Influence of Seed and Root Exudates on Fluorescent Pseudomonads and Fungi in Solarized Soil

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ABSTRACT

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We investigated the effect of soil solarization on seed and root exudates of tomato plants and the role of these exudates in the establishment of fluorescent pseudomonads in soil. Seed and root exudates of tomato grown in solarized soil contained lower amounts of sugars and higher amounts of amino acids and amino compounds and were less favorable for growth of bacteria and fungi in culture compared with seed and root exudates of tomato grown in nonsolarized soil. Addition of exudates from germinating seeds into solarized soil, however, increased populations of fluorescent pseudomonads and decreased populations of fungi compared with nonsolarized soil. A similar effect was obtained with volatile exudates from germinating seeds. Addition of antimicrobial agents to nonsolarized soil supplemented with exudates reduced populations of soil microorganisms and increased populations of fluorescent pseudomonads. We suggest that the rapid establishment of fluorescent pseudomonads in the rhizosphere of plants in solarized soils is due to an improved capacity of these bacteria to compete for exudates.

Additional keywords: beneficial microorganisms, fluorescein diacetate, 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 a well documented phenomenon (3,9,10,12,19,31,39). This was observed in greenhouse experiments and under field conditions. Increased growth is attributed to various chemical, physical, and biotic factors (3,4,10,31,39). Pronounced changes in microbial activities in solarized soil take place, as reflected in the rate of enzymatic activities as determined by fluorescein diacetate (FDA) hydrolysis, in stimulation of antagonistic activity and in induced soil suppressiveness (10,15,19,31,40). Colonization of the rhizosphere and roots by fluorescent pseudomonads increased after solarization of soil or container media (3,9,10,12). Strains of these bacteria improve growth of inoculated plants in greenhouse experiments; in contrast, fungal establishment in the rhizosphere and roots after solarization is suppressed (9,10). Certain fungal species, such as *Penicillium pinophilum* Hedgc., suppress plant growth and are regarded as minor pathogens (10).

Root exudates provide the major energy source in the root zone for microbial activity and colonization of living and nonliving substrates. Content and composition of root exudates are affected by edaphic factors, pesticides, pathogens, and soil microorganisms (16,18,20,22-24,29,32-36,38). In the present study, we investigated the effect of soil solarization on the composition of root and seed exudates; the effect of soluble and volatile root exudates on populations of fluorescent pseudomonads and fungi in soil; and the role of exudates in the establishment of fluorescent pseudomonads in solarized soils.

MATERIALS AND METHODS

Soils. Soils used originated from Rehovot (3.8% clay, 0% silt, 96.2% sand, and 0.4% organic matter; pH 6.9) and Bet HaShitta (55% clay, 17.5% silt, 27.5% sand, and 1.3% organic matter; pH 7.2). Soils were left untreated or disinfested by solarization. Solarization was carried out manually by mulching preirrigated soil with transparent polyethylene sheets (40–50 μm thick) for 6 wk during July–August in 1988 and 1989 (10,15). The typical maximal temperatures of the solarized soils were 48 and 41 C, at depths of 10 and 30 cm, respectively. Temperatures of the corresponding nonsolarized soils were 7–12 C lower. Samples from solarized and nonsolarized soils were collected from the upper 20 cm layer (after removal of the top 2–3 cm) of the soils and kept in containers in the shade until use. In some experiments, sterile acid-washed sea sand (BDH, Poole, England) was also used.

Culture media. The following media were employed: nutrient agar (N) (6) was used for enumeration of total bacteria; colloidal chitin medium (17) was used for the enumeration of actinomycetes; King's B medium (KB) (6) was used for culturing *Pseudomonas putida*; KB medium, modified by the addition of 100 mg/L of cycloheximide, 50 mg/L of ampicillin, and 12.5 mg/L of chloramphenicol (30), and supplemented with 5 mg/L of pentachloronitrobenzene (PCNB) to suppress *Rhizopus* spp. (10) was used for enumeration of fluorescent pseudomonads from soil; potato dextrose agar (PDA) (6) was used for culturing *P. pinophilum*; Martin's agar (6) was used for enumeration of total fungi; Martin's agar (6) supplemented with 5 mg/L of PCNB (10) was used for enumeration of *P. pinophilum*; and Czapek salt agar (Czapek-Dox medium lacking sucrose) (6) was used to test growth of *P. pinophilum* on exudates as the sole carbon source.

Microorganisms. *P. putida* (RS34M) originally was isolated from the rhizosphere of tomato plants grown in solarized Rehovot soil and was defined as a plant growth-promoting rhizobacterium because it significantly increased dry weight of tomato plants by 42% after root inoculation in greenhouse tests (10). This strain was stored in glycerol at −70 C until 24 h before use and then was spotted on KB agar medium and grown for 24 h. *P. pinophilum* (ST50) initially was isolated from rhizosphere of tomato plants grown in nonsolarized Bet HaShitta soil. It was defined as a minor pathogen because it reduced growth of inoculated tomato seedlings by 58% in a greenhouse test (10).

Plant growth. Surface-sterilized (1% NaOCl for 30 s) seeds of tomato (*Lycopersicon esculentum* Mill. 'Rehovot 13') initially were sown in test soil, nonsolarized or solarized, and transplanted 1 day after emergence to new pots (12 cm diameter, five transplants per pot with six replicates) filled with the same soil. Pots were placed in a growth chamber (25 C with 14 h of daily artificial illumination at light intensity of 400 $\mu E \cdot m^{-2} \cdot s^{-1}$), and treatments were arranged in a completely randomized design. At different growth stages, seedlings were removed from the soil, along with their roots and adhering soil. Shoots were cut, and dry weight (70 C for 48 h) was determined. Rhizosphere soil adhering to the roots was collected by shaking in sterile 0.1% water agar supplemented with 0.1% $MgSO_4 \cdot 7H_2O$ (10) and was used for assay of microbial activity. For collection of exudates from germinating seeds, 200 seeds were sown in either nonsolarized or solarized soil moistened to field capacity in glass petri dishes (85 mm diameter) with five replicates. In some experiments, surface-sterilized seeds were placed between moistened sterile filter papers in glass petri dishes. Dishes were placed in a growth chamber (described above) for the indicated period of time.

