

Chemotaxis of Fluorescent Pseudomonads Towards Seed Exudates and Germinating Seeds in Solarized Soil

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ABSTRACT

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We investigated the chemotaxis of fluorescent pseudomonads towards seeds exudates and germinating tomato seeds. Chemotaxis of *Pseudomonas putida* and *P. fluorescens* in a capillary tube was more pronounced towards exudates originating from seeds that were germinated in solarized soil than towards comparable exudates from seeds that were germinated in nonsolarized soil. Movement of these bacteria was enhanced also towards a mixture of either amino acids or amino acids and sugars, but not of sugars alone. Chemotaxis of fluorescent pseudomonads from solar-

ized soil through sterile sand towards exudates originating from seeds germinated in solarized soil was more pronounced than movement of these bacteria from nonsolarized soil. Chemotaxis of streptomycin-resistant pseudomonads in soil also was enhanced towards exudates and germinating seeds in solarized soil. We suggest that the improved capacity of fluorescent pseudomonads to move towards attractants and compete for exudates may contribute to their rapid establishment in the rhizosphere and roots of plants in solarized soils.

Additional keywords: beneficial microorganisms, minor pathogens, plant growth-promoting rhizobacteria.

Increased growth response of plants in fumigated, steam-treated, and solarized soils, even in the absence of known pathogens, is well documented (2,8,9,16,21). This phenomenon is attributed to various chemical, physical, and biotic factors (2,9,16,21). Significant changes in microbial activities in solarized soil take place, as reflected in stimulation of antagonistic activity, induced soil suppressiveness, and the rate of enzymatic activity as determined by fluorescein diacetate hydrolysis (9,10,13,16,21,25). Moreover, colonization of the rhizosphere and roots by fluorescent pseudomonads increases after solarization of soil or container media, already at the early growth stages of the plants (8,9,11). Strains of these bacteria improved growth of inoculated plants in greenhouse experiments.

Root exudates provide the major energy source in the root zone, thus influencing microbial activity and colonization of living and nonliving substrates. Composition of root exudates is affected by edaphic factors, pesticides, pathogens, and soil microorganisms (5,10,12,14,22-24). Root exudates of tomato plants in solarized soils contain higher amounts of amino acids and lower amounts of sugars compared with nonsolarized soil, and these exudates strongly affect microbial activities in solarized and nonsolarized soils (10). Chemotaxis of microorganisms to root exudates is regarded as an important mechanism in the establishment of microorganisms in plant roots (3,6). Chemotaxis ability depends on exudate composition and on gradient flow. In the present study, we investigated the effect of exudates of germinating seeds from different sources on chemotaxis of fluorescent pseudomonads in capillary tubes and in nonsolarized and solarized soils, and the chemotaxis of these bacteria towards seeds that were germinated in nonsolarized and solarized soils.

MATERIALS AND METHODS

Plant material and exudate collection. Soil samples were collected from a field in Rehovot (3.8% clay, 0% silt, 96.2% sand,

and 0.4% organic matter; pH 6.9). Soil was untreated or solarized in experimental field plots as described previously (9). Exudates were collected as described by Gamliel and Katan (10) based on Graham et al (12). Seeds of tomato (*Lycopersicon esculentum* Mill. 'Rehovot 13') were germinated in nonsolarized or solarized soil, or between sterile filter papers. Exudates were collected during 22 h of incubation. Exudate solutions were passed immediately through a sterile 0.45- μ m membrane filter, freeze-dried, and kept frozen until use.

Culture media and bacteria. King's B (KB) medium (7) was used for culturing *P. putida* and *P. fluorescens* and for chemotaxis assays in capillary tubes; a modified KB medium (9) was used for enumeration of fluorescent pseudomonads from soil; nutrient agar (N) (7) was used for enumeration of total bacteria in the exudate collection system and in the chemotaxis assay in soil; and Martin's agar (7) was used for the enumeration of fungi in the assays of chemotaxis in soil.

P. putida (RS34M) and *P. fluorescens* (RS7B) initially were isolated from the rhizosphere of tomato plants grown in solarized Rehovot soil. A strain of *P. putida* (MS2) carrying a spontaneous mutation for resistance to streptomycin (100 μ g/ml) was isolated on modified KB medium supplemented with 100 μ g/ml streptomycin from the rhizosphere of tomato seedlings grown in solarized Rehovot soil. All of the above bacterial strains were found to increase dry weight of tomato plants in greenhouse experiments (9). Bacteria were stored in glycerol at -70 C until 24 h before use and then spotted on KB medium for 24 h. They were then transferred to KB medium and incubated at 28 C for 24 h. Bacterial cells were scraped, suspended in 0.01 M phosphate buffer (pH 7.0), washed twice, and adjusted to the desired concentration by optical density.

