York.
6. **Burford, J. R., R. J. Dowell, and J. M. Lynch.** 1978. Denitrification in British soils, p. 365–377. *In* D. R. Nielsen and J. G. MacDonald (ed.), Nitrogen in the environment, vol. 2. Soil-plant-nitrogen relationships. Academic Press, Inc. New York.
7. **Chet, I., and R. Mitchell.** 1976. Ecological aspects of microbial chemotactic behavior. Annu. Rev. Microbiol. **30**:221–239.
8. **Currier, W. W., and G. A. Strobel.** 1976. Chemotaxis of *Rhizobium* species to plant root exudates. Plant Physiol. **57**:820–823.
9. **Freter, R., and P. C. M. O'Brien.** 1981. Role of chemotaxis in the association of motile bacteria with intestinal mucosa: chemotactic responses of *Vibrio cholera* and description of motile nonchemotactic mutants. Infect. Immun. **34**:215–221.
10. **Freter, R., P. C. M. O'Brien, and S. A. Halstead.** 1978. Adhesion and chemotaxis as determinants of bacterial association with mucosal surfaces. Adv. Exp. Med. Biol. **107**:429–437.
11. **Freter, R., P. C. M. O'Brien, and M. S. Macsai.** 1981. Role of chemotaxis in the association of motile bacteria within intestinal mucosa: in vivo studies. Infect. Immun. **34**:234–240.
12. **Gallucci, K. K., and H. W. Paerl.** 1983. *Pseudomonas aeruginosa* chemotaxis associated with blooms of N_2-fixing blue-green algae (cyanobacteria). Appl. Environ. Microbiol. **45**:557–562.
13. **Gamble, T. N., M. R. Betlach, and J. M. Tiedje.** 1977. Numerically dominant denitrifying bacteria from world soils. Appl. Environ. Microbiol. **33**:926–939.
14. **Grant, M. A., and J. G. Holt.** 1977. Medium from the selective isolation of members of the genus *Pseudomonas* from natural habitats. Appl. Environ. Microbiol. **33**:1222–1224.
15. **Greenwood, D. J.** 1978. Influence of spatial variability in soil on microbial activity, crop growth, and agronomic practice, p. 213–222. *In* D. R. Nielsen and J. G. MacDonald (ed.), Nitrogen in the environment, vol. 1. Nitrogen behavior in field soil. Academic Press, Inc., New York.
16. **Lauffenburger, D., R. Aris, and K. H. Keller.** 1981. Effects of random motility on growth of bacterial populations. Microb. Ecol. **7**:207–227.
17. **Lauffenburger, D., R. Aris, and K. H. Keller.** 1982. Effects of cell motility and chemotaxis on microbial population growth. Biophys. J. **40**:209–219.
18. **Lynch, W. H.** 1980. Effect of temperature on *Pseudomonas fluorescens* chemotaxis. J. Bacteriol. **143**:338–342.
19. **Moulton, R. C., and T. C. Montie.** 1979. Chemotaxis by *Pseudomonas aeruginosa*. J. Bacteriol. **137**:274–280.
20. **Okon, Y., L. Cakmakci, I. Nur, and I. Chet.** 1980. Aerotaxis and chemotaxis of *Azospirillum brasilence*: a note. Microb. Ecol. **6**:277–280.
21. **Payne, W. J.** 1973. Reduction of nitrogenous oxides by microorganisms. Bacteriol. Rev. **37**:409–452.
22. **Pilgram, W., and F. D. Williams.** 1976. Survival value of chemotaxis in mixed cultures. Can. J. Microbiol. **22**:1771–1773.
23. **Sjoblad, R. D., and B. A. Coleman.** 1980. Chemotactic responses of *Thiobacillus thioparus*. Microb. Ecol. **6**:147–151.
24. **Sjoblad, R. D., and R. Mitchell.** 1979. Chemotactic responses of *Vibrio alginolyticus* to algal extracellular products. Can. J. Microbiol. **25**:964–967.
25. **Smith, M. S., and J. M. Tiedje.** 1980. Growth and survival of antibiotic-resistant denitrifier strains in soil. Can. J. Microbiol. **26**:854–856.
26. **Soby, S., and K. Bergman.** 1983. Motility and chemotaxis of *Rhizobium meliloti* in soil. Appl. Environ. Microbiol. **46**: 995–998.
27. **Tiedje, J. M., A. J. Sexstone, D. D. Myrold, and J. A. Robinson.** 1982. Denitrification: ecological niches, competition and survival. Antonie van Leeuwenhoek **48**:569–583.
28. **Walsh, F., and R. Mitchell.** 1978. Bacterial chemotactic responses in flowing water. Microb. Ecol. **4**:165–168.