Collection of seed and root exudates. Root exudate collection was performed essentially as described by Graham et al (13). Plants or germinating seeds were removed from the soil and washed free of soil. Roots or the whole germinating seeds were submerged for 2 h in glass beakers containing 200 ml of aerated $CaCl_2$ solution supplemented with 0.05 g/L of rifampicin and 0.025 g/L of tetracycline (Sigma, St. Louis, MO). Roots or germinating seeds were then rinsed twice with 500 ml of sterile 0.5 mM $CaCl_2$ and immediately submerged again in sterile-aerated 200 ml of 0.5 mM $CaCl_2$ solution for 22 h at 25 C in the dark. Throughout the whole process, beakers were aerated by bubbling fresh air through perforated tubes that were placed in each beaker. Bacterial contamination was monitored throughout the process by spread plating samples from exudate solutions on petri dishes containing N agar. Antibiotic pretreatment reduced bacterial populations by 99%. Roots or germinating seeds were removed from the solution and air dried at 70 C for 48 h. Dry weight was determined. Exudate solutions immediately were passed through a sterile 0.45-μm membrane filter, freeze-dried, and kept frozen at −18 C until used.

Analyses of exudates. Exudates were tested for sugar content and composition, amino acids, amino compounds, and for electrolyte content. Total sugar content was determined using phenol-sulfuric acid reagent (1). Determination of specific sugars (Table 1) was done by high-performance liquid chromatography (HPLC) (Waters, Bedford, MA). Sugars were separated on two reverse-phase Guard Pack and Sugar pack I columns, using distilled water flowing at a rate of 0.5 ml/min at 90 C as the solvent, and detected with a refractive index detector (Waters 410, Bedford, MA). Amino acids were analyzed on a cation exchange column, amino acid analyzer (Biotronik LC-7000, Frankfurt-Maine, Germany), using a sodium citrate buffer system as eluent. Other amino compounds (Table 2) were separated and quantified on a cation exchange column amino acid analyzer (LKB Alpha Plus 4151, Cambridge, England) using lithium citrate buffer as eluent. Content of electrolytes in exudates was determined by measuring the electrical conductivity (EC) of the exudate solution with conductivity meter (Radiometer CDM-83, Copenhagen, Denmark).

Microbial counts by dilutions. Three 5-g soil or sand subsamples of each replicate were added individually to 45 ml of sterile water agar (0.1%) supplemented with $MgSO_4 \cdot 7H_2O$ (0.1%), shaken for 15 min on a reciprocal shaker, and then serially diluted with the same solution. Samples of 0.1 ml (for bacterial counts) or 0.2 ml (for fungal counts) taken from the appropriate dilutions were spread on five petri dishes containing the appropriate selective agar. Dishes were incubated in the dark at 28 C. Colonies were counted after 4–7 days. Results are expressed as colony-forming units (cfu) per gram of soil or sand (dried at 105 C for 48 h).

Growth of microorganisms on seed exudates as the sole carbon source. *P. putida* was cultured on KB for 48 h, and then colonies were suspended in 0.01 mM potassium phosphate buffer (pH 7.0) and washed twice. The bacterial suspension was mixed into sterile sea sand (BDH) (10^4 cfu/g as determined by optical density) supplemented with exudates from seeds (10 mg/g) that were germinated in nonsolarized or solarized soil or on filter paper. Infested sand was moistened to 15% (v/w) and incubated at 28 C for the indicated period of time, after which cfu number of *P. putida* was determined.

P. pinophilum was cultured on PDA for 8 days. Then, 3-mm-diameter disks of mycelium were transferred individually to the center of a petri dish containing Czapek salt agar supplemented with 30 mg/ml of exudates from seeds that were germinated in nonsolarized soil, solarized soil, or on filter paper. Dishes were incubated in the dark at 28 C, and colony area was measured during 9 days of incubation. *P. pinophilum* also was grown on PDA supplemented with exudates as described above for Czapek salt agar to detect any possible toxic effect of exudates. Population density of *P. pinophilum* in the presence of exudates also was tested by mixing washed conidia from culture of 8 days in sterile sand as described above for *P. putida*.

TABLE 1. Effect of soil solarization on composition of sugars in root exudates from tomato grown in Rehovot (R) and Bet HaShitta (BH) soils[a]

		Days after sowing								Days after transplanting					
		2			3			8		7		14		21	
Soil	Compound[b]	NS	S	P	NS	S	P	NS	S	NS	S	NS	S	NS	S
R	Glucose	70	50	39*[c]	85	45*	53*	1,600	500*	450	75*	285	110*	145	15*
	Fructose	10	15	10	20	10	15	300	180*	120	25*	45	28	72	10*
	Sucrose	0	0	0	0	0	0	150	90*	60	0*	15	0*	24	0*
	Others[d]	9	9	11	25	12	5*	432	63*	145	15*	52	38	224	0*
	Total	89	74	60	130	67*	73*	2,482	833*	775	115*	397	181*	282	47*
	NS/S	1.2			1.94			3		6.74		2.2		6	
BH	Glucose	130	85	39*	138	59*	53*	820	390*	430	180*	310	110*	275	18*
	Fructose	30	15	10	30	3*	15	135	65*	120	63*	45	25	62	7*
	Sucrose	20	10	0	25	8*	0	95	30*	33	27	27	10	15	0*
	Others	40	30	11*	57	15*	5*	92	44*	55	24*	68	30*	48	10*
	Total	220	140*	60*	250	85	73*	1,142	529*	650	300*	450	175*	400	35*
	NS/S	1.57			2.94			2.16		2.16		2.57		11.4	

[a] Tomato plants were sown in nonsolarized soil (NS), solarized soil (S), or on filter paper (P) and incubated in a growth chamber for the indicated period of time. After emergence (8 days), seedlings were transplanted to pots with the same soil. Exudates were collected at the indicated periods.
[b] Sugar content was determined by high-performance liquid chromatography and expressed as micrograms per gram of root dry weight.
[c] Asterisks denote significant difference from the corresponding nonsolarized soil according to the Student's t test ($P < 0.05$).
[d] Others = unidentified sugars.