Chemotaxis in capillary tubes. Chemotaxis of bacteria towards attractants in capillary tubes was tested essentially as described by Adler (1), by assessing the number of bacteria attracted to the tested substrate in a capillary tube. *P. putida* (RS34M) and *P. fluorescens* (RS7B) each from 24-h-old culture were suspended in 0.01 M phosphate buffer (pH 7.0) and left for 3 h. Capillary tubes (1 μ l) were sealed at one end and filled with the tested

attractant or with a phosphate buffer, as the control. Attractants tested were: exudates (5 mg/ml) from seeds germinated in non-solarized soil, solarized soil, or between filter papers; a mixture of sugars (100 µg/ml of glucose, 50 µg/ml of fructose, and 50 µg/ml of sucrose); mixture of amino acids (100 nmol/ml of asparagine, 100 nmol/ml of threonine, 10 nmol/ml of methionine, 10 nmol/ml of proline, 10 nmol/ml of leucine, 10 nmol/ml of valine, 10 nmol/ml of alanine, and 10 nmol/ml of glycine); and a mixture of the amino acids and sugars specified above. Concentrations of amino acids were similar to those in exudates from seeds germinating in solarized soil, and concentrations of sugars were similar to those in exudates from seeds germinating in nonsolarized soil (10). Capillary tubes were placed individually in 0.5 ml of bacterial suspension (2×10^7 cfu/ml) on an Adler apparatus (1) for 25 min at 25 C with three replicates for each attractant. Bacterial movement into the tubes was monitored with a dark-field microscope during the assay. After 25 min, the sealed end of each tube was broken, the contents were suspended in phosphate buffer, and further diluted. Aliquots of 0.1 ml from each dilution were spread on five dishes of KB medium. Plates were incubated for 3 days at 28 C, after which the number of colony-forming units per capillary was determined.

Chemotaxis in soil. Chemotaxis of fluorescent pseudomonads to exudates in soil was assayed in a soil chemotaxis apparatus in a glass petri dish (85 mm diameter) essentially as described by Scher et al (20). Ten micrograms of dried exudates from seeds germinated in either nonsolarized or solarized soil or between filter papers was placed in washed and sterilized sea sand (BDH, Poole, England) in the center of a 15-mm-diameter ring (fraction 1). Another ring (10 mm) containing washed and sterilized sea sand without exudates (fraction 2) surrounded the inner ring with exudates. The remaining periphery of the dish (fraction 3) was filled with either nonsolarized or solarized sieved Rehovot soil. The whole apparatus was moistened to 12% (v/w); after 30 min, the glass rings that served to separate the fractions were removed, and the soil fractions were unified by lightly tapping the dishes. The dishes were incubated for 24 h in the dark at 28 C, and then the contents of the inner 15-mm ring were removed, suspended in 0.1% water agar supplemented with $MgSO_4 \cdot 7H_2O$, and serially diluted. Samples of 0.1 ml were spread on five petri dishes of modified KB medium and incubated at 28 C for 5 days. This system served for assessing chemotaxis of native fluorescent pseudomonads and total number of bacteria that moved from the tested soil in the periphery through 10 mm of sterile sand belt to the inner sand fraction with the exudates. Results were expressed as number of cfu per gram of dry sand on dry basis (after heating at 105 C for 48 h).

Chemotaxis of a streptomycin-resistant strain of *P. putida* (MS10) towards exudates in soil also was assayed in the apparatus described above. Ten-microgram exudates were placed in sieved Rehovot soil, either nonsolarized or solarized, in fraction 1 of the dish. The middle fraction (fraction 2) was filled with the same soil. Washed cells of *P. putida*, suspended in 0.01 M potassium phosphate buffer, were mixed with Rehovot soil, either nonsolarized or solarized, as in fractions 1 and 2, and the soil with bacteria (10^7 cells per gram) was placed along the periphery of the apparatus (fraction 3). All the fractions were moistened to 12% (v/w); and after 30 min, the glass rings were removed, and the dishes were lightly tapped to unify the fraction. Dishes were sealed and incubated at 28 C for the indicated period of time. Samples were taken from the soil in fractions 2 and 3 and analyzed for populations of fluorescent pseudomonads as described above. Soil adhering to the germinating seeds in fraction 1 was analyzed for colonization of fluorescent pseudomonads as described (9).