Effect of root exudates and organic compounds on microorganisms in soil. Exudates from tomato seeds that were germinated for 3 days in either solarized or nonsolarized soil or on sterile filter paper were each mixed with sieved Rehovot soil (5 mg/g) in 6-cm-diameter glass petri dishes. Soil was moistened to field capacity and incubated in the dark at 28 C for 4 days. Populations of total fungi and fluorescent pseudomonads were determined by soil dilution.

Mixtures of sugars (100 μg/g of glucose, 50 μg/g each of fructose and sucrose) and amino acids (100 nmol/g each of asparagine and threonine; 10 nmol/g each of alanine, glycine, methionine, proline, leucine, and valine) in quantities similar to those found in root exudates (Tables 1 and 2) were added to soil to test their effect on microbial populations.

The following antimicrobial agents were incorporated into nonsolarized Rehovot soil to follow their effect on the establishment of fluorescent pseudomonads on exudates from germinated seeds: ampicillin (75 μg/g), fuchsin (10 μg/g), mixture of 2,3,5-triphenyltetrazolium (100 μg/g) and nalidixic acid (20 μg/g), PCNB (20 μg/g), and cycloheximide (100 μg/g). Subsequently, exudates from seeds germinated for 3 days in nonsolarized soil also were mixed in treated soil, and the soil was moistened to field capacity. Soils were incubated in the dark at 28 C for 4 days, after which populations of the indicated microorganisms were determined.

Iron chelators were mixed with soil to test the possibility that iron mediates the rapid establishment of fluorescent pseudomonads in solarized soil. Ferum ethylenediaminetetracetate (FeEDTA) (100 mg/g), ethylenediamine di-o-hydroxyphenylacetate (EDDHA) (100 mg/g), 8-hydroxyquinoline (100 mg/g), and pseudobactin (0.5 and 2 mg/g) obtained from *P. putida* (kindly supplied by E. Yurkevitz, Dept. of Plant Pathology and Microbiology, Hebrew University of Jerusalem, Israel) were each mixed with nonsolarized or solarized sieved Rehovot soil. Subsequently, exudates from seeds germinated for 3 days in either nonsolarized or solarized soil were mixed in the treated soils moistened to field capacity. Soils were incubated in the dark at 28 C for 4 days, and then populations of fluorescent pseudomonads and total fungi were determined. Concentrations of Fe^{3+} extractable in diethylenetriaminepentaacetic acid (DTPA) were 150 and 170 μg/kg of soil in nonsolarized and solarized soils, respectively, before adding the chelators.

Volatile exudates from germinating seeds. Effect of volatile exudates from germinating seeds on microorganisms in soil was tested in an apparatus described below. One hundred surface-sterilized tomato seeds were sown in an uncovered glass petri dish (50 mm diameter, 10 mm high) in 20 g of nonsolarized or solarized Rehovot soil, or in sterile sea sand at a depth of 0.5 cm. Soil with seeds was moistened to field capacity, and each dish was placed in the center of a glass petri dish (90 mm diameter, 15 mm high). Ten grams of Rehovot soil at field capacity, either nonsolarized or solarized, was placed around the wall of the inner dish in the space between the two dish walls, without direct contact between the soil and the seeds in the small inner dish. In this system, only volatiles from the germinating seed could pass through to the soil in the periphery. The large dishes were covered, sealed with polyethylene bags, and incubated at 28 C for 4 days. Then, populations of fluorescent pseudomonads and fungi in the soil fraction in the periphery of the outer dish were determined. In another experiment, the inner petri dish with seeds was placed at the rim of a 90-mm petri dish containing PDA medium. A mycelium disk of an 8-day culture of *P. pinophilum* was placed on the agar between the dish with the seeds and the other edge

TABLE 2. Effect of soil solarization on composition of amino compounds in root exudates from tomato grown in Rehovot soil[a]

	Days after sowing										Days after transplanting					
	2			3			5		8		7		14		2	
Compound	NS	S	P	NS	S	P	NS	S	NS	S	NS	S	NS	S	NS	S
Amino acids[b]																
Aspartic acid	9	22	21	7	30*	18*	13	69*	439	614*	75	360*	170	306*	0	75*
Threonine	11	26	39*[c]	8	37*	24*	14	96*	296	357	35	430*	50	170*	0	35*
Serine	15	24	46*	21	35	29	10	30	195	665*	35	165*	140	310*	0	56*
Glutamic acid	13	36*	25	15	140*	45*	25	201*	47	773*	60	770*	27	157*	0	60*
Proline	0	14	7	0	0	33*	5	0	5	0	0	1,050*	0	193*	0	0
Glycine	10	26	32	15	25	26	9	31*	228	466*	30	630*	160	200	85	60
Alanine	12	33	43*	15	91*	31	14	55*	205	345*	45	74	110	165	65	80
Cysteine	0	0	6	0	3	27*	10	13	143	332*	0	0	0	0	0	0
Valine	5	17	20	6	43*	15	8	54*	140	207	0	22*	40	50	0	0
Methionine	5	7	27*	3	38*	9	17	19	67	148*	5	21*	37	75*	0	0
Isoleucine	4	12	16	3	40*	10	6	44*	109	160	0	0	40	93*	25	50
Leucine	6	18	25*	4	39*	11	7	27	130	245*	27	23	36	98*	30	75*
Tyrosine	3	8	8	2	11	6	0	0	0	0	0	10	35	100*	10	0
Phenylalanine	2	8	7	2	18*	4	6	17	50	323*	0	0	0	80*	0	0
Histidine	10	18	34*	10	18	12	19	29	321	448*	25	462*	112	185*	90	7*
Lysine	3	11	12	3	25*	6	9	36*	155	275*	71	0*	35	94*	2	8
Arginine	4	17	9	3	17	8	0	22*	0	227*	140	490*	150	354*	0	0
Total	112	297*	377*	117	727*	314*	172	743*	2,530	5,585*	548	4,507*	1,219	2,630*	307	506*
NS/S	0.38			0.16			0.23		0.45		0.12		0.46		0.61	
Amino compounds																
Asparagine				0	56*	28*			358	531*						
Glutamine				0	60*	42*			0	352*						
α Amino adipic acid				0	22*	12			82	142						
β Amino butyric acid				0	182*	160*			97	0*						
γ Amino butyric acid				560	800*	620			777	833*						
Cystine				0	18*	10			131	596*						
Ethanolamine				0	84*	36*			0	2,400*						
Citruline				0	44*	20*			41	82*						
Ornithine				169	1,900*	809*			309	600*						
Total				729	3,166*	1,737*			1,795	5,536*						
NS/S				0.23					0.32							

[a]Tomato plants were sown in nonsolarized soil (NS), solarized soil (S), or on filter paper (P) and incubated in a growth chamber for the indicated period of time. After emergence (8 days), seedlings were transplanted to pots with the same soil. Exudates were collected at the indicated periods.