Statistical analyses. Experiments were repeated three times. Data of repeated experiments were pooled because variances among trials were homogeneous. Statistical analyses of the results included analyses of variance, Duncan's multiple range test, and calculation of standard error as indicated. All analyses were performed with the SAS program (SAS Institute Inc., Cary, NC) at $P \leq 0.05$.

RESULTS

Chemotaxis of fluorescent pseudomonads to exudates or carbon sources in capillary tubes. Attraction of *P. putida* and *P. fluorescens* to exudates originating from seeds germinated in solarized soil was significantly stronger (8.1- to 10-fold) than those from nonsolarized soil (Table 1). The number of bacteria attracted to exudates that originated from seeds germinated between filter papers was similar to the number attracted to exudates from seeds germinated in solarized soil. Amino acids alone and a mix-

TABLE 1. Chemotaxis of *Pseudomonas putida* and *P. fluorescens* to exudates of tomato seeds and to amino acids and sugars in culture^a

Attractant ^b	Log cfu/capillary	
	<i>P. putida</i>	<i>P. fluorescens</i>
Phosphate buffer	3.0 c ^z	3.0 b
Exudates		
Nonsolarized soil	3.69 c	3.30 b
Solarized soil	4.59 a	4.47 a
Filter paper	4.38 ab	4.41 a
Carbon source		
Sugars	3.24 c	3.25 b
Amino acids	4.30 b	4.29 a
Sugars + amino acids	4.47 ab	4.29 a

^a Attractants were dissolved in phosphate buffer (0.01 mM; pH = 7.0) and placed in capillary tubes. Tubes were placed with their open edge in bacterial suspension, and number of colony-forming units (cfu) of bacteria was determined after incubation for 25 min.

^b Exudates from seeds that were germinated in nonsolarized or solarized soil or between filter papers (10 mg/ml); carbon sources included sugars: a mixture of glucose (50 µg/ml), fructose (50 µg/ml), and sucrose (50 µg/ml); amino acids: a mixture of threonine and asparagine (100 nmol/ml), alanine, valine, leucine, proline, methionine, and glycine (10 nmol/L); sugars + amino acids: a mixture of the above two mixtures.

^z Within columns, figures followed by a common letter do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

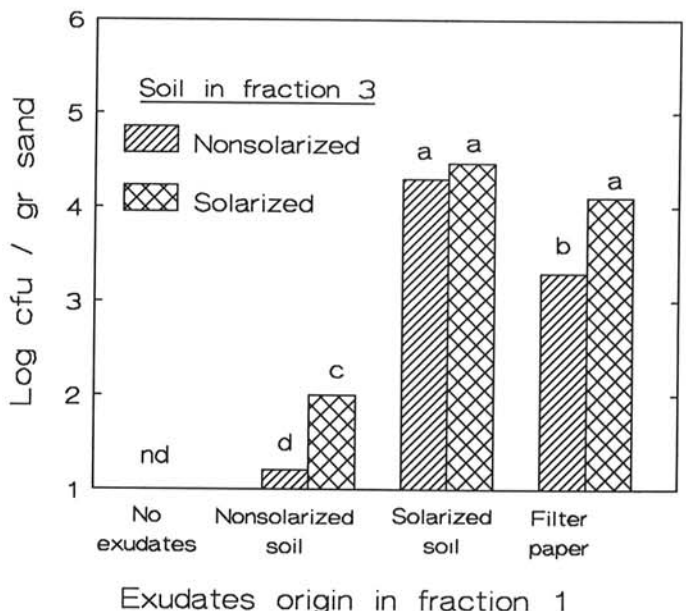


Fig. 1. Chemotaxis of fluorescent pseudomonads towards exudates from soil through a sterile sand belt. Exudates (10 mg) from seeds germinating in either nonsolarized or solarized soil were placed in the center of the inner fraction of the chemotaxis system that contained sterile sand (fraction 1). Fraction 2 contained sterile sand only, and fraction 3 (along the periphery) contained either nonsolarized or solarized soil. The number of bacteria reaching fraction 1 was determined after incubation for 24 h. Different letters indicate significant difference between treatments according to Duncan's multiple range test ($P \leq 0.05$). cfu = Colony-forming units; nd = below detectable level.

ture containing amino acids and sugars enhanced chemotaxis of pseudomonads, whereas sugars did not.