[b]Amino acid and amino compound contents were determined in an amino acid analyzer and are expressed as nanomoles per gram of dry root.

[c]Asterisks denote significant difference from the corresponding nonsolarized soil according to the Student's *t* test ($P < 0.05$).

of the plate. Dishes were covered and incubated at 28 C for 5 days, after which colony area was measured (average diameter was taken from two measurements at right angles).

Determination of microbial activity in soil and rhizosphere. Hydrolysis of FDA was used as an enzymatic assay for the determination of general microbial activity (27,40). Soil samples were taken from either nonsolarized or solarized soil and from the rhizosphere of plants grown on either of the soils. Three 8-g subsamples of soil were preincubated with 50 ml of 60 mM sodium phosphate buffer (pH 7.6) for 30 min on a reciprocal shaker (150 strokes per minute). Subsequently, 0.5 mg of FDA dissolved in 250 μl of acetone was added to each suspension, and the suspensions were further incubated for 1 h by shaking as described. Samples of 0.6 ml of the suspension were transferred to a microfuge tube, and an equal volume of acetone was added thereby terminating the hydrolytic reaction. Tubes were centrifuged for 10 min at 10,000 *g*, and optical density (OD) of the supernatant was determined at 490 nm (Kontron, Uvikon 810, Switzerland). Amount of FDA hydrolyzed was calculated from a standard curve of fluorescein, which was performed according to the procedure by Chen et al (2). Aliquots of 0, 100, 200, 300, 400, or 500 μg of FDA from acetone stock solution were added in three replicates to 5 ml of phosphate buffer in test tubes. Tubes were closed tight and heated in boiling water for 60 min to hydrolyze all the FDA. Hydrolyzed FDA was added to flasks containing 8 g of Rehovot soil to which 45 ml of phosphate buffer was added. The suspension was shaken for 10 min, and then samples of 0.6 ml were transferred to a microfuge tube containing an equal volume of acetone. Tubes were centrifuged, and OD of the supernatant was determined. A linear standard curve of the hydrolyzed FDA was obtained.

Statistical analyses. Greenhouse experiments and microbial analyses were conducted at least three times, and exudate collection and analyses five times, unless otherwise indicated. Data of repeated experiments were pooled because variances between trials were homogeneous. Statistical analyses of the results included analysis of variance, Student's *t* test, or Duncan's multiple range test, as indicated. All analyses were performed with the SAS program (SAS Institute Inc., Cary, NC) at $P \leq 0.05$.

RESULTS

Increased growth response. An increase in growth of tomato seedlings resulting from solarization was observed in both Rehovot and Bet HaShitta soils. Significant ($P \leq 0.05$) increases in dry weight of roots and shoots of plants grown in solarized soil, over the nonsolarized Rehovot soil, reached 57 and 45%, respectively, 21 days after transplanting. The respective increases in Bet HaShitta soil were 46 and 59%.

Root exudates in solarized and nonsolarized soil. Amount and composition of sugars and amino compounds in exudates of tomato roots and seeds germinating in nonsolarized soils were different from those in solarized soils. Glucose was the main sugar found in exudates, usually constituting more than 50% of total sugars (Table 1). The predominant sugar in miscellaneous unidentified sugars was an oligosaccharide that could not be identified by the HPLC separation column that was used. Total sugar content was higher in root exudates from plants grown in nonsolarized soil as compared with solarized soil in both Rehovot and Bet HaShitta soils during all growth stages. This trend was observed 2–3 days after sowing, but was more pronounced at an advanced stage of plant growth. The increase in sugar content in exudates from plants grown in nonsolarized soils was in the range of 20–574% in Rehovot soil and 57–1,040% in Bet HaShitta soil.

Amount of amino acids and amino compounds was higher in root exudates from plants grown in solarized than in nonsolarized Rehovot soil (Table 2). This was evident in germinating seeds 2 days after sowing. The increase in total amino acids in exudates from roots of plants grown in solarized soil over those from plants grown in nonsolarized ranged from 65 to 720%. The increase in aspartic acid was especially pronounced. A similar trend was observed with other amino compounds, especially ethanolamine and ornithine, which were produced in very high amounts (Table 2). Some of the amino acids (e.g., proline, arginine, and tyrosine) were found at very low levels in root exudates of plants grown in nonsolarized soil during all or most growth stages. The above trends regarding amino acids and amino compounds were obtained also with tomato plants in Bet HaShitta soil (results not shown). In Bet HaShitta soil, the solarization-

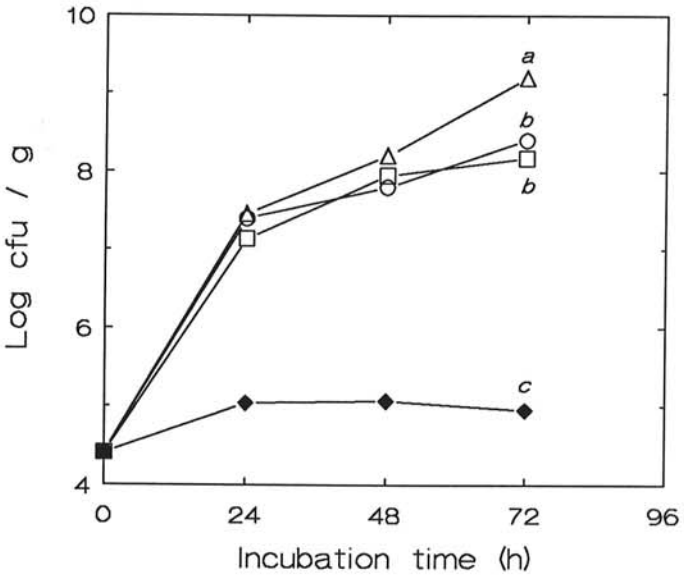

Fig. 1. Effect of exudates from germinating tomato seeds on growth of *Pseudomonas putida* in sterile sand. Sand was mixed with exudates (10 mg/g of sand) moistened to 15% (v/w) with a bacterial suspension (5 $\times 10^4$ cells per gram) and incubated for the indicated periods of time. Different letters indicate significant differences among treatments at $P \leq 0.05$, according to Duncan's multiple range test. ■ = No exudates; △ = exudates from seeds germinated in nonsolarized soil; ○ = exudates from seeds germinated in solarized soil; and □ = exudates from seeds germinated on sterile filter paper. cfu = Colony-forming units.

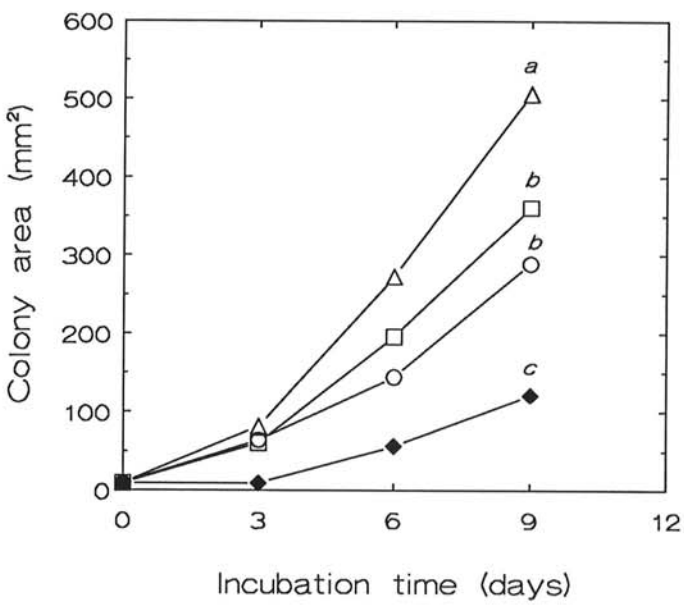

Fig. 2. Effect of exudates from germinating tomato seeds on growth of *Penicillium pinophilum* in culture. Exudates from germinating seeds were added to Czapek salt agar medium (30 mg/ml), and a disk of a culture was placed in the center of each petri dish. Different letters indicate significant differences among treatments at $P \leq 0.05$, according to Duncan's multiple range test. ■ = No exudates; △ = exudates from seeds germinated in nonsolarized soil; ○ = exudates from seed germinated in solarized soil; and □ = exudates from seeds germinated on sterile filter paper.

caused increase in amino acids and amino compounds in exudates of seedlings at emergence was 200 and 368%, respectively. This increase was especially pronounced with aspartic acid, glutamic acid, ethanolamine, and ornithine.

In both solarized and nonsolarized soils, total amount of exudates (sugars and amino compounds) is highest at seedling emergence (8 days after sowing) (Tables 1 and 2). Amount of exudates, especially amino acids, decreased 3 wk after trans-