Chemotaxis of native pseudomonads towards exudates in soil.

Attraction of native fluorescent pseudomonads in soil was very pronounced towards exudates originating from seeds germinated in solarized soil with no difference between tested soils from which the bacteria migrated (Fig. 1). This was reflected in the number of bacteria able to move, within 24 h, at least 10 mm from the soil along the periphery, across the middle belt of sterile sand fraction to the exudates in the center. When exudates originated from seeds germinated in nonsolarized soil were placed in the inner fraction, greater numbers of native fluorescent pseudomonads were attracted to these exudates from solarized than from nonsolarized soil (fraction 3, in the periphery). Attraction of fluorescent pseudomonads to exudates from seeds germinated between filter papers was also high compared with exudates from seeds germinated in nonsolarized soil. In contrast, total number of bacteria that were attracted to the exudate fraction (fraction 1) ranged between 5×10^4 and 9×10^4 cfu/g without any significant difference between treatments. Thus, the majority (82–88%) of bacteria attracted from both soils to exudates from seeds germinating in solarized soil were fluorescent pseudomonads, whereas only 0.5% of the total bacteria attracted from nonsolarized soil to exudates originated from seeds germinated in nonsolarized soil were fluorescent pseudomonads. Fungi were not detected in the inner fraction, indicating that only bacteria could migrate and reach the exudate fraction during the testing period.

Chemotaxis of streptomycin-resistant bacteria towards exudates and seeds in soil. Chemotaxis of streptomycin-resistant mutants of *P. putida* towards exudates originating from seeds germinated in solarized soil was much stronger than that towards exudates originating from nonsolarized soil (Fig. 2). This was reflected in the 11-fold higher number of bacteria reaching, within 24 h, the inner soil fraction containing the exudates as compared with exudates from nonsolarized soil. A significant difference still was evident after 72 h of incubation.

Enhanced chemotaxis of bacteria was recorded also towards tomato seeds, which were placed and germinated in the inner fraction of the system that contained solarized soil (Fig. 3). Numbers of bacteria in the inner fraction in solarized soil were 10- and 19.2-fold higher than in nonsolarized soil, after 48 and 96 h, respectively. No streptomycin-resistant pseudomonads were detected in any of the soil fractions in a control system that was not infested with streptomycin-resistant *P. putida*.

DISCUSSION

Chemotaxis of fluorescent pseudomonads in capillary tubes or in soil towards exudates that originated from seeds germinating in solarized soil was very pronounced. This pattern was observed also when germinating seeds embedded in soil were used as attractants. Enhanced chemotaxis of these bacteria was observed towards a mixture of amino acids but not of sugars. These findings are in accordance with results from other studies that show attraction of *P. putida*, *P. aeruginosa*, and *P. lacrymans* to amino acids but not to sugars (3,15,17,18). They are also in accordance with the findings that exudates from roots and seeds of plants in solarized soil contain higher amounts of amino acids and lower amounts of sugars (10). Apparently, the amino acid fraction is an important component of the enhanced bacterial chemotaxis towards these exudates. Enhanced chemotaxis was obtained also with exudates from seeds that were germinated on filter paper. These exudates also contained higher amounts of amino acids than did exudates from seeds in nonsolarized soils (10), thus further supporting the role of amino acids in chemotaxis. A chemotactic response by *P. syringae* and *P. putida* towards organic acids and aromatic acids in root exudates has been demonstrated (4,15). The role of these compounds and others in root exudates in solarized soil needs to be studied further.

Origin of exudates to which native fluorescent pseudomonads were attracted is the major factor determining the intensity of chemotaxis. Chemotaxis of natural populations of fluorescent