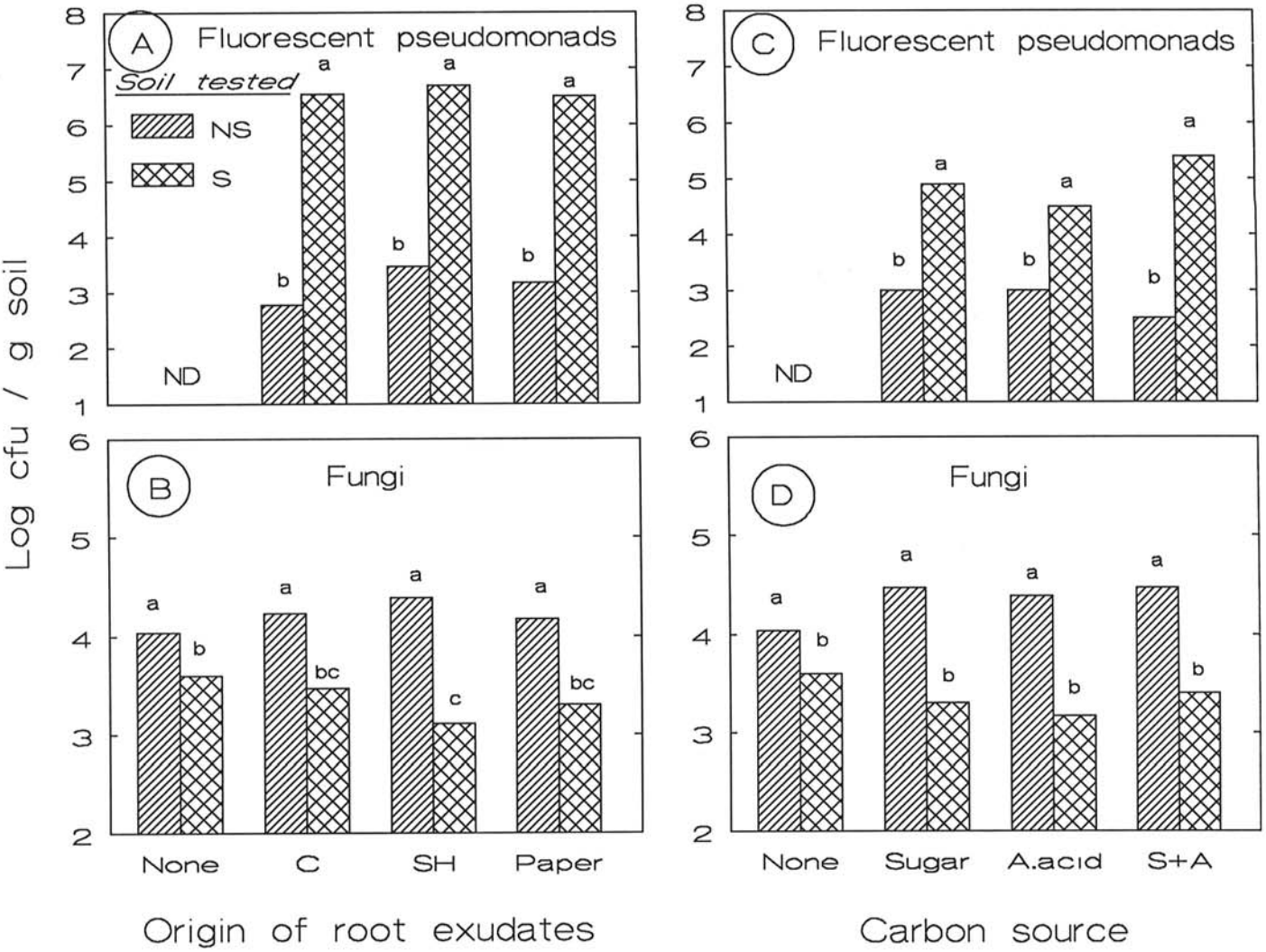

Fig. 3. Effect of seed exudates, sugars, and amino acids on the population density of fluorescent pseudomonads (A and C) and fungi (B and D) microorganisms in soil. **A** and **B,** exudates from seeds that were germinated in nonsolarized soil (C), solarized soil (SH), or on filter paper (paper), and were mixed with nonsolarized (NS) or solarized (S) Rehovot soil (4 mg/g). **C** and **D,** mixture of sugars (Sugar) containing glucose (100 μg/g), fructose, and sucrose (50 μg/g each); mixture of amino acids (A. acids) containing 100 nmol/g each of asparagine and threonine and 10 nmol/g each of alanine, glycine, methionine, proline, leucine, and valine; and a combination of the two mixtures (S+A) mixed in nonsolarized (NS) or solarized (S) soil. Population densities were determined after 4 days of incubation. Within each figure, different letters indicate significant differences among treatments ($P \leq 0.05$), according to Duncan's multiple range test. ND = below detectable level.

TABLE 3. Effect of microbial inhibitors on population densities of soil microorganisms in soil supplemented with root exudates[a]

Treatment[b]	Total bacteria ×10⁶	Fluorescent pseudomonads ×10³	Actinomycetes ×10³	Fungi ×10³
No exudates	15*[c]	0*	15*	14*
Exudates	90	8	40	38
Exudates + AM	25*	200*	16*	50
Exudates + FU	45*	77*	17*	23
Exudates + NA + TTC	18*	80*	20	23
Exudates + PCNB	92	160*	6*	33
Exudates + CY	100	600*	10*	8*

[a] Microbial inhibitors and exudates from seeds germinated for 3 days (4 mg/g of soil) were mixed with nonsolarized Rehovot soil and moistened to field capacity. Population density of microorganisms was determined by the soil dilution method after 4 days of incubation at 28 C. Numbers denote colony-forming units per gram of dry soil (105 C for 48 h). Zero denotes below detectable level.

[b] AM = ampicillin (75 μg/g); FU = fuchsin (10 μg/g); NA = nalidixic acid (20 μg/g); TTC = 2,3,5-triphenyltetrazolium chloride (100 μg/g); PCNB = pentachloronitrobenzene (20 μg/g); CY = cycloheximide (100 μg/g).

[c] Within each group of microorganisms, asterisks denote significant differences between the indicated treatment and the exudate treatment according to Student's *t* test ($P < 0.05$).

planting. Root exudates from seedlings on filter paper contained lower amounts of sugars and higher amounts of amino compounds as compared with exudates from plants in nonsolarized soils. The EC of exudates from germinating seeds or roots of plants was similar in nonsolarized and solarized soils (70 and 75 mmho, respectively), indicating a similar electrolyte content in exudates.

Growth of microorganisms on root exudates as the sole carbon source. The population density of *P. putida* was significantly high after 72 h of incubation when bacteria were grown on exudates from seeds that were germinated in nonsolarized soil as compared with solarized soil or filter paper (Fig. 1). After 72 h, the respective numbers of bacteria were 1.5×10^9, 3×10^8, and 1.5×10^8 cfu/g of sand, respectively. Similarly, growth of *P. pinophilum* was the most rapid on an agar medium supplemented with exudates from seeds that were germinated in nonsolarized soil (Fig. 2). The population densities of *P. pinophilum* grown in sand supplemented with exudates from seeds that were germinated in nonsolarized soil, solarized soil, or filter paper were 8.8×10^4, 3.5×10^4, and 2×10^4 cfu/g of sand, respectively. The difference was significant only between nonsolarized soil and filter paper.

Effect of external substances on microorganisms in soil. Addition of exudates from any origin to solarized soil resulted in a pronounced increase in populations of fluorescent pseudomonads as compared with nonsolarized soil (Fig. 3A). Exudate origin did not have a significant effect on the population of fluorescent pseudomonads. Population densities of these bacteria were below detectable levels in both nonsolarized and solarized soils, if exudates were not added. In contrast, the population density of total fungi in solarized soils was lower than in the corresponding nonsolarized soils, with or without the addition of exudates (Fig. 3). Addition of exudates from seeds that were germinated in solarized soil to solarized soil further decreased fungal populations. Growth of *P. pinophilum* was not inhibited when grown on PDA supplemented with any of these exudates (results not shown), thus excluding the possibility that the slower growth of *P. pinophilum* on exudates from solarized soil is due to the presence of toxic substances.

TABLE 4. Effect of root exudates and iron chelators on populations of fluorescent pseudomonads and fungi in soil[a]

Treatment[b]	Soil treatment	Fluorescent pseudomonads $\times 10^3$[c]	Fungi $\times 10^2$
No exudates	NS[d]	0*[e]	90*
	S	0[f]	20
Exudates (E)	NS	3	630
	S	2,900	10
Exudates + FeEDTA	NS	6	100*
	S	3,000	11
Exudates + EDDHA	NS	0	500
Exudates + pseudobactin	NS	3	190*
Exudates + pseudobactin	NS	5	140*
Exudates + 8-hydroxiquinoline	NS	0*	20*

[a] Iron chelators and exudates from seeds germinated for 3 days (4 mg/g of soil) were mixed with nonsolarized Rehovot soil (moistened at 12%). Population density of microorganisms was determined by the soil dilution method after 4 days of incubation at 28 C. Concentrations of Fe in nonsolarized and solarized soils were 150 and 170 μg/kg, respectively, before adding the chelators.

[b] FeEDTA = ferum ethylenediamine tetracetate (100 μg/g); EDDHA = ethylenediamine di-o-hydroxyphenylacetate (100 μg/g); pseudobactin at 0.5 μg/g and 2 μg/g, respectively; 8-hydroxiquinoline at 100 μg/g.

[c] Numbers denote colony-forming units per gram of dry soil (105 C for 48 h); zero denotes below detectable level.

[d] NS = nonsolarized; S = solarized.

[e] Within each group of microorganisms, an asterisk denotes significant difference between the indicated treatment and the exudate treatment in NS soil according to Student's t test (< 0.05).

[f] Significantly different from the exudate treatment in S soil according to Student's t test ($P < 0.05$).

Addition of a sugar mixture or an amino acids mixture alone or combined to solarized soil resulted in a significant increase in populations of fluorescent pseudomonads and a decrease in fungal populations as compared with nonsolarized soil (Fig. 3). This trend was similar to that observed with exudates.

All the tested microbial inhibitors greatly increased the populations of fluorescent pseudomonads in nonsolarized soil amended with exudates (Table 3). These inhibitors were chosen because they do not affect fluorescent pseudomonads and thus are used in selective media for the isolation of pseudomonads from soil (8,14,30). The most pronounced effect was observed with the antifungal agent cycloheximide, which also reduced fungal populations by 79%. PCNB did not affect number of total bacteria but was strongly inhibitory to actinomycetes, similar to other findings (8), and resulted in a significant increase in populations of fluorescent pseudomonads. The effect of the various antimicrobial agents on fluorescent pseudomonads, total bacteria, actinomycetes, and fungi varied with different agents.

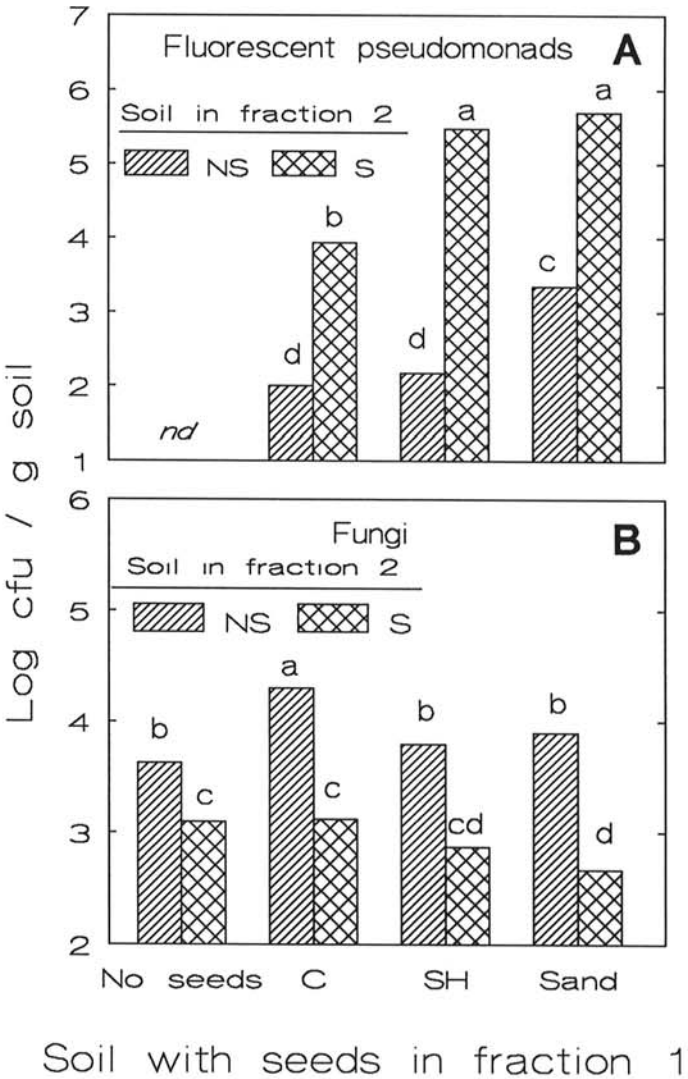

Fig. 4. Effect of volatile exudates from germinating seeds on populations of fluorescent pseudomonads (A) and fungi (B) in soil. Surface-sterilized tomato seeds were sown in a small petri dish (fraction 1) in nonsolarized soil (C), solarized soil (SH), or sterile sand (Sand). The plate with seeds was placed in the center of an 85-mm-diameter petri dish and nonsolarized (NS) or solarized (S) soil was spread around the inner plate (fraction 2). Densities of microbial populations were determined after 4 days of incubation. Within each group of microorganisms, different letters indicate significant differences among treatments ($P \leq 0.05$), according to Duncan's multiple range test. ND = below detectable level; cfu = colony-forming units.

The use of the chelator FeEDTA, which increases iron availability, did not affect the population of fluorescent pseudomonads in either nonsolarized or solarized soil amended with exudates, as compared with their number in the corresponding soil amended only with exudates (Table 4). Increasing iron availability to fluorescent pseudomonads by adding the siderophore pseudobactin or decreasing iron availability to these bacteria by adding EDDHA and 8-hydroxyquinoline in nonsolarized soil amended with exudates had no effect on numbers of fluorescent pseudomonads compared with soil amended with exudates only.

Volatile exudates from germinating seeds. Volatile exudates increased by up to 5,048-fold (Fig. 4) the populations of fluorescent pseudomonads in solarized soil, regardless of the source of volatiles, as compared with nonsolarized soils. Numbers of total fungi were significantly higher in nonsolarized soils exposed to volatiles originating from seeds in nonsolarized soil or sand than from the comparable solarized soil (Fig. 4). The effect of volatile exudates on population density of fungi was less pronounced than on the density of the bacteria. None of the volatiles from either origin exhibited toxicity to *P. pinophilum*, as determined by measuring fungal growth on PDA medium.

Microbial activity in the soil and rhizosphere. Microbial activity in the rhizosphere of tomato plants in solarized soil was significantly lower than in nonsolarized soil until the second week after transplanting (Fig. 5). Microbial activity in the nonrhizosphere soils was lower than in the rhizosphere soil and decreased by 59% after solarization (Fig. 5); it was highest at seedling emergence.