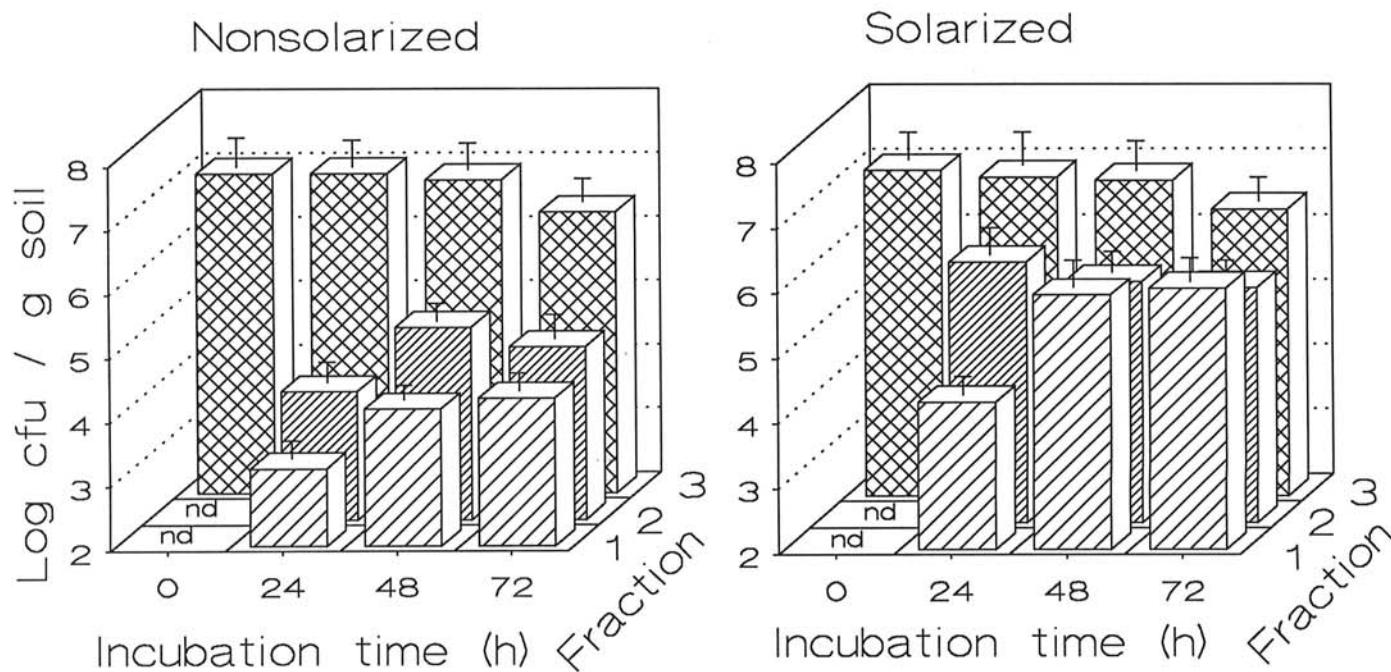


Fig. 2. Chemotaxis of *Pseudomonas putida* (MS10 resistant to streptomycin) to exudates in soil. Exudates (10 mg) from seeds that were germinated in either nonsolarized or solarized soil were placed in the center of the inner fraction of the chemotaxis system containing soil from the same origin. Fraction 2 contained nonsolarized or solarized soil as in fraction 1 without the addition of exudates or bacteria. Fraction 3 (along the periphery) contained nonsolarized or solarized as in fractions 1 and 2, mixed with the bacteria (10^7 colony-forming units [cfu] per gram of soil). Populations of bacteria were determined after incubation for the indicated periods of time. Vertical bars indicate standard error of each treatment ($P \leq 0.05$); nd = below detectable level.

pseudomonads to exudates originating from seeds germinating in solarized soil, across a sterile sand belt, was higher for both nonsolarized and solarized soil compared with the attraction to exudates from seeds germinating in nonsolarized soil. In this system (Fig. 1), most of the bacteria that were attracted to exudates from solarized soil were fluorescent pseudomonads as compared with 0.5% attracted to exudates from seeds germinating in nonsolarized soil.

Streptomycin-resistant *P. putida* exhibited a stronger attraction to exudates originating from seeds germinating in solarized soil as well as to seeds germinating in solarized soil compared with nonsolarized soil. The attraction of bacteria to exudates in the inner ring of the apparatus through soil or sterile sand belts resembles, but cannot entirely mimic, the gradient flow of exudates from roots. However, the similar results obtained with chemotaxis towards the inner ring containing either exudates (Fig. 2) or seeds (Fig. 3) support the reliability of the system. The enhanced chemotaxis of fluorescent pseudomonads towards exudates in capillary tubes reflects chemoattraction only. Enhanced chemotaxis in soil reflects both the attraction to specific components of the exudates and the improved ability to move towards the exudates or the germinating seeds in soil and to reproduce while exposed to competition by the dense populations of soil organisms and to chemical and physical forces operating in soil.