DISCUSSION

The increase in plant growth in disinfested soils, in the absence of known pathogens, has been attributed to abiotic factors (e.g., improved mineral nutrient status) (2,3) and to biotic factors (3,9,10,39). Enhanced colonization of both rhizosphere and roots by plant growth-stimulating fluorescent pseudomonads and the suppression of colonization of the rhizosphere and roots by minor fungal pathogens were both associated with improved yield and quality of the crop in solarized soils or container media (10,12). These microbial changes, which were consistent and reproducible, are likely to be triggered by signals operating in the solarized soil.

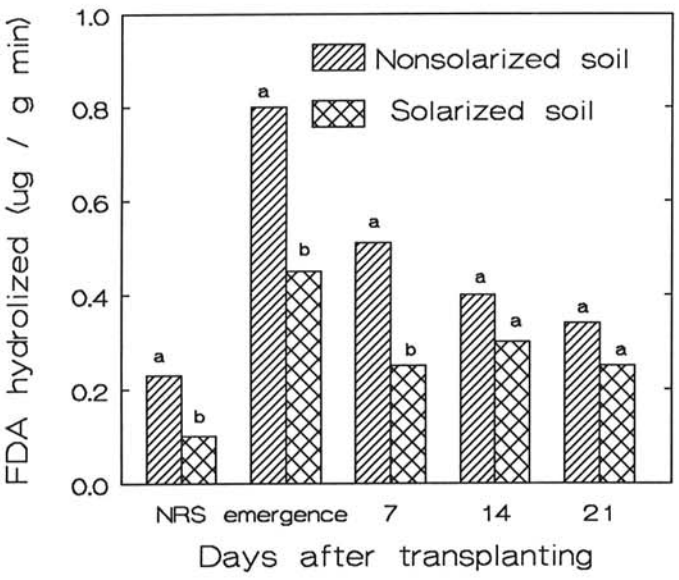

Fig. 5. Effect of soil solarization on microbial activity in the rhizosphere of tomato plants grown in nonsolarized (NS) or solarized (S) Rehovot soil, as determined by fluorescein diacetate (FDA) hydrolysis. Within each time period, different letters indicate significant differences among treatments according to the Student's *t* test. NRS = nonrhizosphere soil collected after the termination of solarization; emergence = rhizosphere soil of seedlings at emergence (8 days after sowing).

Solarization affected exudation both quantitatively and qualitatively. Exudates from roots and seeds in solarized soil contained higher amounts of amino acids and amino compounds and lower amounts of sugars as compared with nonsolarized soils. Some of the amino acid compounds (e.g., proline, phenylalanine, and tyrosine) were found at a very low or even below detectable level in exudates from plants in nonsolarized soil. Higher amounts of sugars and lower amounts of amino compounds in exudates have been reported as resulting from stress, such as nutrient deficiency, water stress deficit, and pathogens (18,20,22,32-34,36,38). Exudate content can be affected also by a brief stress that does not affect plant growth (36). These changes were attributed to alteration in membrane permeability (36). It is possible that the changes in exudation reflect an alleviation by solarization of the mild stress normally existing in untreated soil. Indeed, similar to the trend observed in solarized soil, exudates from seeds that were germinated on sterile filter paper contained lower amounts of sugars and higher amounts of amino acids and amino compounds (Tables 1 and 2). Quantitative and qualitative changes in other compounds that are usually present in exudates, such as volatiles, fatty and organic acids, vitamins, and growth factors (5,28,36,37) should be studied in future research.

Exudates originating from solarized soil were less favorable for multiplication of fluorescent pseudomonads in axenic sand culture than those from nonsolarized soil (Fig. 1) but the opposite was true when these exudates were examined in soil (Fig. 3). Regardless of the origin, addition of exudates (or external nutrients) to solarized soil resulted in a pronounced increase (up to 5,013-fold) in the population of fluorescent pseudomonads over addition to nonsolarized soil (Fig. 3). This is another demonstration of the limited value of results obtained in culture as representatives of microbial activities in soil niches. A stimulatory effect on fluorescent pseudomonads was exerted also by volatile exudates from germinating seeds (Fig. 4), in accordance with another study (26). The most pronounced effect was obtained when volatiles from seeds in solarized soils were applied to solarized soil (Fig. 4). According to Rovira and Sands (25), fluorescent pseudomonads are poor competitors in soil, and thus a reduction in the competition by other microorganisms is expected to favor these pseudomonads. Indeed, microbial inhibitors, especially the antifungal agent cycloheximide, increased populations of fluorescent pseudomonads in nonsolarized soil (Table 3). The reduced microbial activity in the rhizosphere of the solarized soil (Fig. 5; 40) and the reduction in fungal populations (Fig. 3; 10) apparently reflect a similar situation leading to improved competition in favor of the fluorescent pseudomonads. Root exudates originating from plants in solarized soil have a stimulatory effect on chemotaxis of fluorescent pseudomonads to the exudates (11). This may further contribute to an improved ability of fluorescent pseudomonads to compete in solarized soil. The addition of exudates to soil is a useful method for studying their effect on microorganisms. However, this system cannot mimic the gradient nature of exudate flow from the root to the rhizosphere. This is especially relevant to chemotaxis as related to the establishment of fluorescent pseudomonads on plant roots. Iron was suggested to play a key role in competition (7,21). However, changes in iron availability in both nonsolarized and solarized soil do not affect the establishment of fluorescent pseudomonads (Table 4).

Changes in root exudation in the solarized soil may stem from abiotic agents (e.g., mineral nitrogenous nutrients [4]) or biotic agents that are stimulated or suppressed in the solarized soil. These changes may affect membrane permeability or cellular metabolism in the plant tissues or both. It is possible that the enhanced growth of the plant in solarized soil results in intensive translocation from the roots leading to reduced sugar content in the roots and to reduced sugar exudation. Prikryl and Vancura (22) have reported an increase in amino acid content in root exudates of plants that were inoculated with *P. putida* in axenic culture. The relevance of this phenomenon to soil conditions has yet to be studied. Stimulation of fluorescent pseudomonads in the rhizosphere and roots in solarized soil is a widespread

phenomenon (10). It was verified in all 11 tested solarized soils and was evident already 2 days after planting (10). Elucidation of the mechanisms involved in the stimulation of fluorescent pseudomonads in these soils is important beyond solarization. Such a knowledge will be of great value for the application of beneficial microorganisms.

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