Increased growth response of plants in solarized soils is associated with enhanced colonization of the rhizosphere and plant roots by beneficial fluorescent pseudomonads (8,9). These bacteria are regarded as poor competitors in soil (10,19), thus limiting their establishment in the root zone of plants in a natural undisturbed soil. Solarization improves the competitive ability of fluorescent pseudomonads for root exudates by reducing the populations of other microorganisms (2,8,9,11,16,21). The stronger chemotaxis of fluorescent pseudomonads towards exudates originating from seeds in solarized soil indicates that root exudates play a role in the improved establishment of fluorescent pseudomonads in the root zone in solarized soils. These bacteria are potential biocontrol agents (2,6,9,20; A. Gamliel and J. Katan,

unpublished data). Thus, the improved chemotactic ability also may play a role in the induced suppressiveness found in solarized soils (2,13,16).

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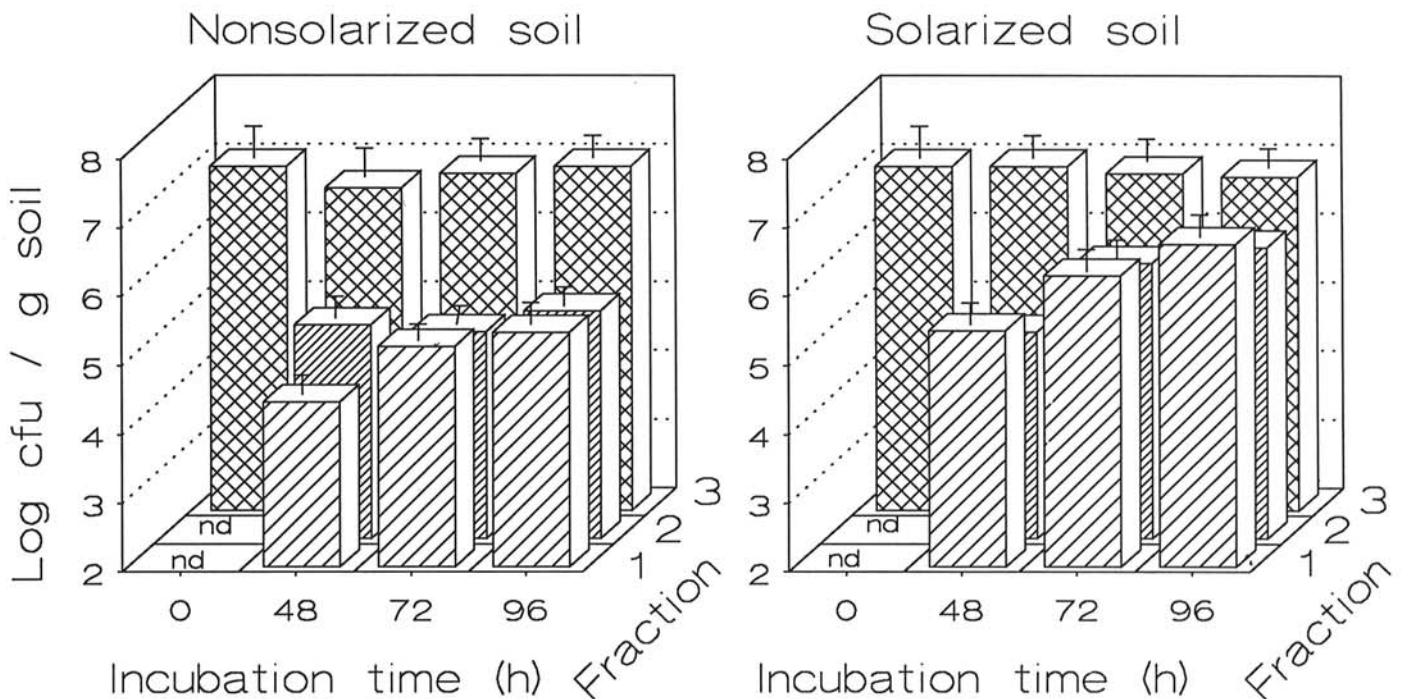


Fig. 3. Chemotaxis of *Pseudomonas putida* (MS2 resistant to streptomycin) to germinating seeds in soil. Ten surface-sterilized tomato seeds were embedded in the center of the inner fraction of chemotaxis system containing either nonsolarized or solarized soil. Fraction 2 contained nonsolarized or solarized soil as in fraction 1 without addition of seeds or bacteria. Fraction 3 (along the periphery) contained nonsolarized or solarized soil as in fractions 1 and 2, mixed with tested bacteria (10^7 colony-forming units [cfu] per gram of soil). Populations of bacteria were determined after incubation for the indicated periods of time. Vertical bars indicate standard error of each treatment ($P \leq 0.05$); nd = below detectable level.

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